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Antiseptics and Disinfectants

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Introduction

Cleansers, antiseptics, and disinfectants play critical roles in preventing infectious disease transmission in veterinary medicine. From use as a presurgical scrub to disinfection after an outbreak, these products are relied upon by veterinarians for safe and effective germicidal activity. The beneficial effects of cleansing or disinfecting practices have been known for many years; the efficacy of hand washing was demonstrated as early as the 1840s by Ignaz Semmelweis, a Hungarian obstetrician. Following Pasteur's identification of infective agents as the cause of disease, Joseph Lister suggested the use of antiseptics in the field of surgery. His treatment of the hands with 1 : 20 carbolic lotion and his initiation of methods for chemical sterilization of bandages, dressings, and surgical instruments and for antisepsis of wounds began aseptic surgery.

Cleansers, antiseptics, and disinfectants are differentiated by their intended use and characteristic properties, not by their chemical content. A cleanser aids in physical removal of foreign material and is not necessarily a germicide. An antiseptic is a biocide applied to living tissue, whereas a disinfectant is a biocide applied to inanimate objects. Because certain antiseptics may be inactivated on inanimate surfaces and because certain disinfectants are hazardous to living tissue, the two should not be used interchangeably; however, these products may still have a very similar chemical content. Even products with the identical active chemical moiety may be formulated in such a way (e.g., exposure time, concentration) as to prevent their interchangeable use. Products formulated as disinfectants (and sanitizers or sterilants) to be used on inanimate surfaces, objects, or instruments are regulated by the Environmental Protection Agency (EPA). Antiseptics for use on living tissue must be registered with the Food and Drug Administration (FDA), along with some chemicals used on critical and semicritical devices.

Different cleaning, antiseptic, and disinfectant protocols exists for many different clinics, farms, procedures, and uses in veterinary medicine; no one compound is applicable, appropriate, or effective for every use.

Cleansers

Cleansers contain surfactants or detergents that remove dirt and contaminating organisms by solubilization and physical means. Cleansers are often a critical step to proper disinfection or antisepsis as removing gross contamination from an area prior to disinfection or antisepsis treatment maximizes their efficacy. Depending on the application and use, cleansing may be sufficient.

Cleansers can be classified into three types based on the presence and charge of the hydrophilic portion of the molecule: anionic, cationic, and nonionic. Soaps are anionic surfactants of the general structure R-COO−Na^+. Dissociation in water to R-COO− liberates a molecule with both a hydrophilic and a hydrophobic portion, which can emulsify and solubilize hydrophobic dirt, fat, and protoplasmic membranes. Once solubilized, this contamination can be rinsed away with water. The ability to solubilize membranes renders soaps antibacterial against gram-positive and acid-fast bacteria. The anionic nature of soaps, however, causes them to be inactivated in the presence of certain positive ions such as free Ca^+ in hard water and in the presence of cationic detergents. The mixture of soaps and quaternary ammonium compounds forms a precipitate, which terminates the activity of both compounds. Inclusion of antiseptic compounds in soap preparations has given them a wider antibacterial spectrum.

The quaternary ammonium compounds (QACs) are examples of cationic surfactants with germicidal activity. These compounds have been widely used as disinfectants (see Section Examples of disinfectant use in Veterinary Medicine). Cationic surfactants combine readily with proteins, fats, and phosphates and are thus of limited value in the presence of serum, blood, and other tissue debris (Huber, 1988). In addition, use with materials such as gauze pads and cotton balls makes them less germicidal owing to absorption of the active ingredients.

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Antiseptics

An antiseptic is a chemical agent that reduces the microbial population on skin and other living tissues. Because in most cases its mechanism of action involves nonspecific disruption of cellular membranes or enzymes, caution must be taken not to harm host tissue. An ideal antiseptic would have a broad spectrum of activity, low toxicity, high penetrability, would maintain activity in the presence of pus and necrotic tissue, and would cause little skin irritation or interference with the normal healing process.

The use of antiseptics has been suggested in situations which require maximal reduction of bacterial contamination (Larson, 1987) such as when defense mechanisms are compromised after surgery, during catheterization or insertion of other invasive implants, and in immunocompromised states due to immune defects, cytotoxic drug therapy, extreme old or young age, or extensive skin damage (burns and wounds).

Disinfectants

Disinfection is a process that eliminates most, if not all, pathogenic organisms, excluding spore forms, from an inanimate object. Disinfection is sometimes incorrectly confused with sterilization, a process that completely eliminates all microbial forms by a physical or chemical means. True chemical sterilization necessitates the use of an EPA-registered agent capable of killing all infective organisms, including fungal and bacterial spores, usually within 10 hours. Sometimes, however, chemical sterilants can be considered disinfectants when shorter exposure periods are used. The treatment of objects that are too large to soak in disinfectant, such as cabinets, exam tables, chairs, lights, and cages, is considered surface disinfection. Immersion disinfection is the immersion of smaller objects in disinfectant for sufficient time to kill the majority of contaminating, pathogenic organisms.

The ideal characteristics of a disinfectant includes a broad spectrum, fast action, activity in the presence of organic material (including blood, sputum, and feces), compatibility with detergents, low toxicity, low cost, ease of use, and residual surface activity. They should not corrode instruments or metallic surfaces or disintegrate rubber, plastic, or other materials, and should be odorless and economical (Molinari et al., 1982).

The ability to kill different classes of microorganisms further categorizes disinfectants into high, intermediate and low levels. High-level disinfection destroys all microorganisms except high concentrations of bacterial spores. Intermediate-level disinfection inactivates acid-fast microorganisms, including *Mycobacterium tuberculosis*, most viruses and fungi, but not necessarily bacterial spores. Low-level disinfection kills most bacteria, some viruses, and some fungi, but not tubercle bacilli or bacterial spores. In addition, low-level disinfection usually occurs in less than 10 minutes.

A second classification system is intended to divide instruments and patient-care items into three categories based on risk of infection involved in their use (Spaulding, 1968). In this system, items are classified as: (i) critical – those that enter or penetrate skin or mucous membranes (e.g., needles, scalpels), usually at a sterile site; (ii) semicritical – those that touch intact mucous membranes (e.g., anesthesia equipment, endoscopes); and (iii) noncritical – those that do not touch mucous membranes but may contact intact skin (e.g., stethoscopes, cages, tables, food bowls). In general, items classified as critical should be sterilized, semicritical items require high-level disinfection, and noncritical items require low to intermediate-level disinfection.

Popular Antiseptic and Disinfecting Agents

Alcohol

Alcohols are one of the most popular antiseptic and disinfecting products, used every day in veterinary clinics and laboratories. Although many alcohols are germicidal, the two most commonly used as disinfecting agents are ethyl and isopropyl alcohol. These compounds are both lipid solvents and protein denaturants. They kill organisms by solubilizing the lipid cell membrane and by denaturing membrane cellular proteins. Alcohols are most effective when diluted with water to a final concentration of 70% ethyl or 50% isopropyl alcohol by weight. It is thought that at greater concentrations, initial dehydration of cellular proteins makes them resistant to the denaturing effect (Molinari and Runnel, 1991). Alcohols have excellent antibacterial activity against most vegetative gram-positive, gram-negative, and tubercle bacillus organisms but do not inactivate bacterial spores. They are active against many fungi and viruses, principally enveloped viruses due to alcohol's lipid-solubilizing action.

The alcohols are not recommended for high-level disinfection or chemical sterilization due to their inactivity against bacterial spores and reduced efficacy in the presence of protein or other bioburden. Blood proteins are denatured by alcohol and will adhere to instruments being disinfected. Fatal *Clostridium* spp. infections have occurred postoperatively that were the result of contaminated surgical instruments that had been disinfected with alcohol containing bacterial spores (Nye and Mallory, 1923). After repeated and prolonged use, alcohols can damage the shellac mounting of lensed instruments, can swell or harden rubber and certain

plastic tubing (Rutala, 1990), and can be corrosive to metal surfaces. Alcohols are flammable; thus caution must be taken in their storage and when used prior to electrocautery or laser surgery. In deciding between ethyl and isopropyl alcohol, it is important to consider isopropyl's inactivity against hydrophilic viruses, its less corrosive nature, and the abuse potential for ethyl alcohol (grain alcohol).

Both isopropyl and ethyl alcohol are also commonly used, effective antiseptics, with only subtle differences in their action. Because their effectiveness is drastically reduced by organic matter such as feces, mucus, and blood, they are most effective on "clean" skin. They produce rapid reduction in bacterial counts (Lowbury et al., 1974), with contact times of 1–3 minutes, resulting in elimination of almost 80% of organisms. Rapid evaporation limits contact time; however, residual decreases in bacterial counts are seen to occur after the alcohol has evaporated from the skin. Although alcohols are among the safest antiseptics, toxic reactions have been reported in children. Alcohol can be drying to the skin and can cause local irritation. In efforts to minimize this drying effect, emollients such as glycerine have been added with good results (Larson et al., 1986).

Halogens

Iodine and chlorine both demonstrate antimicrobial activity and are used as antiseptics or disinfectants. Elemental iodine has germicidal activity against gram-positive and gram-negative bacteria, bacterial spores, fungi, and most viruses. It exerts these lethal effects by diffusing into the cell and interfering with metabolic reactions and by disrupting protein and nucleic acid structure and synthesis. Iodine has a characteristic odor and is corrosive to metals. It is insoluble in water and thus is prepared in alcohol (tincture) or with solubilizing surfactants ("tamed" iodines). Tincture of iodine, used as early as 1839 in the French Civil War, is most effectively formulated as a 1–2% iodine solution in 70% ethyl alcohol. In this form, most (approximately 90%) bacteria are killed within 3 minutes of application. The antibacterial activity of this combination is greater than that of the alcohol alone. Tincture of iodine, however, is irritating and allergenic, corrodes metals, and stains skin and clothing. It is also painful when applied to open wounds and is harmful to host tissue; therefore, it can delay healing and increase the chance of infection. For these reasons, this preparation has fallen out of favor as an antiseptic or disinfectant. Strong tinctures of iodine have been used as blistering agents in the equine industry.

Efforts to reduce the undesirable aspects of tinctures while retaining the powerful killing action of iodine have led to the introduction of tamed iodines known as iodophors. In this preparation, iodine is solubilized by surfactants, which allow it to remain in a dissociable form. Application of this product allows for slow continual release of free iodine to exert its germicidal effects. The iodophors have a similar spectrum of activity to aqueous solution; are less irritating, allergenic, corrosive, and staining; and have prolonged activity after application (4–6 hours). Common solubilizing carriers include polyvinylpyrrolidone (called PVP-iodine or povidone-iodine, PI) as well as other nonionic surfactants, making iodophors excellent cleansing agents as well as antiseptics and disinfectants. Iodophor solutions retain their activity in the presence of organic matter at pH <4 (Huber, 1988). The water-soluble carriers have been postulated to interact with epithelial surfaces to increase tissue permeability, thereby enhancing iodine's killing efficacy.

Proper dilution to 1% iodine is necessary for maximum killing effect and minimal toxicity. More-concentrated solutions are actually less efficacious, presumably due to stronger complexation preventing free iodine release. It takes approximately 2 minutes of contact time for release of free iodine (Lavelle et al., 1975). Literature reports indicate that iodophors are quickly bactericidal, virucidal, and mycobactericidal but may require prolonged contact times to kill certain fungi and bacterial spores. Iodophors formulated as antiseptics are not suitable as hard-surface disinfectants, due to insufficient concentrations of iodine.

Consideration must be taken of iodine's ability to be systemically absorbed through the skin and mucous membranes. The extent of absorption is related to the concentration used, frequency of application, and status of renal function (the principal excretory route) (Swaim and Lee, 1987). Complications of iodophor absorption include increased serum enzyme levels, renal failure, metabolic acidosis (Pretsch and Meakins, 1976), and increased serum free iodide. If renal function is normal, serum iodine concentrations quickly return to normal. Clinical hyperthyroidism and thyroid hyperplasia have been reported after treatment with PI (Scheider et al., 1976; Altemeier, 1976).

Chlorine-containing solutions were first introduced by Dakin in the early 1900s in the chemical form of sodium hypochlorite. They are effective bactericidal, fungicidal, virucidal, and protozoacidal agents. The chemical forms most commonly used today include the hypochlorites (sodium and calcium) and organic chlorides (chloramine-T). In either form, the germicidal activity is due to release of free chlorine and formation of hypochlorous acid (HOCl) from water. The mechanisms of action of these compounds include inhibition of cellular enzymatic reactions, protein denaturation, and inactivation of nucleic acids (Dychdala, 1983). Dissociation of HOCl to the less microbicidal hypochlorite ion (OCl^-) increases as pH increases, and thus the solution may be

rendered ineffective above pH 8.0 (Weber, 1950). Mixing NaOCl with acid liberates toxic chlorine gas, and NaOCl decomposes when exposed to light.

Low concentrations of free chlorine are active against M. tuberculosis (50 ppm) and vegetative bacteria (<1 ppm) within seconds. Concentrations of 100 ppm destroy fungi in less than 1 hour, and many viruses are inactivated in 10 minutes at 200 ppm. Household bleach is 5.25% (52,500 ppm); thus dilutions of 1 : 100 to 1 : 250 should result in effective germicidal concentrations although more-concentrated solutions are often recommended (1 : 10 to 1 : 100).

The use of the hypochlorites as disinfectants are limited by several characteristics. Chlorine solutions are corrosive to metals and destroy many fabrics. Because chlorine solutions are unstable to light, they must be prepared fresh daily. Hypochlorites are inactivated by the presence of blood more so than are the organic chlorides (Bloomfield and Miller, 1989). They have a strong odor and are not suitable for enclosed spaces. In addition, hypochlorites may lead to irritation of mucous membranes and may form toxic bioproducts when interacting with other chemicals. Despite these shortcomings, chlorine solutions are commonly used as low-level disinfectants on dairy equipment, animal housing quarters, hospital floors, and other noncritical items. Of 12 disinfectant solutions evaluated for their ability to kill the dermatophyte *Microsporum canis*, those containing hypochlorite were most effective. Also found effective were benzalkonium chloride and glutaraldehyde-based products; phenolics and anionic detergents were considered inadequate (Rycroft and McLay, 1991). The hypochlorites are not recommended for routine use as antiseptics because they are very irritating to skin and other tissues and they delay healing. There is, however, research to suggest diluted household bleach can be applied to control superficial pyoderma in dogs.

Several compounds from a class called N-halamines (oxazolidinones or imidazolidinones) have been developed, which are water-soluble solids that have been shown to be bactericidal, fungicidal, virucidal, and protozoacidal in water disinfection at low total halogen concentrations (1–10 mg/l). They are noncorrosive and tasteless and odorless in water. They are extremely stable in water even in the presence of organic loads. Their potential use in poultry processing to control *Salmonella* has been evaluated (Smith et al., 1990).

Biguanides

Chlorhexidine (Chx) is popular synthetic cationic antiseptic compound (1-1'-hexamethylenebis[5-(p-chlorophenyl)biguanide]) with better activity against gram-positive than against gram-negative organisms. The compound lacks sporicidal activity. Chlorhexidine kills bacteria by disrupting the cell membrane and precipitating cell contents. It has also been suggested that membrane-bound adenosine triphosphatases, specifically inhibition of the F1 ATPase, may be a primary target for Chx (Gale et al., 1981). It is active against fungi, fairly active against *M. tuberculosis*, but poorly active against viruses. The antibacterial activity of Chx is not as rapid as that of the alcohols; however, as a 0.1% aqueous solution, significant killing action is evident after only 15 seconds. Additionally, Chx solutions have the longest residual activity, remaining chemically active for 5–6 hours and retaining their activity in the presence of blood and other organic material. Being cationic, it is inactivated by hard water, nonionic surfactants, inorganic anions, and soaps. Dilution with saline causes precipitation and its activity is pH dependent. It has extremely low toxicity even when used on intact skin of newborns (O'Neill et al., 1982). Chlorhexidine is available in a detergent base as a 4% solution or as a 2% liquid foam. Traditionally, it has widely been used as a presurgical antiseptic, wound flush, and teat dip. Formulations of chlorhexidine and alcohol have also been described and appear to improve efficacy. Its use as a disinfectant are not well described.

Polyhexamethylene biguanide (PHMB) is a polymeric biguanide with activity against gram-positive and gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus equi*. PHMB rapidly kills bacteria by disrupting the cytoplasmic membrane resulting in leakage and precipitation of cellular contents (Broxton et al., 1983). PHMB has been used to treat infections in the eye, mouth, and vagina and has been formulated in contact lens disinfectants and mouth rinses. It was shown to be nontoxic as a component of an ear flush for dogs (Mills et al., 2005) and when impregnated in a gauze wound dressing, reduced growth of underlying gram-positive and gram-negative bacteria in vitro (Lee et al., 2004).

Aldehydes

Two related aldehyde disinfectants are formaldehyde and glutaraldehyde (GLT). Formaldehyde has antimicrobial activity both as a gas and in liquid form. Formalin, the aqueous form, is 37% formaldehyde by weight. It inactivates microorganisms by alkylating the amino and sulfhydryl groups of proteins and ring nitrogen atoms of purine bases (Favero, 1983). Formaldehyde is an effective but slow bactericide, virucide, and fungicide, requiring 6–12 hours contact time. It is effective against *M. tuberculosis*, bacterial spores, and most animal viruses, including foot-and-mouth disease virus. Its action is not

affected by organic matter and it is relatively noncorrosive to metals, paint, and fabric. Formaldehyde alone is considered a high-level disinfectant and in combination with alcohol can be used as a chemical sterilant for surgical instruments. However, due to irritating fumes and pungent odor at low concentrations (approximately 1 ppm), and because the National Institute for Occupational Safety and Health requires it to be handled as a potential carcinogen, thereby limiting worker exposure time, formaldehyde's use as a disinfectant has been limited.

Glutaraldehyde, a saturated dialdehyde, is similar to formaldehyde but without some of its shortcomings. It has better bactericidal, virucidal, and sporicidal activity than formaldehyde. Its biocidal activity is related to its ability to alkylate sulfhydryl, hydroxyl, carboxyl, and amino groups affecting RNA, DNA, and protein synthesis (Scott and Gorman, 1983). Acidic GLT solutions are not sporicidal; thus, they must be "activated" by alkalinizing agents to a pH between 7.5 and 8.5. Once activated, these solutions have a limited shelf life (14 days) due to polymerization of the GLT molecules (Rutala, 1990). Newer formulations (stabilized alkaline GLT, potentiated acid GLT, GLT-phenate) have increased shelf life (28–30 days) and excellent germicidal activity (Pepper, 1980). GLT has gained wide acceptance in high-level disinfection and chemical sterilization due to several favorable properties, including wide spectrum of activity. Low surface tension allows GLT to penetrate blood and exudate without coagulating proteins. It retains its biocidal activity in the presence of organic matter. It is noncorrosive to metal, rubber, and plastic and does not damage lensed instruments. GLT solutions must be used in well-ventilated areas, since air concentrations of 0.2 ppm are irritating to the eyes and nasal passages (CDC, 1987). Contact times of less than 2 minutes for vegetative bacteria, 10 minutes for fungi, and 3 hours for bacterial spores were necessary using a 2% aqueous alkaline GLT solution (Stonehill et al., 1963). Activity against the tubercle bacillus was found to be somewhat variable; at least 20 minutes at room temperature is needed to reliably kill these organisms with 2% GLT. When used as a high-level disinfectant, a minimum of 1% GLT should be used. GLT-phenate formulations should be used with caution since they were shown to be less effective than other aldehyde solutions in decreasing bacterial counts from some medical instruments (Ayliffe et al., 1986). GLT disinfectants were found to more effectively reduce duck hepatitis B virus infectivity when they contained additives such as alcohol, an ammonium chloride derivative, and a surfactant (Murray et al., 1991). The caustic nature of both formaldehyde and GLT makes them inappropriate as antiseptics, and in fact, protective gloves should be worn when using the aldehyde disinfectants.

Gluteraldehyde and QAC combinations have been formulated (e.g., Synergize^{TM}, Preserve International, Reno, NV) and largely marketed as a cleaner and disinfectant for use in animal (e.g., swine and poultry) production facilities.

Oxidizing Compounds

Conflicting reports concerning hydrogen peroxide's efficacy as a germicide make evaluating its utility in disinfection and antisepsis difficult. Although it has been reported to have bactericidal (Schaeffer et al., 1980), virucidal (Mentel and Schmidt, 1973), and fungicidal (Turner, 1983) activity, the activity of hydrogen peroxide is nonpenetrable and short lived. For this reason hydrogen peroxide antiseptic use is most valuable in the initial treatment of recently contaminated wounds. Because 3% hydrogen peroxide has been shown to be damaging to tissues, including fibroblasts (Lineweaver et al., 1982), it is not considered suitable for routine wound care. It is, however, considered a stable and effective disinfectant and is used in the disinfection of soft contact lenses. More recently, accelerated hydrogen peroxide products have been formulated to also contain a surfactant and stabilizer, which improve antimicrobial activity. These products are being implemented in many veterinary clinic settings for use as a disinfectant.

Other oxidizing agents include potassium peroxymonosulfate (PPMS), an oxidizing agent used in disinfection systems of pools and hot tubs. More recently, it has been formulated with potassium chloride and organic acids and salts (i.e., sulphamic acid, malic acid, sodium hexametaphosphate, and sodium dodecyl benzene sulphonate) resulting in a disinfectant effective against over 580 infectious agents including viruses, gram-positive and gram-negative bacteria, fungi (molds and yeasts), and mycoplasma (EPA Master Label). It is marketed as a powder because it is stable in solution for approximately 1 week. It is not inactivated by organic challenge and has been found to be user friendly to both humans and animals. It is widely used as a high-level disinfectant for surfaces in laboratories, dental care facilities, and hospitals; for decontaminating laundry; for air disinfection; and in food processing and transport. Use of peracetic acid, sodium perborate, benzyl peroxide, and potassium permanganate have also been reported in human and veterinary literature.

Phenols

Carbolic acid, a phenol, is the oldest example of an antiseptic compound. However, due to severe toxicity, it is

no longer appropriate for use as an antiseptic. These agents act as cytoplasmic poisons by penetrating and disrupting microbial cell walls. Most commercially available phenolic products contain two or more compounds that act synergistically, resulting in a wider spectrum of activity, including against *M. tuberculosis*. Sodium o-phenylphenol is effective against staphylococci, pseudomonads, mycobacteria, fungi, and lipophilic viruses, and against ascarids, strongyles, and tichurids. Cresols are substituted phenols and are more bactericidal and less toxic and caustic than phenols. Phenolics are not recommended for disinfection of anything other than noncritical items, because of residual disinfectant on porous materials causing tissue irritation even when the items have been thoroughly rinsed, because of strong odors, and because of absorption into feed.

Triclosan (Irgasan DP 300; 2,4,4′ trichloro-2′-hydroxydiphenyl ether) is a chlorinated diphenyl ether or bisphenol that possesses high antibacterial activity particularly against many gram-positive (e.g., *Bacillus subtilis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*, *Salmonella enterica* serotype Typhimurium, *Shigella flexneri*) as well as fungi and yeasts (Stewart et al., 1999). Triclosan has been used for over 30 years and was first introduced in the health care industry in a surgical scrub in 1972. However, recently there has been a rapid increase in the use of triclosan-containing products including soaps, disinfectants, deodorants, shampoos, and medical supplies. In addition, it can be incorporated into plastics (e.g., children's toys) and fibers to retard decomposition.

The mechanisms of action of triclosan have been debated, but it is likely that they are concentration dependent. At low concentrations, triclosan acts as a competitive inhibitor of bacterial enoyl-acyl carrier protein reductase, which is involved in the bacterial fatty acid elongation cycle. At higher concentrations, because of its lipophilicity, triclosan has been shown to incorporate into bacterial membranes to alter the physicochemical properties of the lipid bilayer, including perturbation of the packing and interaction between membrane phospholipids (Guillen et al., 2004). Resistance due to specific mutations (Heath et al., 1999) of the bacterial carrier protein has been demonstrated in *S. aureus* and *E. coli* (Fan et al., 2002). The clinical significance of this decreased sensitivity remains questionable since concentrations achieved during triclosan use are likely high enough for the generalized bactericidal activity to prevail. Decreased sensitivity has been demonstrated in bacteria that overexpress the AcrAB efflux pump (Wang et al., 2001). The possibility and data suggesting that these resistance mechanisms may not only confer resistance to triclosan but also to other antibiotics has led to concern about the ubiquitous use of this compound in detergents, toothpaste, and other household items.

Gases

Technologies using ethylene oxide or hydrogen peroxide gas plasma for sterilization have been described. These products may be toxic and are not appropriate for antisepsis but could be useful in the treatment of temperature-sensitive medical devices or equipment. Ethylene oxide (C_2H_4O) is a water-soluble flammable gas. Mixing ethylene oxide with carbon dioxide or fluorocarbons reduce its flammability. Ethylene oxide kills bacteria, fungi, yeasts, viruses, and spores. Bacterial spores are only two to ten times more resistant to the cidal activity than are vegetative cells. It has been shown that the relative humidity of the microenvironment is critical to microbial susceptibility to ethylene oxide. Activity is decreased in the presence of organic matter due to interaction with proteins and nucleic acids. Care must be used to contain the gas as it has an irritant effect on the skin and eyes and may cause headaches and nausea. Hydrogen peroxide gas plasma has also been reported to have efficacy against a broad range of microorganisms, including bacterial spores.

Formaldehyde gas inactivates viruses, fungi, bacteria, and bacterial spores. Its activity is dependent on relative humidity and its efficacy is thought to peak at less than 50% relative humidity. Formaldehyde has been used for disinfection of hospital linen and for terminal disinfection in certain food-producing industries (see Section *Salmonella*). Propriolactone, methyl bromide, and propylene oxide have also been used as gas disinfectants.

Factors Affecting Efficacy of Antiseptics and Disinfectants

As indicated above, many factors can influence the efficacy of antiseptics and disinfectants. Often, more than one of these factors is contributing to the efficacy of an antiseptic or disinfecting product in a clinical setting.

Concentration

In general, the time needed to kill an organism is inversely dependent upon antimicrobial concentration; however, for certain compounds a small decreases in concentration may result in large increases required for killing whereas other compounds are less sensitive to changes in concentration. Alcohols and phenolics are very concentration dependent, whereas QACs, aldehydes, and chlorhexidine are less sensitive.

Table 31.1 Q_{10} coefficients for selected disinfectant/antiseptic compounds

Compound	Q_{10} Coefficient
Formaldehyde	1.5
β-propriolactone	2–3
Ethylene oxide	2.7
Phenol and cresol	3–5
Aliphatic alcohols	30–40

Exposure Time

A minimum contact time is required for efficacy of most antiseptics and disinfectants. All registered EPA disinfectants are labeled with an appropriate contact time. Reducing the exposure time below recommended times could lead to incomplete germicide activity.

Temperature

Increased temperature results in increased antimicrobial activity. This relationship can be described by $Q_{(T2-T1)}$ = (time to kill at T_1) / (time to kill at T_2), where T_2 and T_1 are two different temperatures in centigrade degrees. This equation is commonly referred to as the Q_{10} coefficient and describes the change in activity caused by a 10°C rise in temperature. Table 31.1 lists the Q_{10} coefficient for certain disinfectant compounds.

pH

The pH at the site of action may affect a compound's activity by two mechanisms, influencing the compound or the microbial cell. Molecules such as phenols, and certain acids, including hypochlorous acid (bleach), are effective only in the unionized form, thus as pH increases they become less efficacious. Glutaraldehyde is more potent at alkaline pH but is more stable at acid pH. Increased pH results in higher numbers of negative charges on cell surfaces with which positively charged molecules, such as QACs and chlorhexidine, can interact, thereby increasing their activity. Lastly, in a process similar to absorption through any cell membrane, pH can effect partitioning from the bathing solution into the cell's interior.

Contamination

The most important step to maximize antiseptic or disinfectant efficacy is thorough cleaning and washing of the site or area prior to application. Organic matter such as blood, pus, feces, soil, food, and milk, are believed to directly reduce the activity of antimicrobial compounds via a chemical reaction which results in a smaller amount of compound available for killing microorganisms or by spatial nonreaction (the inability of the disinfectant molecule to get to the organism). Certain compounds (hypochlorites and iodines) are more susceptible to this type of interference than others. Glutaraldehyde is less affected by organic contamination than other compounds and is therefore useful for instruments whose surface or design make it impossible to thoroughly clean. In a veterinary setting, contamination can make disinfection of large animal facilities difficult and may require removal of surface layers of soil and bedding for complete treatment. The presence of inorganic ions, Ca^{+2}, Mg^{+2}, Na^{+}, and Cl^{-} may be physically incompatible with certain antiseptics/disinfectants and therefore dilution with either hard water or saline solutions may render these formulations ineffective.

Organism Type

The sensitivity of different organism types (bacteria, virus, fungi, and spores) have been previously discussed for levels and types of disinfecting agents. Within an organism type, differences between genera and species also exist, which may render a particular disinfection process ineffective against certain microbes yet effective against others. Gram-positive bacteria are in general less resistant to disinfectant and antiseptic compounds than are gram-negative organisms due to a less complex and less lipid-rich cell wall. Staphylococci are less susceptible to alcohols, glycols, and ethylene oxide than are other cocci. Of the gram-negative bacteria, *Pseudomonas aeruginosa* have been identified as more resistant to antimicrobial agents, especially QACs, than other species. Mycobacteria, due to the unusual and hydrophobic nature of their cell wall, are highly resistant to many compounds. Bacterial sporicides include the aldehydes, hydrogen peroxide, hypochlorites, iodine, acid alcohol, and ethylene oxide. By inhibiting germination or spore outgrowth, phenols, QACs, biguanides, and alcohols are sporostatic. The efficacy of most germicides against bacterial spores increases with temperature; however, the most effective method against bacterial spores is moist heat (115°C autoclaving). Fungi are sensitive to chlorine, phenols, iodine compounds, ethylene oxide, and the aldehydes, whereas QAC are fungistatic. Fungal spores are resistant to most disinfectants. The sensitivity of viruses to disinfectant compounds relates to the composition of the viral envelope. The lipid-enveloped viruses are readily inactivated by lipophilic agents such as ether, chloroform, phenols, QACs, and even detergents. The nonenveloped viruses are resistant to these agents but are sensitive to chlorine and the aldehydes. Formaldehyde and β-propriolactone are used to inactivate viruses in the production of viral vaccines utilized in veterinary medicine.

Formation of Biofilms

Bacteria present on metal or other surfaces may form a biofilm (Mafu et al., 1990) that is an adherent slimy layer consisting of an organic polymer matrix in which microbes are embedded. In addition, the intercellular matrix contains products of cellular metabolism including ions, nutrients, and enzymes such as polysaccharases, proteases, and β-lactamases. Differing cellular densities and extracellular concentrations of factors results in diverse cellular phenotypes with regard to growth rate, nutrient deprivation, etc. Bacteria in biofilm are less sensitive to disinfectant inactivation than are those grown in culture broth (i.e., planktonic). Proposed reasons for this increased resistance include decreased diffusion of disinfectant solution through polymer matrix preventing centrally located cells from being exposed to lethal concentrations of the compound. Chemical or enzymatic modification by extracellular components or decreased inherent microbe susceptibility due to slow growth rate or starvation responses may also contribute to increased bacterial survival in biofilms (Gilbert et al., 2002).

Resistance to Antiseptics and Disinfectants

Similar to resistance towards traditional antibiotics, bacteria can acquire genes that are associated with resistance toward antiseptic and disinfectant compounds. In general, acquisition of a genetic element (e.g., plasmid, transposon) or a chromosomal mutation results in bacteria with reduced susceptibility or increased tolerance; acquired resistance may not mean failure of an antiseptic or disinfectant, however. Concentrations of disinfectants in practice are generally much higher than the cidal concentration required to kill bacteria in vitro.

Staphylococcus aureus has been shown to be resistant to triclosan, quaternary ammonia compounds, and chlorhexidine (Heath and Rock, 2000; Suller and Russell, 1999, 2000). There are also reports of low-level resistance to QACs and chlorhexidine in *Pseudomonas* species (Mechin et al., 1999; Bamber and Neal, 1999; Tattawasart et al., 1999). However, this resistance has been considered unstable and not clinically significant (Russell, 2000). The possibility that the mechanisms of resistance developed against a disinfectant or antiseptic could confer resistance to an antibiotic, however, is considered quite possible and potentially clinically disastrous. It may also be possible that resistance genes associated with disinfecting or antiseptic agents are located on the same genetic element as genes encoding for antibiotic resistance.

The mechanisms of action of antibiotics are well known and in most cases, take advantage of a single specific target (e.g., inhibitors of peptidoglycan, protein, and nucleic acid synthesis, inhibitors of RNA polymerase, DNA gyrase) in their ability to kill or suppress the growth of bacteria. This is in contrast to mechanisms of action of disinfectants and antiseptics, which are less well understood and often involve more general and multiple cellular targets (Denyer and Stewart, 1998). These include interactions with the cell wall or the envelope, disruption of membrane integrity, interruption of the proton-motive force, and inhibition of membrane enzymes, or as alkylating, cross-linking, and intercalating agents. Similarly, the mechanisms of resistance to antibiotics have been better characterized than those to biocides. Changes in the drug's target (e.g., methylation of the ribosome, penicillin-binding protein alterations), impermeability to the drug (e.g., intrinsic gram-negative resistance, biofilm formation), enzymatic modification or destruction of the drug (e.g., β-lactamases), and increased efflux of the drug can confer resistance to antibiotics.

As mentioned, there is evidence of resistance to disinfectants developing or being measured in vitro; however, because of redundancy in the number of targets for activity and the ability to achieve very high concentrations of biocide chemicals at the site of contamination, in vivo or clinical resistance to these compounds is not thought to be prevalent. However, because several of the mechanisms that allow for this resistance are common to those that provide resistance to antibiotics (efflux, impermeability, modification of target sites), the possibility for decreased efficacy to antibiotics is real.

β-lactam resistance in association with resistance to quaternary ammonia compounds has been demonstrated in *S. aureus* (Akimitsu et al., 1999). Triclosan-resistant mycobacteria were also resistant to isoniazid (McMurry et al., 1999), and there are many reports of biocide–antibiotic cross resistance in gram-negative bacteria. For example, *E. coli* that are resistant to triclosan (McMurry et al., 1998) or pine oil (Moken et al., 1997) have been shown to display the multiple antibiotic resistance (mar) phenotype. It is generally believed that in *E. coli* the mar phenotype is attributable to increased efflux due to up-regulation of the efflux pump (e.g., AcrAB-ToIC) (Okusu et al., 1996), which can cause resistance to β-lactams, chloramphenicol, fluoroquinolones, and tetracyclines.

The discovery of ciprofloxacin-resistant *E. coli* on farms with no previously reported quinolone exposure suggests that a disinfectant caused antibiotic resistance (Randall et al., 2005). Based on laboratory investigations designed to induce antibiotic resistance by repeated exposure to three different disinfectants, these authors conclude that, although bacteria became less sensitive to fluoroquinolones, this mechanism could not produce clinically resistant strains from fully susceptible ones.

They further conclude that the risk of disinfectant exposure giving rise to multiple antibiotic resistant bacteria is outweighed by the value of sanitation provided by these compounds.

Examples of Antiseptic Use in Veterinary Medicine

Several resources, including the Centers for Disease Control and Prevention and World Health Organization, outline the role of antiseptics and disinfectants in human health care settings; many of the principals discussed in those references could be applied to veterinary hospitals. Discussions and research on antiseptic and disinfectant practices specific to veterinary medical settings are on-going and recommendations are expected to become more refined. Below are several specific examples of the role of antiseptics and disinfectants in veterinary medicine.

Presurgical Skin Cleansers

Skin cleansers are important in the presurgical antisepsis of both the surgeon and the patient. Historically, the recommendations for presurgical antisepsis of the surgeon include two alternatives. The first involves an initial water-and-soap cleansing followed by use of an alcohol-based rub for at least 5 minutes. The second and more traditional method consists of a 5-minute chlorhexidine or iodophor hand scrub. Alcohol hand rubs are effective in immediately eliminating pathological bacteria on the hand skin surface and have a prolonged period of action superior to traditional antiseptics. Chlorhexidine techniques have the advantage that the active agents have residual bactericidal activity under surgical gloves; however, they have been shown to have negative effects on the skin of health care workers, resulting in tissue disruption, elimination of beneficial deeper microflora, and predisposition to colonization with pathogenic bacteria. For this reason, the World Health Organization (WHO) now recommends alcohol-based handrubs as the gold standard (WHO, 2009). The presence of organic material and dirt can decrease the effectiveness of most antiseptics; thus removal of gross contamination should precede any antiseptic scrub. Additionally, an important reservoir of dirt and bacteria that needs to be specifically addressed is the subungual space (McHinley et al., 1988).

Preoperative preparation of the veterinary patient varies depending on the surgical environment, yet attempts to achieve the optimal antiseptic cleansing can aid in limiting postsurgical infections. Contrary to human surgery, hair removal from the operative site is almost always a necessity with animal patients. Clipping hair is superior to shaving since it causes less damage and less favorable conditions for bacterial colonization of the surgical skin site (Alexander et al., 1983). Removal of gross contamination and dirt should precede use of antiseptics for previously mentioned reasons. Gentle antiseptic scrubbing should begin at the incision site and move outward over the entire surgical area. Consideration of proper antiseptic contact times should be made. A final antiseptic spray is often applied and left to dry on the surgical site. Despite even the most careful presurgical preparation, up to 20% of skin-resident bacteria may be unaffected by skin antiseptic cleansing (Smeak and Olmstead, 1984). Characteristics of an ideal skin antiseptic include broad spectrum, rapid killing, persistent lethal effect, cleansing effect, lack of skin irritation, noninhibition of healing, and activity in the presence of organic material.

In one study, three antiseptic combinations were evaluated for surgical preparation of canine paws (Swaim et al., 1991): 7.5% PI scrub/10% PI solution, 2% Chx acetate scrub/2% Chx diacetate solution, and tincture of green soap/70% isopropyl alcohol combinations were each shown to effectively reduce bacterial colony counts. The first two combinations were also effective in residual killing when applied under a sterile bandage for 24 hours. However, no significant advantage of applying the antiseptics 24 hours prior to surgery was shown. This is in contrast to results in human patients, where antiseptic cleaning the night prior to surgery has resulted in fewer wound infections (Garibaldi et al., 1988). A similar technique involving prophylactic antiseptic cleansing and wrapping of a limb overnight has been shown to reduce contamination of equine orthopedic surgical sites (Stewart, 1984). For surgery of the foot, additional reduction in bacteria load was achieved by removal of the superficial layer of the hoof; however, counts remained above a level that might predispose the surgical site to infection (Hennig et al., 2001). Antibacterial agents found in shampoos were shown to prevent infections caused by *S. intermedius* in a skin infection model in beagles. Shampoo containing 3% benzoyl peroxide was most effective, followed by shampoos containing 0.5% Chx acetate and iodine (1.0% polyalkyleneglycol-iodine) (Kwochka and Kowalski, 1991).

Another study compared the presurgical efficacy of chlorhexidine to a stabilized glutaraldehyde compound. Glutaraldehyde is most commonly associated with disinfection of inanimate objects; however, in its stabilized form it was noncorrosive, nonvolatile, nontoxic, biodegradable, stable, and highly microbiocidal at neutral pH. Stabilized glutaraldehyde, with and without alcohol, and chlorhexidine with alcohol had similar and significant ability to reduce and maintain surface bacteria levels and therefore were recommended for presurgical antiseptic prophylaxis in elective (noncontaminated) procedures (Lambrechts et al., 2004).

Treatment of Open Wounds

The treatment of open wounds is an important procedure in veterinary medicine. The processes involved in wound healing and proper wound care have been reviewed (Swaim and Wihalf, 1985; Berk et al., 1992). Issues involved in the decision of how to properly treat a wound include patient age and general health status, and the age, cause, size, and extent of contamination of the wound. Treatment options include surgical closure, bandaging (of different types), and irrigation or application of a varied group of topical agents, including saline, antiseptics, antibiotics, and local anesthetics. It is important to recognize that each wound has different characteristics and thus treatment must be individualized. For all wounds, however, a basic principle to which all caregivers should adhere is "above all, do no harm"; that is, any agent chosen should not impede the healing process. When treating a wound topically, a general guideline would be not to apply anything that should not be placed in the patient's conjunctival sac (Peacock, 1984).

The literature is divided concerning the utility of antiseptics in routine wound care. Some authors contend that this practice reduces the incidence of infections as a complication (Zukin and Simon, 1987), while others believe that any benefit is outweighed by the potential for these agents to cause tissue damage (Oberg and Lindsey, 1987). Once the healing process has begun, however, the use of more-benign agents may be indicated. Saline has been shown to be an effective means of eliminating debris and lowering bacterial counts (Stevenson et al., 1976). Hypertonic saline has also been proposed as a wound dressing (Lowthian and Oke, 1993). Archer et al. (1990) report that surface colonization of wounds does not impede healing and thus recommend a move away from potentially damaging antiseptics.

Many reports in the literature discuss potential toxic and harmful effects of antiseptics on fragile healing tissues, making their use controversial. A 5% PI solution inhibited local leukocyte migration, fibroblast activity, and wound cellularity (Viljanto, 1980). In vitro, neutrophil migration was inhibited at concentrations greater than 0.05% (Tvedten and Till, 1985), whereas 1% PI killed fibroblasts and resulted in weaker wound breaking strength (Lineweaver et al., 1985). Detergent scrubs containing PI and other surfactants were found to damage wound tissue and therefore are not recommended for wound care (Rodheaver et al., 1982). A maximum of 1% PI solution has been recommended as the most effective and least tissue-toxic dilution for wound irrigation (Swaim and Lee, 1987). Because antibacterial activity lasts 4–6 hours, repeated treatment is necessary for optimal results.

Chlorhexidine's residual activity (possibly by binding to proteins of the stratum corneum) and its activity against many organisms, make it a useful wound treatment. In an experimental wound infection model, wounds irrigated with 0.05–1% Chx diacetate solution had fewer infections than those treated with 0.1–0.5% PI. Concentrations of Chx gluconate 0.5% or greater were effective against *S. aureus* in vitro; however, concentrations above 0.05% were lethal to equine fibroblasts (Redding and Booth, 1991) and in a wound model in pigs. Unfortunately, it also delayed healing to a greater extent than other solutions tested, including PI (Archer et al., 1990).

Chlorine solution, such as sodium hypochlorite, was used as an effective wound flush in World War I. Full strength Dakin's solution (0.5% NaOCl) kills bacteria and fibroblasts, as well as retarding epithelialization in vivo in rats (Lineweaver et al., 1985). Other studies have shown low concentrations (0.025–0.0025%) to be toxic to neutrophils, fibroblasts, and endothelial cells, prompting one author to recommend abandoning the use of NaOCl as an irrigant (Kozol et al., 1988). In contrast, a concentration of 0.025% NaOCl was shown to be bactericidal while having no in vitro or in vivo tissue toxicity, suggesting a modified Dakin's solution may be a safe and effective fluid dressing (Heggers et al., 1991). Chloramine-T (Chlorazene) was shown to reduce in vitro *Pseudomonas aeruginosa* growth and the ability of the bacteria to colonize experimentally created wounds in guinea pigs. Additionally, Chlorazene was seen not to delay the healing of these wounds at a concentration of 0.03% (Henderson et al., 1989). Thus, it was concluded that this preparation should have no effect on healing of wounds when used to sanitize hydrotherapy units. More recently, sodium hypochlorite solutions have been proposed and evaluated for treatment of atopic dermatitis and recurrent pyoderma in dogs, as it is highly effective against *Staphylococcus* species in vitro. The use of this and other topical antiseptic products is being increasingly investigated as veterinarians continue to struggle with antibiotic resistant (including methicillin-resistant *Staphylococcus* spp.) organisms associated with skin infections.

Examples of Disinfectant Use in Veterinary Medicine

Disinfectants are widely used in veterinary medicine on floors, tables, walls, surgical equipment and other instruments before storage, and for disinfection of animal housing facilities. For effective germicidal activity, manufacturer recommendations regarding contact time, dilution, and useful life of a disinfectant solution

should be followed. The best disinfectant for a particular situation will depend on the surface's shape, structure, chemical reactivity, and use, as well as on the type of contaminating organisms anticipated. In addition, in almost all instances, disinfection is only effective after the removal and cleaning of organic debris.

Although detailed descriptions of guidelines for disinfectant use in all circumstances is beyond the scope of this chapter, it is justified to provide several examples of their use in situations involving microbes that cause significant health concerns or that can be easily transmitted.

Salmonella

Salmonella species are well known by veterinarians and controlling transmission of the organism is important in a variety of settings. There are many *Salmonella* serotypes, some of which may be associated with an increased concern for transmission. An important food-borne pathogen, control of *Salmonella* on production animal facilities, even when animals are asymptomatically shedding the organism in feces, is important for public health and consumer confidence. Zoonotic transmission in a veterinary clinic setting from a cat to technical staff has also been reported (Cherry et al., 2004). In other situations, patients are symptomatic with a *Salmonella* infection and efforts to prevent transmission to other patients, particularly in hospital or boarding facilities, is critical. Several highly publicized *Salmonella* outbreaks have caused interruptions in services at veterinary teaching hospitals, prompting the search for effective methods of detection, prevention, and disinfection. Environmental contamination (Patterson et al., 2005) or affected individuals (Schott et al., 2001) may have been the initiating events; however, housing of sick and immunocompromised patients as well as incomplete disinfection likely contributed to routine shedding developing into epidemics. Sites and methods of sampling for monitoring for *Salmonella* contamination have been proposed and a high frequency of positive results have been recorded (~50%). However, this high percentage may reflect the sampling and detection method and not necessarily disease risk. The ability to decontaminate a veterinary hospital quickly, efficiently, and effectively is paramount to preventing loss of income and public confidence and to ensuring high quality treatment of the veterinary population. Following one outbreak (in 1996 at Colorado State Veterinary Teaching Hospital) the facility was at least partially closed for 3 months to allow manual decontamination/disinfection.

Disinfectant footbaths have been used as a hygiene barrier to prevent spread of microbes in veterinary hospital environments. Footbath efficacy has been shown to be dependent upon the disinfectant used and the compliance with which it is utilized. In one study, a peroxygen compound was shown to be more effective than a QAC; however, a maximal reduction in contamination of only 75% was observed (Morley et al., 2005). These results suggest that footwear hygiene can be improved through appropriate use of disinfectant footbaths, but it should not be relied on as the only method of controlling the spread of infectious agents. Mist application of a 4% peroxymonosulfate compound was shown to be an effective method of eliminating artificially induced contamination of an animal holding facility (Patterson et al., 2005).

Glutaraldehyde was found to be the most effective compound in reducing *Salmonella enteritidis* and *S. senftenberg* bacterial load in a study designed to mimic worst-case conditions in disinfecting poultry houses (Gradel et al., 2004). Four types of materials (e.g., concrete, wood) were contaminated with bacteria mixed with several types of organic matter (e.g., feed, egg yolk) and disinfection was attempted at high and low temperatures. Formaldehyde was considered effective even at low temperatures despite reports that a minimum temperature of 16°C is required for activity, whereas a peroxygen compound was found to be least effective except for one material/organic matter combination. This lack of efficacy was attributed to peroxygen compounds inactivation in the presence of organic matter.

In a similar study that investigated disinfection of poultry transport containers, real-world conditions were created by testing five compounds against bacteria growing isolated and in a biofilm. Although halogen compounds and QAC were effective against artificially contaminated surfaces, after the biofilm had matured, only sodium hypochlorite or an iodine-containing disinfectant was able to achieve 100% reduction. In the ultimate test of disinfectant activity in the face of organic matter, sodium carbonate, ammonia, and sodium hydroxide were shown to reduce food-borne pathogen load in cattle manure (Park and Diez-Gonzalez, 2003).

Avian Influenza

The spread of avian influenza (including highly pathogenic avian influenza, HPAI) among poultry populations and is very concerning and problematic. As an enveloped virus, the orthomyxoviridae, including influenza viruses, are very sensitive to most detergents and disinfectants. They are readily inactivated by pH, heating, and drying. The US Environmental Protection Agency currently reports approximately 200 products registered for disinfection use against avian influenza. These products are typically intended to be used by poultry producers to disinfect their facilities after an

outbreak. Classes of disinfectants considered effective at destroying avian influenza virus include alcohols, phenolics, oxidizing agents, and dilute acids. However, flu viruses are well protected from inactivation by organic material, and infectious virus can be recovered from manure for up to 105 days. It is therefore suggested that complete removal of all organic material is part of any effective disinfection procedure. Contaminated litter and manure should be composted or buried to ensure that it does not spread infectious virus.

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32

Sulfonamides and Potentiated Sulfonamides

Mark G. Papich

The sulfonamides are one of the oldest groups of antimicrobial compounds still in use today. Sulfanilamide, an amide of sulfanilic acid, was the first sulfonamide used clinically. It was derived from the azo dye Prontosil. Other sulfonamides also share the same structure and the "sulfonamide" structure is prevalent among other drug classes, including nonsteroidal antiinflammatory drugs (NSAIDs), anticonvulsants, and diuretics. Sulfonamide antimicrobials have been in clinical use for 50 years, but resistance is common when these drugs are used alone (without addition of trimethoprim or ormetoprim). Previous editions of this textbook should be consulted for a review of this extensive historical database. Clinical use of sulfonamides in dogs, cats, horses, and some exotic and zoo animals usually relies on the addition of trimethoprim (trimethoprim–sulfonamide) or ormetoprim (e.g., ormetoprim–sulfadimethoxine) to broaden the spectrum and increase antibacterial activity against bacteria that are resistant to either drug used alone. Technically, trimethoprim and ormetoprim are chemically called *diaminopyrimidines,* but they will be referred to by their respective names in this chapter. In companion animals, trimethoprim–sulfonamide combinations have all but replaced single or combination sulfonamide (triple-sulfas) treatment regimens. Sulfonamide administration is restricted in food animals, particularly dairy cattle, because of a concern for drug residues.

Pharmacology of Sulfonamides

All sulfonamides are derivatives of sulfanilamide (structurally similar to para-aminobenzoic acid), which was, in the 1940s, the first sulfonamide discovered to have antimicrobial activity. Note that in some countries and certain formularies outside the United States, different spellings have been used for sulfonamides (e.g., *sulphamethoxazole* for sulfamethoxazole; *sulphadiazine* for sulfadiazine; *sulphadimethoxine* for sulfadimethoxine, and so forth). This textbook uses the United States Adopted Names (USAN) and United States Pharmacopeia (USP) official names throughout.

Many structural derivatives of sulfanilamide with differing pharmacokinetic and antimicrobial spectrums have been used in veterinary medicine to treat microbial infections of the respiratory, urinary, gastrointestinal, and central nervous systems (Figure 32.1). Susceptible organisms include many bacteria, coccidia, chlamydia, and protozoal organisms, including *Toxoplasma* spp. Treatment of protozoa infections is discussed in more detail in Chapter 42 of this book.

Sulfonamides are white crystalline powders that are weak organic acids, with solubility in water that varies among the specific drugs (ranging from slightly soluble to practically insoluble), and have a wide range of pK_a values, as shown in Table 32.1. The pK_a values of these compounds and their ionization are important because – among other properties – the antibacterial activity, solubility, and protein binding have been associated with the pK_a value (Mengelers et al., 1997). Drugs with high pK_a are less soluble and exhibit lower protein binding; drugs with low pK_a tend to have higher protein binding. The sulfonamides all share a similar structure, which contains a $–SO_2$ group linked to a benzene ring, and a para NH_2- group on N-4. An attached pyrimidine ring may contain zero, one, or two methyl groups (sulfamethazine, sulfamerazine, and sulfadiazine, respectively), which may undergo hydroxylation during metabolism. The other major site of metabolism is acetylation of the para-NH_2, which can vary among species (for example, dogs do not acetylate, which is discussed in Section Metabolism). Acetylated forms of the drug tend to be less soluble.

The sulfonamides exhibit large variation in the extent to which they bind to plasma proteins. In general, the plasma protein binding is higher than other antimicrobials (>70% in many animals), and ranges from 90% (sulfadimethoxine in some species) to as low as 50% (sulfamethazine in some species). In horses, the protein binding of trimethoprim was 20–30% and for sulfadiazine was 18–30% (Winther et al., 2011). Because they

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Companion Website: www.wiley.com/go/riviere/pharmacology

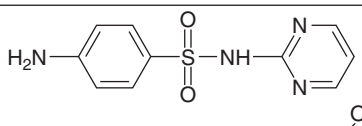
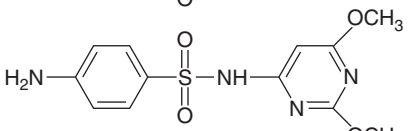
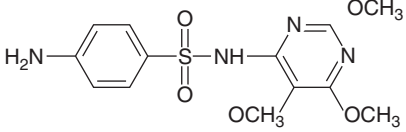
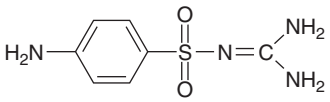
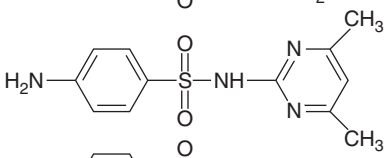
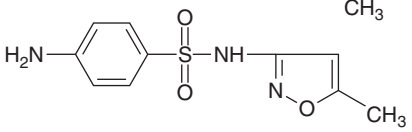
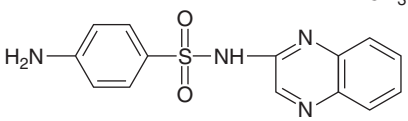
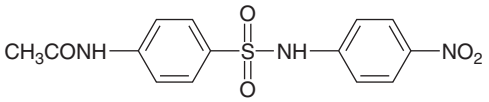
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Sulfadiazine	4-amino-*N*-2-pyrimidinylbenzenesulfonamide ($C_{10}H_{10}N_4O_2S$) [250.28]	
Sulfadimethoxine	4-amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_4S$) [310.33]	
Sulfadoxine	4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_4S$) [310.34]	
Sulfaguanidine	4-amino-*N*-(aminoiminomethyl)-benzenesulfonamide ($C_7H_{10}N_4O_2S$) [214.24]	
Sulfamethazine	4-amino-*N*-(4,6-dimethoxy-2-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [278.32]	
Sulfamethoxazole	4-amino-*N*-(5-methyl-3-isoxazolyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [253.31]	
Sulfaquinoxaline	4-amino-*N*-2-quinoxalinyl-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [300.33]	
Sulfanitran	4′-[(*p*-nitrophenyl)sulfamoyl]acetanilide ($C_{12}H_{14}N_4O_2S$) [300.33]	

Figure 32.1 Sulfonamides and their structures.

Table 32.1 Physical chemistry properties of sulfonamides, trimethoprim, and ormetoprim

Drug	pK_a	Log P
Sulfanilamide	10.1	−0.072
Sulfadimidine	7.7	0.691
Sulfamerazine	7.0	0.812
Sulfadiazine	6.4, 6.5, 6.6	0.631
Sulfadimethoxine	6.3, 6.2	1.648
Sulfachlorpyridazine	6.1, 6.0	1.305
Sulfamethoxazole	5.7, 5.9, 6.0	1.396
Sulfisoxazole	5.0, 4.9	2.259
Sulfadoxine	6.1, 6.3	1.271
Sulfaquinoxaline	5.5	1.68
Trimethoprim	7.12, 7.6	0.91
Ormetoprim	na	1.23

The pK_a is the dissociation rate constant. For some drugs, more than one pK_a value is listed because of variation among sources. For pK_a values, all sulfonamides are weak acids; trimethoprim and ormetoprim are weak bases. Log P is the logarithm of the partition coefficient between an organic solvent (oil) and water. The higher the Log P, the more lipophilic is the drug. Some values are from Mengelers et al. (1997) and van Duijkeren et al. (1994a).

are weak acids, sulfonamides are more soluble in alkaline than in neutral or acidic pHs; water solubility is enhanced when the sulfonamides are formulated as sodium salts or when in solution in more alkaline environments. Some sulfonamide solutions have pHs between 9 and 10, prohibiting extravascular use. Because solubility is decreased in acidic pH, they may become particularly insoluble and crystallize in renal tubules when urine pH is low, especially when high doses are administered, or animals are dehydrated or acidemic. To minimize crystalluria, yet allow administration of high doses, they have been formulated in combination with other sulfonamides. Each sulfonamide in a mixture of sulfonamides exhibits its own solubility in solution (law of independent solubility); that is, sulfonamides do not significantly affect the solubility of each other, but the antimicrobial effect is additive; thus the use of "triple-sulfas" (three sulfonamides formulated in solution together) allows increased efficacy without a significant increased risk of adverse effects (Bevill, 1988).

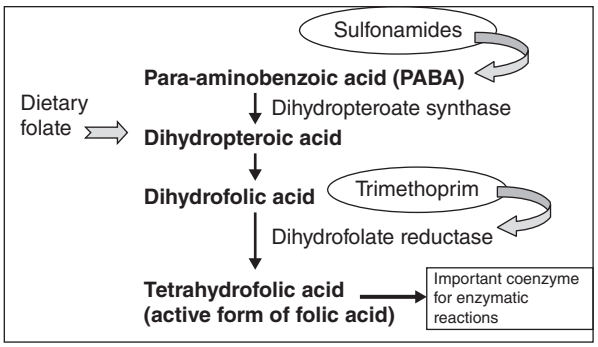


Figure 32.2 Simplified pathway for the action of trimethoprim–sulfonamide combinations. Sulfonamides provide a false substrate for para-aminobenzoic acid (PABA) inhibiting the synthesis to dihydropteroic acid, a precursor for synthesis to dihydro- and tetrahydrofolic acid. Trimethoprim inhibits the enzyme dihydrofolate reductase, an enzyme critical to the synthesis of tetrahydrofolic acid.

Mechanism of Action

Sulfonamides rely on the requirement of susceptible organisms to synthesize folic acid as a precursor of other important molecular molecules in the cell. Sulfonamides act as false substrates in the synthesis of folic acid. Trimethoprim and ormetoprim (diaminopyrimidines, discussed in Section Potentiated Sulfonamides) produce a synergistic effect when used together by inhibiting the enzyme dihydrofolate reductase.

Folic acid metabolism is presented in Figure 32.2. Para-aminobenzoic acid (PABA), pteridines, glutamic acid, and the enzyme dihydropterate synthase interact to form dihydropteroic acid, the immediate precursor to dihydrofolic acid. Dihydropteroic acid is enzymatically converted to dihydrofolic acid by dihydrofolate synthase, followed by another enzymatic conversion of dihydrofolic acid to tetrahydrofolic acid (THFA) via dihydrofolate reductase (DHFR). The combination of sulfonamides and trimethoprim inhibits formation of tetrahydrofolic acid at two steps. This action is synergistic and increases activity against organisms that could otherwise be resistant. Tetrahydrofolate is a coenzyme in a number of complex enzymatic reactions and also is a coenzyme in the synthesis of thymidylic acid (a nucleotide), which is a building block of DNA. Trimethoprim and sulfonamides are bacteriostatic by themselves; together, they can be bactericidal. Bacteria are more susceptible to this combination than to either drug when tested alone (White et al., 1981).

Trimethoprim–sulfonamides are formulated in a ratio of 1:5 (trimethoprim:sulfonamide). In the animal, it is usually cited that the optimum ratio to produce antibacterial activity is 1:20 (Bushby, 1980; van Duijkeren et al., 1994b). Testing for susceptibility using approved CLSI methods (CLSI, 2015) uses a ratio of 1:20 trimethoprim:sulfonamide. However, this ratio is often much lower in animals because the trimethoprim component is excreted faster than the sulfonamide and the optimum ratio may actually be much wider than the value of 1:20 cited in human medical references, and may be as low as 1:40.

Sulfonamide action is dependent on the chemical similarity with PABA. Therefore, sulfonamides act as a false substrate in this reaction and synthesis of THFA is inhibited. The sulfonamides are relatively safe to mammalian cells because mammals utilize dietary folate for the synthesis of dihydrofolic acid, and they do not require PABA. The enzyme dihydrofolate reductase of bacteria has a much higher affinity (50,000 to 60,000-fold, and in some references as high as 100,000-fold) for trimethoprim than mammalian dihydrofolate reductase.

The mechanism of action of sulfonamides on bacteria does not entirely explain the activity against protozoa. Sulfonamides may inhibit protozoal dihydrofolate synthetase. Protozoal dihydrofolate reductase also is susceptible to the action of trimethoprim, which may explain some of the effect to support the use of these drugs for protozoal infections (treatment of protozoa infections is discussed in Chapter 42).

Clinical Uses and Microbial Susceptibility

The spectrum of activity for the sulfonamides is broad, affecting gram-positive, gram-negative, and many protozoal organisms. Sulfonamides have been used clinically for approximately 50 years and many organisms once susceptible to the sulfonamides are now resistant. To increase the activity, most of the sulfonamides used in clinical practice are combinations with either trimethoprim or ormetoprim (diaminopyrimidines). These combinations (referred to in this chapter as *trimethoprim–sulfonamides,* but also referred to in clinical practice as *trimethoprim–sulfa* or simply abbreviated as *TMP/SU*) have increased the activity.

Administration of a single sulfonamide, or combination of sulfonamides, continues to be used in some livestock practices. In the United States, there are no approved formulations of trimethoprim–sulfonamides available for food animals, but trimethoprim–sulfadoxine is available in some countries.

The susceptibility/resistance patterns of sulfonamides and the trimethoprim–sulfamethoxazole combination against the most commonly encountered veterinary pathogens has been reported (van Duijkeren et al., 1994a, 1995; Bade et al., 2009; Winther et al., 2011). The activity of these agents has allowed for treatment of common respiratory infections, urinary tract and soft tissue infections, and intestinal infections (intestinal protozoa). Susceptible organisms include *Arcanobacterium*,

Bacillus spp., *E. rhusiopathiae, L. monocytogenes, Streptococcus* spp., (*Streptococcus equi* subsp. *zooepidemicus* from horses), and protozoa (coccidia and *Pneumocystis carinii*).

The wild-type strains of following organisms are usually susceptible to the trimethoprim–sulfonamide (or ormetoprim–sulfonamide) combination: *Pasteurella* spp., *Proteus* spp., *Salmonella* spp., *Histophilus* (formerly *Hemophilus*), the protozoa *Toxoplasma,* and coccidia. Other bacteria that may be susceptible, but for which resistance can develop, include *Staphylococcus* spp., *Corynebacterium, Nocardia asteroides, Stenotrophomonas maltophilia,* and bacteria of the Enterobacteriaceae (*Klebsiella, Proteus, Enterobacter,* and *Escherichia coli*).

The organisms that are consistently resistant to trimethoprim–sulfonamide combinations include: *Pseudomonas* spp., *Chlamydia spp.,* and *Bacteriodes.* One should cautiously interpret trimethoprim–sulfonamide susceptibility for *Enterococcus spp*. Although *Enterococcus* may appear susceptible to trimethoprim–sulfonamides using in vitro tests, it escapes the antifolate activity of the drug in vivo by its unique ability to incorporate preformed exogenous folates (Wisell et al., 2008). Sulfonamides alone are not active against *Enterococcus* spp. Clinical failures are reported despite in vitro susceptibility and microbiology laboratories should not report the susceptibilities of *Enterococcus* to trimethoprim–sulfonamides.

The activity of trimethoprim–sulfonamides against anaerobic bacteria can be variable. When measured in vitro, trimethoprim–sulfonamides have good activity against anaerobic bacteria (Indiveri and Hirsh, 1986), but clinical results are not as good (Dow, 1988) because thymidine and PABA (inhibitors of trimethoprim–sulfonamide activity) may be present in anaerobic infections.

Trimethoprim–sulfonamides have been used to treat infections caused by protozoa (including *Toxoplasma gondii*) and intestinal coccidia. Trimethoprim–sulfonamide combinations have also been used to treat equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*. (Use of pyrimethamine for treating EPM and treatment of protozoa infections is discussed in Chapter 42.)

Interactions Affecting Antimicrobial Activity

Components found in some tissue environments may inhibit trimethoprim–sulfonamide activity. For example, thymidine and PABA present in infected tissue – may interfere with activity. This has been demonstrated in tissue cages in horses. Ensink et al. (2005) showed an inability to eliminate the infection in an infected environment, despite in vitro sensitivity. They cited inhibitors – such as PABA and thymidine – present in abscessed and infected tissues that may inhibit the effects of these drugs. In another study in which trimethoprim–sulfadoxine was administered to cattle with infected tissue cages (Greko et al., 2002), it was shown that high levels of thymidine in the tissue cage fluid inhibited trimethoprim and compromised the ability to eradicate the infection.

Susceptibility Testing

For susceptibility testing, trimethoprim–sulfamethoxazole (1:20 ratio of trimethoprim:sulfamethoxazole) should be used, even when trimethoprim–sulfadiazine is used for therapy (CLSI, 2013, 2015). There are no quality control (QC) ranges developed for trimethoprim–sulfadiazine, and tests using trimethoprim–sulfamethoxazole are expected to give equivalent results. Winther et al. (2011) showed that there were no significant differences observed between the minimal inhibitory concentration (MIC) of sulfadiazine and sulfamethoxazole for individual bacterial strains, confirming that sulfamethoxazole is an effective surrogate for susceptibility testing of sulfadiazine. The CLSI susceptibility testing standards state that Mueller–Hinton agar containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and of trimethoprim, which may result in false-resistant reports (CLSI, 2013). Susceptibility testing agar that is as thymidine free as possible should be used. The current CLSI interpretive categories (CLSI, 2015) do not provide veterinary-specific interpretations; therefore, the human breakpoint is used by laboratories to predict susceptibility. For *Staphylococcus* spp. and the Enterobacteriaceae the susceptible breakpoint is $\leq$2/38 (trimethoprim/sulfonamide) and for *Streptococcus* spp. the breakpoint is $\leq$0.5/9.5 (trimethoprim/sulfonamide).

Drug Resistance

Resistance by many bacterial and protozoal organisms has become widespread due to the extensive use of sulfonamides over many years (Huovinen, 2001). Resistance occurs via efflux pumps, failure to penetrate the organism, and changes in target enzymes. Resistance can be transferable. Chromosomal resistance tends to occur slowly and confers resistance via impaired drug penetration into the microbial cell, producing an insensitive dihydropteroate enzyme and an increased production of PABA. Plasmid-mediated resistance, the most commonly encountered form of sulfonamide resistance, occurs quickly and manifests itself via the impaired drug penetration mechanism in addition to producing sulfonamide-resistant dihydropteroate synthase enzymes. If an organism becomes resistant to one sulfonamide, it is generally resistant to all other

sulfonamides. Resistance to trimethoprim occurs via overproduction of the dihydrofolate reductase enzyme or synthesis of an enzyme that resists binding of the drug.

Pharmacokinetics of Sulfonamides

Pharmacokinetics of sulfonamides, trimethoprim, and related drugs used in veterinary medicine are listed in Tables 32.2, 32.3, 32.4, 32.5, and 32.6.

Oral Absorption

In dogs, absorption is excellent and not affected by feeding (Sigel et al., 1981). There has been considerable interest in the oral absorption of trimethoprim–sulfonamide combinations in horses and the effect of feeding. When trimethoprim-sulfonamides are administered to a horse that has not been fed, rapid absorption occurs, but is not as complete as for dogs or people. Nevertheless, oral administration is sufficient in horses to produce effective results. The fraction absorbed for trimethoprim was reported to be 67%, and for sulfadiazine 58%, but for both components the variability was high (van Duijkeren et al., 1994c). Oral absorption in another study in horses was 90.2% for intragastric administration and 74.45% for the oral paste (Winther et al., 2011). For trimethoprim in the same study it was 71.5% oral absorption for the intragastric administration and 46% for the oral paste (Winther et al., 2011). In that study the absorption of trimethoprim–sulfadiazine was likely diminished by feeding. When trimethoprim–sulfadiazine was administered to horses as an oral suspension and compared to the equine paste, the absorption from the suspension was higher for both drugs compared to the paste, that is 136% and 118% of the paste AUC concentrations for sulfadiazine and trimethoprim, respectively (McClure et al., 2015). In another study (van Duijkeren et al., 1994c) the oral paste was compared to two compounded formulations (mixed with syrup and water or carboxymethylcellulose gel). In this comparison, all three formulations were judged to be equivalent. When administered to horses that have been fed or when it is added to the horses' feed concentrate, a delayed and biphasic absorption is observed (van Duijkeren et al., 2002, 1995). When trimethoprim sulfachlorpyridazine was administered to horses, oral absorption was delayed, with the first peak appearing 1 hour after dosing and the second appearing 8–10 hours postdosing. Dual absorption peaks were not found after nasogastric administration (van Duijkernen et al., 1995). The best explanation for this phenomenon is that that there is an initial peak of absorption in the small intestine where much of drug absorption is known to occur. However, the drug that is bound to feed (adsorption) is unavailable for absorption until it travels to the cecum and, after digestion of the carbohydrates, the drug is released, producing a delayed and biphasic peak in absorption. Trimethoprim–sulfachlorpyridazine can bind to equine cecal contents 60–90%, which supports the theory of the "double peak". Feeding also decreased the systemic availability from 70% when fasted to 45% when fed (van Duijkernen et al., 1996).

In ruminants, age and diet can markedly affect trimethoprim and oral sulfadiazine disposition in calves (Guard et al., 1986; Shoaf et al., 1987). Orally administered sulfadiazine (30 mg/kg) was absorbed very slowly in those calves fed milk diets, with absorption slightly higher in ruminating calves. Trimethoprim was absorbed in preruminant calves, but not absorbed in mature ruminants after oral administration (Shoaf et al., 1987), probably because of inactivation in the rumen.

Sulfasalazine is not used for the antibacterial properties, but is used to treat inflammatory disease of the large intestine in small animals (discussed in more detail in Chapter 46). It is not absorbed as a whole molecule but rather is cleaved into two more active compounds by native resident colonic bacteria.

Distribution

Sulfonamides distribute to most body fluids, but are not distributed to tissues as extensively as trimethoprim. Generally, sulfonamide tissue concentrations are lower than plasma concentrations (approximately 20–30% of corresponding tissue concentration), but distribution to extracellular fluids is generally high enough to produce effective concentrations against susceptible pathogens. High protein binding affects the distribution and markedly increases the half-life of sulfonamides.

Sulfonamides are weak acids and trimethoprim is a weak base (Table 32.1). The ionization affects distribution, which favors the distribution and ion trapping of trimethoprim in tissues (intracellular environment is typically more negative than plasma). Therefore trimethoprim has a higher volume of distribution than sulfonamides. Also, because sulfonamides are weak acids, the pH-partition hypothesis shows that these drugs do not attain therapeutic concentrations in milk; however, enough passive diffusion occurs to limit their use in dairy cattle.

The prostate is another example in which pH-dependent distribution is known to occur (Robb et al., 1971). Sulfadiazine being a weak acid, penetrated the prostate to approximately 11% that of the mean plasma concentration. Because trimethoprim is a weak base (pK_a of 7.3) the concentrations in the prostate are higher owing to ion trapping. The concentration in the prostatic

Table 32.2 Some pharmacokinetic parameters of sulfamethazine (sulfadimidine) in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$t^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Cattle	107	IV	0.346	NR	NR	Bevill et al., 1977a
Cattle (male)	200	IV	0.37	5.82	45	Witcamp et al., 1992
Cattle (female)	200	IV	0.24	3.64	54	Witcamp et al., 1992
Calves (62–70 days old)	10	IV	NR	5.2	NR	Nouws et al., 1988c
Calves (68–76 days old)	100	IV	NR	5.7	NR	Nouws et al., 1988c
Cows (4–5 years old)	10	IV	NR	4	NR	Nouws et al., 1988c
Cows (3–5 years old)	100	IV	NR	5.9	NR	Nouws et al., 1988c
Cows (5–6 years old)	200	IV	NR	5.5	NR	Nouws et al., 1988c
Pigs (9 weeks old)	50	IV	0.51	16	21	Sweeney et al., 1993
Pigs (10 weeks old)	20	IV	0.604	10	42	Nouws et al., 1989a
Pigs (10 weeks old, given in drench)	20	PO	NR	11.9	NR	Nouws et al., 1989a
Pigs (10 weeks old, given in medicated feed)	20	PO	NR	16.6	NR	Nouws et al., 1989a
Pigs (male, 18–32 kg)	20	IV	0.55	12.4	25	Nouws et al., 1989a
Gilts (12–13 weeks old)	107.5	IA	0.493	15.61	NR	Duffee et al., 1984
Barrows (12–13 weeks old)	107.5	IA	0.614	17.7	NR	Duffee et al., 1984
Boars (12–13 weeks old)	107.5	IA	0.542	16.63	NR	Duffee et al., 1984
Pigs (normal castrated males and intact females)	50	IV	0.50	15	23	Yuan et al., 1997
Pigs (castrated males and intact females infected with *S. suum*)	50	IV	0.52	20	17	Yuan et al., 1997
Goat	100	IV	0.316	2.77	81	Elsheikh et al., 1991
Goats (adult and fed)	100	IV	0.9	4.75	135.6	Abdullah and Baggot, 1988
Goats (adult and fasted)	100	IV	0.897	7.03	69.6	Abdullah and Baggot, 1988
Goats (adult male)	20	IV	0.28	8.7	20	Witcamp et al., 1992
Goats (adult female)	20	IV	0.18	2.13	70	Witcamp et al., 1992
Goats (12 weeks old)	100	IV	0.43	1.97	134	Nouws et al., 1989b
Goats (18 weeks old)	100	IV	0.507	2.56	106	Nouws et al., 1989b
Sheep	100	IV	0.297	4.72	44.6	Elsheikh et al., 1991
Sheep (male)	100	IV	0.4	4.5	90	Srivastava and Rampal, 1990
Ewes	100	IV	0.474	9.51	35.07	Youssef et al., 1981
Ewes (dosed in summer months)	100	IV	0.37	3.64	63	Nawaz and Nawaz, 1983
Ewes (dosed in winter months)	100	IV	0.49	3.92	85	Nawaz and Nawaz, 1983
Sheep (ewes and rams)	100	IV	0.41	10.8	41	Bulgin et al., 1991
Sheep (ewes and rams)	100	PO	NR	4.3	NR	Bulgin et al., 1991
Sheep (ewes and rams)	391	PO	NR	14.3	NR	Bulgin et al., 1991
Sheep (ewes and rams)	100	IV	0.37	3.64	NR	Bulgin et al., 1991
Sheep (ewes and rams)	107.5	IV	0.293	5.87	NR	Bulgin et al., 1991
Sheep (ewes and rams)	107.5	IV	0.327	7.09	NR	Bulgin et al., 1991
Ponies (breed unknown)	160	IV	0.63	11.4	42.1	Wilson et al., 1989
Ponies (Shetland)	20	IV	0.33	5.4	55.2	Nouws et al., 1987
Mare (2 years old)	20	IV	0.47	5	65	Nouws et al., 1985a
Mare (2 years old)	200	IV	0.56	6	67	Nouws et al., 1985a
Mare (22 years old)	20	IV	0.38	9.5	28	Nouws et al., 1985a
Mare (22 years old)	200	IV	0.36	14.6	27	Nouws et al., 1985a
Stallion (1.5 years old)	20	IV	0.44	9.5	32	Nouws et al., 1985a
Stallion (1.5 years old)	200	IV	0.65	11	41	Nouws et al., 1985a
Horse	20	IV	0.33	5.4	54	Nouws et al., 1987
Horse	160	IV	0.63	11.4	48	Wilson et al., 1989
Horse	60	IV	0.74	9.8	NR	
Dogs (normal)	100	IV	0.628	16.2	22.4	Riffat et al., 1982
Dogs (febrile)	100	IV	0.495	16.7	20.2	Riffat et al., 1982
Rabbits (male)	35	IV	0.42	0.4	73.6	Witcamp et al., 1992
Rabbits (female)	35	IV	0.23	0.39	40.8	Witcamp et al., 1992
Carp (10°C)	100	IV	1.15	50.3	16.14	van Ginneken et al., 1991
Carp (20°C)	100	IV	0.9	25.6	24.66	van Ginneken et al., 1991
Rainbow trout (10°C)	100	IV	1.2	20.6	41.1	van Ginneken et al., 1991
Rainbow trout (20°C)	100	IV	0.83	14.7	39.9	van Ginneken et al., 1991
Camel	50	IV	0.73	13.2	40	Younan et al., 1989
Camel	100	IV	0.394	7.36	40.9	Elsheikh et al., 1991
Buffalo (female)	200	IV	1.23	12.36	193.2	Singh et al., 1988

NR, not reported; IV, intravenously; IA, intraarterially; PO, orally; Vd (volume of distribution); $t^1/_2$ (half-life).

Table 32.3 Some pharmacokinetic parameters of sulfadiazine in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Pigs	25/5[a]	PO	NR	3.1–4.31	NR	Soli et al., 1990
Pigs	20	IV	0.54	4.0[b]	140	Nielsen and Gyrd-Hansen, 1994
Pigs (fed)	40	PO	NR	11.5[b]	NR	Nielsen and Gyrd-Hansen, 1994
Pigs (fasted)	40	PO	NR	8.1[b]	NR	Nielsen and Gyrd-Hansen, 1994
Carp (10°C)	100/20[a]	IV	0.53	47.1	7.9	Nouws et al., 1993
Carp (20°C)	100/20[a]	IV	0.60	33	12.2	Nouws et al., 1993
Ewes	100	IV	0.39	37.15	38.75	Youssef et al., 1981
Dogs	100/20[a]	PO	NR	9.84	NR	Sigel et al., 1981
Calves (milk diet, 7 weeks)	25/5[a]	SC	NR	3.4	NR	Shoaf et al., 1987
Calves (milk diet, 13 weeks)	25/5[a]	SC	SC	3.4	NR	Shoaf et al., 1987
Calves (grain diet, 7 weeks)	25/5[a]	SC	NR	4.4	NR	Shoaf et al., 1987
Calves (grain diet, 13 weeks)	25/5[a]	SC	NR	3.6	NR	Shoaf et al., 1987
Calves (8–20 days)	20	IV	NR	6.2	NR	Nouws et al., 1988c
Calves (0.5 years)	100	IV	NR	7	NR	Nouws et al., 1988c
Cattle (5 years)	10	IV	NR	4.1	NR	Nouws et al., 1988c
Calves (male, 1 day)	25/5[a]	IV	0.72	5.78	5.8	Shoaf et al., 1989
Calves (male, 7 days)	25/5[a]	IV	0.67	4.4	102	Shoaf et al., 1989
Calves (male, 42 days)	25/5[a]	IV	0.59	3.6	112.8	Shoaf et al., 1989
Calves (7 days, with synovitis)	25/5[a]	IV	28.7	24.44	102	Shoaf et al., 1986
Horses (adult)	20/4[a]	PO	NR	7.8	NR	FOI summary (FDA)
Horses	12.5	IV	0.52	2.7	NR	Brown et al., 1983
Horses	20	IV	0.4	3.8	138	Nouws et al., 1987
Horses	25	PO	NR	7.4	NR	Sigel et al., 1981
Horses (adult)	25	IV	0.58	5.37	100	Winther et al., 2011
Horses (adult)	25	PO (paste, fed)	NR	14.03	NR	Winther et al., 2011
Horses (adult)	25	PO (intragastric, fed)	NR	12.3	NR	Winther et al., 2011
Ponies	25	PO	NR	12.08	NR	Van Duijkeren et al., 2002
Horses (adult)	12.5	IV	0.50	4.6	90	Gustafsson et al., 1999
Horses (adult)	25	PO (fed)	NR	8.2	NR	Gustafsson et al., 1999
Horse (adult)	25	PO (fasted)	NR	8.15	NR	Van Duijkeren et al., 1994c
Horse (adult)	25	IV	0.58	4.65	115.2	Van Duijkeren et al., 1994c

NR, not reported; IV, intravenously; PO, orally; SC, subcutaneously; Vd (volume of distribution); $T^1/_2$ (half-life).
[a]Sulfadiazine–trimethoprim dose.
[b]Reported as mean residence time (MRT).

fluid has been measured to be 380% higher than that of plasma. Consequently, trimethoprim–sulfonamide combinations are an acceptable choice for treating infections of the prostate.

In horses, studies have been conducted to examine tissue concentrations in urine, peritoneal fluid, endometrium, and synovial fluid (Brown et al., 1983, 1988, 1989) of trimethoprim or ormetoprim–sulfonamide combinations. In each tissue, drug concentrations were adequate for treating infections in these sites. Urine concentrations – as expected because of the route of elimination – were much higher than plasma, but, otherwise, the plasma concentration and tissue concentration curves were parallel. The only tissue in

Table 32.4 Some pharmacokinetic parameters of sulfamethoxazole in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Horse	2.5	IV	0.301	3.9[a]	90	Peck et al., 2002
Horse	12.5	IV	0.33	3.53	78.2	Brown et al., 1988
Donkey	2.5	IV	0.335	2.7[a]	132	Peck et al., 2002
Mule	2.5	IV	0.337	5.9[a]	60	Peck et al., 2002

NR = not reported; IV = intravenously; SC = subcutaneously; PO = orally; Vd (volume of distribution); $T^1/_2$ (half-life).
[a]Reported as mean residence time (MRT).

Table 32.5 Some pharmacokinetic parameters of trimethoprim in animals

Species	Dose[a] (mg/kg)	Route	Vd (l/kg)	$T^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Cows	8/40	IV	NR	1.18	NR	Davitiyananda and Rasmussen, 1974
Pigs	4	IV	1.8	3.3[b]	0.55	Nielsen and Gyrd-Hansen, 1994
Pigs (fed)	8	PO	NR	10.6[b]	NR	Nielsen and Gyrd-Hansen, 1994
Pigs (fasted)	8	PO	NR	6.5[b]	NR	Nielsen and Gyrd-Hansen, 1994
Calves (male, 1 day old)	5/25	IV	1.67	8.4	2.8	Shoaf et al., 1989
Calves (male, 7 days old)	5/25	IV	2.23	2.11	2.0	Shoaf et al., 1989
Calves (male, 42 days old)	5/25	IV	2.36	0.9	28.9	Shoaf et al., 1989
Calves (7 weeks old, milk diet)	5/25	SC	NR	3.4	126.0	Shoaf et al., 1987
Calves (13 weeks old, milk diet)	5/25	SC	NR	3.4	124.8	Shoaf et al., 1987
Calves (7 weeks old, grain diet)	5/25	SC	SC	4.4	105.6	Shoaf et al., 1987
Calves (13 weeks old, grain diet)	5/25	SC	NR	3.6	112.2	Shoaf et al., 1987
Calves (7 days old)	5/25	IV	28.72	4.44	102.0	Shoaf et al., 1986
Carp (10°C)	20/100	IV	3.1	40.7	47.0	Nouws et al., 1993
Carp (20°C)	20/100	IV	4.0	20.0	141.0	Nouws et al., 1993
Broilers	4/2[c]	PO	NR	0.63	NR	Dagorn et al., 1991
Quail (*Coturnix coturnix japonica;* male and female)	10	PO	NR	2.98	NR	Lashev and Mihailov, 1994
Quail (*Coturnix coturnix japonica;* male and female)	4	IV	2.99	2.38	1.129	Lashev and Mihailov, 1994
Pigs	5/25 (Tribrissen 12%)	PO	NR	3.35	NR	Soli et al., 1990
Pigs	5/25 (Trimazin 12%)	PO	NR	4.86	4.86	Soli et al., 1990
Pigs	5/25 (Trimazin Forte 24%)	PO	NR	5.92	NR	Soli et al., 1990
Horses (adult)	4/20[a]	PO	NR	3	NR	FOI summary (FDA)
Horse	2.5–8	IV	2	3	720	Van Duijkeren et al., 1994b (mean values from summary of 7 studies)
Horse (adult)	5	IV	2.22	2.43	650	Winther et al., 2011
Horse (adult)	5	PO (paste, fed)	NR	3.33	NR	Winther et al., 2011
Horse (adult)	5	PO (intragastric, fed)	NR	3.2	NR	Winther et al., 2011
Horse (adult)	2.5	IV	1.82	1.5[b]	1224	Peck et al., 2002
Donkey	2.5	IV	1.43	1[b]	1680	Peck et al., 2002
Mule	2.5	IV	1.35	1.4[b]	942	Peck et al., 2002
Horse (adult)	2.5	IV	1.96	2.8	530	Gustafsson et al., 1999
Horse (adult)	5	PO (fed)	NR	5.1	NR	Gustafsson et al., 1999
Horse (adult)	5	IV	1.68	2.74	509.4	Van Duijkeren et al., 1994c
Horse (adult)	5	PO (fasted)	NR	2.58	NR	Van Duijkeren et al., 1994c
Horse (adult)	5	IV	1.51	2.57	463.8	Van Duijkeren et al., 1995
Horse (adult)	25	PO (fasted)	NR	3.11	NR	Van Duijkeren et al., 1995
Horse (adult)	25	PO (mixed with concentrate)	NR	6.46	NR	Van Duijkeren et al., 1995

NR, not reported; IV, intravenously; SC, subcutaneously; PO, orally; Vd (volume of distribution); $T^1/_2$ (half-life).

[a] First dose is trimethoprim; second dose is sulfadiazine (except for Davitiyananda and Rasmussen, 1974, in which the sulfonamide is sulfadoxine).

[b] Reported as mean residence time (MRT).

[c] Dose reported in mg/kg/24 h.

Table 32.6 Some pharmacokinetic parameters of aditoprim, ormetoprim, tetroxoprim, and metioprim in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Aditoprim:						
Calves (80 kg, milk fed)	5.0	IV	10.44	13.0	11.03	Sutter et al., 1993
Calves (80 kg, conventionally fed)	5.0	IV	9.72	14.8	8.20	Sutter et al., 1993
Calves (160 kg, milk fed)	5.0	IV	9.64	10.7	12.17	Sutter et al., 1993
Calves (160 kg, conventionally fed)	5.0	IV	6.29	8.8	10.29	Sutter et al., 1993
Calves (210 kg, conventionally fed)	5.0	IV	7.16	7.2	13.75	Sutter et al., 1993
Calves (80 kg, milk fed)	5.0	PO	NR	11.6	NR	Sutter et al., 1993
Calves (80 kg, conventionally fed)	5.0	PO	NR	11.60	NR	Sutter et al., 1993
Calves (160 kg, milk fed)	5.0	PO	NR	10.2	NR	Sutter et al., 1993
Calves (160 kg, conventionally fed)	5.0	PO	NR	NR	NR	Sutter et al., 1993
Calves (210 kg, conventionally fed)	10.0	PO	NR	16.6	NR	Sutter et al., 1993
Dairy cows (3–7 years old)	5.0	IV	6.28	7.26	820.0	Lohuis et al., 1992
Dairy cows (3–7 years old, mammary endotoxin)	5.0	IV	12.25	about 7 h	1000.0	Lohuis et al., 1992
Horses	5	IV	7.8	12	300	Fellenberg et al., 1990
Ormetoprim:						
Calves (6–8 months old)	5.5/27.5[a]	IV	1.450	1.37	13.71	Wilson et al., 1987
Mare[b]	9.2/45.8[a]	IV	1.66	1.19	671.0	Brown et al., 1989
Tetroxoprim:						
Dogs	5.0	IV	NR	5.45	NR	Vergin et al., 1984
Metioprim:						
Dogs	5.0	IV	NR	3.07	NR	Vergin et al., 1984

NR, not reported; IV, intravenously; PO, orally; Vd (volume of distribution); $T^1/_2$ (half-life).
[a] First dose is trimethoprim; second dose is sulfadimethoxine.
[b] One mare studied.

which drug concentrations are low is the central nervous system (Brown et al., 1988). Although trimethoprim–sulfonamides can be used to treat CNS infections, higher doses may be required in order to reach effective concentrations (Brown et al., 1988). Administration of dimethylsulfoxide (DMSO) concurrently does not increase the penetration across the blood–brain barrier (Green et al., 1990).

Metabolism

Metabolism and elimination have been examined in several of the veterinary species. One phenomenon that is apparent from these studies is that herbivores metabolize sulfonamides and trimethoprim at a faster rate and more extensively than carnivores or omnivores. This may be caused by a higher metabolic capacity among herbivores – because of the nature of their diet and compounds to which they are exposed – compared to carnivores. Metabolic pathways are discussed in more detail by Nouws et al. (1988c, 1987). Acetylation of the NH_2 group on N-4 is a major mechanism of metabolism. Hydroxylation of the methyl group on the pyrimidine ring, in addition to carboxylation, also occurs. The extent to which these metabolites are produced is drug- and species-dependent. Acetylation and hydroxylation increases the polarity of the sulfonamides, which increases excretion (Nouws et al., 1988c). Acetylation (mainly occurring in the liver and lung) is the major pathway by which sulfonamides are metabolized in most species. Acetylated metabolites are the major urinary metabolites in cattle, sheep, and swine. The canine (dogs and other canine species) lacks the ability to acetylate aromatic amines, relying on alternative metabolic pathways to convert sulfonamides to less active forms. Acetylated metabolites are less soluble than the parent compounds and increase the risks of renal tubular injury caused by precipitation and crystal formation. Glucuronide conjugation and aromatic hydroxylation are two additional metabolic pathways by which sulfonamides are metabolized in animals. Glucuronide metabolites are water soluble and are excreted in urine, decreasing the risk of precipitation in renal tubules. Deacetylation, oxidation, deamination, conjugation with

sulfate, and cleavage of heterocyclic rings of sulfonamide molecules have also been reported (Bevill, 1988). Regardless of the metabolic pathway taken, metabolites have either reduced therapeutic activity (hydroxy metabolite) or are therapeutically inactive (N4-acetyl metabolite).

Excretion

Sulfonamides that are capable of obtaining therapeutic blood concentrations are excreted by the kidneys, either as the parent compound or as metabolites via glomerular filtration (unbound to plasma proteins). Subsequently, urine concentrations are consistently higher than the corresponding plasma drug concentrations (approximately 10 times higher for trimethoprim and 30 times higher for the sulfonamides), which aids in the treatment of lower urinary tract infections. There also is some active carrier-mediated proximal tubular excretion and passive absorption of the nonionized drug from the distal tubular fluid. Small concentrations of sulfonamides are also excreted in the tears, feces, bile, milk, and sweat. Low urine pHs favor tubular reabsorption and hence longer half-lives of the sulfonamides, whereas alkalinization of the urine increases urinary excretion by slowing this pH-dependent passive reabsorption in the tubules. Tubular reabsorption is responsible for the long half-lives observed for some sulfonamides. Nonabsorbed sulfonamides intended for intestinal activity are primarily eliminated via the feces, with little of the active or metabolized drug being absorbed systemically to be excreted by these renal mechanisms.

Adverse Effects Caused by Sulfonamides

Sulfonamides can produce a variety of adverse effects in animals. Likewise, when trimethoprim- or ormetoprim–sulfonamide combinations are administered, the adverse effects are primarily attributed to the sulfonamide component.

Crystalluria

Crystalluria, hematuria, and renal tubule blockage can occur owing to precipitation of the sulfonamide in the glomerular filtrate of the kidney. Subsequently, crystals of sulfonamides can form in the renal tubules. This problem is not as important as it once was because it was caused primarily from older insoluble preparations. Sulfadiazine is the least soluble and can precipitate in renal tubules at acidic pH. Even though this complication is rare with the current use of sulfonamides, one should ensure that patients are well hydrated when receiving sulfonamides because renal failure caused by sulfonamide crystals has been reported in human patients that are dehydrated. The tubular blockage has been anecdotally reported in animals, but with current formulations it is not considered an important clinical problem. It is noteworthy that this problem is more likely with acetylated metabolites of sulfonamides, which are not formed in dogs.

Keratoconjunctivitis Sicca

Keratoconjunctivitis sicca (KCS), also known as "dry eye", is a lack of adequate tear production resulting in ocular inflammation, irritation, and susceptibility to infection. Several cases of sulfonamide-induced KCS have been reported in dogs treated with sulfasalazine, sulfadiazine, and sulfamethoxazole (Morgan and Bachrach, 1982; Slatter and Blogg, 1978; Collins et al., 1986). The reaction is seen most commonly after chronic treatment, but cases have been reported that received only short-term administration. Berger et al. (1995) observed 33 dogs of various breeds for the occurrence of KCS after trimethoprim–sulfadiazine treatment, as characterized by changes in the Schirmer tear test values. There has been disagreement as to whether this effect is caused by an intrinsic dose-related effect, or is idiosyncratic. The prognosis appears to depend on the animal's age and duration of exposure (Morgan and Bachrach, 1982). Dogs treated with sulfonamides should have tear production checked periodically.

The reaction apparently is caused by a lacrimotoxic effect of the sulfonamide component (toxic to the lacrimal acinar cells). The lacrimotoxic effect may be caused by the nitrogen-containing pyridine ring on the lacrimal acinar cells (Collins et al., 1986; Slatter and Blogg, 1978). Reversal of KCS may or may not occur once sulfonamide therapy has been discontinued.

Hypersensitivity

A delayed hypersensitivity reaction has been described primarily in dogs (Trepanier, 1999; Trepanier et al., 2003). The reaction may be caused by either sulfadiazine, sulfadimethoxine, or sulfamethoxazole. Doberman Pinschers may be more susceptible than other breeds (Giger et al., 1985; Cribb, 1989; Cribb and Spielberg, 1990). This may be a serum sickness reaction (type III hypersensitivity) or involve another mechanism of cytotoxicity and hypersensitivity, or may be idiosyncratic (Trepanier, 2004). The lesions include, but are not limited to, glomerulopathy, polymyositis, polyarthritis, skin rash, skin eruptions, fever, hepatotoxicity, thrombocytopenia, neutropenia, and anemia (Giger et al., 1985; Cribb, 1989; Rowland et al., 1992). The reaction is caused by the sulfonamide component rather than trimethoprim (Giger et al., 1985). In affected dogs, the clinical signs quickly resolved after the sulfonamide was discontinued. However, some problems such as hepatopathy did not resolve

in dogs after the initial drug-induced injury (Trepanier et al., 2003). There is some evidence that a reaction to a metabolite of the sulfonamide, rather than an immunological reaction to the parent drug is responsible for these signs (see Section Effect of Acetylator Status on Adverse Reactions).

Hepatic Necrosis

A component of the hypersensitivity reaction is hepatic necrosis. Trimethoprim–sulfadiazine and trimethoprim–sulfamethoxazole combination therapy in dogs has resulted in hepatic necrosis (Twedt et al., 1997; Dodds, 1997). Hepatotoxicity may be caused by a hypersensitivity reaction or a result of an abnormal metabolic pathway, which allows the production or accumulation of hepatotoxic metabolites.

Blood Clotting Disorders

Hypoprothrombinemia has been reported in dogs (Neer and Savant, 1992; Patterson and Grenn, 1975), in coyote pups (Brown et al., 1982), and in Leghorn chickens (Daft et al., 1989) given sulfaquinoxaline. Sulfaquinoxaline is unique among the sulfonamides in that it can induce hypoprothrombinemia in animals within 24 hours after dosing by lengthening prothrombin times. It is thought that this adverse effect is unrelated to the individual sulfonamide or to the quinoxaline portion of the sulfaquinoxaline molecule but occurs when the two entities are combined into a single molecule. Sulfaquinoxaline is not an anticoagulant in vitro, nor does it destroy or otherwise inactivate prothrombin. Nevertheless, sulfaquinoxaline can be an inhibitor of vitamin K epoxide reductase, and this inhibition is the most likely reason for the hypothrombinemic reaction seen in the reported cases of sulfaquinoxaline toxicosis. Treatment is by vitamin K_1 administration for 4–7 days, and recovery is usually uneventful.

Blood Dyscrasias

Anemia and thrombocytopenia have been associated with administration of sulfonamides (Weiss and Adams, 1987; Weiss and Klausner, 1990; Stockner, 1993). Mammals derive their folic acid preformed, either in the diet or from bacteria that produce the vitamin in the intestinal tract. The anemia induced by trimethoprim–sulfonamide combinations may be caused by decreased serum folate reductions, presumably by inhibiting the folate production by intestinal bacteria or by blocking its reduction to tetra- and dihydrofolate, resulting in lowered serum folate concentrations in the animal's serum, which eventually induce an anemia. Folate deficiency anemia is rare but should be monitored with long-term use. Some veterinarians administer folic acid or folinic acid (vitamin B supplements) to patients receiving trimethoprim–sulfonamides. Whether this is routinely necessary, or whether this is effective, is controversial and unproven.

Thrombocytopenia has been reported in animals and in humans (Sullivan et al., 1992; Dodds, 1993). The thrombocytopenia in animals, as in humans, is probably associated with an immune-mediated or hypersensitivity reaction. The thrombocytopenia usually resolves after the drug is discontinued.

Thyroid Metabolism Disorders

Both sulfamethoxazole and sulfadiazine have been associated with hypothyroidism in dogs. The effect is probably caused by the ability of sulfonamides to inhibit thyroid peroxidase activity. Studies have demonstrated that administration of trimethoprim–sulfamethoxazole at a dose of 30 mg/kg, q 12 h for 6 weeks, or 15 mg/kg q 12 h for as short as 3 weeks decreased thyroxine (T_4) levels in dogs and decreased thyroid stimulating hormone (TSH) response (Frank et al., 2005; Hall et al., 1993; Williamson et al., 2002). The hypothyroidism is reversible, and can return to normal in as short as 1 week, or in most dogs by 3 weeks, after discontinuing the drug (Hall et al., 1993; Williamson et al., 2002). One study (Panciera and Post, 1992) produced conflicting evidence in which administration of trimethoprim–sulfadiazine at a dose of 15 mg/kg, q 12 h for 4 weeks had no effect on total T_4, free-T_4, or TSH tests. Sulfadimethoxine has also been implicated as being goitrogenic to swine fetuses in late gestation (Blackwell et al., 1989).

Skin Reactions

Sulfonamides are among the most common drugs implicated in skin eruptions in people, especially the drug-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (Roujeau et al., 1995). In dogs, skin reactions (drug eruptions) also are possible (Medleau et al., 1990). The skin reactions in dogs are believed to be a manifestation of the hypersensitivity reaction described in Section Hypersensitivity (Trepanier, 1999).

Effect of Acetylator Status on Adverse Reactions

Adverse effects in people have been associated with acetylator status. In slow acetylators a greater proportion of the drug may be directed to conversion of a more toxic metabolite, sulfonamide–hydroxylamine or nitroso compounds, which are more toxic to cells. Ordinarily,

these metabolites are detoxified by glutathione conjugation, but some patients may lack this ability. Dogs lack ability to acetylate drugs; therefore, they may be more susceptible to adverse effects than other animals. There is evidence that some dogs are more susceptible to the adverse effects of the hydroxylamine–sulfonamide metabolite than mixed-breed dogs because of decreased ability to handle the metabolite, which may explain the higher incidence of adverse effects reported in Dobermans (Cribb and Spielberg, 1990). (Other drugs that are acetylated in people include dapsone and isoniazid and these also may present a higher risk of toxicity in dogs.)

Diarrhea

Diarrhea has been associated with trimethoprim–sulfonamide therapy in horses. However, this effect may not be any more common from trimethoprim–sulfonamides than from other orally administered antimicrobials in horses. When healthy horses were administered doses of 25 to 100 mg of sulfadiazine in combination with 5 to 20 mg/kg of trimethoprim there was no evidence of increased *Clostridium perfringens*–associated colitis (White and Prior, 1982). In another study, Gustafsson et al. (1999) administered trimethoprim–sulfadiazine twice daily for 5 days to horses and measured the effect on intestinal flora. There was an initial decline in bacterial numbers, but these rebounded and the authors concluded that this treatment did not produce a disturbance of the intestinal bacterial flora.

Carcinogenesis

Sulfamethazine has been demonstrated to induce thyroid hyperplasia in rats (Astwood et al., 1943; MacKenzie and MacKenzie, 1943; Swarm et al., 1973) and induce specific types of thyroid cancer in both mice and rats. Fullerton et al. (1987) found that male and female Fischer 344 rats fed diets containing 1200 or 2400 ppm of sulfamethazine had thyroid weights that were increased significantly more than controls and that these increased weights were most likely due to thyroid hyperplasia related to increased thyroid-stimulating hormone levels. Littlefield et al. (1989) fed sulfamethazine to mice and induced follicular cell adenomas of the thyroid gland after 24 months of continuous feeding at a 4800 ppm dose with focal follicular cell hyperplasia and other organ aberrations being noted at some of the lower doses of sulfamethazine. In a similar study, there was a statistically significant increase in the incidence of thyroid follicular cell adenocarcinomas in rats sacrificed after 24 months of continuous feeding of sulfamethazine, with other nonneoplastic lesions of the thyroid also being reported in other treatment groups. There are no reports that have associated sulfonamide administration with cancer in domestic animals.

Potassium Regulation

Trimethoprim has been associated with hyperkalemia in people and laboratory animals, but except for anecdotal accounts, this has not been well documented in veterinary species. The mechanism of hyperkalemia appears to be caused by inhibition of renal Na-K-ATPase in the face of intact H-K-ATPase activity. The effects of trimethoprim can mimic amiloride, a potassium-sparing diuretic. These effects could be potentiated by coadministration of angiotensin converting enzyme inhibitors (ACE inhibitors), such as enalapril or benazepril, or administration of an angiotensin receptor blocker.

Sulfonamides in Veterinary Medicine

Sulfadimethoxine

Sulfadimethoxine (Figure 32.1) is a long-acting sulfonamide that has been used alone or in combination with ormetoprim (ormetoprim–sulfadimethoxine) for the treatment of susceptible microbial infections of cattle, swine, horses, poultry, fish, and dogs, in addition to other vertebrate and invertebrate animals. The combination is discussed in Section Sulfadimethoxine–Ormetoprim.

Sulfadimethoxine pharmacokinetics have been reported for many species. In dogs, the oral absorption is 49%, with a half-life of 13.1 hours (Baggot et al., 1976). Peak serum concentrations in dogs, at a dose of 55 mg/kg oral, was 67 μg/ml (mean). Systemic clearance in dogs is via the kidneys.

Sulfadimethoxine pharmacokinetics in cattle has been described by many investigators. Bourne et al. (1981) administered adult cattle with 107 mg/kg either intravenously (IV) or orally. In the IV study, sulfadimethoxine plasma concentrations peaked at 0.5 hours after administration and slowly declined over time, with the parent compound, acetylsulfadimethoxine, and a "polar" metabolite being found in the urine for at least 48 hours after the IV dose. The volume of distribution (Vd) was 0.315 l/kg in those cattle. In the oral study, plasma concentrations of sulfadimethoxine started low at 0.5 hours and gradually peaked at 10 hours after dose and then began to drop, with detectable levels of parent compound and all metabolites being found in the urine for at least 84 hours after dosing. Bioavailability of sulfadimethoxine was calculated to be 59.1%. Boxenbaum et al. (1977) administered 55 mg/kg IV sulfadimethoxine (40% solution) or 55 mg/kg orally to cattle, followed by 27.5 mg/kg sulfadimethoxine administered orally at

24, 48, and 72 hours after the initial loading dose. After IV injection, the half-life of sulfadimethoxine was determined to be 12.5 hours, with a volume of distribution of 0.31 l/kg. This study also confirmed that adequate plasma concentrations (>50 μg/ml) were maintained throughout the oral-dosing study, and this method could be used when IV administration was not possible. By comparison, a study by Wilson et al. (1987) demonstrated that sulfadimethoxine (27.5 mg/kg) in combination with ormetoprim (5.5 mg/kg) administered IV to cattle had a shorter half-life, 7.91 hours, and volume of distribution of 0.185 l/kg. When given the same dose orally, bioavailability of sulfadimethoxine was 56.6%.

Studies by Righter et al. (1979) examined the pharmacokinetics of sulfadimethoxine in mature, growing, and suckling pigs. Mature pigs dosed with 20, 50, or 100 mg/kg of sulfadimethoxine IV had volume of distribution values of 0.178, 0.258, and 0.331 l/kg and total body clearance of 4.21, 5.54, and 7.37 ml/kg/h, respectively. The pharmacokinetic parameters of 55 mg/kg sulfadimethoxine given IV to growing and suckling pigs have also been reported. Suckling pigs (1–2 weeks old) had sulfadimethoxine half-lives of 16.16 hours, volume of distribution of 0.483 l/kg, and total body clearance of 20.9 ml/kg/h. In contrast, growing pigs (11–12 weeks old) had sulfadimethoxine half-lives of 9.35 hours, volume of distribution of 0.347 l/kg, and total body clearance of 26.1 ml/kg/h, indicating an age-related effect of sulfadimethoxine pharmacokinetics in young pigs. Weanling pigs consuming water dosed with 0.05 g sulfadimethoxine/100 ml showed mean plasma concentrations of 80 ppm 12 hours after introduction of the medicated water, with plasma concentrations declining to approximately 50 ppm thereafter. Total water consumption was not affected, which indicated that sulfadimethoxine may be of therapeutic use in swine provided that water consumption is maintained throughout the medication period. Mengelers et al. (1995) dosed 34–40 kg healthy and febrile (inoculated endobronchially with *Actinobacillus pleuropneumoniae* toxins) pigs with 25 mg/kg sulfadimethoxine and 5 mg/kg trimethoprim intravenously. Sulfadimethoxine plasma half-lives for both healthy and pneumonic pigs were not significantly different (approximately 13 hours). Trimethoprim half-lives were not significantly different between healthy and pneumonic pigs (approximately 2.7 hours); however, the half-lives were significantly shorter than the half-life of sulfadimethoxine. In addition, the volume of distribution values of healthy and pneumonic pigs receiving sulfadimethoxine were not significantly different (approximately 0.25 l/kg), but trimethoprim did show significant differences between healthy (1.21 l/kg) and pneumonic (1.49 l/kg) pigs. The mean area under the curve (AUC) of trimethoprim was decreased and the total body clearance was increased in the febrile pigs, but with no significant changes in these sulfadimethoxine pharmacokinetic parameters.

Sulfadimethoxine is available in a concentrated solution (e.g., Albon 12.5%) for cattle or poultry; oral suspension (e.g., 5% Albon Suspension), and tablets and boluses for dogs, cats, and cattle; extended release tablets (Albon SR); 40% injection used in dogs, cats, and cattle; and soluble powder that can be added to drinking water for cattle and poultry. The approved clinical use for this formulation is for treatment of intestinal coccidiosis, bacterial enteritis, fowl cholera, bacterial pneumonia, pododermatitis in cattle, skin and soft tissue infections in dogs and cats, and bacterial cystitis in dogs. On the product labels for treating bacterial infections, it states "for treatment of susceptible bacteria causing these infections." Because resistance can be common, some of the conditions listed above may not respond appropriately to treatment and may be outdated.

The clinical use of sulfadimethoxine has been described for turkeys (Epstein and Ashworth, 1989), dogs (Yagi et al., 1981; Fish et al., 1965; Dunbar and Foreyt, 1985; Imamura et al., 1986, 1989), primates (Adamson et al., 1970; Bridges et al., 1968), lobsters (James and Barron, 1988), channel catfish (Squibb et al., 1988), and rainbow trout (Kleinow and Lech, 1988). Detection of violative levels of sulfadimethoxine residues in channel catfish has also been reported (Walker and Barker, 1994).

Sulfamethazine (Sulfadimidine)

Sulfamethazine (sulfadimidine) (Figure 32.1), like many sulfonamides, has been utilized for decades in veterinary medicine; hence, the veterinary literature contains many reports on its usage in a wide variety of animals, including cattle, horses, swine, poultry, small ruminants, and rabbits (among others). Table 32.2 summarizes some of the pharmacokinetic parameters of sulfamethazine in animals.

Sulfamethazine has been administered to cattle and swine, and is formulated for use in drinking water (Church et al., 1979), as a feed additive, an extended-release bolus, and an IV preparation. Sulfamethazine has been marketed as a sole treatment and in combination with other antimicrobials, such as other sulfonamides, tylosin, chlortetracycline, and procaine penicillin G.

The product labels for sulfamethazine products include uses for treating intestinal coccidiosis, bacterial enteritis, pneumonia, and pododermatitis in cattle. Sulfamethazine is available as an oral solution for calves, poultry, pigs, and cattle. It is available as a 12.5% solution to be added to drinking water. It is also available as a powder to be added to the drinking water of these

species. Tablets (Sulmet 2.5 and 5-gram tablets) are also available for large animals, as well as extended-release tablets (e.g., Sulfa-Max) in a range of sizes for calves and adult cattle. Triple-sulfa products containing sulfamethazine (sulfamethazine, sulfanilamide, and sulfathiazole) as well as sulfamethazine–sulfathiazole combination oral powder are no longer available in the United States, but may still be available in other countries.

The basic pharmacokinetic parameters of sulfamethazine in cattle have been reported by Bevill et al. (1977a) and Nouws et al. (1988c), among many others. Of particular interest are the oral forms of sulfamethazine that have been formulated in extended-release (sustained-release) form for cattle. Several reports on the efficacy and clinical uses of the extended-release form of sulfamethazine in cattle are available (Clark et al., 1966; Ellison et al., 1967; Miller et al., 1969; Carlson et al., 1976). This sustained-release formulation has been reported to achieve blood concentrations sufficient for susceptible bacteria within 6–12 hours after oral administration and to maintain or exceed that level for 2–5 days after dosing. The sustained-release formulation of sulfamethazine has been reported to be effective for treating bovine respiratory disease, diphtheria, and pneumonia in cattle (Carlson et al., 1976; Clark et al., 1966). Clearance of sulfamethazine and its metabolites in cattle are age and dose dependent (Nouws et al., 1986a, 1985b, 1983; Lapka et al., 1980). Several metabolites of sulfamethazine have been identified and described in both adult cattle and calves (Nouws et al., 1988c).

The pharmacokinetics of sulfamethazine and its metabolites are of particular interest in swine. Sulfamethazine and its metabolites were often associated with violative levels in pork products because of sulfamethazine's widespread use as a swine feed additive. Sulfamethazine has been used extensively to treat a host of susceptible microbial infections in swine, including *Salmonella typhisuis* (Fenwick and Olander, 1987) and *Bordetella bronchiseptica* (Kobland et al., 1984). Sulfonamides were one of the most common causes of food-residue violations reported by the US Food Safety Inspection Service, with swine being the food-animal species with the greatest number of residue violations (Sweeney et al., 1993).

Pharmacokinetic parameters for swine have been described by Sweeney et al. (1993) and others (see Table 32.2), including pharmacokinetics of metabolites (Nouws et al., 1989a, 1986b). Several studies have used radiolabeled (Mitchell et al., 1986; Mitchell and Paulson, 1986) and nonradiolabeled (Biehl et al., 1981; Ashworth et al., 1986) sulfamethazine to determine the elimination patterns of sulfamethazine and its metabolites from the tissues in swine. Other studies have shown that the major metabolites produced from sulfamethazine metabolism in swine are N_4-acetylsulfamethazine, N_4-glucose conjugate of sulfamethazine, and desaminosulfamethazine (Mitchell et al., 1986). Studies using pigs fed 110 ppm of ^{14}C-sulfamethazine in the feed for 3–7 days, euthanized, and their tissues examined for total radioactivity and metabolite content found the highest concentration of radioactivity in the gut (undigested feed). Blood, kidney, urine, and liver all had high concentrations of radioactivity (i.e., parent compound and metabolite). Adipose tissue contained the least amount of radioactivity of all tissues assayed (Mitchell et al., 1986). Specific metabolites found in these and other tissues of swine given ^{14}C-labeled sulfamethazine in the feed have been reported by Mitchell and Paulson (1986).

Other studies have also reported on sulfamethazine residues in swine (Ashworth et al., 1986; Biehl et al., 1981). In addition, sulfamethazine and its metabolites have been described in pigs using physiological based pharmacokinetic (PBPK) models (Buur et al., 2005, 2006; Mason et al., 2008). These studies demonstrated significant amount of variability in disposition and metabolism, which would suggest potential for tissue residue violations with minor differences in dosing, environmental contamination, or in the presence of disease.

Cattle and swine are the two major species in which sulfamethazine is approved for use, with fewer reports in other species. Pharmacokinetic parameters and/or tissue-depletion kinetics of sulfamethazine and metabolites have been established in ponies (Wilson et al., 1989; Nouws et al., 1987) and horses (Nouws et al., 1985a). Studies on pharmacokinetics of sulfamethazine in goats (Abdullah and Baggot, 1988; Witcamp et al., 1992; Nouws et al., 1988b, 1989b; Elsheikh et al., 1991; Youssef et al., 1981; van Gogh et al., 1984; Witcamp et al., 1993), sheep (Srivastava and Rampal, 1990; Bourne et al., 1977; Bevill et al., 1977c; Bulgin et al., 1991; Nawaz and Nawaz, 1983), dogs (Riffat et al., 1982), chickens (Righter et al., 1971; Nouws et al., 1988a; Goren et al., 1987), rabbits (Yuan and Fung, 1990), mice (Littlefield et al., 1989), buffalo (Singh et al., 1988), camels (Younan et al., 1989), and carp and rainbow trout (van Ginneken et al., 1991) also have been published.

Lashev et al. (1995) described altered pharmacokinetics in roosters treated with a single 50 mg/kg IV dose of sulfadimidine only or IV sulfadimidine after 2 weeks of four 3.5 mg/kg subcutaneous (SC) testosterone treatments. Normal and castrated roosters provided no significant differences in half-life values, which ranged from 7.62 hours (castrated) to 9.38 hours (intact). Roosters pretreated with testosterone and then dosed with sulfadimidine had half-lives of 23.85 hours, as well as significantly decreased clearance and volume of distribution. Chickens metabolize sulfamethazine in relatively equal parts through hydroxylation and

acetylation. It was hypothesized in this study that the acetylation pathway of sulfamethazine metabolism was retarded by the testosterone treatments and resulted in the prolonged half-lives.

Sulfaquinoxaline

Sulfaquinoxaline (Figure 32.1) has been used primarily for control of coccidia and some susceptible bacterial diseases in poultry. The veterinary literature also contains a few reports of sulfaquinoxaline use in rabbits (Eppel and Thiessen, 1984; Joyner et al., 1983) and dogs (Brown et al., 1982; Patterson and Grenn, 1975), but clinical use in these species is rare today.

Sulfaquinoxaline is available as an oral solution in a range of concentrations (20–32%). These solutions are intended to be mixed with drinking water. In some countries (but not the US) there are combinations of pyrimethamine and sulfaquinoxaline solution for administration to drinking water.

For the use in poultry, sulfaquinoxaline has been administered to control coccidiosis. Mathis and McDougald (1984) described the therapeutic effectiveness of sulfaquinoxaline and sulfaquinoxaline–pyrimethamine against several coccidia species of *Eimeria*. It was determined from that study that both sulfaquinoxaline and sulfaquinoxaline–pyrimethamine were highly effective against *E. acervulina* but less effective against *E. tenella*. In addition, the potentiated mixture was determined to be more effective against *E. tenella* than sulfaquinoxaline alone, although neither mixture was found to be particularly effective against any cecal coccidia. Amprolium was efficacious against cecal–dwelling forms of coccidia; hence amprolium has been combined with sulfaquinoxaline or sulfaquinoxaline–pyrimethamine to enhance the spectrum of activity. Ineffectiveness of sulfaquinoxaline–pyrimethamine against *E. tenella* has also been documented in another study (Chapman, 1989), underlining the importance of correct coccidia species identification before instituting anticoccidial therapy with sulfaquinoxaline or any other sulfonamide.

Banerjee et al. (1974) reported on the blood concentrations after administration of sulfaquinoxaline, which were in the therapeutic range. In that same study, sulfaquinoxaline was found in high concentrations in the liver, kidney, and cecum, with the lowest concentrations found in the yolk sac and brain. A single oral dose of ^{35}S-labeled sulfaquinoxaline in 1-week-old chicks showed rapid uptake from the gastrointestinal tract and wide distribution throughout the body, including crossing of the blood–brain barrier. At 0.5 hours after dosing, autoradiography showed that all tissues (brain, lung, liver, kidney, fat, and muscle), except the lens of the eye had measurable concentrations of sulfaquinoxaline. Similar findings resulted from IV administration of ^{35}S-labeled sulfaquinoxaline, and it was also found that excretion of sulfaquinoxaline by the bile and secretion by the cecal mucosa, crop, and gizzard probably occur. Oral absorption was higher (3.5 times) in birds infected with *E. acervulina* and *E. tenella* compared to uninfected birds (Williams et al., 1995). A study by Li et al. (1995) found that in 7- to 8-week-old male and female broilers given a single 200 mg/kg oral dose of sulfaquinoxaline, peak concentration times in plasma and liver were similar (4 hours) but were longer in the heart, kidney, and muscle (6 hours). The half-life of sulfaquinoxaline was shortest in the muscle (4.5 hours), with significantly longer half-lives in the heart (10 hours), plasma (11 hours), liver (13 hours), and kidney (18 hours).

The safety and efficacy of sulfaquinoxaline alone or in combination with trimethoprim (trimethoprim:sulfaquinoxaline ratio is 1:3) have been reported in poultry (White and Williams, 1983; Piercy et al., 1984; Sainsbury, 1988). A total dose of 30 mg/kg/day PO satisfactorily controlled experimentally induced colisepticemia and pasteurellosis in addition to five species of coccidia (White and Williams, 1983). A wide margin of safety has been shown for the 1:3 combination of trimethoprim:sulfaquinoxaline in poultry, although decreased appetite and water consumption and lowered egg production, egg weight, and hatchability were noted when these antimicrobials were incorporated in the feed or water in higher than recommended concentrations (Piercy et al., 1984).

Toxicosis from sulfaquinoxaline use in animals is rare. Toxicity from sulfaquinoxaline has occurred in Leghorn chickens (Daft et al., 1989), where a mortality of 47% was reported in a commercial flock given a 0.05% concentration of sulfaquinoxaline in the feed. Lesions included mildly enlarged livers; swollen and pale livers; hemorrhages on the epicardium, kidney, oviduct, small intestine, and cecum; pale bone marrow; gangrenous dermatitis; and some lung involvement was present. Patterson and Grenn (1975) reported on 12 adult Miniature Poodles that received 3.16 g/l of sulfaquinoxaline in the drinking water as treatment for coccidiosis suffered similar lesions as described above in poultry, in addition to depressed body temperature, pale mucous membranes, microscopic hemorrhages of the jejunum and ileum, and prolonged prothrombin times. Although the exact mechanism has not been reported, sulfaquinoxaline possesses an ability to produce a marked hypothrombinemia (see Section Adverse Effects Caused by Sulfonamides), even in animals receiving balanced diets containing adequate amounts of vitamin K. These animals also responded to vitamin K treatment. It is thought that this adverse effect is not related to the individual sulfonamide or quinoxaline portion of the sulfaquinoxaline molecule but occurs only when the two entities are combined. A

similar toxicosis has also been reported in coyote pups treated with sulfaquinoxaline (Brown et al., 1982).

Sulfamerazine

Sulfamerazine (Figure 32.1) has primarily been utilized in adult sheep and lambs to treat susceptible microbial infections. In these ruminants, sulfamerazine has been used alone or in combination with other antibiotics (tylosin) and other sulfonamides (sulfamethazine, sulfadiazine).

The pharmacokinetics of sulfamerazine has been described for ewes and lambs. Hayashi et al. (1979) described the pharmacokinetics of sulfamerazine in ewes after either IV or oral administration of 107 mg/kg. After oral administration, systemic availability was 81 ± 19%. Urinary concentrations of parent compound and metabolites were also reported for both IV and PO dosing studies. Both routes produced appreciable concentrations of sulfamerazine and three metabolites in the urine (described as a polar metabolite acetylsulfamerazine). Intravenous sulfamerazine produced more parent compound and fewer metabolites were found in the urine, which is attributed to lack of rumen metabolism, while more metabolite than parent compound was found in the PO study because of conversion by rumen metabolism.

The pharmacokinetics of sulfamerazine has also been reported in neonatal and young lambs (Debacker et al., 1982). Lambs from birth to 16 weeks of age were administered either IV or PO, 100 mg/kg of sulfamerazine. In the IV study, it was found that the sulfamerazine half-life was longest in the first week of life (9–14 hours) and decreased to 4–7 hours by 9–16 weeks of age. The volume of distribution was highest during the first week of life and steadily decreased with age, while clearance of sulfamerazine was lowest in the first week of life (20–40 ml/kg/h) and steadily increased with age up to 9–16 weeks of age (50–80 ml/kg/h).

Sulfathiazole

Sulfathiazole use in veterinary medicine has declined. It has been formulated in combination with chlortetracycline HCl and procaine penicillin G, but reports of its use are rare and earlier editions of this text should be consulted for more details.

Sulfathiazole pharmacokinetics in sheep has been outlined by Koritz et al. (1977), and sulfathiazole tissue residues in sheep have been described by Bevill et al. (1977b). When 36 or 72 mg/kg of 5% aqueous solution of sulfathiazole sodium IV was given to ewe lambs, it cleared quickly from the plasma, with a low volume of distribution of 0.34 and 0.59 l/kg and half-lives of 1.2 and 1.4 hours, respectively. Ewes given 214 mg/kg orally of a 12.5% aqueous solution of sulfathiazole sodium had systemic bioavailability of approximately 73%, with a half-life of approximately 18 hours. Both oral and IV routes produce acetylsulfathiazole, and a third "polar" metabolite in the urine of these sheep. Sulfathiazole residues in sheep are highest in the kidney (308 ppm), followed by the liver (40 ppm), heart (34 ppm), shoulder muscle (23 ppm), leg and loin muscle (22 ppm), body fat (11 ppm), and omental fat (6.7 ppm). Residues quickly dropped to very low (<0.13 ppm) or to nondetectable levels by 24 hours after dosing in all tissues tested.

Pharmacokinetic parameters have also been reported for swine. Pigs given 72 mg/kg of sulfathiazole sodium IV had quick plasma elimination of the drug, with mean Vd of 0.54 l/kg and a biological half-life of 1.39 hours, similar to those for sheep. After a dose of 214 mg/kg orally, sulfathiazole had a Vd of 0.32 l/kg and a systemic bioavailability of 73%, identical to that of sheep.

Sulfasalazine (Salicylazosulfapyridine)

Sulfasalazine (Figure 32.3) was originally developed as a possible treatment for rheumatoid arthritis in humans. It was found, however, to be more effective in the treatment of inflammatory bowel disease. The most frequent use is to treat various forms of colitis (Aronson and Kirk, 1983). The gastrointestinal use is discussed in more detail in Chapter 46 of this book. Inflammatory bowel diseases (most commonly ulcerative colitis and Crohn's disease) have been treated with sulfasalazine in humans.

Sulfasalazine consists of two components, 5-aminosalicylic acid and sulfapyridine, which are linked by an azo bond. This bond is broken by the bacterial enzymes in the colon (Figure 32.3). After oral administration, the sulfonamide component is absorbed systemically, but the 5-aminosalicylic acid component produces a local inflammatory effect in the colon. 5-aminosalicylic acid is also known as *mesalamine* and there are now other forms of mesalamine (e.g., olsalazine) or enteric-coated (pH-sensitive) tablets that release mesalamine in the colon, while avoiding the systemic effects of the sulfonamide component.

The intestinal antiinflammatory effects may be due to prostaglandin inhibition (Hoult and Moore, 1978), inhibition of leukotrienes, decreased intestinal oxygen free radicals (Del Soldato et al., 1985), or sulfhydryls (Garg et al., 1991).

Sulfadiazine

The most commonly used formulation containing sulfadiazine is a trimethoprim–sulfadiazine combination (e.g., Tribrissen and others). This combination will be discussed in more detail in Section Trimethoprim–Sulfadiazine, because of its common use. Sulfadiazine

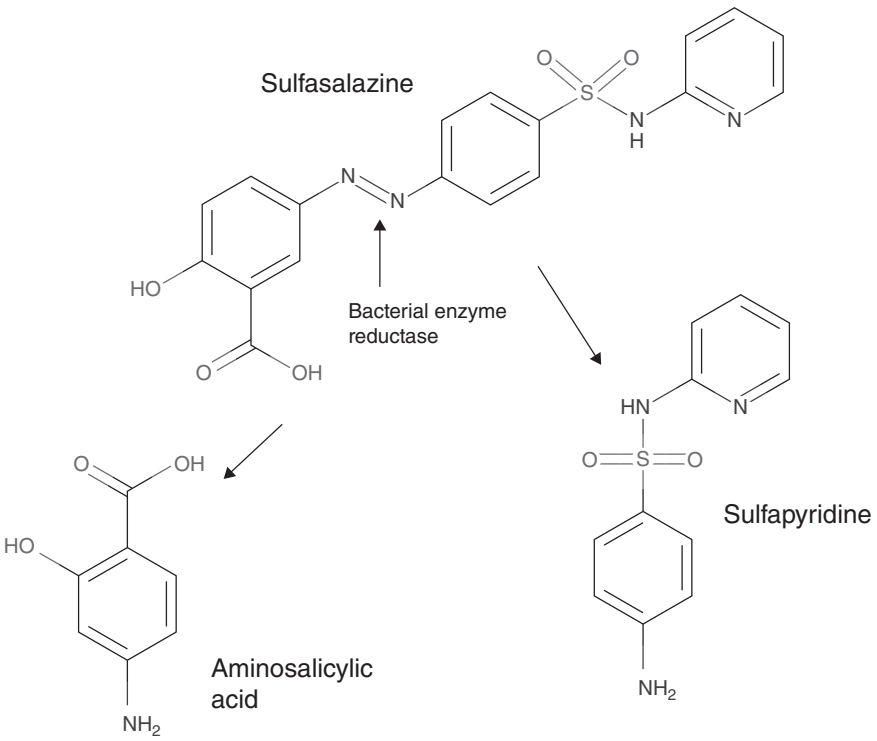


Figure 32.3 Structure of sulfasalazine, showing the reduction by bacterial enzymes in the colon (breaking the azo bond) to form 5-aminosalicylic acid and sulfapyridine. Sulfapyridine can be absorbed systemically but has no significant therapeutic effects; aminosalicylic acid has local antiinflammatory effects in the bowel.

(Figure 32.1) use alone has been reported in some studies. The pharmacokinetics are listed in Table 32.3. Sulfadiazine has been used to control plaque and gingivitis in dogs (Howell et al., 1989) and has attained concentrations in the cerebrospinal fluid (when administered IV) above the reported MIC values for many of the Enterobacteriaceae family (Vergin et al., 1984). In pigs, sulfadiazine administration was reported from various studies (Soli et al., 1990; Guise et al., 1986).

Sulfabromomethazine

Sulfabromomethazine is the brominated derivative of sulfamethazine and is considered a long-acting sulfonamide that is now rarely used. Sulfabromomethazine has a lower solubility than sulfamethazine, and single oral doses of the drug have been used to treat calf diphtheria and pneumonia, metritis, foot rot, and septic mastitis in cattle, with a repeated dose 48 hours later sometimes required. Use of sulfabromomethazine during the last 3 months of pregnancy should be avoided (Bevill, 1988).

Sulfaethoxypyridazine

Sulfaethoxypyridazine also is rarely used today. It is rapidly absorbed after oral administration to swine, sheep, and cattle and is extensively bound to plasma proteins. The parent compound and the N4-acetylated metabolite and another unidentified glucuronide conjugate seem to be the major urinary excretion products (Bevill, 1988). Sulfaethoxypyridazine can induce cataracts at some doses when fed to dogs and rats over a period of 27 and 118 weeks, respectively (Ribelin et al., 1967).

Sulfisoxazole

Sulfisoxazole has limited use today but has been used in the treatment of urinary tract infections in the dog and cat caused by susceptible bacteria (Bevill, 1988). The pharmacokinetics of sulfisoxazole has been studied in dogs, swine, and humans (Suber et al., 1981), as well as its delivery across the skin using iontophoresis (Inada et al., 1989). A formulation of combined trimethoprim–sulfisoxazole is available in some countries for injection of cattle.

Sulfachlorpyridazine

When used alone, sulfachlorpyridazine is administered most often to calves and pigs. Sulfachlorpyridazine powder, to be mixed into an oral solution (Vetisulid powder) or tablets (Vetisulid 2-gram tablets), has been administered to calves and pigs for treatment of enteritis at a dose of 33–49.5 mg/kg for calves and 44–77 mg/kg per day to pigs. There is also an injectable solution available (Vetisulid injection 200 mg/ml) for intravenous use in

calves. Sulfachlorpyridazine has also been used in combination with trimethoprim and this combination is discussed in more detail in the section on potentiated sulfonamides.

Sulfachlorpyridazine is rapidly eliminated from the plasma following IV administration. Intramuscular injections in swine result in maximum blood concentrations within 30 minutes after injection, which are maintained for up to 3 hours (Bevill, 1988). A single 50 mg/kg IV dose of sulfachlorpyridazine demonstrated significantly different volume of distribution in cocks (0.34 l/kg) versus hens (0.36 l/kg), with the sulfonamide being more slowly excreted in hens (Lashev et al., 1995).

The pharmacokinetics of sulfachlorpyridazine after oral and intracardiac administrations has also been described in the channel catfish (*Ictalurus punctatus*), and the drug has been found to have a potential use in aquaculture (Alavi et al., 1993).

Other Sulfonamides

This chapter has discussed the major sulfonamides in use in veterinary medicine today, and briefly some minor sulfonamides even though their used has declined significantly. However, other sulfonamides exist that are not currently, or are no longer, used in the US markets. Other sulfonamides that may be of interest, either historically or because of use in other countries, include sulfadimethoxypyrimidine (Walker and Williams, 1972), sulfasomidine and sulfamethomidine (Bridges et al., 1969), sulfamethoxypyridazine (Garg and Uppal, 1997), sulfamethoxydiazine (Weijkamp et al., 1994), and sulfamethylphenazole (Austin and Kelly, 1966). The combination of sulfadimethoxine–sulfamethoxazole use in healthy and pneumonic pigs (Mengelers et al., 1995) has also been reported.

Previous editions of this textbook or the individual references listed here may be consulted for more in-depth information on the older and less commonly used sulfonamides not discussed in this chapter.

Potentiated Sulfonamides

The combination of sulfonamides with trimethoprim or ormetoprim (Figure 32.4) produces greater antibacterial activity than either drug used alone. Consequently, in humans, dogs, cats, horses, and occasionally other animals, these formulations are used more commonly than sulfonamides alone.

Sulfonamide and trimethoprim combinations have been reviewed in some depth by Bushby (1980) and van Miert (1994). An extensive review of trimethoprim–sulfonamide combinations in the horse is also available (van Duijkeren et al., 1994b). Although these reviews are

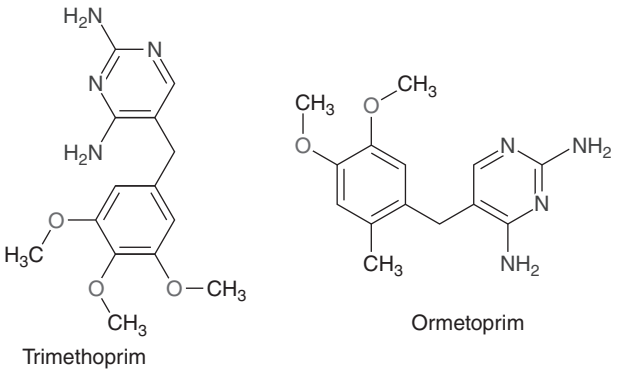


Figure 32.4 Structure of trimethoprim (left) and ormetoprim (right).

several years old, the pharmacology and use in veterinary medicine has not changed substantially in 30 years.

The pharmacokinetic parameters of diaminopyrimidines (trimethoprim, ormetoprim, and others) have been established for some species and are listed in Tables 32.5 and 32.6. Specific properties of these diaminopyrimidines are also discussed by Ascalone et al., 1986; Mengelers et al., 1990; Lohuis et al., 1992; Sutter et al., 1993; Wilson et al., 1987; Brown et al., 1989; Iversen et al., 1984; Vergin et al., 1984; Aschhoff, 1979.

The full range of combinations possible include a sulfonamide with trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl) pyrimidine), aditoprim (2,4-diamino-5-[4-(dimethylamino)-3,5-dimethoxybenzyl] pyrimidine), ormetoprim (2,4-diamino-5-[4,5-dimethoxy-2-methylbenzyl] pyrimidine), or tetroxoprim (2,4-diamino-5-[3,5-dimethoxy-4(2-methoxy ethoxy)benzyl] pyrimidine). These are commonly referred to as *potentiated sulfonamides*.

Trimethoprim–Sulfadiazine

Sulfadiazine is most often administered in combination with trimethoprim (e.g., Tribrissen and other veterinary products). This combination has been used for respiratory infections, urinary tract infections, urogenital infections, protozoal infections, bone and joint infections, and skin and soft-tissue infections. It is available as an oral paste (400 mg per gram, e.g., Tribrissen®) and oral suspension (Equilsul-SDZ®, 400 mg/ml). It also is available as a powder (e.g., Tucoprim®, Uniprim™) to be added to feed for horses. Feeding does not affect absorption of sulfadiazine in horses, but it may delay the absorption of the trimethoprim component. Tablets of sulfadiazine and trimethoprim (5:1 ratio) have been available in a range of sizes for small animals, but the availability of the small animal products has diminished in recent years. Injectable formulations have become less available, but

24% and 48% injections (e.g., Tribrissen®) have been used in a range of species.

Trimethoprim–sulfadiazine combination in a 1:5 ratio has been among the most popular antimicrobials in dogs, cats, and horses as a general "first-line" antimicrobial that can be useful for treating a wide variety of pathogens, in particular *Staphylococcus* spp., *Streptococcus* spp., and some gram-negative organisms, such as *Proteus mirabilis* spp., *Pasteurella* spp., and *Klebsiella* spp. However, resistance among the gram-negative bacilli *Enterobacteriaceae* can be common.

Dosing Recommendations

It is difficult to correlate plasma drug levels (and plasma elimination rates) with clinical efficacy and dosing intervals because trimethoprim is excreted faster (shorter half-life) than the sulfadiazine component; however, trimethoprim persists longer in some tissues than in the plasma and it is possible that tissue are maintained higher than plasma drug concentrations. Among the various species, doses range from 15 mg/kg twice daily, to 30 mg/kg twice daily. (See specific species recommendations.) Doses are generally listed as the combined product; that is, 30 mg/kg is equivalent to 5 mg/kg trimethoprim + 25 mg/kg of the sulfonamide.

The safety is generally acceptable for use in dogs, except for general concerns discussed for sulfonamides in this chapter. Specific toxicological studies have been conducted in both dogs and cats (Craig and White, 1976). In the Craig and White study, dogs were administered up to 300 mg/kg/day orally (10 times the recommended dose) of trimethoprim–sulfadiazine for as long as 20 days with no abnormal clinical signs or blood or serum chemistry abnormalities reported. Cats were more sensitive to adverse effects. When cats were administered 30 to 300 mg/kg/day orally for 10 to 30 days, the high dose (300 mg/kg) produced signs of lethargy, anorexia, anemia, leukopenia, and altered blood urea nitrogen (BUN).

Equine Use

Trimethoprim–sulfadiazine use in horses is common. Pharmacokinetics have been reported for horses (Tables 32.3 and 32.5) and dosing protocols have been established (McClure et al., 2015; White and Prior, 1982; Divers et al., 1981; Bertone et al., 1988). A common use in horses includes treatment of *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi* from horses (McCandlish and Thompson, 1979; McClure et al., 2015; van Duijkeren et al., 1994c). However, in tissue cages placed in horses, trimethoprim–sulfadiazine did not eliminate infection of *Streptococcus zooepidemicus* (Ensink et al., 2005). The inability to eliminate the infection in an infected environment may be caused by inhibitors – such as PABA and thymidine – present in abscessed and infected tissues that may inhibit the effects of these drugs.

For horses, the proper dosing regimen was reviewed by van Duijkeren et al. (1994c) and drug concentrations reported by Winther et al. (2011), and McClure et al. (2015). Drug concentrations of the oral paste are not as high as intragastric administration or the oral liquid suspension, but high enough for treatment. The conclusion reached from most studies in horses is that twice daily administration is needed because of the rapid elimination in horses – particularly of the trimethoprim component – and the need to maintain concentrations above the MIC throughout most of the dosing interval. This was confirmed in the studies by Winther et al. (2011) and McClure et al. (2015) for respiratory infections. In horses with joint infections the optimum dosage is 30 mg/kg q 12 h (Bertone et al., 1988). van Duijkeren et al. (2002) also concluded from an analysis of plasma concentrations that twice-daily administration to horses at 30 mg/kg (25 sulfadiazine; 5 trimethoprim) was necessary.

Small Animal Use

Trimethoprim–sulfadiazine combination was effective for treating urinary tract infections caused by *Staphylococcus intermedius* (now referred to as *S. pseudintermedius*) (Turnwald et al., 1986) as well as the more common pathogens such as *E. coli, Proteus mirabilis, Klebsiella pneumoniae,* and *Streptococcus* spp. (Ling and Ruby, 1979; Ling et al., 1984). Beagle dogs treated with trimethoprim–sulfadiazine (240 mg total of a 1:5 combination) once a day or the same daily dose divided into twice daily had high concentrations of both sulfadiazine and trimethoprim in their urine that greatly exceeded the MIC values for most susceptible pathogens (Sigel et al., 1981). The effectiveness for urinary tractions caused by *Enterococcus* spp. is doubtful based on the analysis by Wisell et al. (2008).

An overall success rate of 85% was reported in dogs and cats treated with a trimethoprim–sulfadiazine combination for microbial diseases involving the alimentary, respiratory, urogenital, skin, and other systems (Craig, 1972). A common use of trimethoprim–sulfadiazine is for the treatment of bacterial skin infections. Success rates of 90% (skin diseases either cured or improved) in bacterial skin infections; foot infections; interdigital abscesses; anal abscesses; and infections of the eye, ear, and mouth in dogs have been reported. A similar success rate was reported in cats (89%) with infections caused from bite wounds and other infections. Although good activity is expected against wild-type strains of *Staphylococcus pseudintermedius* isolated from dogs, methicillin-resistant strains are usually resistant.

Pharmacokinetic studies in dogs have reported that 30 mg/kg (25 sulfadiazine + 5 trimethoprim) administered once daily, should be adequate for most infections

caused by susceptible organisms (Sigel et al., 1981). In a specific study in which response for treating skin infections was examined (Messinger and Beale, 1993), there was no difference in response between once- or twice-daily administration. However, the authors acknowledged that the sample size may have been too small to detect a significant difference. Supporting a once daily schedule, a study in dogs with skin infections showed that 30 mg/kg once daily is adequate (Pohlenz-Zertuche et al., 1992). These authors reported that 30 mg/kg of trimethoprim–sulfadiazine orally at 12- or 24-hour intervals were found to attain therapeutically useful concentrations of both trimethoprim and sulfadiazine in the skin (Pohlenz-Zertuche et al., 1992).

In dogs, it also has been used for *Bordetella bronchiseptica* (Powers et al., 1980), ocular infections (Sigel et al., 1981), and prostate infections. The distribution into the prostate is discussed in Section Distribution. The use for treatment of protozoan infections is discussed in more detail in Chapter 42 of this book.

Cattle Use

Trimethoprim–sulfadiazine has been used to treat infections in cattle (Slaughter, 1972), but because there are no longer any approved formulations of trimethoprim–sulfadiazine for cattle, the use has diminished. Nevertheless, the pharmacokinetics have been described in calves with values reported for tissue fluids (Shoaf et al., 1986). The pharmacokinetics of sulfadiazine and trimethoprim are reported for calves and in cattle in Tables 32.3 and Table 32.5. Calves given sulfadiazine subcutaneously (30 mg/kg) had a rapid absorption of the drug; age and diet had no effect on sulfadiazine or trimethoprim disposition in those calves (Shoaf et al., 1987). In another study by Guard et al. (1986), calves 1 day of age showed higher serum and synovial fluid concentrations of trimethoprim and sulfadiazine than did calves of 1 week or 6 weeks of age. Trimethoprim–sulfadiazine concentrations also have been documented in the cerebrospinal fluid of neonatal calves (Shoaf et al., 1989). Although rarely administered orally to cattle, as indicated in Section Oral Absorption, trimethoprim is absorbed in preruminant calves, but not absorbed in mature ruminants after oral administration (Shoaf et al., 1987).

Pig Use

Trimethoprim (8 mg/kg) –sulfadiazine (40 mg/kg) was administered orally to pigs to determine bioavailability and other pharmacokinetic parameters (Tables 32.3 and Table 32.5). Bioavailability of sulfadiazine was 89% and 85% in fasted and fed pigs, respectively, while the trimethoprim resulted in bioavailability values of 90% and 92%. After IV administration of trimethoprim (4 mg/kg) –sulfadiazine (20 mg/kg), sulfadiazine was detectable in plasma up to 30 hours after administration, while the trimethoprim was found in the plasma only during the first 12 hours after dosing (Nielsen and Gyrd-Hansen, 1994). Other species in which trimethoprim–sulfadiazine was investigated include carp (Nouws et al., 1993) and ewes (Youssef et al., 1981).

Trimethoprim–Sulfamethoxazole

The most common human formulation is trimethoprim–sulfamethoxazole (Bactrim and Septra) and it is sometimes referred to as *cotrimoxazole*. Although there are no registered veterinary formulations, the generic human formulation is inexpensive and has been used commonly in dogs and horses for oral administration.

Trimethoprim–sulfamethoxazole human formulations are available as liquid suspensions (48 mg/ml) or oral tablets (480 or 960 mg). The injectable formulation is 96 mg/ml (80 mg sulfamethoxazole + 16 mg trimethoprim). The injectable form should be given slowly IV. It has been used at a dose of 43.5 mg/kg (combined drugs) to treat CNS infections in foals.

Because of the availability of the human generic formulation of trimethoprim–sulfamethoxazole, it is often used in horses, dogs, and other species for oral administration. Many veterinarians consider trimethoprim–sulfamethoxazole to be interchangeable with trimethoprim–sulfadiazine. Although there are no side-by-side comparisons in clinical studies, there is no reason to doubt this assumption. For susceptibility testing, trimethoprim–sulfamethoxazole can be used as a surrogate to test susceptibility of trimethoprim–sulfadiazine. The pharmacokinetics have been examined in horses (Brown et al., 1988; Peck et al., 2002) (Table 32.4) and the pharmacokinetics are favorable for clinical use and dosage regimens that essentially mirror that of the use of trimethoprim–sulfadiazine.

Trimethoprim–Sulfachlorpyridazine

Even though this product is rarely used, the studies of trimethoprim–sulfachlorpyridazine have been primarily in horses. Horses given 5 mg/kg trimethoprim and 25 mg/kg sulfachlorpyridazine IV revealed an elimination half-life of 2.57 hours (trimethoprim) and 3.78 hours (sulfachlorpyridazine) and a volume of distribution of 1.51 l/kg (trimethoprim) and 0.26 l/kg (sulfachlorpyridazine) (van Duijkeren et al., 1995). Bioavailability of the same dose of sulfachlorpyridazine in a powder formulation administered in the feed was about 46%. The dose of trimethoprim–sulfachlorpyridazine of 30 mg/kg (combined drug) twice daily is recommended for most indications in horses (Van Duijkeren et al., 1995).

Sulfadimethoxine–Ormetoprim

Sulfadimethoxine has been formulated with ormetoprim to enhance the spectrum of antimicrobial activity in a similar manner to trimethoprim's enhancement of the activity of other sulfonamides. Commercial preparations of ormetoprim–sulfadimethoxine are available for dogs (Primor tablets), poultry (Rofenaid premix for feed), and for fish (Romet). These combinations offer the same advantages as trimethoprim–sulfonamide combinations because the ormetoprim component produces the same synergistic effect as trimethoprim. Pharmacokinetic parameters of ormetoprim are listed in Table 32.6.

Sulfadimethoxine–ormetoprim is available as a premix for medicated feed (Rofenaid). In this form, sulfadimethoxine–ormetoprim is used for prevention of coccidiosis in chickens and turkeys caused by susceptible *Eimeria* species and for prevention of fowl cholera. A similar formulation (Romet) also is available for treating furunculosis in salmon and trout. Sulfadimethoxine–ormetoprim oral tablets for dogs (Primor) have been used for skin and soft tissue infections, urinary tract infections, and intestinal coccidia infections. It has been administered once daily for these indications.

There are no commercially available cattle formulations of sulfadimethoxine–ormetoprim in the US However, this combination has been shown to be highly effective in treating calves with experimentally induced *Mannheimia hemolytica* pneumonia. Wilson et al. (1987) investigated the potential efficacy of a sulfadimethoxine–ormetoprim combination administered orally and IV to treat *Moraxella bovis* infections in cattle. In cattle, sulfadimethoxine–ormetoprim administered IV was effective in maintaining sufficiently high concentrations of both drugs in the tears to exceed the known MICs of 13 *Moraxella bovis* isolates and in maintaining those concentrations for approximately 6 hours. However, when the same concentration of sulfadimethoxine–ormetoprim was administered orally, sulfadimethoxine appeared in low concentrations and ormetoprim in very low or trace concentrations in the tears, indicating this combination of drugs when administered orally is not suitable for treating *Moraxella bovis* infections in cattle.

In horses, pharmacokinetics were described by Brown et al. (1989). That study administered sulfadimethoxine–ormetoprim (45.8 mg/kg:9.2 mg/kg) orally, followed by lower oral doses (22.9 mg/kg:4.6 mg/kg) at 24-hour intervals, to healthy adult mares. Sulfadimethoxine produced peak plasma concentrations 8 hours after the initial dose, and plasma concentrations above 50 μg/ml were maintained for the entire dosing schedule. Significant concentrations were also found in the synovial fluid, peritoneal fluid, endometrium, and urine, with a small amount (2.1 mg/ml) appearing in the cerebrospinal fluid approximately 100 hours after the initial dose.

The pharmacokinetic parameters of sulfadimethoxine–ormetoprim were determined in 1- to 3-day-old foals given a sulfadimethoxine–ormetoprim dose (17.5 mg/kg:3.5 mg/kg) orally (Brown et al., 1993). In the foals, sulfadimethoxine concentrations peaked at 8 hours (55 μg/ml) after the oral dose and declined to 37.6 μg/ml 24 hours after the dose.

Residues in Food Animals

Tissue residues from sulfonamide use in food-producing animals are a concern of both US government agencies and the end consumers (Bevill, 1989). FARAD (Food Animal Residue Avoidance Databank), a computerized databank of scientific and regulatory data, has specific information on the withdrawal times for sulfonamides used extralabel (www.farad.org) in food-producing animals (Riviere et al., 1986). Chapter 61 of this book address the issues of residues in food-producing animals. Detection methods for sulfonamides and metabolites have also been described (Sharma et al., 1976; Agarwal, 1992).

Sulfonamide residues were a problem in the United States for at least 30 years, having produced more drug-residue violations than any other drug, with the highest incidence occurring in pork, followed by veal and poultry. Residues in animal tissues consumed by humans are considered to be potential health hazards to humans. Toxic or allergic reactions to the sulfonamide class of antimicrobials have been reported in humans receiving therapeutic doses of sulfonamides. However, we are aware of no reports in the open literature about toxicity or other adverse reactions in humans consuming animal products containing trace amounts of sulfonamides or its metabolites. Evidence indicating that sulfonamides (in particular, sulfamethazine) may be carcinogenic in humans consuming small amounts over long periods of time (based on in vivo rat and mouse data) heightened the Food Safety Inspection Service's (FSIS) concern for controlling sulfonamide residues in food animals (USDA, 1988).

The highest rate of sulfonamide–residue violations has historically occurred in swine. Sulfamethazine and sulfathiazole are the two most commonly used sulfonamides in swine feeds today. However, sulfamethazine was responsible for most of the sulfonamide–residue violations (97%) because it had been commonly added to swine feeds and its longer half-life when compared to that of sulfathiazole (12.7 versus 1.2 hours). The primary reasons for the occurrence of violative levels of sulfonamides in pork were failure to observe drug withdrawal time, improper feed mixing, and improper cleaning of feed-mixing equipment, causing a cross-contamination of feed (Bevill, 1984, 1989). During the late 1970s, 13% of swine livers were found to be in violation of federal sulfonamide tissue concentrations. At that time the

maximum amount of sulfonamide (parent compound) permitted in animal tissues was 0.1 ppm, with a 7-day withdrawal period. Drug manufacturers at this time increased the withdrawal time for sulfonamides used in animal feed from 7 to 15 days, and, by 1980, the violation rate in liver tissue had fallen to 4%. In 1987, the rate was reported to be 3.8% (Augsburg, 1989), with the rate decreasing significantly by the end of the 1990s. Part of the persistence of even these low levels may also be related to the ease of cross contamination seen after water medication (Mason et al., 2008).

In veal calves presented for slaughter, similar problems with sulfamethazine residues have been reported. The prevalence rate of sulfamethazine violations in veal calves was 1.9% in 1979 and 2.9% in 1981. Reasons for violations in this species include administering the drug to calves by individuals unaware of the drug withdrawal time constraints, unknowingly selling calves treated with sulfonamides, not following drug label directions, not seeking professional advice regarding drug use, and failing to maintain drug use records (Bevill, 1989).

Legal control of veterinary drugs is discussed in Chapter 55 of this book, and more information about residues in food-producing animals is presented in Chapter 61 of this textbook and also in previous editions. Several references are also available on this subject (Kaneene and Miller, 1992; Bevill, 1984; Dalvi, 1988; Rosenberg, 1985).

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33

β-Lactam Antibiotics: Penicillins, Cephalosporins, and Related Drugs

Mark G. Papich

In 1928, Alexander Fleming observed that a *Penicillium* mold contaminating a Petri dish culture of staphylococci colonies was surrounded by a clear zone free of growth. Fleming cultured the contaminating mold on a special medium and demonstrated that the culture broth contained a potent antibacterial substance that was relatively nontoxic to animals, but was active against a variety of gram-positive organisms. He named the substance *penicillin*. In 1940, penicillin was isolated in the form of a brown, impure powder and was the most powerful chemotherapeutic agent known at that time. Since then, more than 40 penicillins have been identified. Some occur naturally; others are biosynthesized (semisynthetic penicillins).

In 1945, *Cephalosporium acremonium* was isolated from raw sewage. The first cephalosporin, cephalosporin C, was derived from this fungus. All other cephalosporins are semisynthetic antibiotic derivatives of cephalosporin C. The first cephalosporin was available for clinical use in 1964. Since those first cephalosporins, they have developed through first, second, third, and fourth generations, all of which are used in veterinary medicine. There is now a commercially available *fifth* generation used in humans. The reader may notice differences in spelling. The older cephalosporins, derived from the fungus are spelled *ceph*, whereas the newer semisynthetic derivatives are spelled *cef*.

Although penicillins, penicillin derivatives, and cephalosporins are still the most commonly used β-lactam antibiotics, much progress has been made in the development of new β-lactams during recent years. Most notably, these include the β-lactamase inhibitors (e.g., clavulanic acid), new cephalosporins, the carbapenems, also known as *penems* (e.g., imipenem, meropenem, ertapenem, doripenem), and the monobactams (e.g., aztreonam) (Abraham, 1987).

Mechanism of Action of β-Lactam Antibiotics

β-lactam antibiotics exert their effects by preventing bacterial cell wall synthesis and disrupting bacterial cell wall integrity. These drugs are considered *bactericidal* in a time-dependent manner. They kill bacteria by inhibiting or weakening the cell wall. The cell wall of bacteria consists of alternating units of *N-acetylglucosamine* and *N-acetylmuramic acid,* which are cross-linked by short strands of peptides. A transpeptidation reaction is responsible for cross-linking the strands to form a strong, netlike structure. Inhibition of this transpeptidation reaction by acetylating the enzyme is one of the sites of action for β-lactam antibiotics. Interference with transpeptidation results in a weak cell wall and rupture of the bacteria.

The binding sites for β-lactam antibiotics are called penicillin-binding proteins (PBPs), which are the enzymes that form the cell wall. There can be anywhere from two to eight distinct PBPs in bacteria, which are numbered according to their molecular weight. The most common PBPs affected by β-lactams are PBP-1, -2, or -3. Some of the variation in spectrum of action and bactericidal action of β-lactam antibiotics can be related to their relative affinity for different PBPs. For example, inhibition of PBP-1a and -1b generally cause lysis, PBP-2 results in rounded cells called *spheroblasts,* and PBP-3 produces long filamentous forms. The drugs that cause rapid lysis (for example, carbapenems) are the most bactericidal and have highest affinity for PBP-1.

On the other hand, mutations in the enzyme, produced by the resistance gene *mecA*, produces PBP-2a, a penicillin-binding protein that resists binding of the β-lactam drugs and renders bacteria with this gene resistant. The PBP-2a target is the basis for resistance to

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methicillin and oxacillin, known as *methicillin-resistant staphylococci (MRS)* (Weese, 2005).

In order to reach the site of action, these drugs first must penetrate the outer layer of the bacteria. They penetrate the outer layer of bacteria through a pore (a porin protein) that is ordinarily present in bacteria to allow nutrients to enter the cell. It is generally easier to reach the target site in gram-positive bacteria and they usually are more susceptible to the effects of β-lactam antibiotics than gram-negative bacteria. Gram-negative bacteria may have a thick outer membrane and have pores more difficult to penetrate. The drugs most effective against gram-negative bacteria are those that can rapidly penetrate the outer membrane of the cell wall.

Pharmacokinetic–Pharmacodynamic (PK-PD) Properties

The β-lactam antibiotics are time dependent in their activity (Turnidge, 1998). β-lactam antibiotics are slowly bactericidal because of the slow rate of acetylation of the PBP, and the time of drug concentration above the minimum inhibitory concentration (MIC) (T > MIC) is important to clinical success (Drusano, 2004). At low exposure of β-lactams, not all of the available PBP are acetylated and bacterial stasis occurs (bacteriostatic effect). As the maximum number of PBP become acetylated, bactericidal activity occurs. Once maximum acetylation of the enzymes occurs the killing rate does not increase further, which explains the time-dependent effect of β-lactams, rather than a concentration-dependent effect. In some cases, drug concentrations can fall below the MIC for treating staphylococcal infections and still attain a cure because of a postantibiotic effect (PAE), but a PAE does not occur against gram-negative bacilli (Zhanel et al., 1991). Additionally, since the MICs are lower for gram-positive bacteria, longer dose intervals may be possible for infections caused by gram-positive as compared to gram-negative bacteria because it is easier to keep the plasma concentration above the MIC.

The optimum duration of plasma concentrations above the MIC has varied among studies, but a general assumption is that the drug concentration should be above the MIC for 50% – and perhaps less – of the dosing interval (Turnidge, 1998; Drusano, 2004). This may vary depending on the immune competence of the animal and specific drug class. The carbapenem group of drugs (for example, imipenem and meropenem), are used with increasing popularity in small animal practice. These drugs are more bactericidal than penicillins and cephalosporins and the T > MIC for successful therapy may be less for these drugs than other β-lactams (for example, 30% of the dose interval). When immune competence is a question – for any of the β-lactams – increasing the time above MIC is advised.

Dosage regimens for the β-lactam antibiotics should consider these pharmacodynamic relationships. Therefore, for treating a gram-negative infection, especially a serious one, it is necessary to administer many of the penicillin derivatives and cephalosporins three to four times per day if they have short half-lives. Some of the third-generation cephalosporins have long half-lives and less frequent regimens have been used for some of these drugs (e.g., ceftiofur–Naxcel, cefovecin–Convenia, and cefpodoxime proxetil–Simplicef). For highly susceptible gram-positive infections, less frequent administration may be acceptable. Note that the indications on the FDA-approved label for veterinary β-lactams may be aimed at these highly susceptible gram-positive pathogens and may not be appropriate for gram-negative bacteria with higher MIC values. Specific examples to illustrate this point are provided in this chapter.

Microbial Resistance to β-Lactam Antibiotics

Three independent factors determine the bacterial susceptibility to β-lactam antibiotics: (i) production of β-lactamases, (ii) decreased penetration through the outer cell membrane to access the cell wall enzymes (which includes efflux pump mechanisms), and (iii) the resistance of the target (PBP) to binding by β-lactam agents (Gold and Moellering, 1996; Frere et al., 1991). An example of an altered target (PBP) is the one found in methicillin-resistant isolates, mentioned in Section Mechanism Of Action Of β-Lactam Antibiotics.

β-Lactamase Enzymes

The elaboration of β-lactamases, enzymes that inactivate the drugs by hydrolyzing the β-lactam ring, is the major mechanism of drug resistance (Jacoby and Munoz-Price, 2005). Bacteria produce β-lactamases that possess a range of physical, chemical, and functional properties. Some β-lactamases are specific for penicillins (penicillinases), some are specific for cephalosporins (cephalosporinases), and others have affinity for both groups.

The genes that code for β-lactamases can occur via mutations in chromosomes, or they may be transferred by genetic elements. The genetic elements carrying these genes – which may be transferred among bacteria – include plasmids, which may be organized into integrons or carried on tranposons. A transposon may move (transpose) from DNA to plasmids, and vice versa. Integrons may be part of a transposon. The integrons may also contain genes that code for resistance to other drugs – thus producing multidrug resistance.

There are many β-lactamase enzymes that are capable of hydrolyzing the cyclic amide bond of the β-lactam structure and inactivating the drug. Investigators have described over 190 unique β-lactamase proteins that have this ability. Classification schemes may vary, and β-lactamase enzymes have been categorized according to molecular structure and substrate, bacterial type (gram-negative versus gram-positive), transmission (plasmid coded versus chromosomal coded), and whether they are inducible or constitutive. The Ambler Class (Class A, B, C, and D), which uses amino acid sequencing, is now widely accepted (Rice and Bonomo, 2000; Jacoby and Munoz-Price, 2005). Class A enzymes are the most important in veterinary medicine. These include those listed in the following sections.

Staphylococcal β-Lactamase

These are produced by coagulase-positive *Staphylococcus* sp. and some coagulase-negative strains. The synthesis of these enzymes is determined by specific resistance genes and the enzymes are exocelluar. These enzymes typically do not inactivate cephalosporins and antistaphylococcal penicillins (e.g., isoxazolyl penicillins such as oxacillin or dicloxacillin). These β-lactamases can be inactivated by β-lactamase inhibitors (e.g., clavulanate acid, tazobactam, and sulbactam).

Gram-Negative β-Lactamases

This is a very diverse group that can arise through mutation or via transferable genetic elements. These enzymes are more wide spectrum and can hydrolyze penicillins (penicillinases), cephalosporins (cephalosporinases), or both. β-lactamases produced by Enterobacteriaceae such as *E. coli* belong to the TEM, SHV, and CTX-M, families, as well as other less common groups. Some, but not all, of these enzymes are inhibited by β-lactamase inhibitors (e.g., clavulanate, sulbactam). Gram-negative bacteria secrete small amounts of β-lactamases into their periplasmic space, allowing for optimal location of the enzyme to degrade the β-lactam upon entry into the organism. The advantage of newer drugs such as third-generation cephalosporins (also called *oxyimino-β-lactams*) and carbapenems is their ability to remain stable to gram-negative β-lactamases.

Among the most serious of the gram-negative β-lactamases are the ESBLs – extended-spectrum β-lactamases. These β-lactamases will cause resistance to even the most active (extended-spectrum) cephalosporins. These enzymes are generally acquired through horizontal gene transfer (plasmids or transposons), but resistant clones also can spread in the local environment or hospital. Regardless of the mechanism, it presents a challenge to clinicians. Bacteria positive for ESBL can be difficult to treat because of limited drug options, and many are resistant to other classes of antibiotics. A more recently emerging β-lactamase resistance is the production of carbapenemase. This enzyme will inactivate the valuable carbepenem class of drugs. These bacteria have been identified as *carbapenem-resistant Enterobacteriaceae* (CRE), and include the *Klebsiella pneumoniae* carbapenemase (KPC). Bacteria that carry this resistance are very difficult to treat because there are so few available treatment options. Fortunately, this mode of resistance has not yet become a clinical problem in veterinary medicine, but there are concerns about its occurrence in animals (Abraham et al., 2014).

Resistance Caused by Reduced Access to Binding Sites

Gram-negative bacteria can produce a cell wall with a modified outer membrane that is no longer permeable to β-lactam antibiotics. These impenetrable porin proteins can resist entry by β-lactam drugs. Reducing the permeability of the outer membrane limits antibiotic entry into the bacteria by down-regulation of the porin protein, or by replacement of the porin with a channel that resists entry by the drug. The porins can also delay or slow the rate of entry, thus making the drugs more susceptible to attack by β-lactamase enzymes. Therefore, this mechanism can enhance resistance produced by the elaboration of β-lactamases.

Another mechanism that inhibits access to target sites is the efflux of β-lactams out of the bacteria. This mechanism actively transports the antibiotic out of the cell. When these pumps are expressed, it can produce a high level of resistance and can affect multiple drug classes – thus conferring multidrug resistance (MDR). The MDR efflux pumps may be carried on chromosomes or transferred by plasmids.

Penicillins

General Pharmacology

Penicillin G is historically important because it was the first antibiotic introduced in medicine. Alexander Fleming discovered penicillin in 1928, but it was not used clinically until the early 1940s. Much of the early supply was designated for military use during World War II. It remains a popular antibiotic and is still the drug of choice for initial treatment of many infections in horses and cattle. It has little usefulness in small animals because of the high incidence of resistance.

Unitage

Penicillin is one of the few antibiotics that is still measured in terms of units rather than weight in mg or μg. One unit of penicillin represents the specific activity in

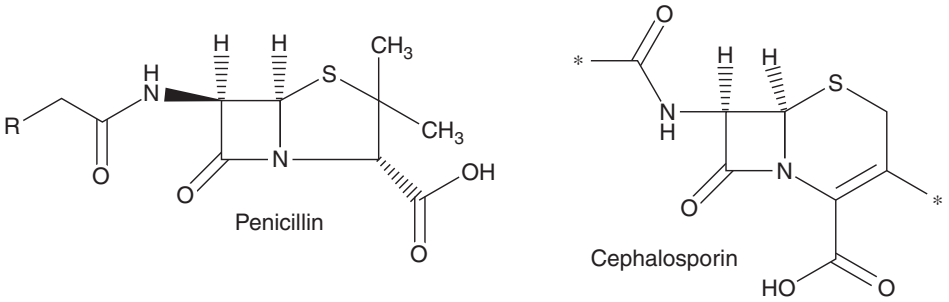

Figure 33.1 Structure of penicillins (left) and cephalosporins (right). Penicillins are composed of a five-member thiazolidine ring; cephalosporins are composed of a six-member dihydrothiazine ring. The structures can be modified into different drugs by replacing the R-groups or asterisk (*) with other functional groups. The most critical feature of the structure to exert its antibacterial action is the β-lactam ring. If the β-lactam ring is broken (hydrolyzed), the drug is inactive.

0.6 µg of sodium penicillin based on the International Standard for Penicillin. Thus, one mg of penicillin sodium represents 1,667 units of penicillin. Doses of other β-lactams are expressed in weight (mg) rather than units.

Structure

Penicillin contains a fused ring system, the β-lactam thiazolidine (Figure 33.1). The physical and chemical properties, especially solubility, of penicillins are determined by the acyl side chain and the cations used to form salts.

Hydrolysis is the main cause of penicillin degradation, which can occur via bacterial enzymes (β-lactamases). This reaction also can occur from drug interactions (for example, in the syringe when penicillin is mixed with another drug). Some penicillins are poorly orally absorbed because they are unstable in the stomach and hydrolyzed by gastric acid. Penicillin is incompatible with heavy metal ions, oxidizing agents, and strong concentrations of alcohol.

The penicillins are listed in categories based on their synthesis and spectrum of action. These include the natural penicillins (e.g. penicillin G), aminopenicillins (e.g. ampicillin, amoxicillin), antistaphylococcal penicillins (e.g. oxacillin), and the extended-spectrum penicillins (e.g. piperacillin). These are discussed in more detail in specific sections later in the chapter.

Antimicrobial Activity

The natural penicillins are active against many *Streptococcus* spp. and nonpenicillinase-producing *Staphylococcus* spp. They are active against some gram-positive, but only selected gram-negative bacteria, which include *Arcanobacterium* (formerly *Actinomyces)*, *Listeria monocytogenes,* and *Pasteurella multocida*. Therefore, these drugs are narrow-spectrum drugs that are active against non–β-lactamase–producing gram-positive bacteria but few gram-negative bacteria. Many anaerobic bacteria are susceptible, including *Fusobacterium, Peptococcus, Peptostreptococcus*, and some strains of *Bacteroides* (except *Bacteriodes fragilis* group) and *Clostridium*. These drugs are also active against most spirochetes, including *Leptospira* and *Borrelia burgdorferi*. Natural penicillins are consistently inactive against *Pseudomonas*, most Enterobacteriaceae, and penicillinase-producing *Staphylococcus* spp.

Aminopenicillins are active against the bacteria that are susceptible to penicillin G. They can penetrate through the outer membrane of gram-negative bacilli better than penicillin, which increases the spectrum to include some Enterobacteriaceae, including strains of *E. coli, Proteus mirabilis*, and *Salmonella;* however, most are resistant. Aminopenicillins are inactive against *Pseudomonas, Bacteroides fragilis*, and penicillinase-producing *Staphylococcus* spp. There are only subtle differences in antimicrobial activity between amoxicillin and ampicillin, and for susceptibility testing purposes, they are considered equivalent (CLSI, 2015).

The antistaphylocccal penicillins include methicillin and nafcillin as well as the isoxazolylpenicillins (oxacillin, cloxacillin, and dicloxacillin). The isoxazolylpenicillins are considered as a group because of their structural similarity. These semisynthetic penicillins are active against many penicillinase-producing *Staphylococcus* spp. that would ordinarily be resistant to penicillin G and the aminopenicillins. They also have some activity against other gram-positive and gram-negative bacteria and spirochetes. They are rarely, if ever, used clinically except for intramammary products that contain cloxacillin.

The extended-spectrum penicillins have the most activity against gram-negative aerobic and anaerobic bacteria of all of the penicillin groups. The drugs are active against many strains of Enterobacteriaceae and some strains of *Pseudomonas*. Carbenicillin and ticarcillin are active against some strains of *E. coli, Morganella morganii, Proteus* spp., and *Salmonella*. In addition to these organisms, mezlocillin and piperacillin are active

against some strains of *Enterobacter, Citrobacter, Klebsiella*, and *Serratia*. These extended-spectrum penicillins have some activity against gram-positive aerobic and anaerobic bacteria but have no advantages against these organisms compared to penicillin G and aminopenicillins. Extended-spectrum penicillins are generally more active against *Bacteroides fragilis* than are other available penicillins. Of this group, few are available and used clinically. Ticarcillin and ticarcillin–clavulanate, once popular for injectable use, is no longer available. Carbenicillin is no longer available. Piperacillin–tazobactam is the most popular and widely available of this group.

Susceptibility Testing

The CLSI has approved breakpoints for animals for most of the penicillins and derivatives (CLSI, 2013, 2015). These breakpoints often reflect the accepted clinical use of the drug and not necessarily the approved label indication. For example, the breakpoint for procaine penicillin G was established using a dose of 22,000 U/kg IM, consistent with good clinical practice, rather than the FDA-approved label dose of 7,500 U/kg. CLSI approved breakpoints are listed in Table 33.1.

Pharmacokinetics

Ampicillin and amoxicillin have been the most studied of the penicillin group and Table 33.2 lists the pharmacokinetic parameters in several domestic species. Penicillin G pharmacokinetics are presented in Table 33.3. When sodium salts of these drugs are injected, maximum blood concentrations occur usually within an hour.

Absorption

For the penicillin G formulations, distinct absorption patterns are observed, depending on which form is administered, the formulation, and the site of injection. The long-acting formulations (procaine- or benzathine–penicillin) are given IM or SC (never IV). The slow absorption from the injection site prolongs the plasma concentration. Injections of penicillin G IM to horses produced prolonged plasma concentrations, even when the sodium salt of penicillin was administered (Uboh et al., 2000). The half-life was longer from the procaine formulation, but at 24 hours, concentrations were similar in horses, regardless of whether the procaine or potassium formulation of penicillin G was administered. Benzathine formulations produce lower, but much longer, plasma concentrations because of insolubility and extremely slow absorption from the injection site (Papich et al., 1994).

For all the penicillins, as expected, the maximum plasma concentration will be lower, and the time to reach maximum concentration delayed, from an IM route versus IV route. More rapid absorption occurs from IM injection than SC. Also, there are differences depending on the location in large animals, with an injection in the neck muscle being absorbed faster than an injection in gluteal muscle (Papich et al., 1993). This pattern of absorption has been shown for other penicillins in cattle and horses (Firth, 1986), in which injections in the neck muscle are absorbed more rapidly and completely than injections in the rear leg.

For ampicillin and amoxicillin, these can be administered IM or IV as sodium salts, or they may be formulated as the trihydrate form that is more stable in aqueous solutions and may be administered SC or IM to produce a more prolonged absorption phase and a longer duration of activity (Traver and Riviere, 1982). Sodium salts of the other penicillin derivatives are no longer commercially available for IM or IV administration (e.g., ticarcillin sodium, piperacillin sodium, etc.).

Penicillin is easily inactivated in the acidic pH of the stomach (a pH of 6–6.5 is optimum for chemical stability), and therefore is not absorbed orally (except the acid-stable penicillin V, which is discussed under the specific formulations below). Because oral absorption of penicillin G is not sufficient to be of therapeutic value in animals this route is not used.

Amoxicillin and ampicillin are often administered to small animals. Amoxicillin differs from ampicillin only by the addition of a hydroxyl group (Figure 33.2). This decreases the lipophilicity of amoxicillin, but increases the oral absorption, whereby systemic availability of amoxicillin administered orally is higher than a similar dose of ampicillin (Watson et al., 1986). For example, absorption of ampicillin in dogs and cats has been reported to range from 30 to 40%, and for amoxicillin 64 to 68% (Küng and Wanner, 1994). Ampicillin had wide variation of oral absorption in cats (18% for suspension; 42% for capsule), depending on the dosage form (Mercer et al., 1977). Amoxicillin also has twice the systemic bioavailability of ampicillin when administered orally in pigs and preruminant calves. Other penicillin derivatives may have low oral absorption, which limits their clinical usefulness. Cloxacillin is absorbed poorly in dogs (Watson et al., 1986; Dimitrova et al., 1998) and with its short half-life is unsuitable for therapy in dogs.

The effect of feeding on oral absorption of these drugs has been debated, and results have varied, depending on the conditions of the study. The most complete study on this subject was reported by Watson et al. (1986). They found that feeding dogs inhibited oral absorption of amoxicillin, ampicillin, penicillin V, and cloxacillin. The effect was modest for ampicillin and amoxicillin (feeding decreased ampicillin tablets by 38%, but only 20–25% for amoxicillin). Feeding had a large effect for cloxacillin, decreasing oral absorption by 74%.

Aminopenicillins are absorbed poorly in horses and ruminants following oral administration.

Table 33.1 Susceptibility breakpoints for penicillin and derivatives (as approved by CLSI, 2013, 2015).

Species	Body site	Antimicrobial agent	MIC interpretive criteria (µg/ml)			Comments
			S	I	R	
Dogs	Skin, soft tissue (*E. coli*)	Ampicillin	≤ 0.25	0.5	≥ 1.0	Ampicillin may be used to test for amoxicillin. Systemic breakpoint derived from microbiological, PK-PD data. For dogs, the dose of amoxicillin was 22 mg/kg every 12 hours orally.
Dogs	Skin, soft tissue (gram-positive cocci)	Ampicillin	≤ 0.25	–	≥ 0.5	Ampicillin is used to test for susceptibility to amoxicillin and hetacillin. Systemic breakpoint derived from microbiological, PK-PD data. For dogs, the dose of amoxicillin was 22 mg/kg every 12 hours orally.
Dog	UTI	Ampicillin	≤8	–	–	A breakpoint of ≤8 can be used for uncomplicated UTI to account for high concentration in urine.
Dogs	Skin, soft tissue	Amoxicillin–clavulanate	≤0.25/0.12	0.5/0.25	≥1/0.5	Amoxicillin–clavulanate breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and PK-PD analysis of amoxicillin in dogs. The dosage regimen used for PK-PD analysis of amoxicillin was 11 mg/kg administered every 12 hours orally.
Dogs	UTI	Amoxicillin–clavulanate	≤8/4			A breakpoint of ≤8/4 can be used for uncomplicated UTI to account for high concentration in urine.
Dogs	Skin, soft tissue, UTI	Piperacillin–tazobactam	≤8/4	≤16/4	≤32/4	Breakpoint was derived from an examination of MIC distribution data, and PK-PD analysis of piperacillin in dogs at a dosage of 350 mg/kg every 6 hours, intravenously, or 3.2 mg/kg/hour constant rate IV infusion.
Cats	Skin, soft tissue, UTI	Amoxicillin–clavulanate	≤0.25/0.12	0.5/0.25	≥1/0.5	Amoxicillin–clavulanic acid breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and PK-PD analysis of amoxicillin in cats at a dosage of 12.5 mg/kg (amoxicillin) administered every 12 hours orally.
Horse	Respiratory, soft tissue	Penicillin G	≤ 0.5	1	≥ 2	Breakpoints derived from microbiological, PK data (using accepted clinical, but extralabel doses), and PD data. The dose of procaine penicillin G modeled was 22000 U/k, IM, every 24 hours.
Horse	Respiratory Disease	Ampicillin	≤ 0.25	–	–	For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed.
Swine	Lung (SRD)	Ampicillin	≤ 0.5	1	≥ 2	Breakpoints derived from microbiological data using ampicillin, PK data from a dose of 15 mg/kg IM of amoxicillin once daily, and PD data.
Swine	Lung (SRD)	Penicillin G	≤ 0.25	0.5	≥ 1	Breakpoints derived from microbiological, pharmacokinetic data (using accepted clinical, but extralabel doses), and pharmacodynamic data. The dose of procaine penicillin G modeled was at a dose of 33,000 U/kg, IM by needle in the neck, every 24 hours.
Cattle	Lung (BRD)	Penicillin G	≤ 0.25	0.5	≥ 1.0	Breakpoints derived from microbiological, PK data (using accepted clinical, but extralabel doses), and PD data. The dose of procaine penicillin G modeled was 22000 U/k, IM, every 24 hours.
Cattle	Mastitis	Penicillin–novobiocin	≤ 1/2	2/4	≥4/ 8	Mastitis use only.

BRD, bovine respiratory disease; SRD, swine respiratory disease; PK, pharmacokinetic; PD, pharmacodynamic; R, resistant; I, intermediate; S, susceptible; UTI, urinary tract infection.

Table 33.2 Ampicillin and amoxicillin pharmacokinetics (mean values)

Species	Dose (mg/kg)	$T_{1/2}$ (hour)	Vd (l/kg)	CL (ml/kg/hour)	C_{max} (μg/ml)	T_{max} (hour)	F (%)	Reference
Ampicillin trihydrate IM administration								
Calves	11				2.62			Martinez et al., 2001
Pigs	6.6				3.25			Martinez et al., 2001
Horse	11				1.48	1		Beech et al., 1979
Horse	22				2.9	6		Beech et al., 1979
Horse	20				2.49	6		Brown et al., 1982
Cattle	17	6.66	4.49	467.8				Gehring et al., 2005
Ampicillin sodium IV								
Horses		0.62	0.18	210	6.7–9.7			Sarasola and McKellar, 1993
Horses	10	0.725	0.303	268	59.9			Sarasola and McKellar, 1992
Horses		1.55						Durr, 1976
Horses		1.41	0.17					Bowman et al., 1986
Horses		0.75	0.21					Horspool et al., 1992
Horses	15	1.72	0.705	285				Ensink et al., 1992
Horses	10	0.7	0.2628	365.4				Sarasola and McKellar, 1995
Cats		1.22						Mercer et al., 1977
Pigs		0.55						Galtier and Charpenteau, 1979
Dogs	15	1.35	0.679	387				ten Voorde et al., 1990
Sheep	10	0.78	0.156	372.6				Oukessou and Toutain, 1992
Ampicillin Sodium IM								
Cows	10	2.3			6.18	1.5		Nelis et al., 1992
Horses	15	2.3	0.71	209.8	31.1	0.32		Van Den Hoven et al., 2003
Horses	11				10	0.5		Beech et al., 1979
Horses	22	2			12.88	0.5		Traver and Riviere, 1982
Dogs	15	5.2–5.5				0.92–1.03		ten Voorde et al., 1990
Ampicillin Oral								
Dogs	14.5	0.9			4.6			Nelis et al., 1992
Dogs	10	0.96			3.9	1.6		Watson et al., 1986
Foal	20				5.0	1		Brown et al., 1984
Cat							42	Mercer et al., 1977
Horse	15				0.84	0.69	2	Ensink et al., 1996
Amoxicillin								
Foals (30 days) IM	22	0.991	0.986	691	23.21			Carter et al., 1986
Foals (6–7 days) IV	20	0.74	0.369	343.2				Baggot et al., 1988
Foals (6–7days) oral	20	1.09			6.23	2	36.2	Baggot et al., 1988
Horses IV	10	0.657	0.325	340.8				Wilson et al., 1988
Horses IM					3.9–11.9			Evans et al., 1971
Horses oral	20	0.85			11.05	0.3	10.4	Wilson et al., 1988
Horses oral	20	0.75			2.03		5	Ensink et al., 1992
Horses IV	10	1.43	0.556	273				Ensink et al., 1992
Dogs IV	20	1.3	0.312	204				Küng and Wanner, 1994
Dogs IV	15	1.18	0.449	270				ten Voorde et al., 1990
Dogs IM	15	6.98–9.02			7.64–8.13	1.61–1.89		ten Voorde et al., 1990
Pigs IV	8.6	1.8	0.55, 0.63	370, 520				Agersø and Friis, 1998
Pigs IM	14.7	15.5			5.1	2	83	Agersø and Friis, 1998
Sheep IV	10	0.77	0.22	606				Craigmill et al., 1992
Sheep IV	20	1.43	0.18	90				Carceles et al., 1995
Goats IV	10	1.15	0.47	684.6				Craigmill et al., 1992
Goats IV	20	1.13	0.18	110				Carceles et al., 1995
Dogs oral		1.06	0.284	182				Marier et al., 2001
Dogs oral	16.9	1.52	0.71	460	11.4	1.38		Vree et al., 2003
Dogs oral	21	1.5			18–21	1.4–2	64–77	Küng and Wanner, 1994
Dogs oral	10	1.4		8.1	1.6			Watson et al., 1986
Pigs oral	10	9, 9.9	2.25		0.8, 1.6	1.9, 3.6	28, 33	Agersø et al., 1998
Pigs oral	20	4.2			7.5	1.5		Jensen et al., 2004

Vd, volume of distribution; CL, systemic clearance; $T_{1/2}$, half-life; C_{max}, peak concentration; T_{max}, time of peak concentration after absorption; F, fraction of dose absorbed.

Table 33.3 Pharmacokinetic parameters for penicillin in animals

Species	Form	Dose (Units/kg)	Route/Site	C_{max} (μg/ml)	T_{max} (hours)	$T_{1/2}$ (hours)	Reference
Calves (6–9 months)	Potassium	10,000	IM/Neck	4.71 ± 3.86	1 to 1.5	–	Bengtsson et al., 1989
Calves (6–9 months)	Procaine	30,000	IM/Neck	1.55 ± 0.33	1.5 to 6	–	Bengtsson et al., 1989
Cattle	Procaine	66,000	IM/Neck	4.24 ± 1.08	6.00 ± 0.00	8.9	Papich et al., 1993
Cattle	Procaine	66,000	SC/Neck	1.85 ± 0.27	5.33 ± 0.67	17	Papich et al., 1993
Cattle After 5-day administration	Procaine	24,000	IM/Gluteal	0.99 ± 0.04	5.33 ± 0.67	17	Papich et al., 1993
Cattle	Procaine	66,000	IM/Gluteal	2.63 ± 0.27	6.00 ± 0.00	17	Papich et al., 1993
Cattle	Procaine					9.37 ± 3.4	Craigmill et al., 2004[a]
Horses	Sodium	10,000	IV/Jugular				Love et al., 1983
Horses		20,000	IV/Jugular				
Horses		40,000	IV/Jugular				
Horses	Procaine	10,000	IM/Gluteal				Sullins et al. 1984
Horses		20,000	IM/Gluteal				
Horses		40,000	IM/Gluteal				
Horses	Procaine	22,000	IM/Gluteal	1.42 ± 0.22	3		Stover et al., 1981
Horses	Procaine	22,000	IM/neck	1.8	3.5	24.7	Uboh et al., 2000
Horses	Potassium	22,000	IM/neck	5.8	1	12.9	Uboh et al., 2000
Horses	Procaine	20,000	IM	1.57 ± 0.44	4.77 ±0.26	16.4 ± 8.0	Mean of 3 studies[b]
Foals (0–7 day)	Procaine	22,000	IM Semimembranous	2.17 ± 0.27	2		Brown et al., 1984
Pigs	Procaine	15,000	IM neck	1.47	1.20	7.78	Papich (unpublished)
Pigs	Procaine	66,000	IM neck	5.40	1.0	14.13	Papich (unpublished)

IM, intramuscular; IV, intravenous; SC, subcutaneous; C_{MAX}, peak concentration; T_{MAX}, time of peak concentration after absorption.
[a]Craigmill et al. (2004). Craigmill's analysis used 18 published papers, 28 data sets, and 288 data points. They also reported a volume of distribution/F of 13 l/kg (± 7.46).
[b]Mean values (± standard deviation) is available for more than one study.

They can be absorbed in preruminant calves, but not ruminating (6 weeks of age) calves (Soback et al., 1987a). Oral absorption of ampicillin in adult horses has been reported to be only 2–3.5% (Ensink et al., 1996; Sarasola and McKellar, 1994). Systemic availability of oral amoxicillin is higher than ampicillin in adult horses but is only 2–10% (Wilson et al., 1988; Ensink et al., 1992, 1996; Baggot, 1988; Sarasola and McKellar, 1994); still too low to be practical for dosing. In foals, oral absorption of amoxicillin has been higher at 36.2–42.7% (Baggot et al., 1988), but this is a seldom-used route of administration.

Elimination

The elimination half-life for IV penicillin is short (0.5 to 1.2 hours). Slow-release formulations are designed to lengthen this half-life. For example, because of slow release from the injection site, procaine penicillin may produce a terminal half-life of 20 hours or more and can maintain concentrations against susceptible bacteria for 24 hours after a single injection. The prolonged terminal half-life is caused by slow absorption (see the Section Absorption), rather than slow elimination, which is referred to as the "flip-flop effect" determining the plasma profile. All penicillins rely on renal elimination (primarily tubular secretion) and reach high drug concentrations in the urine.

Distribution

Penicillins diffuse into extracellular fluid easily unless protein binding is high. Protein binding is low to moderate in most species, ranging from approximately 30 to

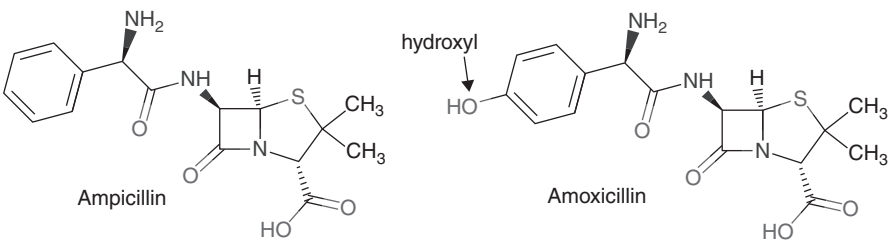

Figure 33.2 Structure of ampicillin (left) and amoxicillin (right).

60%. Penicillin is a weak acid with a pK_a of 2.7, therefore it is mostly ionized in the plasma and the volume of distribution is moderate. For example, volume of distribution (Vd) listed in some studies is 0.2 to 0.3 l/kg. In some studies, and in some species, however, it may be as high as 0.6 to 0.7 l/kg. These values represent a distribution to extracellular fluid, and possibly reaching moderate intracellular concentrations. Sufficient concentrations are attained for susceptible bacteria in kidneys, synovial fluid, liver, lung, skin, and soft tissues (Stover et al., 1981; Brown et al., 1982; Beech et al., 1979). Penicillins do not penetrate the blood–brain barrier to reach concentrations in the central nervous system (CNS) to a large extent, but they have been used to treat infections of the CNS when administered at high doses.

Metabolism

Penicillin G, penicillin V, nafcillin, ticarcillin, and the aminopenicillins are metabolized to some extent by hydrolysis of the β-lactam ring. The metabolites are microbiologically inactive. Penicillins and their metabolites are excreted in the urine by tubular secretion. Most of the drug is excreted in the urine within 1 hour of IM injection of sodium or potassium penicillin in aqueous solution. Probenecid competitively inhibits renal tubular secretion of penicillins and can prolong the half-life of penicillin. However, probenecid is rarely used for this purpose in clinical situations.

Summary of Penicillins and Derivatives

Natural Penicillins

The only natural penicillins still in use are penicillin G and penicillin V. Penicillin V has a phenoxymethyl group that provides more acid stability in the stomach, allowing for oral administration. It has been used orally in people, but it is of limited value in animals. It had low oral absorption and limited spectrum in calves (Soback et al., 1987b). In dogs, oral administration of penicillin V tablets produces maximum plasma concentrations of 3.5–4.8 μg/ml, but the concentrations decline quickly and were above 0.5 μg/ml for only approximately 3 hours (Watson et al., 1987a, 1987b).

Penicillin formulations: There are three injectable formulations. Pharmacokinetics of these formulations are provided in Table 33.3:

1) *Na^+ and K^+ salts of penicillin, also called crystalline penicillin:* These are water soluble solutions and may be injected intravenously (IV), intramuscularly (IM), or subcutaneously (SC). They achieve rapid, but short-lived, plasma concentrations.
2) *Procaine penicillin G (Crysticillin, Pen-Aqueous):* This compound is a poorly soluble salt in an aqueous vehicle suspension that is slowly absorbed following intramuscular (IM) or subcutaneous (SC) injection. *Do not administer IV.*
3) *Benzathine penicillin G (Benza-pen, Durapen, Flocillin):* This preparation is the so-called "long-acting penicillin". It is absorbed more slowly than the procaine salt because of its insolubility. It produces persistent, but low, plasma concentrations. Most formulations of long-acting penicillin contain 50% procaine penicillin G, and 50% benzathine penicillin G. *Do not administer IV.*

Additional formulations and route of administration are the formulations of penicillin G administered via intramammary routes to treat bovine mastitis.

The doses of penicillin G vary greatly depending on the formulation, the species of animal, and the disease treated. It is best to consult references related to the specific disease being treated. In general, Na^+ or K^+ penicillin G are administered IM, or IV at doses of 20,000 to 50,000 U/kg every 4 to 6 hours. Procaine penicillin G is administered IM or SC at dosages of 22,000 to 70,000 U/kg, every 12 to 24 hours. Treatment of streptococcal infections may use a lower dose, but for some infections such as those caused by *Arcanobacterium* (formerly called *Actinomyces*) doses as high as 100,000 U/kg have been recommended.

The United States FDA–approved label dose for cattle is 7,500 U/kg, but the approved withdrawal time for food animals – 10 days – applies only to an approved food animal dose. Since the doses used in food animals are extralabel, extended withdrawal times must be applied. Food animal residues and withdrawal times are discussed further in Chapter 61 of this book.

Aminopenicillins

Ampicillin and amoxicillin have been used in the treatment of a variety of diseases in domestic animals. The half-life of all aminopenicillins is short (Table 33.2), requiring frequent administration for some infections, particularly gram-negative pathogens that may have high MIC values.

Aminopenicillins are popular because they have a broader spectrum of activity compared to penicillin G, can be administered orally, and are relatively inexpensive and safe. The aminopenicillins differ from penicillin by the addition of an amino group, and amoxicillin has a parahydroxy group that ampicillin lacks. Compared to penicillin G, these compounds have two ionization points (pK_a 2.7 and 7.3). Ampicillin is more soluble than amoxicillin, and is also more lipophilic (Log P ampicillin 1.35; Log P amoxicillin 0.87), but amoxicillin is better absorbed by a factor of approximately two in most animals.

Compared to penicillin G, the aminopenicillins can penetrate the outer layer of gram-negative bacteria better than penicillin G; therefore, they have a spectrum of activity that includes those listed for penicillin but is extended to include some of the gram-negative bacteria (e.g., susceptible Enterobacteriaceae). However, acquired resistance can be common and this group is still quite susceptible to β-lactamase. To overcome resistance the β-lactamase inhibitors clavulanic acid and sulbactam have been added to amoxicillin (Clavamox) and ampicillin (Unasyn), respectively, to increase the spectrum. These are discussed in section β-Lactamase Inhibitors.

Aminopenicillin formulations: The formulations of aminopenicillins used in veterinary medicine (examples of brand names in parentheses) that are available include:

1) *Sodium ampicillin:* This formulation is used for injection IM, IV, SC (Omnipen®).
2) *Ampicillin trihydrate (Polyflex®):* This is a poorly soluble, slow-release aqueous suspension. Absorption of this formulation is erratic, and it produces prolonged, but low, blood concentrations.
3) *Amoxicillin trihydrate:* (Amoxi-inject®).
4) *Ampicillin:* These are oral products such as tablets, capsules, and liquid suspensions (for example, Omnipen®).
5) *Amoxicillin:* These are oral products, such as tablets and liquids (Amoxi-Tabs®, Amoxi-Drops®, and others).
6) *Intramammary preparations:* These include Amoxi-Mast, which is used for treating mastitis.

The elimination of the aminopenicillins is slightly longer than that reported for sodium salts of penicillin, but this difference does not appear to relate to a difference in clinical efficacy. Common doses are listed in Table 33.4, and listed for breakpoints in Table 33.1. A common formulation used in animals is ampicillin trihydrate (Polyflex), a poorly soluble preparation. After IM injection, the half-life is 6.7 hours in cattle, which is adequate for once-daily administration (Gehring et al., 2005). The IM injection route in horses also prolongs the plasma concentration (Table 33.2).

Table 33.4 Common dosages for ampicillin and amoxicillin

Drug	Dose and species
Amoxicillin oral	Dogs, cats 6.6–20 mg/kg q 8–12 h
Ampicillin trihydrate injection	Cattle 6.6–22 mg/kg q 8, 12, or 24 h (24 h most common); the approved label dose in cattle is 4.4–11 mg/kg IM, q 24 h Dogs, cats: 10–20 mg/kg, q 12–24 h, IM, SC
Ampicillin sodium	Horses IV 10–20 mg/kg q 6–8 h
Ampicillin sodium	Horses IM 10–22 mg/kg q 12 h
Ampicillin sodium	Dogs, cats: 10–20 mg/kg, q 8 h, IV, IM, or SC

An important difference between the aminopenicillins and penicillin G is that the aminopenicillins are not inactivated by gastric acid and may be administered orally. There appears to be a saturable transport process for absorption of aminopenicillin in the intestine. Oral absorption of the penicillins in various species and effect of feeding and age is discussed in the Section Absorption.

In addition to the formulations listed above, there are ester derivatives of ampicillin, but these are not currently available. The advantage of these esters is that they are stable in the gastrointestinal tract and are absorbed intact, but esterases release the active drug after absorption across the intestinal mucosa. They are absorbed much better than the parent drugs. Examples of these drugs are *pivampicillin* and *bacampicillin* (Sarasola and McKellar, 1994; Ensink et al., 1996).

Antistaphylococcal Penicillins

Also called the β-lactamase stable penicillins, this group of antibiotics includes the isoxazolylpenicillins (e.g., oxacillin, cloxacillin, and dicloxacillin) and synthetic derivatives of penicillin (e.g., methicillin and nafcillin). These drugs are rarely used clinically in veterinary medicine (except for intramammary forms). These drugs have the disadvantage of poor or inconsistent oral absorption in animals and short half-life. More details on these drugs are provided in earlier editions of this book. They are resistant to the β-lactamase of *Staphylococcus* spp. They have minimal activity against gram-negative bacteria because they do not exhibit good penetration of the outer layer of these bacteria.

One of the drugs in this group is methicillin. If *Staphylococcus* shows phenotypic resistance to methicillin, it is a marker for resistance mediated by the *mecA*-gene, which codes for a resistant PBP protein. When it occurs in *S. aureus,* this type of resistance is called *methicillin-resistant Staphylococcus aureus*–MRSA. If the organism is *Staphylococcus pseudintermedius,* it is referred to as MRSP. Today, oxacillin is used to test for this resistance, even though the resistant strains are still referred to as "methicillin-resistant" (CLSI, 2013).

Preparations available: The only drug from this group that is used clinically is cloxacillin, which is used to treat staphylococcal and streptococcal mastitis. It is also available as an intramammary infusion for dry cows (cloxacillin benzathine).

Extended-Spectrum Penicillins

The extended-spectrum penicillins have also been called the *antipseudomonas penicillins* because they are among

the few drugs active against *Pseudomonas*. This group includes the carboxypenicillins – carbenicillin and ticarcillin – because of a substitution of a carboxy group for the amino group on ampicillin, and the ureidopenicillins, which include piperacillin and azlocillin. The carboxy group decreased activity against *Streptococcus*, but improved penetration through the outer cell membrane of gram-negative bacteria. Substitution of a ureido for the carboxy group produced ureido penicillins, which maintained ampicillin's activity against *Streptococcus* and also produced good penetration through the outer membrane of gram-negative bacteria.

The value of this group of penicillins, particularly the ureidopenicillins, is their broad-spectrum activity and that they are able to penetrate the outer wall of *Pseudomonas* and some other gram-negative bacteria (e.g., *Proteus*, *Providencia,* and *Enterobacter*) better than other penicillins. Like the other penicillins, they are susceptible to β-lactamase inactivation, but the addition of tazobactam to piperacillin reduces this inactivation (discussed further in Section β-lactamase Inhibitors). This group has good synergistic activity when administered with the aminoglycosides (e.g., gentamicin, amikacin). The ureido penicillins also have good activity against anaerobic bacteria, and piperacillin may have some activity against enterococci.

Pharmacokinetic features: Despite advantages listed above, the disadvantage of this group of penicillins is the short half-life, which necessitates frequent administration. The half-life of these drugs is approximately 1 hour or less in most animals (VanCamp et al., 2000; Garg et al., 1987; Tilmant et al., 1985). The apparent volume of distribution is between 0.2 and 0.4 l/kg for most drugs in this class, much like other penicillins.

Clinical use: Ticarcillin and ticarcillin–clavulanate (Timentin®) was once a popular injectable antibiotic used in veterinary medicine and there was a formulation used in horses as an intrauterine flush diluted in saline (VanCamp et al., 2000). However, ticarcillin and the combination with clavulanate are no longer commercially available. Likewise, there are no longer formulations of carbenicillin available. More details about both of these drugs can be found in earlier editions of this book.

One drug from this group that still remains commercially available is piperacillin–tazobactam. (Piperacillin as a single agent is not available.) Piperacillin–tazobactam is discussed in more detail with the β-lactamase inhibitors.

Adverse Effects

Penicillins are very safe drugs, with relatively few adverse effects reported. Like most β-lactams, adverse effects are rare. The most common, and often most serious, adverse effects are attributed to allergy – immune-mediated reactions or allergic reactions. These are common in people from penicillins (approximately 15% of the human population) and are seen in veterinary species. Treatment may require administration of medications to attenuate the allergic response and avoidance of future use. Coombs-positive hemolytic anemia has been reported in horses following penicillin administration (Blue et al., 1987; Step et al., 1991).

After oral administration, there can be disruption of intestinal bacteria. *Clostridium* bacterial intestinal overgrowth from oral administration is a risk in guinea pigs, hamsters, gerbils, and rabbits.

Central nervous system reaction can be produced by penicillins (and other β-lactam antibiotics). At high concentrations these drugs can inhibit GABA (an inhibitory neurotransmitter) and cause excitement and seizures. Procaine, which is in some preparations, also causes excitement in some animals (horses) (Neilsen et al., 1988). There may be free procaine in some formulations of aqueous procaine penicillin G. When injected IM (or inadvertently IV) it can elicit an excitatory response. Because procaine can mask pain, and produce excitation in horses, its use is regulated in racing horses (see Chapter 57 on control of drugs in racing animals for additional information). Injections of procaine-penicillin in a horse may cause a positive procaine test reaction at the race track for as long as 2 weeks. Oral administration of ampicillin, amoxicillin, and similar formulations may cause vomiting at high doses.

Special Species Considerations

The penicillins are excreted similarly in all mammalian animals. In general, doses and intervals are similar among mammals, except that in larger species, dose intervals may be longer because of slower renal clearance. Renal clearance can be scaled allometrically to show that the larger body weight is associated with slower renal clearance.

In reptile species, clearance of all penicillin drugs is slow. The half-lives are much longer in reptiles, which allows for infrequent dosing intervals of once every 3–5 days. In birds, renal clearance and metabolic rate is high. This difference results in high doses and frequent intervals. Because of the need for frequent dosing, and because injectable drugs of this group may cause intramuscular pain, these drugs may be impractical in most clinical situations involving birds.

β-Lactamase Inhibitors

The β-lactamase inhibitors are a specific class of drugs with little antibacterial effects of their own, but they act

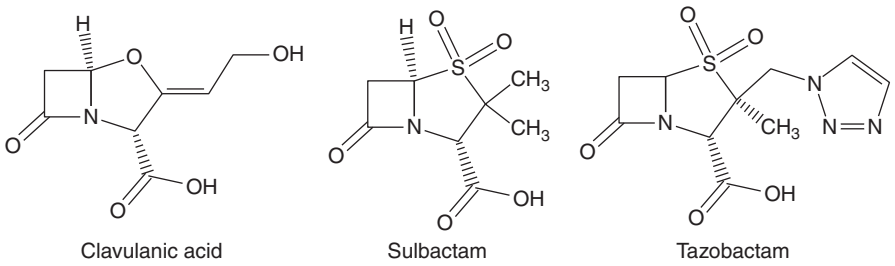

Figure 33.3 β-lactamase inhibitors: Structure of clavulanic acid (left), sulbactam (center), and tazobactam (right).

to inhibit the β-lactamase enzyme. They have structures that resemble the β-lactam antibiotics with an intact β-lactam ring structure (Figure 33.3). They are always combined with another active drug of the β-lactam class. These combinations, particularly amoxicillin–clavulanate, have been popular in veterinary medicine (Mealey, 2001). The primary drugs of this group are clavulanic acid (also called *potassium clavulanate*), combined with amoxicillin, sulbactam (combined with ampicillin), and tazobactam (combined with piperacillin).

Mechanism of Action

The β-lactamase inhibitors produce antibacterial effects only at high concentrations (Díez-Aguilar et al., 2015). They bind to the β-lactamase enzyme that is produced by gram-negative or gram-positive bacteria. This usually is an irreversible, noncompetitive binding. Clavulanate is considered an irreversible, suicide inhibitor. A wide range of β-lactamases are inhibited by clavulanate, including class B β-lactamases, TEM and SHV enzymes found in Enterobacteriaceae, many of the extended-spectrum β-lactamases, and various chromosomally mediated enzymes (Finlay et al., 2003). AMP-C β-lactamases are usually not inactivated by these agents.

When an inactive enzyme complex is formed from these inhibitors, the coadministered antibiotic (e.g., amoxicillin or ampicillin) can exert its antibacterial effect. All β-lactamase inhibitors are not equal with respect to potency and ability to bind β-lactamase enzymes. For example, compared to clavulanate, sulbactam is less active against β-lactamase of *Staphylococcus*, *Bacteroides,* and some *E. coli*. However, whether these differences are important clinically is not known.

Pharmacokinetics

The pharmacokinetics of clavulanate in animals has been studied more than the other inhibitors (Bywater et al., 1985; Vree et al., 2003). Clavulanate is notoriously unstable and should be protected from moisture. Tablets are packaged in foil-protective packaging. Although clavulanate is absorbed orally (the only one of the β-lactamases absorbed orally), the absorption is variable and can vary highly among animals administered similar doses (Vree et al., 2003). There is evidence that high doses of amoxicillin inhibit the absorption of clavulanate in dogs and people (Vree et al., 2003). Clavulanate is susceptible to enzymatic degradation and is excreted by glomerular filtration, whereas amoxicillin is eliminated by renal tubular excretion.

Examples and Clinical Use

Amoxicillin–clavulanic acid (Clavamox®, Synulox): This is one of the most popular oral antibiotics used in small animals. Amoxicillin–clavulanate, sometimes called potentiated amoxicillin and co-amoxiclav, extends the spectrum of amoxicillin to include many of the β-lactamase–producing bacteria. There is an equivalent drug used in people (Augmentin), which is one of the most popular drugs in human medicine. The human drug is not entirely equivalent because the proportion of amoxicillin: clavulanate may be different. Clavamox has a 4 : 1 ratio, whereas augmentin is either in a 4 : 1 ratio or a 7 : 1 ratio, depending on the tablet size. The dose administered to animals is a fixed ratio of 4 : 1 (amoxicillin : clavulanate), but because of lower absorption of clavulanate and more rapid excretion, the ratio in the body can be highly variable and as low as 20 : 1. For susceptibility testing, CLSI (CLSI, 2015) uses a fixed ratio of 2 : 1 (amoxicillin : clavulanate) (Table 33.1).

In small animals Clavamox® has been used to treat infections in almost all tissues (except CNS). It has been successful for skin infections and urinary tract infections (Bywater et al., 1985; Senior et al., 1985; Weese et al., 2011; Hillier et al., 2014). It is particularly useful to treat infections caused by β-lactamase–producing staphylococci. Amoxicillin–clavulanate also is useful for treating anaerobic infections in dogs and cats, such as those of the oral cavity (Indiveri and Hirsh, 1985).

In large animals, amoxicillin–clavulanate has not been an important drug. As reported previously, the oral absorption of amoxicillin in horses is small and unlikely to reach therapeutic concentrations. The oral administration has also been examined in calves (Soback et al., 1987a). There is sufficient absorption of amoxicillin–clavulanate in preruminant calves, but three-times-daily administration was recommended (Soback et al., 1987a), which is impractical. Oral absorption was not high enough in ruminant calves – because of degradation in the rumen – to produce therapeutic concentrations.

Sulbactam–ampicillin (Unasyn®): This is a human drug, but veterinary preparations exist in other countries. This drug has been administered IM, IV, and SC to dogs, cats, horses, and cattle. (Sulbactam is not absorbed orally.) In some countries (e.g., Canada) this combination is used as an intramuscular drug (ampicillin trihydrate + sulbactam, Synergistin) in cattle for the treatment of diseases such as pneumonic pasteurellosis (Risk and Bentley, 1987; Risk and Cummins, 1987; Girard et al., 1987).

Ticarcillin–clavulanic acid (Timentin®): This product is no longer available. Information about this preparation may be found in earlier editions of this book.

Piperacillin–tazobactam (Zosyn®, also referred to as "Pip-Taz"): This is one of the most popular intravenous (IV) antibiotics for in-hospital use in people. Piperacillin is an extended-spectrum penicillin and tazobactam has essentially the same activity as clavulanate. The use includes septicemia, urinary tract infections, skin, soft-tissue, respiratory infections, intraabdominal infections, and gynecological infections. Targeted organisms include bacteria of the Enterobacteriaceae (*Escherichia coli, Klebsiella pneumonia*), including some ESBL-producing strains, and *Pseudomonas aeruginosa*. It also has activity against *Streptococcus* spp. and *Staphylococcus* spp. Formulations are typically in 8 : 1 ratio of piperacillin : tazobactam available in injectable vials reconstituted with sterile water, 0.9% saline solution, or 5% dextrose in water, and further diluted to desired volume for intravenous fluid administration.

The pharmacokinetics have been studied in dogs. The combined analysis of seven different studies showed that it has a half-life of only 0.55 (± 0.11) hours and a volume of distribution of 0.276 (± 0.05) l/kg. The short half-life requires frequent administration (every 6 hours), or as a constant rate infusion (CRI). In immunocompetent animals it may be administered at a dose of 50 mg/kg every 6 hours IV (higher doses are needed in immunosuppressed animals), or as a CRI of 3.2 mg/kg/hour after a loading dose of 2.25 mg/kg. The breakpoint is listed in Table 33.1.

New β-lactamase inhibitor combinations: There are new β-lactamase inhibitor combinations, but there is no record of their use in veterinary medicine. Ceftazidime–avibactam (Avycaz) and ceftolozane–tazobactam (Zerbaxa) are approved for IV treatment of infections in people. They are third-generation cephalosporin/ β-lactamase inhibitor combinations (discussed in Section Cephalosporins) with a spectrum that includes many ESBL-producing bacteria and *Pseudomonas aeruginosa.* Ceftazidime–avibactam also has activity against some carbapenemase-producing *Klebsiella pneumoniae*. These drugs are used in people primarily for complicated urinary tract and intraabdomnial infections caused by bacteria resistant to other drugs.

Cephalosporins

Cephalosporins that are veterinary labeled, as well as drugs approved for humans (e.g., ceftazidime, cefotaxime, cefazolin), have commonly been used in veterinary medicine for many infections, including pyoderma, urinary tract infection, pneumonia, soft-tissue infection, osteomyelitis, and pre- and postsurgical use. They are often considered first-line treatments, often employed empirically for routine outpatient and in-hospital use. One of the advantages of the use of the generic formulations intended for humans is their low cost. For more resistant infections caused by *Pseudomonas aeruginosa* or the Enterobacteriaceae such as *E. coli,* extended-spectrum drugs of the third and fourth generation have been used.

General Pharmacology

The spectrum includes most bacteria susceptible to amoxicillin and ampicillin, but also includes some β-lactamase-producing bacteria, depending on the specific generation of cephalosporin. Many in this class have greater activity against gram-negative bacteria than amoxicillin or ampicillin. In general (exceptions are noted in Section Classification), the cephalosporins owe their usefulness to activity against *Staphylococcus* (β-lactamase–positive, but not methicillin-resistant strains), streptococci (but not enterococci), and gram-negative bacteria, except *Pseudomonas* (exceptions noted in Section Classification). Although cephalosporin antibiotics show activity against some anaerobic bacteria, they are ordinarily *not* considered a drug of choice for the gram-negative anaerobes. The cephamycins (a subclass of cephalosporins), however, have good anaerobic activity.

Cephalosporins contain a 7-aminocephalosporanic acid nucleus, which is composed of a β-lactam ring fused with a six-membered dihydrothiazine ring (see Figure 33.1). Additions of various groups (shown by the asterisk in Figure 33.1) form derivatives with differences in antimicrobial activity, stability against β-lactamases, protein binding, intestinal absorption, metabolism, and toxicity.

The cephalosporin antibiotics are extremely important in veterinary medicine. They were first produced by a fungus isolated from raw sewage from the sea in Sardinia. Although the antibiotic was first isolated from *Cephalosporium acremonium* in 1948, it was not available commercially until 1962. There are now over 30 cephalosporin antibiotics on the market (most on the

human pharmaceutical market), but newer ones have been introduced to veterinary medicine. Although the cephalosporins are widely used in many animal species, the extralabel dosages of these drugs in food-producing animals is not allowed in the United States. Regulatory control of antibiotics in the USA is discussed in Chapter 52 and 55 of this book.

Classification

The cephalosporins are broadly classified into first-, second-, third-, and fourth-generation cephalosporins. There is also a new group active against methicillin-resistant staphylococci that has been called a *fifth* generation (e.g., ceftobiprole and ceftaroline) but there is no record of their use in veterinary medicine. This classification system is somewhat arbitrary, depending on when they were synthesized. This classification is largely based on activity against gram-negative bacteria and susceptibility to β-lactamase. Various other classifications of cephalosporins have been proposed (Williams et al., 2001). For this chapter, we retain the classification categories listed by the CLSI (CLSI, 2015) provided in Table 33.5.

First Generation

The first-generation drugs are effective against almost all gram-positive bacteria, except *Enterococcus*, and their activity includes β-lactamase-positive staphylococcus. They also have greater activity against members of the Enterobacteriaceae than penicillin G. Compared to others in this group, cefazolin has the greatest gram-negative activity (Petersen and Rosin, 1995), and it has been grouped with the second-generation drugs in some references based on this activity (Williams et al., 2001). Gram-negative bacteria may develop resistance by inhibiting penetration and by producing β-lactamase enzymes.

Second Generation

In general, these drugs have greater activity against many gram-negative bacteria that are resistant to the first-generation drugs (e.g., resistant *E. coli, Klebsiella, Proteus, Enterobacter*), but are no more active against the gram-positive bacteria. Improved activity against gram-negative bacteria compared to first-generation drugs is attributed to an increased resistance to β-lactamases. Cefoxitin and cefotetan belong to the cephamycin group and have been used clinically because of good activity against anaerobic bacteria (e.g., *Bacteroides fragilis,* and the *Bacteroides fragilis* group). Cefotetan is no longer available in the USA, but cefoxitin is still used in veterinary patients. Cefaclor (Ceclor®), cefprozil (Cefzil®), and cefuroxime axetil (Cefetin®) can be administered orally, but their use has not been reported for small animals.

Third Generation

This group of antibiotics has more activity against gram-negative bacteria than the earlier generations of cephalosporins. Only ceftazidime and cefoperazone have good activity against *Pseudomonas aeruginosa*, with ceftazidime having the greatest activity. For this reason, ceftazidime has been an important drug for some infections in small animals.

The third-generation drugs, in general, are less active against gram-positive cocci, but there is considerable variability in the activity against staphylococci and

Table 33.5 Classification of cephalosporins

First generation		Second Generation		Third generation	
Drug name	**Brand name**	**Drug name**	**Brand name**	**Drug name**	**Brand name**
cephalexin	generic, Keflex[a]	cefamandole	Mandol	cefoperazone	Cefobid
cephalothin	Keflin	cefmetazole	Zefazone	cefotaxime	Claforan
cefadroxil	Cefa-Tabs[a]	cefonicid	Monocid	ceftazidime	Fortaz
cephapirin	Cefadyl	cefprozil	Cefzil[a]	ceftizoxime	Cefizox
cefazolin	Kefzol	cefotetan	Cefotan	ceftriaxone	Rochephin
cephradine	Velosef[a]	cefoxitin	Mefoxin	moxalactam	Moxam
cefaparin	Cefa-Lak and Cefa-Dri	cefuroxime	Kefurox	cefixime	Suprax[a]
		cefuroxime axetil	Ceftin[a]	cefdinir	Omnicef[a]
		cefaclor	Ceclor[a]	ceftiofur	Naxcel
				cefpodoxime proxetil	Vantin[a]
					Simplicef[a]
				Cefovecin	Convenia

[a]Oral drugs.
Fourth-generation drugs not listed include cefquinome (Cobactan) (veterinary drug) and cefepime (Maximime).

streptococci among this group. For example, cefotaxime has the highest activity against streptococci, but others have less activity. Some of the third-generation agents are more active against *Staphylococcus* spp. than cephalexin. There are only three that can be administered orally. Of these, two have been used in veterinary medicine, cefixime (Suprax®) (Lavy et al., 1995), and cefpodoxime proxetil (Simplicef veterinary formulation and Vantin human formulation). Cefixime is no longer available in the USA.

The human-labeled injectable third-generation drugs have been used in veterinary medicine when resistance has been shown to other drugs. An exception is ceftiofur (Naxcel®), which has been used extensively in cattle, pigs, and horses, and is also approved for use in dogs. The activity of the major metabolite, desfuroylceftiofur, is similar to cefotaxime, which is considered a typical third-generation cephalosporin. Cefovecin (Convenia), is an injectable formulation that has an extremely long half-life in dogs and cats compared to other cephalosporins. Specific agents are discussed in more detail in Section Clinical Features and Specific Drugs Used in Veterinary Medicine.

Fourth Generation

The fourth generation of cephalosporins include the human drug cefepime (Maxipime®), and the veterinary drug cefquinome (Cobactan). The use of cefepime has not been reported in veterinary medicine except for some experimental studies. Cefquinome is approved in other countries, but not the USA. These drugs are discussed in more detail in Section Clinical Features and Specific Drugs Used in Veterinary Medicine.

Mechanism of Action

Similar to other β-lactam antibiotics, the cephalosporins bind to PBPs and disrupt the cell wall. They are usually bactericidal and most often bind the PBP-2 and PBP-3.

Pharmacokinetics

The pharmacokinetic features of specific drugs are provided in Table 33.6.

Pharmacokinetics–Pharmacodynamics

Pharmacokinetic–pharmacodynamic (PK-PD) relationships for cephalosporins are the same as for other β-lactam antibiotics discussed in this chapter. Like other β-lactam antibiotics, cephalosporins are considered to be bactericidal in action; they kill bacteria if the drug concentrations are maintained above the MIC for a critical period during the dosing interval (Turnidge, 1998). Thus the important parameter is considered time above MIC (T > MIC). It is the duration of exposure, rather than the magnitude of the concentration above the MIC that determines efficacy of cephalosporins. Dosage regimens for the cephalosporins have been formulated to consider these PK-PD relationships (Craig, 1995, 2001; MacGowan, 2001; Turnidge, 1998). Among the β-lactams, penicillins are not as bactericidal as carbapenems, and cephalosporins are not as bactericidal as penicillins. Therefore, among the β-lactams, cephalosporins should be maintained above the MIC longer than others in this class. Although the optimum time above the MIC has not been determined for most cephalosporins used in companion animals, in humans and laboratory animals the optimum time above the MIC is regarded as approximately 50% of the dosing interval. However, for treating gram-negative infections, maximum bactericidal effect occurs at 60–70% of the dosing interval, and as the duration of the T > MIC increases, improved clinical outcomes are possible. In some experimental studies the T > MIC may be less than 50%. For example, when four cephalosporins were examined to determine the T > MIC necessary for optimum dosing, the T > MIC was 30–40% of the dosing interval for Enterobacteriaceae and *Streptococcus*, but less than 30% for *Staphylococcus*.

Because the half-lives of most cephalosporins in mammals are short, many regimens for cephalosporins use require an administration frequency of three to four times per day. Alternatively, some of the third-generation cephalosporins have long half-lives, and less frequent regimens have been used for some of these drugs (for example cefpodoxime, cefovecin, cefotaxime, and ceftiofur). However, the long half-life for ceftriaxone in people does not occur in animals because of differences in drug–protein binding (Popick et al., 1987).

The dosing regimen that produces the greatest T > MIC is the CRI, and superior efficacy has been reported from CRI regimens rather than intermittent dosing (Zeisler et al., 1992). Constant rate intravenous infusions have also been calculated for some third-generation cephalosporins for dogs (Moore et al., 2000).

Gram-positive organisms are more susceptible to the bactericidal effect of cephalosporins than are gram-negative bacteria. Additionally, since the MICs are lower for gram-positive bacteria, and antibacterial effects occur at concentrations below the MIC for *Staphylococcus* (postantibiotic effect, PAE), longer dose intervals may be possible for infections caused by gram-positive as compared to gram-negative bacteria. For example, cephalexin or cefadroxil have been used successfully to treat staphylococcal infections when administered twice daily (discussed further in Section Clinical Features and Specific Drugs Used in Veterinary Medicine). Some studies have reported efficacy for cephalexin treating staphylococcal pyoderma in dogs with administration of only once daily (although twice-daily

Table 33.6 Pharmacokinetic parameters of selected cephalosporins in domestic species

Drug	Species	Vd[a] (l/kg)	Clearance (ml/kg/min)	Hallf-life (hour)	Reference
Cephapirin	Foals[b]	1.06	18.4	0.7	Brown et al., 1987
	Horses	0.17	10		Brown et al., 1986a
	Cows[c]		12.7		Prades et al., 1988
	Dogs	0.32	8.9	0.42	Cabana et al., 1976
Cephalothin	Horses	0.15	13.6	0.25	Ruoff and Sams, 1985
Cefadroxil	Horses	0.46	7.0	0.77	Wilson et al., 1985
Cefazolin	Foals	0.45	0.4	1.37	Duffee et al., 1989
	Horses	0.27 (0.03)	5.07 (1.23)	0.62 (0.07)	Multistudy[f]
	Calves	0.17	5.8	0.62	Soback et al., 1987c
	Dogs	0.27 (0.13)	2.89 (0.92)	1.04 (0.46)	Multistudy[e]
Cephalexin	Calves	0.32	1.9	2	Garg et al., 1992
	Cows	0.39	10.5	0.58	Soback et al., 1988
	Sheep	0.17	5.0	1.2	Villa et al., 1991
	Dogs	0.92 (Vd/F) (0.48)	3.14 (CL/F) (0.87)	2.74 (1.6)	Multistudy[d]
	Horse[g]	9.92 (Vd/F)	86.4 (CL/F)	1.64	Davis et al., 2005
Cefoxitin	Calves			1.12	Soback, 1988
	Horses	0.12	4.3	0.82	Brown et al., 1986b
Ceftriaxone	Dogs			0.85	Matsui et al., 1984
	Sheep	0.3	3.7		Guerrini et al., 1985
	Calves			1.4	Soback and Ziv, 1988
Ceftazidime	Dogs			0.82	Matsui et al., 1984
	Sheep	0.36		1.6	Rule et al., 1991
Cefoperazone	Calves			0.89	Carli et al., 1986
	Sheep	0.16	2.7		Guerrini et al., 1985
Moxalactam	Calves			2.4	Soback, 1989

[a]Vd, volume of distribution; [b]neonatal; [c]lactating; [d]analysis of 8 studies with oral dosing and 52 observations. Mean (standard deviation) shown; [e]analysis of 4 studies and 35 observations. Mean (standard deviation) shown; [f]analysis of 3 studies and 17 observations. Mean (standard deviation) shown. [g]oral absorption was only 5% in the cephalexin equine study.

administration is recommended to obtain maximum response).

Susceptibility Testing

Breakpoints for susceptibility testing have been approved by CLSI for testing many of the cephalosporins used in veterinary medicine. These breakpoints are based on PK-PD analysis, MIC distributions, and clinical efficacy (Table 33.7).

Tissue Concentrations and Protein Binding

Cephalosporins are relatively polar antibiotics. They are minimally lipid soluble and have poor intracellular penetration. The volume of distribution is generally in the range of 0.2 to 0.3 l/kg and rarely exceeds 0.5 l/kg. However, they have good distribution into the extracellular fluid of most tissues, except prostate and the CNS. They do not reach effective intracellular concentrations. Their ability to penetrate the epithelial lining fluid of the respiratory tract varies among drugs and across species. Specific features of each drug will be discussed in more detail later in this chapter.

Pharmacokinetic–pharmacodynamic-based dosing regimens use plasma concentrations of the unbound drug as the surrogate marker for determining the optimum dose and interval. Only protein-unbound drug is microbiologically active. Protein binding varies across species and among the drugs. Some cephalosporins are highly protein bound, but for others it is low. There are differences between animals and people that affect their use. For example, ceftriaxone has high protein binding of 90–95% in people, which restricts clearance and causes a long half-life (Popick et al., 1987). But the same drug in dogs has protein binding of only 25% at low concentrations to 2% at high concentrations. Cefazolin has high protein binding in people (85%), but low protein binding in dogs (19%), which favors rapid distribution from plasma to interstitial fluid. The most highly protein-bound cephalosporin in animals is cefovecin, which is greater than 99% bound in dogs and cats, but much lower in some other animal species. This property, in addition to other factors, prolongs the plasma concentrations in dogs and cats.

The effect of protein binding on drug distribution was demonstrated for cephalexin and cefpodoxime, two orally administered cephalosporins used in dogs (Papich et al., 2007). Protein binding is higher for cefpodoxime in dogs (>80%), which prolongs the half-life compared to

Table 33.7 Susceptibility breakpoints for cephalosporins (as approved by CLSI 2013, 2015)

Test/report group	Body site	Antimicrobial agent	MIC interpretive criteria (µg/ml)			Comments
			S	I	R	
Dogs	Skin, soft tissue	Cephalexin	≤2	4	≥8	Cephalexin breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and PK-PD analysis of cephalexin. The dosage regimen used for PK-PD analysis of cephalexin was 25 mg/kg administered every 12 hours orally.
Dogs	Skin, soft tissue, UTI, respiratory	Cefazolin	≤2	4	≥8	Cefazolin breakpoints were determined from an examination of MIC distribution of isolates and PK-PD analysis of cefazolin. The dosage regimen used for PK-PD analysis of cefazolin was 25 mg/kg administered every 6 hours IV in horses and dogs.
Dogs	Wounds, abscesses	Cefpodoxime	≤2	4	≥8	Approved for dogs at a dose of 5–10 mg/kg once daily orally.
Horses	Respiratory, genital tract	Cefazolin	≤2	4	≥8	Cefazolin breakpoints were determined from an examination of MIC distribution of isolates and PK-PD analysis of cefazolin. The dosage regimen used for PK/PD analysis of cefazolin was 25 mg/kg administered every 6 hours IV in horses.
Horses	Respiratory disease	Ceftiofur	≤0.25	–	–	The susceptible only category is used for populations of organisms (usually one species) for which regression analysis (disk vs. MIC) cannot be performed. This breakpoint will permit detection of strains with decreased susceptibility as compared to the original population.
Swine	Lung (SRD)	Ceftiofur	≤ 2	4	≥ 8	Approved for treating swine respiratory disease.
Cattle	Lung (BRD)	Ceftiofur	≤2	4	≥8	Approved for treating bovine respiratory disease.
Cattle	Bovine mastitis	Ceftiofur	≤ 2	4	≥ 8	Mastitis treatment only.

BRD, bovine respiratory disease; SRD, swine respiratory disease; PK, pharmacokinetic; PD, pharmacodynamic; R, resistant; I, intermediate; S, susceptible; UTI, urinary tract infection. Note that some laboratories have used cephalothin as a test for the first-generation cephalosporins such as cephalexin.

cephalexin. The free drug concentration for cefpodoxime in tissue fluid represented the unbound drug fraction in plasma, reflecting the effect of protein binding to restrict drug diffusion from capillaries into tissues. This phenomenon has also been observed in humans (Liu et al., 2002).

Oral Absorption

Many of the cephalosporins are absorbed orally. Cefadroxil and cephalexin of the first-generation group are well absorbed in small animals, but not in large animals. Oral absorption of the ester formulations (cefpodoxime proxetil) is enhanced. This feature will be discussed in more detail for individual drugs in Section Clinical Features and Specific Drugs Used in Veterinary Medicine. For cefadroxil, but not cephalexin, absorption was enhanced somewhat with food (Campbell and Rosin, 1998).

Oral absorption of cephalosporins is generally too low to be effective in horses and ruminants. However, cefadroxil is absorbed better in the foal than in adult horses (Wilson et al., 1985; Duffee et al., 1989). Oral absorption of cephalexin is low in horses (5%) (Davis et al., 2005) but at 30 mg/kg orally q 8 h, concentrations can be maintained above the MIC of highly susceptible bacteria.

Metabolism

Cephalosporins are minimally metabolized by the liver, but degree of metabolism can vary widely among the various drugs. Ceftiofur is transformed almost completely to the metabolite desfuroylceftiofur, which is responsible for its antibacterial efficacy. Most cephalosporins rely on renal elimination.

Elimination

The cephalosporins are eliminated rapidly after systemic administration. The route of elimination is primarily renal, and concentrations in the urine are usually high. This feature makes cephalosporins good choices for treatment of urinary tract infections.

In general, the cephalosporins have half-lives of 1 to 2 hours, but some (particularly the third-generation cephalosporins) may have longer half-lives, which may allow for infrequent dosing. For example, ceftiofur is metabolized to an active metabolite and has a half-life of approximately 3–6 hours in cattle, 4 hours in dogs, and 2.5 hours in horses.

Clinical Features and Specific Drugs Used in Veterinary Medicine

The drugs in the first-generation group have a spectrum of activity that includes staphylococci and streptococci. Resistance among gram-negative bacteria develops easily, primarily from synthesis of β-lactamase enzymes that can hydrolyze these drugs. Resistance has been demonstrated in clinical studies in which samples were collected from dogs and cats (Thungrat et al., 2015; Oluoch et al., 2001; Walker et al., 2000; Cooke et al., 2002). Some older studies may have underestimated resistance because older breakpoints were higher than current values (Table 33.7). Most of the enteric gram-negative isolates are resistant to first-generation cephalosporins because most wild-type bacteria of the Enterobacteriaceae have MIC values above the susceptible breakpoint for cephalexin. The second, third, and fourth generation have higher activity against the gram-negative pathogens. The situations in veterinary medicine in which extended-spectrum cephalosporins are most often used are for treatment of bacterial infections that are resistant to other drugs. The bacteria often identified in these resistance problems have been *Escherichia coli, Klebsiella pneumoniae, Enterobacter* species, *Proteus* species (especially indole positive), and *Pseudomonas aeruginosa*.

First-Generation Cephalosporins

Veterinarians are familiar with the cephalosporins commonly referred to as the *first-generation cephalosporins* represented by the oral drugs cephalexin (Keflex, Rilexine, and generic forms) and cefadroxil (Cefa-Tabs, Cefa-Drops), and the injectable drug cefazolin (generic). Cefadroxil and cephalexin have been the most extensively used of the oral first-generation cephalosporins in dogs. (Older drugs such as cephradine are no longer available.) Cefadroxil is more lipophilic than cephalexin and has the advantage of being better absorbed orally. The differences between cephalexin and cefadroxil were illustrated in the study by Campbell and Rosin (1998) in which they examined the oral absorption of each drug and the influence of food in dogs after 30 mg/kg every 12 hours. Being more lipophilic, cefadroxil was absorbed better when administered with food and attained higher concentrations in plasma. Cephalexin was less influenced by the presence of food. Every 12-hour dosing is appropriate to maintain concentrations above the MIC (Campbell and Rosin, 1998; Papich et al., 2007).

Cefadroxil: Cefadroxil is available as oral suspension (50 mg/ml) and oral tablets (although the availability of some formulations has diminished in recent years). In cats, cefadroxil pharmacokinetics are similar to that in dogs (Chatfield et al., 1984) and it has a half-life of 2.5 to 2.7 hours. Clinical trials in cats showed that cefadroxil was effective for dermal infections with cure rates of 88% at 10–20 mg/kg and 100% cure rate at 20 mg/kg twice daily. Cefadroxil also is used for urinary tract infections in cats at a dose of 20 mg/kg once daily.

In dogs, cefadroxil has been effective for treatment of urinary tract infections, skin infections, and respiratory infections (Chatfield et al., 1984; Angarano and MacDonald, 1989; Barsanti et al., 1985). The dose for which efficacy has been demonstrated for pyoderma has been 22 mg/kg every 12 hours orally for 21 to 30 days, but efficacy at a once-daily dose of 20 mg/kg once daily also has been reported (Scarampella et al., 2000). Cefadroxil at a dose of 22 mg/kg every 12 hours for 21 days was effective in an experimental model of canine cystitis (Barsanti et al., 1985) and has been approved for dogs for the treatment of urinary tract infections.

Cephalexin: Cephalexin is perhaps the most common oral cephalosporin administered to dogs. There is a chewable canine formulation (Rilexine) and approved formulations available in other countries. Human generic formulations also are administered to animals. Pharmacokinetics are listed in Table 33.6, which summarizes the results from several studies. Its oral absorption ranges from 57% to 73–79% (Lavy et al., 1997; Carli et al., 1999). Another study (Wackowiez et al., 1997), reported oral absorption of 91% in dogs. Most methicillin-susceptible *Staphylococcus* spp. and *Streptococcus* spp. are susceptible and cephalexin is appropriate for these infections in dogs and cats. One of the most common uses is staphylococcal pyoderma in dogs, for which there is established efficacy at a dose of 22–25 mg/kg q 12 h, oral. However,

as noted above, most wild-type organisms of the Enterobacteriaceae (e.g., *E. coli, Klebsiella* spp.) are resistant. All *Pseudomonas aeruginosa* are resistant.

Oral absorption of cephalexin in cats is about 56%, with a half-life of 2.25 hours (Wackowiez et al., 1997). At usual recommended doses, this will maintain concentrations for pathogens in cats that cause dermal or urinary tract infections with 12-hour dosing. Cephalexin oral absorption in horses is only 5% (Davis et al., 2005), but can be administered at 30 mg/kg q 8 h orally to achieve effective plasma concentrations above 0.5 µg/ml.

Cefazolin: Cefazolin is the injectable cephalosporin administered often to companion animals. It is inexpensive, has a broad spectrum of activity, and is stable after reconstitution for 1 week if refrigerated (Bornstein et al., 1974). It has been administered IV, IM, and SC to dogs. There are several papers that have examined its activity and pharmacokinetics in animals (Petersen and Rosin, 1995; Rosin et al., 1989, 1993; Dickson et al., 1987; Marcellin-Little et al., 1996). Pharmacokinetics are shown in Table 33.6. It is more active against *E. coli* than cephalothin or cephalexin, and after standard doses of 20–22 mg/kg IV, concentrations can be maintained during surgical procedures. Cefazolin has low plasma protein binding (19% in dogs, which is much lower than in humans) and diffuses into tissue fluid to reach concentrations that parallel those in plasma (Rosin et al., 1989, 1993). Cefazolin also penetrated normal and osteomyletic bone in concentrations similar to plasma concentrations (Daly et al., 1982). Distribution was not impaired in osteomyletic bone. This advantage of good penetration has allowed it to be used for prevention and treatment of bone infections (Daly et al., 1982) and as a common antibiotic to use prophylactically prior to orthopedic surgery (Rosin et al., 1993). Richardson et al. (1992) showed that at a dose of 22 mg/kg IV every hour, cefazolin concentrations in bone were above the MIC_{90} for pathogens causing common postoperative infections. Concentrations in bone of dogs paralleled the plasma concentrations and the optimum dose for orthopedic surgery was determined by Marcellin-Little et al. (1996). To maintain cefazolin concentrations above 20 µg/ml (10× the MIC_{90} of susceptible organisms a dose of 22 mg/kg administered IV every 2 hours or 8 mg/kg administered IV every hour was determined. During surgery, disease, anesthesia, and blood loss may affect distribution and clearance of some drugs. However, when cefazolin was administered to dogs with hemorrhagic shock, the clearance was slower, but it was offset by an increased volume of distribution (Dickson et al., 1987). Consequently, the plasma concentrations were not different in dogs when compared before and after shock. Some cephalosporins affect blood clotting and platelet function in animals and may be risky to use prior to surgery. However, when cefazolin was compared to cephalothin and cefmetazole in dogs, the investigators showed that cephalothin decreased platelet aggregation, and cefmetazole prolonged bleeding time (Wilkens et al., 1994), but cefazolin caused no adverse effects on platelet aggregation, bleeding time, platelet count, platelet size, or bleeding times.

Cefazolin is used occasionally in horses as an injectable preoperatively or perioperatively. The current doses are derived from pharmacokinetics and susceptibility data (Table 33.6, 33.7). Cefazolin has a slower terminal half-life from IM than from IV administration. The IM injection is thought to have a longer half-life because of slower absorption from muscle (caused by the flip-flop effect). Subsequent doses of 10 to 20 mg/kg can be administered q 8 h IM or q 6 h IV.

Cephapirin: Cephapirin is not used very often for systemic use in animals, but there are dry cow and lactating cow preparations (Cefa-Dri®, Cefa-Lak®, respectively) of cephapirin for intramammary infusion. Cephapirin is used for treatment of mastitis caused by *Streptococcus* or *Staphylococcus*. Cephapirin benzathine is used for dry cow treatment 300 mg/10 ml, administered in each quarter at the time of drying. Cephapirin sodium 200 mg/10 ml is infused 200 mg to each affected quarter every 12 hours.

Second-Generation Cephalosporins

Of the second-generation cephalosporins, the one used most often in veterinary medicine is cefoxitin (Petersen and Rosin, 1993). Cefotetan was once used, but is no longer available commercially. The use has been valuable for treating organisms resistant to the first-generation cephalosporins or in cases in which there are anaerobic bacteria present. Anaerobic bacteria such as those of the *Bacteroides fragilis* group can become resistant by synthesizing a cephalosporinase enzyme, but cefoxitin and cefotetan, which are in the cephamycin group, are resistant to this enzyme. Therefore, this group has been valuable for some cases such as septic peritonitis that may have a mixed population of anaerobic bacteria and gram-negative bacilli.

There are no reports of clinical use of oral second-generation cephalosporins in small animals, but doses have been extrapolated from human studies. Cefaclor was shown to have 75% oral bioavailability in dogs and there are anecdotal accounts of its use (Waterman and Scharfenberger, 1978). Interstitial drug levels were lower than serum, but urine concentrations were high for 4 hours after dosing.

Third-Generation Cephalosporins

The third-generation cephalosporins are the most active of the cephalosporins against gram-negative bacteria,

Table 33.8 Dose, formulations and indications for ceftiofur in animals

Species	Formulation	Dose
Cattle	Ceftiofur crystalline free acid (Excede)	6.6 mg/kg, with a single SQ injection in the middle third of the posterior aspect of the ear.
Horses and Foals	Ceftiofur crystalline free acid (Excede)	6.6 mg/kg IM in neck muscle (15 ml per 1,000 pounds). Administer a second dose in 4 days. Do not administer more than 20 ml in one site.
Pigs	Ceftiofur crystalline free acid (Excede)	5.0 mg/kg IM injection in the postauricular region of the neck.
Cattle	Ceftiofur hydrochloride (Excenel)	1.1–2.2 mg/kg q 24 h for 3 days IM or SQ. Intrauterine (retained fetal membranes): 1 g ceftiofur diluted in 20 ml sterile water infused in uterus once at 14–20 days after calving. Treatment of postpartum metritis: 2.2 mg/kg once daily for 5 days SQ or IM.
Pigs	Ceftiofur hydrochloride (Excenel)	3–5 mg/kg q 24 h for 3 days IM.
Cattle	Ceftiofur sodium (Naxcel)	Bovine respiratory disease (BRD): 1.1–2.2 mg/kg (0.5–1.0 mg/pound) q 24 h for 3 days IM. Additional doses may be given on days 4 and 5 if necessary. In cattle, these doses also may be administered SQ, which is bioequivalent.
Horses	Ceftiofur sodium (Naxcel)	2.2–4.4 mg/kg q 24 h IM or 2.2 mg/kg q 12 h IM for as long as 10 days. Treatment of some gram-negative infections may require doses at the higher range and up to 11 mg/kg/day IM has been given to horses. Foals: 5 mg/kg q 12 h IV, or CRI of 1 mg/kg/hour IV.
Pigs	Ceftiofur sodium (Naxcel)	Respiratory infections: 3–5 mg/kg (1.36–2.27 mg/pound) q 24 h for 3 days IM.
Sheep, goats	Ceftiofur sodium (Naxcel)	1.1–2.2 mg/kg (0.5–1.0 mg/pound) q 24 h for 3 days IM, SQ. Additional doses may be given on days 4 and 5 if necessary.
Dogs	Ceftiofur sodium (Naxcel)	Urinary tract infection: 2.2 to 4.4 mg/kg q 24 h SQ. Dose not established for cats but has been extrapolated from canine dose.

especially enteric organisms that are resistant to other cephalosporins. The injectable drugs are administered IV, SC, or IM. The SC route is often used for convenience (Moore et al., 2000; Guerrini et al., 1986). Veterinarians have observed that the IM or SC administration of some of these drugs can be irritating and painful. Cefotaxime (Claforan, and generic) is one of the typical members of this group to which others are compared. It has activity against most enteric gram-negative bacteria and some streptococci. Except for a few pharmacokinetic studies (Guerrini et al., 1986; McElroy et al., 1986), there are no published studies in which cefotaxime has been evaluated in veterinary patients. However, the pharmacokinetics between dogs and humans are similar enough that doses, as well as clinical uses, have been extrapolated from human medicine. Generally, cefotaxime is administered IV, IM, or SC to dogs and cats at a dose of 30 mg/kg every 8 hours. When administered SC to dogs, and IM to cats, the absorption was high (McElroy et al., 1986; Guerrini et al., 1986).

Ceftiofur: Ceftiofur (Naxcel, Excenel, Excede) has been approved for veterinary use for many years. It is unique because it is not used in human medicine. It is available in three forms: (i) ceftiofur sodium – Naxcel, (ii) ceftiofur hydrochloride suspension (Excenel), and (iii) ceftiofur crystalline free acid (Excede). After injection it is converted to an active metabolite, desfuroylceftiofur. The differences in activity between ceftiofur and its metabolite were reported by Salmon et al. (1996). Ceftofur has greater activity than the desfuroyl metabolite against *Staphylococcus* spp. (four to eight times difference) and slightly greater activity against *Streptococcus* spp. Both the parent drug and metabolite are highly active against gram-negative bacteria that cause bovine and porcine respiratory disease, but less so against gram-negative bacteria of the Enterobacteriaceae (MIC 0.5–1 μg/ml). The CLSI (CLSI, 2015) breakpoint for ceftiofur use in cattle and swine is ≤2.0 μg/ml (Table 33.7), but lower for the use in horses. Doses are listed in Table 33.8.

For dogs ceftiofur sodium is approved only for treating urinary tract infections caused by gram-negative bacilli of the Enterobacteriaceae at a dose of 2.2 mg/kg SC once a day. Bacteria with higher MIC values may require larger doses or more frequent administration (Brown et al., 1995). The use of ceftiofur for treating systemic infections in small animals has not been reported; but, on the basis of the pharmacokinetic profile of plasma concentrations (Brown et al., 1995), it appears that the frequency of administration should be greater than once a day to maintain the drug concentrations above the MIC for a sufficient duration. In dogs, anemia and thrombocytopenia are possible if ceftiofur is administered at doses of three times and five times the registered dose of 2.2 mg/kg.

Ceftiofur is the most frequently used cephalosporin in horses. Ceftiofur was approved for use in horses for treatment of respiratory tract infections caused by *Streptococcus equi* subsp. *zooepidemicus* at a dose of 2.2 to 4.4 mg/kg q 24 h IM. Higher doses or more frequent intervals have been recommended for treating gram-negative organisms (e.g., *Klebsiella, Enterobacter, Salmonella*). Because these organisms are inherently more resistant, higher plasma concentrations are needed for efficacy. Studies in foals indicated that a dose of 2.2 to 6.6 mg/kg could be given to foals IM or IV q 12 h for treatment of neonatal sepsis. Based on pharmacokinetic studies (Jaglan et al., 1994) a dose of 4.4 mg/kg injected q 12 h will produce plasma concentrations above the MIC to meet the criteria for effective therapy. Toxicity studies have shown that horses tolerate ceftiofur doses up to 11 mg/kg/day IM, with pain at the injection site and decreased feed consumption as the most common adverse effects at the highest dose.

An important use of ceftiofur is for respiratory and other infections in cattle and pigs. An advantage of ceftiofur is that concentrations quickly fall below the allowed tolerance and withdrawal times for slaughter and milk are short compared to other antibiotics. Use of this drug in food animals is discussed further in Chapter 52 of this book. It has been used for treating bovine respiratory disease (BRD) in cattle and swine respiratory disease (SRD) in pigs. It has activity against bovine and swine respiratory pathogens such as *Actinobacillus pleuropneumoniae, Mannheimia haemolytica, Histophilus somni*, *Salmonella choleraesuis, Haemophilus*, and *Streptococcus*. This formulation also is approved for treating foot rot in cattle (interdigital necrobacillosis) caused by *Fusobacterium necrophorum, Porphyromonas levii,* and *Bacteriodes melaninogenicus*. It is approved for treatment of acute metritis in dairy cattle via a two-dose regimen. The crystalline-free acid (Excede) is a slow-releasing drug that is injected at the base of the ear in cattle and in the neck of pigs. Ceftiofur hydrochloride and ceftiofur crystalline-free acid have also been administered intramammary to dairy cattle (Spectramast).

Ceftazidime: Compared to other cephalosporins, ceftazidime is the most active against *Pseudomonas aeruginosa*, against which all the other cephalosporins, except cefoperazone, have little or no activity. Ceftazidime has been studied in dogs (Moore et al., 2000; Matsui et al., 1984; Acred, 1983) and it has a short half-life (less than 1 hour) and volume of distribution similar to that in humans. Dosages have ranged from 20–30 mg/kg every 12 hours for Enterobacteriaceae, to 30 mg/kg administered every 4 hours for *Pseudomonas aeruginosa* (Moore et al., 2000).

In vitro activity of ceftazidime is good against most gram-negative bacilli (Martin Barrasa et al., 2000). Isolates of *Pseudomonas aeruginosa* from otitis media showed that 97% were susceptible to ceftazidime (Colombini et al., 2000). In a study that isolated *Pseudomonas aeruginosa* from the skin and ears of dogs, a similar pattern of susceptibility was reported (Petersen et al., 2002). In a study that examined 183 isolates of *Pseudomonas aeruginosa* from various sites in dogs (1993–2000), Seol et al., 2002), 77% were susceptible to ceftazidime.

Because of the good activity against Enterobacteriaceae and *Pseudomonas aeruginosa*, ceftazidime has been used in exotic and zoo animals. In a killer whale, the half-life was greater than 6 hours after IM administration and therapeutic concentrations were maintained after doses of 20 mg/kg every 24 hours, IM (unpublished observations by the author, MGP). In reptiles, cephalosporins are excreted slowly. Ceftazidime pharmacokinetics in sea turtles determined that a half-life of 20 hours allowed for dosing of 20 mg/kg as infrequently as every 72 hours (Stamper et al., 1999). In Eastern box turtles the half-life was 42 hours, which allows for a dose of 20 mg/kg IM every 5 days to maintain concentrations above a therapeutic range (author's data; not yet published).

Cefovecin: In December 2006, cefovecin (Convenia) was introduced to small animal medicine in Europe. The same drug and formulation were available in Canada in October 2007 and in the US in 2008. Pharmacokinetic studies have shown the unique differences between cefovecin and other cephalosporins in dogs and cats (Stegemann et al., 2006b, 2006c). Efficacy studies and clinical field trials have shown its efficacy (Stegemann et al., 2007a, 2007b; Passmore et al., 2007). In the clinical studies, cefovecin was compared to another active antimicrobial (cefadroxil, cephalexin, or amoxicillin–clavulanate) and was found noninferior to these other drugs.

In dogs and cats, cefovecin is approved for treatment of skin and soft-tissue infections. In some countries it is also registered for urinary tract infections. The approved label dose in the US allows for a repeat injection at 7 days at a dose of 8 mg/kg SC. However, concentrations are maintained against some bacteria for 14 days, and the approved labeling in Canada and Europe lists a 14-day dose interval. The studies published show efficacy with a 14-day interval for administration. The injection may be repeated if longer than 14 days is needed for a cure (e.g., canine pyoderma).

The long duration of cefovecin is attributed to the long half-life in dogs and cats. Cefovecin is >99% protein bound in cats and >98% in dogs. With such a small fraction unbound (fu) there is little drug available for excretion and some tubular reabsorption may also occur. Subsequently, the terminal half-life is approximately 7 days

in cats and 5 days in dogs. Effective concentrations can be maintained in the tissue fluid for a 14-day interval or longer (Stegemann et al., 2006b, 2006c). Cefovecin is classified as a third-generation cephalosporin and has a low MIC values for many bacteria. Against pathogens from Europe and the US (Stegemann et al., 2006a), cefovecin MIC_{90} values were 0.25 μg/ml for *Staphylococcus pseudintermedius* compared to 2 μg/ml for cephalexin and cefadroxil. It has greater activity against gram-negative bacteria than first-generation cephalosporins, as was demonstrated by the MIC_{90} values of 1 μg/ml compared to 16 μg/ml for cephalexin and cefadroxil. Many other MIC comparisons are provided in the tables in the paper by Stegemann et al. (2006a). But compared to other third-generation cephalosporins such as cefotaxime, it is not as active against gram-negative bacteria of the Enterobacteriaceae.

Oral Third-Generation Cephalosporins

Because the drugs mentioned above are all injectable, there has been a need for an oral extended-spectrum cephalosporin. Cefixime (Suprax) was once used in small animals, but is no longer available (Lavy et al., 1995; Bialer et al., 1987).

Cefpodoxime proxetil: Cefpodoxime proxetil is the oral third-generation cephalosporin used most often in veterinary medicine. It is a prodrug ester (Borin, 1991) that is designed to remain stable in the stomach, but the prodrug is converted to the active cefpodoxime by intestinal brush border enzymes. As a lipophilic ester, it is anticipated that oral absorption will be enhanced if the drug is administered with food, which has been confirmed in people, but not specifically reported for dogs. Cefpodoxime has similar gram-negative in vitro activity as cefixime, but greater activity against *Staphylococcus*.

In dogs pharmacokinetics have been studied to show good oral absorption and a long half-life (4.7 and 5.6 hours) compared to other cephalosporins that allow for once-daily administration at 5–10 mg/kg (Brown et al., 2007; Klesel et al., 1992; Papich et al., 2007). Cherni and colleagues (2006) reported that cefpodoxime proxetil administered once a day (5 mg/kg) to dogs with pyoderma was as effective as twice-daily (26 mg/kg) administration of cephalexin (Cherni et al., 2006). In horses, cefpodoxime proxetil oral absorption was good enough that a dose of 10 mg/kg q 6–12 h produced plasma concentrations that would potentially treat infections in horses (Carrillo et al., 2005), but clinical use in horses has not been reported.

Fourth-Generation Cephalosporins

The only fourth-generation cephalosporin available in the US at this time is cefepime (Maxipime), which is approved for people and occasionally used in animals. It is unique because of its broad spectrum of activity that includes gram-positive cocci, enteric gram-negative bacilli, and *Pseudomonas aeruginosa*. It has the advantage of activity against some extended-spectrum β-lactamase (ESBL)-producing strains of *Klebsiella* and *E. coli* that have become resistant to other β-lactam drugs and fluoroquinolones. Except for investigations in dogs, adult horses, and foals, the use of cefepime has been limited in veterinary medicine (Gardner and Papich, 2001). In the study in dogs, there was a short half-life of 1 hour, and to maintain drug concentrations above an MIC value of 8 μg/ml for 67% of the dosing interval, a dose of 40 mg/kg IV every 6 hours would be necessary. However, this dose would maintain the concentration above an MIC of 2 μg/ml for 100% of the dosing interval and bacteria with lower MIC values (MIC ≤2 μg/ml) could be treated with longer dose intervals. In foals and mares this drug possibly could be used for infections resistant to other drugs. A cefepime dose for foals of 11 mg/kg IV q 8 h (Gardner and Papich, 2001) and for adults of 2.2 mg/kg IV q 8 h (Gardner and Papich, 2001; Guglick et al., 1998) is recommended. When cefepime was administered to horses orally, signs of colic were observed (Guglick et al., 1998).

Cefquinome (Cobactan) has been licensed for use in cattle and horses in Europe since 1994. It is not approved for use in the USA. It is approved for treatment of infections in horses and cattle caused by *Streptococcus equi subsp. zooepidemicus*, septicemia caused by *Escherichia coli*, and respiratory diseases caused by *Pasteurella multocida* and *Mannheimia haemolytica*, digital dermatitis, infectious bulbar necrosis and acute interdigital necrobacillosis (foul in the foot). It is also approved as an intramammary product for treatment of mastitis.

Adverse Reactions

Cephalosporins have a high therapeutic index and have been administered to small animals safely. Some of the adverse reactions are listed in the following sections.

Hypersensitivity Reactions

Hypersensitivity allergic reactions (type I, II, or III) have been observed in small animals after administration, but they are infrequently reported. There appears to be some cross-sensitivity with penicillins, but the incidence has not been reported. One should not assume that, if animals are sensitive to penicillin drugs, they will have adverse effects from cephalosporins. Sensitivity to penicillins may increase the risk of sensitivity to cephalosporins by a factor of 4 (Kelkar and Li, 2001), but many patients who are sensitive to penicillins can receive cephalosporins safely. Cephalexin has a side chain identical to that of amoxicillin, so animals with sensitivity to

ampicillin should be administered cephalexin cautiously. Likewise, cefadroxil has the same identical side chain as amoxicillin.

Gastrointestinal

Some dogs vomit after receiving oral cephalosporins (e.g., cefadroxil, cephalexin), particularly at high doses. Dogs also may vomit after rapid injections of intravenous cephalosporins (Petersen and Rosin, 1993). In clinical studies with oral cephalosporins, vomiting and diarrhea are the most common adverse reaction (Frank and Kunkle, 1993). Cephalexin and cefadroxil were the third and fifth most common oral drugs to cause adverse events in dogs according to one survey (Kunkle et al., 1995). In this survey, the most common adverse effects associated with oral cephalosporins were gastrointestinal (vomiting, diarrhea, and loss of appetite). It is believed to be caused by irritation to the stomach mucosa, but the exact mechanism has not been investigated.

Blood Disorders

Cephalosporin-induced hemolysis has been reported in people (Ehmann, 1992). Such a disorder has not been reported from use of cephalosporins in small animals. A positive Coombs test reaction can occur with patients receiving cephalosporins, but it is not associated with hemolytic anemia. High doses of ceftiofur in dogs can cause anemia and thrombocytopenia.

Bleeding Disorders

Bleeding disorders have been reported with some cephalosporins in humans because they may produce a prolongation of the prothrombin bleeding time. Even though this effect can be demonstrated in experimental dogs, it has not been reported to be a clinical problem in veterinary medicine, probably because it is associated only with a few of the cephalosporins that are rarely used in animals. Cephalothin was shown to prolong mucosal bleeding times and adenosine diphosphate (ADP)-induced platelet aggregation in dogs, but did not affect platelet numbers or platelet aggregation from collagen (Schermerhorn et al., 1994). This is a moot point for cephalothin because it is no longer used clinically in dogs. Cefazolin is often administered to dogs, cats, and horses. When it was compared to cephalothin and cefmetazole in dogs, the investigators showed that cephalothin decreased platelet aggregation, and cefmetazole prolonged bleeding time (Wilkens et al., 1994). However, cefazolin caused no adverse effects on platelet aggregation, bleeding time, platelet count, platelet size, or bleeding times.

In people, only the cephalosporins with NMTT (*N*-methylthiotetrazole) side chains are prone to producing bleeding problems. *(The NMTT drugs include cefoperazone, cefotetan, and cefamandole.)* Bleeding problems appear to be related to vitamin K antagonism and/or platelet dysfunction.

Glycosuria

Cephalosporins may cause a false-positive glucose test on a urine sample test, but this occurs only if the test employs the copper-reduction test. Others such as the glucose enzymatic tests are not affected. This is of little clinical significance.

Special Species Considerations

In zoo hoofstock, ceftiofur and other cephalosporins are important injectable drugs. Pharmacokinetics are similar as in other large animals, and doses for the cephalosporins are similar among the large zoo species. Drugs such as ceftiofur crystalline free acid are important in these animals because they can be treated without the need for frequent injections.

In reptiles, cephalosporins are excreted slowly. Ceftazidime pharmacokinetics in sea turtles determined that a half-life of 20 hours allowed for dosing of 20 mg/kg as infrequently as every 72 hours (Stamper et al., 1999). Regimens for cephalosporins in other reptiles have been published that also allow for long dosing intervals (Jacobson, 1999).

In birds, rapid elimination and poor oral absorption are a problem. This requires high doses and frequent administration for cephalosporins (Flammer, 1998). Doses for cephalexin and cefotaxime in birds has been listed as high as 100 mg/kg, q 8 h.

Carbapenems (Penems)

Carbapenems (also called *penems*) include imipenem, doripenem, ertapenem, and meropenem. They have the broadest antibacterial action in comparison to other β-lactams, even surpassing third-generation cephalosporins. The carbapenems have become valuable antibiotics because of a broad spectrum that includes many bacteria resistant to other drugs (Edwards and Betts, 2000). Carbapenems are not active against methicillin-resistant staphylococci or resistant strains of *Enterococcus faecium*. The high activity of the carbapenem group of β-lactams is attributed to its stability against most of the β-lactamases (including ESBL) and ability to penetrate porin channels that usually exclude other drugs (Livermore, 2001). Resistance to carbapenems has been extremely rare in veterinary medicine, but as discussed earlier carbapenemase-producing bacteria have been identified in animals (Abraham et al., 2014).

The carbapenems have been used primarily for serious, resistant infections that would otherwise require

multiple drugs, including aminoglycosides. They are more bactericidal than other β-lactam antibiotics against gram-negative bacteria because they affect PBP-1 and PBP-2 and produce postantibiotic effects (PAE) that are not seen with other β-lactams. The rapid bactericidal activity is less likely to induce release of endotoxin in patients from gram-negative sepsis during treatment. The bactericidal activity can be maintained with a shorter time above the MIC than other β-lactam antibiotics (Turnidge, 1998). In veterinary medicine, their use has been limited to serious infections caused by bacteria resistant to other antibiotics. Imipenem and meropenem are the most commonly used of this group. The breakpoints for susceptibility testing are not established for animals. For humans the susceptible breakpoint is ≤1 μg/ml for Enterobacteriaceae (≤0.5 μg/ml for ertapenem) and ≤2 μg/ml for *Pseudomonas aeruginosa.*

Imipenem (Primaxin®)

Imipenem has been used occasionally for treating serious infections in veterinary medicine. Imipenem is ordinarily metabolized extensively by the renal tubules (a brush border enzyme) to a potentially toxic compound. The drug cilastatin inhibits the renal enzymes and imipenem is combined with cilastatin in the product Primaxin® to avoid renal toxicity and to achieve high urine concentrations of active drug.

Some disadvantages of imipenem are the inconvenience of administration, short shelf-life after reconstitution, and high cost. It must be diluted in fluids prior to administration. A common dose for small animals is 10 mg/kg q 8 h or 5 mg/kg q 6 h. This dose must be given by constant rate infusion over 30–60 minutes, but it has been administered subcutaneously. One of the adverse effects caused from imipenem therapy is seizures. Another problem is the risk of renal injury, which should be minimized by the addition of cilastatin (Barker et al., 2003).

Meropenem (Merrem®)

Meropenem is a newer-generation carbapenem. It has antibacterial activity approximately equal to, or greater than, imipenem. Its advantage over imipenem is that it is more soluble and can be administered in less fluid volume and more rapidly. For example, small volumes can be administered subcutaneously with almost complete absorption. There also is a lower incidence of adverse effects to the central nervous system, such as seizures (Edwards and Betts, 2000). Based on pharmacokinetic experiments (Bidgood and Papich, 2002), the recommended dose in dogs for Enterobacteriaceae and other susceptible organisms is 8.5 mg/kg SC every 12 hours, or 24 mg/kg IV every 12 hours. For infections caused by *Pseudomonas aeruginosa*, or other similar organisms that may have MIC values as high as 1.0 μg/ml, the dose is 12 mg/kg q 8 h, SC, or 25 mg/kg q 8 h, IV. For susceptible organisms in the urinary tract, 8 mg/kg, SC, every 12 hours can be used. In the experience of the author, these doses have been well-tolerated except for slight hair loss over some of the SC dosing sites. The dose for cats, based on pharmacokinetic studies, is 10 mg/kg twice daily, SC, IM, or IV.

Ertapenem (Invanz)

Ertapenem is one of the newest of the carbapenems. Ertapenem has good activity against most gram-negative organisms, except *Pseudomonas aeruginosa.* It has a longer half-life in people, allowing for once-daily administration. However, in dogs the protein binding was only 46% and the half-life is not prolonged as it is in people. The half-life after a SC injection of 20 mg/kg was 1.3 hours with high systemic clearance. A dose of 30 mg/kg every 12 hours SC in dogs will maintain concentrations in the therapeutic range for dogs. The dose should be increased to every 8 hours in immunocompromised animals.

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34

Tetracycline Antibiotics

Mark G. Papich and Jim E. Riviere

General Pharmacology of Tetracyclines

The tetracycline antibiotics were initially discovered in 1944, with the first one being chlortetracycline. These were isolated from the *Streptomyces* species (*S. rimosus* and *S. aureofaciens*), and later expanded to include the various semisynthetic products that include tetracycline, doxycycline, and minocycline. Oxytetracycline was discovered in 1948, tetracycline in 1953, doxycycline in 1967, and minocycline in 1972. The newest development is the glycylcyclines, which are derivatives of minocycline. Tigecycline is the only available representative of this group, which possesses better antimicrobial activity than older drugs (Agwuh and MacGowan, 2006). Tigecycline use in veterinary medicine has not been reported.

The tetracyclines are a group of four-ringed amphoteric compounds (Figure 34.1) that differ by specific chemical substitutions at different points on the rings. As a group, the tetracyclines are acidic, hygroscopic compounds in aqueous solutions and easily form salts with acids and bases, with which they are commonly formulated. The most common salt form is the hydrochloride formulation, as is the case with oxytetracycline. However, some tetracyclines, especially oxytetracycline, are formulated with vehicles (excipients) to prolong absorption from the injection site. Some of the chemical and physical properties of the tetracyclines used in veterinary medicine today are listed in Table 34.1.

Tetracyclines have activity against both gram-positive and gram-negative bacteria, but resistance occurs frequently. They also have activity against atypical pathogens such as *Mycoplasma,* blood-borne pathogens (hemoplasma), and organisms such as *Rickettsia* transmitted by ticks and other parasites. Clinically accepted indications include abscesses, enteritis, *Leptospirosis*, pneumonia, bovine and swine respiratory disease, pododermatitis, treatment of tick-borne pathogens, skin and soft tissue infections, canine heartworm disease, and uterine infections.

Many formulations have been administered in medicated water and for feed for production purposes (growth promotion). The US Food and Drug Administration (FDA) announced that as of 2017 the production uses of these antibiotics will be voluntarily withdrawn from livestock use. The tetracyclines comprise the largest group of antibiotics affected by this FDA order. The FDA believes that production use indications such as "increased rate of weight gain" or "improved feed efficiency" are no longer appropriate for the approved conditions of use for medically important antimicrobial drugs. These regulatory changes are provided in the Guidance for Industry (GFI) documents #209 and #213, which may be obtained from the FDA. These medications ordinarily added to feed and water of livestock will not be considered in this chapter because of their future status, and the levels administered are considered subtherapeutic. In addition, the pharmacokinetics of these formulations in the target species is incomplete.

Mechanism of Action

Tetracyclines possess antimicrobial activity by binding to the 30S ribosomal subunit of susceptible organisms. After binding to the ribosome, the tetracyclines interfere with the binding of aminoacyl-tRNA to the messenger RNA molecule/ribosome complex, thereby interfering with bacterial protein synthesis in growing or multiplying organisms (Gale and Folkes, 1953; Suzuka et al., 1966). Tetracyclines require an energy-dependent process to enter bacteria. One of the reasons for their selectivity against microorganisms is that mammalian cells lack this transport mechanism. Tetracyclines also have less affinity for mammalian ribosomes than bacterial ribosomes. Because the binding to the ribosome target is a reversible process, the drug concentrations must be maintained throughout the dose interval and these drugs are generally considered bacteriostatic. Details are described in Section Pharmacokinetic–Pharmacodynamic Properties.

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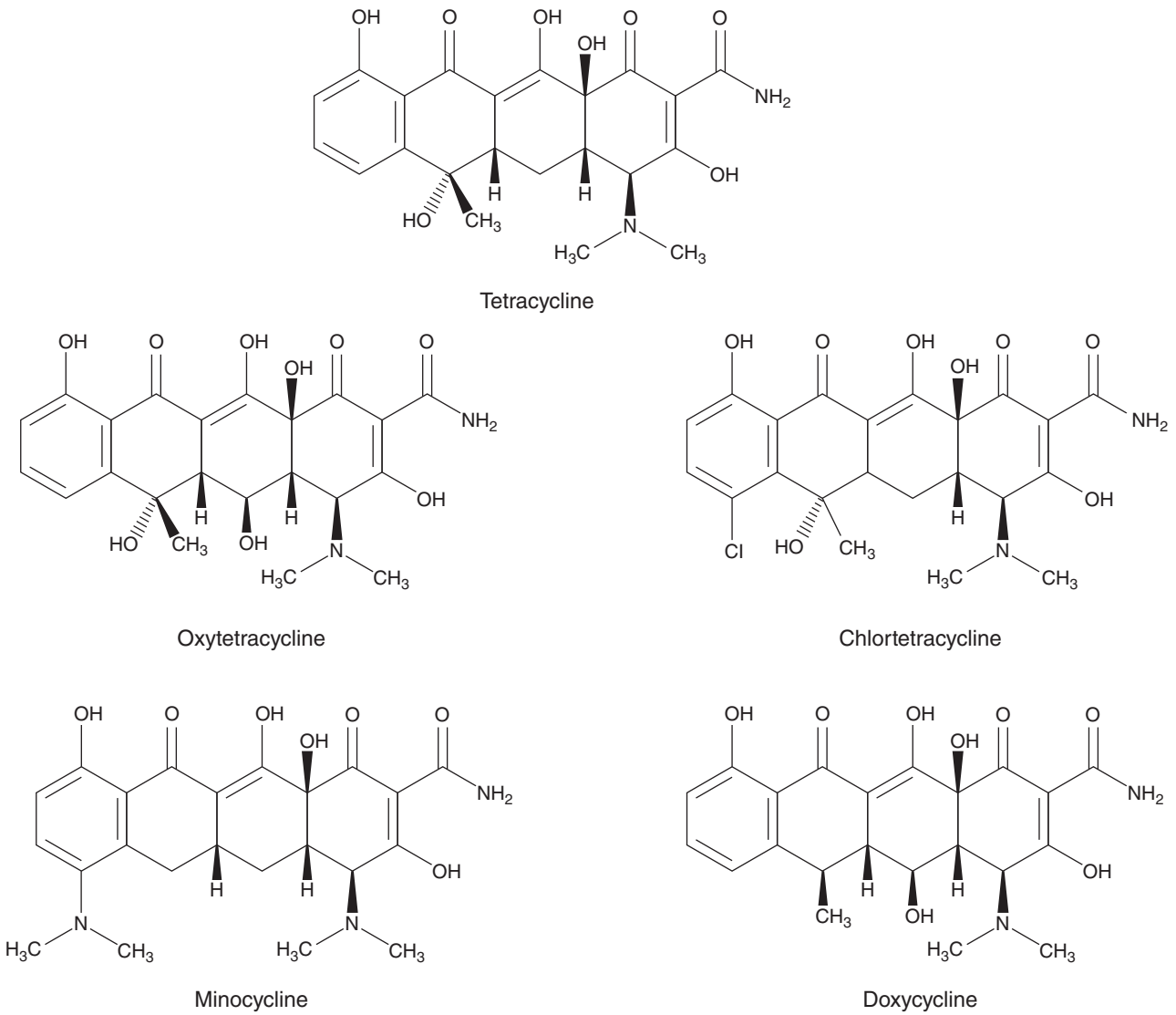

Figure 34.1 Tetracyclines and other tetracycline structures.

Resistance: Because the tetracyclines have been used over many years in veterinary and human medicine, resistance is common and occurs across all groups of bacteria. The mechanisms of acquired resistance include: (i) energy-dependent efflux of antibiotic (membrane efflux proteins), or (ii) altered target whereby the ribosome is protected from binding of tetracyclines (Chopra et al., 1992). A third mechanism whereby the drug is attacked by enzymes liberated by the bacteria is possible. The genes mediating resistance may be carried on plasmids or transposons. Resistance to one tetracycline will generally produce cross-resistance to the others in the group. One of the exceptions is for *Staphylococcus* spp., which is discussed in Section Antimicrobial Spectrum and Clinical Uses. The newest tetracycline, tigecycline, is active against many organisms (e.g., methicillin-resistant staphylococci) that are resistant to older tetracyclines (Agwuh and MacGowan, 2006).

Table 34.1 Chemical and physical properties of tetracyclines

Drug	Molecular weight	pK_a
Chlortetracycline	478.88	3.3, 7.4, 9.3
Doxycycline	462.46	7.75
Minocycline	457.48	8.25
Oxytetracycline	460.44	7.75
Tetracycline	444.43	8.3, 10.2

Susceptibility testing: For susceptibility testing, tetracycline may be used to test all others in the class (CLSI, 2013; CLSI, 2015). Specific breakpoints are also available for minocycline and doxycycline in dogs, and doxycycline in horses (Table 34.2). For veterinary isolates, the

Table 34.2 Tetracycline breakpoints for susceptibility testing. Source: CLSI, 2015.

Drug	Bacteria	Animal	S (μg/ml)	I (μg/ml)	R (μg/ml)
Tetracycline[a]	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*	Cattle	≤ 2	4	≥ 8
Tetracycline[a]	*Actinobacillus pleuropneumoniae* *Pasteurella multocida* *Streptococcus suis*	Pigs	≤ 0.5	1	≥ 2
Tetracycline	*Staphylococcus pseudintermedius*	Dog	≤ 0.25	0.5	≥ 1
Doxycycline	*Staphylococcus pseudintermedius*	Dog	≤ 0.12	0.25	≥ 0.5
Doxycycline	*Staphylococcus aureus, Streptococcus equi ssp. zooepidemicus, Streptococcus equi ssp. equi, Escherichia coli*	Horse	≤ 0.12	0.25	≥ 0.5
Minocycline	*Staphylococcus pseudintermedius*	Dog	≤ 0.5	1	≥ 2
Tetracycline	Enterobacteriaceae	Human	≤ 4	8	≥ 16
Tetracycline	*Staphylococcus, Streptococcus*	Human	≤ 2	4	≥ 8

S, susceptible; I, intermediate; R, resistant.
[a]The results of tetracycline susceptibility tests are used to predict susceptibility for chlortetracycline and oxytetracycline. Isolates susceptible to tetracycline are susceptible to minocycline and doxycycline.

susceptible breakpoint varies among species and is lower than the human breakpoints, which should be considered when interpreting susceptibility tests. The breakpoint for susceptible organisms in humans is ≤4 μg/ml for all organisms, except streptococci, which is ≤2 μg/ml.

Pharmacokinetic–Pharmacodynamic Properties

Based on an evaluation of tetracycline pharmacokinetic–pharmacodynamic properties (PK-PD), the effectiveness is best expressed as a ratio of the area-under-the-curve for a 24-hour interval to the minimum inhibitory concentration (AUC_{24}/MIC) (Agwuh and MacGowan, 2006). The optimal AUC/MIC ratio is in the range of 25–40, with Andes and Craig (2007) suggesting a value of 25. In veterinary studies this has been explored insufficiently. One study (Prats et al., 2005) examined PK-PD parameters after administration of doxycycline to swine in drinking water at 10 mg/kg. They reported high AUC/MIC ratios ranging from 60 for *Pasteurella,* 155 for *Mycoplasma,* and 585 for *Bordetella*. For *Actinobacillus,* which exhibits a higher MIC, the AUC/MIC was only 13. However, these ratios did not factor the protein binding, which is at least 90% (Table 34.3). Using a fraction unbound (*fu*) value of 0.1, reduces these ratios substantially. Ideal AUC/MIC ratios from their study would be achieved only for *Bordetella bronchiseptica*.

For *Staphylococcus* isolates from dogs, substantial PK-PD analysis was performed for doxycycline and minocycline (Maaland et al., 2013, 2014; Hnot et al., 2015a). It was concluded that AUC/MIC was the parameter that should be used for analysis, and a AUC/MIC ratio of 25 of the *unbound fraction* was optimum for predicting susceptibility and deriving dosages administered.

Table 34.3 Protein binding of tetracyclines in various species (references provided in text)

Drug	Species	% Protein binding
Chlortetracycline	Cows	47–51
	Sheep	46–50
Doxycycline	Calves	92
	Cats	98
	Horses	82
	Dogs	91–92
	Pigs	93
	Sheep	84–90
	Turkeys	70–85
Oxytetracycline	Buffalo	42
	Cows	18–22
	Horses	50
	Pigs	75.5
	Sheep	21–25
	Trout	55
Tetracycline	Cows	31–41
	Sheep	28–32
Minocycline	Dog	65.8
	Sheep	80

Absorption

Tetracyclines can be administered intravenously (most tetracyclines) or intramuscularly (oxytetracycline). The oral route also has been used (Table 34.4). Although some formulations (e.g., chlortetracycline and tetracycline) have been administered in feed and water for pigs, cattle, and poultry, the systemic effects of this route of administration may be much less than anticipated. In a study in which oxytetracycline was fed to pigs (Hall et al., 1989) the authors concluded that feeding this medication at a rate of 0.55 g/kg of feed resulted in plasma concentrations so low that only highly susceptible

Table 34.4 Comparison of oral absorption of tetracyclines in animals (mean values of studies reported; references listed in text)

Drug	Species	Systemic absorption (F%)
Chlortetracycline	Chickens	1
	Turkeys	6
	Pigs	6, 11, 19 (depending on the study and fasting conditions)
Oxytetracycline	Pigs	3–5
	Fish	6
	Turkeys	9–48
Tetracycline	Pigs	5, 8, 18, 23 (depending on the study and fasting conditions)
	Dogs	40
	Cats	50
Doxycycline	Pigs	21.2
	Chickens	41.3
	Turkeys	25, 37, 41, 63.5 (depending on age)
	Horses	17.3 (intragastric); 6 as a top dressing; higher in foals
	Dogs	53 (hyclate), 33.5 (monohydrate)
	Dogs	61.85 (summary of 4 studies)
	Calves	70
Minocycline	Dogs	50.3
	Cats	62

bacteria would be inhibited, with plasma concentrations reaching only one-tenth of the MIC for most pathogens. Several studies have examined use of tetracyclines added in subtherapeutic concentrations to feed rations (in particular, chlortetracycline) (Zinn, 1993; Jones et al., 1983; Dawson et al., 1983; Williams et al., 1978; Quarles et al., 1977; Richey et al., 1977; Nivas et al., 1976). The explanation for low oral absorption is not clear, but may be multifactorial. Tetracyclines are zwitterions and ionized at physiological pH values (see Table 35.1). Although the tetracyclines are relatively lipophilic drugs, they are ionized in the gastrointestinal tract and may not cross membranes easily. The oral absorption of tetracycline can be reduced in the presence of food (Nielsen and Gyrd-Hansen, 1996; Hnot et al., 2015b). Tetracyclines can easily chelate to polyvalent cations, which decreases the absorption several-fold. Thus, tetracycline oral absorption can be decreased with the coadministration of food, dairy products, polyvalent cations (i.e., Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}), kaolin/pectin preparations, iron-containing supplements, and antacids (Weinberg, 1957; Waisbren and Hueckel, 1950; Harcourt and Hamburger, 1957; Neuvonen et al., 1970; Hägermark and Hoglund, 1974; Gothoni et al., 1972; KuKanich et al., 2014; KuKanich and KuKanich, 2015). When doxycycline or minocycline were administered with sucralfate (containing aluminum) oral absorption was significantly reduced unless the sucralfate was administered 2 hours after the tetracycline (KuKanich et al., 2014; KuKanich and KuKanich, 2015).

Distribution

Once absorbed, tetracyclines bind to plasma proteins to varying degrees in each species with doxycycline being greater than 80% in most animals (Riond and Riviere, 1989b) (Table 34.3). The high protein binding for some drugs may limit the microbiologically active fraction in the plasma. For the agents that are not restricted by protein binding, tetracyclines are widely distributed throughout most tissues of the body, including intracellular sites. Tables 34.5–34.9 show the pharmacokinetic variables for some of these drugs. High protein binding can limit tissue distribution. Bidgood and Papich (2003) showed that plasma protein binding for doxycycline was high (91.75%), which drastically limited the distribution into the tissue fluid. The same property was shown with doxycycline in horses (Davis et al., 2006). The plasma protein binding of 82% in horses reduced distribution to tissue fluid. Maaland et al. (2014) showed that plasma protein binding of minocycline limited the distribution to approximately 50% of the dose administered. Protein binding has less of an effect on distribution to intracellular sites and joint fluid (see below in this section).

Tetracyclines are moderately lipophilic (depending on the pH) compared to some other classes of antibiotics (e.g., β-lactams, and aminoglycosides); therefore, for the fraction not restricted by protein binding, at physiological pH they are capable of crossing lipid membranes. Some tetracyclines penetrate tissues better than others.

Table 34.5 Pharmacokinetic parameters of chlortetracycline in some food-animal species

Species	Dose (mg/kg)	Route	Vd (l/kg)	$t_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Turkey	0.9	IV	0.2284	0.877	3.77	Dyer, 1989
Pigs	11.0	IV	1.3883	NR	0.3071	Kilroy et al., 1990
Pigs	10	IV	0.7	3.6 (MRT)	3.33	Nielsen and Gyrd-Hansen, 1996
Pigs	39.9	Oral	—	8.7 (MRT)	—	Nielsen and Gyrd-Hansen, 1996
Calves (milk fed)	11.0	IV	3.34	8.89	260.52 l/h/kg	Bradley et al., 1982
Calves (conventionally fed)	11.0	IV	1.93	8.25	162.12 l/h/kg	Bradley et al., 1982

NR, information not reported; IV, intravenous; MRT, mean residence time.

Table 34.6 Pharmacokinetic parameters of tetracycline in some species

Species	Dose (mg/kg)	Route	Vd (l/kg)	$t_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Gilts	11	IA	1.06	NR	0.4	Kniffen et al., 1989
Chickens	65	IV	0.174	2.772	1.632	Anadon et al., 1985
Rabbits (male and female)	10	IV	1.047	2	6.1	Percy and Black, 1988
Channel catfish (*Ictalurus punctatus*) (27°C)	4	IV	0.513	16.5	0.365	Plakas et al., 1988
Pigs	11	IV	4.5	16	3.1	Kniffen et al., 1989
Pigs	9.6	IV	1.2	5.6 (MRT)	3.5	Nielsen and Gyrd-Hansen, 1996
Pigs	46.4	Oral	—	9.0 (MRT)	—	Nielsen and Gyrd-Hansen, 1996

NR, information not reported; IV, intravenous; IA, intraarterial; MRT, mean residence time.

Table 34.7 Pharmacokinetic parameters of oxytetracycline in some species

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Horses	10	IV	0.6728	12.953	0.6583	Horspool and McKellar, 1990
Ponies	10	IV	1.0482	14.949	1.013	Horspool and McKellar, 1990
Donkeys	10	IV	0.7765	6.464	1.523	Horspool and McKellar, 1990
Horses (adult)	2.5	IV	1.35	10.5	NR	Pilloud, 1973
Pigs	10	IV	1.49	5.99	2.88	Pijpers et al., 1991
Pigs (normal)	50	PO	1.44	5.92		Pijpers et al., 1991
Pigs (pneumonia)	50	PO	1.9	14.1		Pijpers et al., 1991
Pigs	20	IV	5.18	3.68	4.15	Mevius et al., 1986b
Cows (adult)	2.5	IV	1.04	9.12	NR	Pilloud, 1973
Dairy cows	5	IV	0.917	2.63	1.24	Nouws et al., 1985a, 1985b
Dairy cows[a]	5.23	IV	1.01	2.58	1.45	Nouws et al., 1985a, 1985b
Veal calves	40	IV	18.144	7.34	2.246	Meijer et al., 1993a
Veal calves	20	IV	18.541		2.167	Meijer et al., 1993a
Calves (3 weeks old)	7.54	IV	2.48	13.5		Nouws et al., 1983
Calves (12 weeks old)	6.88	IV	1.52	8.8		Nouws et al., 1983
Calves (14 weeks old)	17	IV	1.83	10.8		Nouws et al., 1983
Buffalo calves (female)	22	IV	0.32	3.6	1.02	Varma and Paul, 1983
Dogs	5	IV	2.096	6.02	4.23	Baggot et al., 1977
Rabbits	10	IV	0.668	1.32	14.6	McElroy et al., 1987
Turkeys	1	IV	3.622	0.7298	3.6579	Dyer, 1989
Rainbow trout	5	IV	2.988	81.5	0.423	Black et al., 1991
African catfish	60	IV	1.33	80.3	0.19	Grondel et al., 1989
Red-necked wallaby	40	IV	2.041	11.4	NR	Kirkwood et al., 1988
Foal	59	IV	1.95–2.2	6.7–7.3	3.3	Papich et al., 1995
Cattle	20	IM	3.34	21.6	—	Craigmill et al., 2004 (meta-analysis)
Cattle (young)	20	IV	0.94	5.67	—	Toutain and Raynaud, 1983
Calves	40	IM	—	23.9	—	TerHune and Upson, 1989
Calves (healthy)	11	IV	2.32	11.8	3.35	Ames et al., 1983
Calves (disease)	11	IV	3.6	14.8	4.01	Ames et al., 1983
Pigs	9.5	IV	1.4	6.5 (MRT)	3.67	Nielsen and Gyrd-Hansen, 1996
Pigs	45.5	Oral	—	10.3 (MRT)	—	Nielsen and Gyrd-Hansen, 1996
Sea turtles	25	IV	18.4	66.1	4.8	Harms et al., 2004
Sea turtles	25	IM	28.5 (Vd/F)	61.9	5.3 (CL/F)	Harms et al., 2004
Alligator	10	IV	0.77	74.1	0.12	Helmick et al., 2004

NR, or blank space, information not reported; IV, intravenous; PO, per os; MRT, mean residence time.
All formulations were reported to be or are assumed to be HCl unless otherwise noted.
Vd, apparent volume of distribution (Vd/F for non-IV dose forms); $T_{1/2}$, elimination half-life, or terminal half-life (for multicompartment models); Clearance, systemic clearance (CL/F for non-IV dose forms).
[a]Oxytetracycline dihydrate formulation tested.

Table 34.8 Some pharamcokinetic parameters of doxycycline in some species

Species	Dose (mg/kg)	Route	C_{max} (µg/ml)	Vd (l/kg)	$T_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Pigs (9 weeks old)	20	IV		0.53	4.04	1.67	Riond and Riviere, 1989
Pigs	12	Oral		—	7.2	—	Prats et al., 2005
Pigs	10.5	IV		0.89	4.2	2.8	Baert et al., 2000
Pigs	10.5	Oral		0.97	2.9	2.9	Baert et al., 2000
Horses	20	Oral	0.91	—	11.8	—	Davis et al., 2006
Horses	10	Oral	2.54	–	8.5	–	Womble et al., 2007
Horses	20	Oral	2.89	–	11.9	–	Womble et al., 2007
Horses	10	Oral	0.48	–	13.8	–	Winther et al., 2011
Horses	5	Oral	0.37	–	15.08	–	Schnabel et al., 2010
Calves	5	IV		–	9.5	1.2 (mg/l)	Meijer et al., 1993b
Calves (functional rumen)	20	IV		1.31	14.9	1.07	Riond et al., 1989
Calves (nonfunctional rumen)	20	IV		1.81	9.9	2.2	Riond et al., 1989
Cats	5	IV		0.34	4.56	1.09	Riond et al., 1990
Dogs	5	IV		0.93	6.99	1.72	Riond et al., 1990
Dogs	5	IV		1.468	10.36	1.68	Wilson et al., 1988
Dogs	0.1 CRI mg/kg/h	IV		0.65	4.56	1.66	Bidgood and Papich, 2003
Dogs	5–10	Oral	4.53	1.67	12.6	1.68	(summary of 3 studies)
Goats (lactating)	5	IV		9.78	16.63	6.91	Jha et al., 1989

CRI, constant rate infusion; IV, intravenous; NR, information not reported, C_{max}, peak concentration; Vd, apparent volume of distribution (Vd/F for oral dose); $T_{1/2}$, half-life (terminal half-life for multicompartment models). Clearance, systemic clearance (CL/F) for non-IV dose forms. All values listed are means from the study. Blank cells indicates that information was not available.

For example, minocycline and doxycycline, being more lipophilic (Barza et al., 1975), penetrate brain, ocular tissues, spinal fluid, and prostate better than other tetracyclines, such as oxytetracycline or chlortetracycline. Tetracyclines are commonly reported to concentrate intracellularly, and doxycycline has a higher affinity for intracellular accumulation than other tetracyclines (Gabler, 1991; Forsgren and Ballahsene, 1985; Davis et al., 2006). In vitro analysis of the penetration of radiolabeled doxycycline into isolated human polymorphonuclear leukocytes revealed a cellular-to-extracellular concentration ratio of 13 (Forsgren and Ballahsene, 1985). In horses the ratio was 17 at peak concentrations (Davis et al., 2006). These high leukocyte concentrations may contribute to the reported antiinflammatory effects. Minocycline is found in high concentrations in the bronchial secretions (Kelly and Kanegis, 1967a; MacCulloch et al., 1974); prostate (Fair, 1974); brain (Barza et al., 1975); thyroid, saliva, and tears (Hoeprich and Warshauer, 1974). Doxycycline is distributed to the epithelial lining fluid of the airways is high with 87% penetration after an intragastric dose. Distribution into joint fluid of horses has been shown, which has implications for both treating infections of the joint, and also for controlling joint inflammation (Schnabel et al., 2010, 2012; Maher et al., 2014). Distribution of

Table 34.9 Some pharmacokinetic parameters of minocycline HCl in some species

Species	Dose (mg/kg)	Route	C_{max} (µg/ml)	Vd (l/kg)	$T_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Dogs (2-compartment model)	5	IV	NA	1.95	6.93	3.347	Wilson et al., 1985
Dogs (3-compartment model)	5	IV	NA	2.0	7.24	3.424	Wilson et al., 1985
Sheep (normal)	2.2	IV	NA	1.32	2.58	5.94	Wilson and Green, 1986
Sheep (hypoprotein-emic)	2.2	IV	NA	1.67	2.91	5.60	Wilson and Green, 1986
Cats	14 50 mg /cat	Oral	4.77	2.52	6.3	4.61	Tynan et al., 2015
Cats	5	IV	–	1.54	6.66	2.87	Tynan et al., 2015
Dogs	10	Oral	3.44	2.52	4.14	5.4	Maaland et al., 2014
Dogs	5	IV	–	1.46	6.02	2.85	Maaland et al., 2014
Dogs	6	Oral	3.44	2.17	5.8	4.27	Hnot et al., 2015a
Horse	4	Oral	0.67	14.95	11.5	12.03	Schnabel et al., 2012

IV, intravenous; NR, information not reported, C_{max}, peak concentration; Vd, apparent volume of distribution (Vd/F for oral dose); $T_{1/2}$, half-life (terminal half-life for multicompartment models).

Table 34.10 Formulations of tetracyclines used in animals

Formulations approved by US FDA for animals
Oxytetracycline hydrochloride soluble powder: added to drinking water for poultry, cattle, pigs
Oxytetracycline for medicated feed: added to feed for cattle, poultry, fish, pigs
Oxytetracycline tablets: oral treatment for calves
Oxytetracycline injection: IM injection for cattle and pigs. These products are occasionally used in horses and other species. There is both a conventional and long-acting formulation. The long-acting formulation contains a viscosity excipient used to prolong the absorption from the injection site
Tetracycline bolus: oral treatment for cattle
Tetracycline hydrochloride soluble powder: added to drinking water for cattle, pigs and poultry
Tetracycline oral suspension: oral treatment for cats and dogs
Chlortetracycline hydrochloride soluble powder: added to drinking water for poultry, calves, and pigs
Chlortetracycline for medicated feed: premix added to feed for pigs, cattle, poultry
Formulations approved for humans, but used off-label in animals
Doxycycline capsules and tablets: used in dogs, cats, birds, horses, and some exotic animals
Minocycline capsules, tablets, and injectable solution used in dogs, cats, and horses

doxycycline and minocycline to equine joint fluid was 4.6 times, and 2 times the plasma concentrations for doxycycline and minocycline, respectively.

Metabolism, excretion, and elimination

The elimination rates and half-lives are presented in the pharmacokinetic tables for each drug and various species (Tables 34.5–34.9). Although it varies considerably from species to species, the half-life is long enough for once or twice-daily administration in most animals. The intramuscular administration of formulations that contain viscosity excipients (e.g., 2-pyrrolidone) (Table 34.10) may prolong the terminal half-life because of a "flip-flop" effect. This is discussed in more detail in Section Oxytetracycline.

A high percent of the administered dose is eliminated in the urine via glomerular filtration, with the rest eliminated in the feces. The high concentrations in the urine can be effective for treatment of urinary tract infections caused by susceptible bacteria.

An examination of Tables 34.5–34.9 indicates that systemic clearance is similar to, or somewhat higher than GFR. Tetracyclines are also excreted in the bile, with up to 20 times the plasma concentration of tetracyclines being present in the bile (Kunin and Finland, 1961; Schach von Wittenau and Twomey, 1971).

Antimicrobial Spectrum and Clinical Uses

The use and dosages of specific agents in this group are discussed in this chapter for each drug. The value of the tetracyclines is their activity against susceptible gram-positive and gram-negative bacteria, as well as other atypical pathogens transmitted by ticks and other parasites, and blood-borne pathogens.

Tetracyclines in general have good or moderate activity against the respiratory pathogens listed in Table 34.2, but resistance can occur. Tetracyclines are usually active against *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae, Listeria monocytogenes,* streptococci, *Actinobacillus* spp., *Leptospira* spp., *Actinomyces* spp., and some anaerobes. The family Rickettsiaceae includes *Rickettsia* and *Ehrlichia,* and tetracyclines, particularly doxycycline, are considered the first drug of choice for these infections.

In birds, doxycycline is the drug of choice for treatment of *Chlamydophila psittaci* (formerly called *Chlamydia psittaci*). Tetracyclines are also useful against organisms that lack a cell wall, which would ordinarily be resistant to β-lactam antibiotics, for example, *Mycoplasma*, as well as other Mycoplasma organisms such as *Mycoplasma haemofelis* (formerly called *Haemobartonella felis*).

Resistance is common among *Enterococcus* species and members of the family Enterobacteriaceae (*Enterobacter* spp., *E. coli, Klebsiella* spp., *Proteus* spp., *Salmonella* spp.), and treatment should not be considered without a susceptibility test. Anaerobes (such as *Bacteroides* spp. and *Clostridium* spp.) have shown variable susceptibility. Commonly resistant to the tetracyclines are those infections involving *Mycobacterium* spp., *Proteus vulgaris, Pseudomonas aeruginosa,* and *Serratia* spp. Most *Streptococcus* spp. are susceptible, as are many *Staphylococcus* spp.; however, resistance can occur and a susceptibility test is advised before administering a tetracycline (e.g., doxycycline, minocycline) for treating staphylococcal infections in animals. Activity of minocycline against methicillin-resistant *Staphylococcus* has been shown. Staphylococci develop resistance through the efflux pump mediated by the gene *tetK.* These bacteria will be resistant to the other tetracyclines, including doxycycline, but not minocycline, which can still be used to treat some of these resistant infections (Hnot et al., 2015a; Weese et al., 2013; Maaland et al., 2014).

One of the clinical uses that has become common is administration of doxycycline in combination with other agents for treatment of canine heartworm disease. Tetracyclines, particularly doxycycline, are considered the first drug of choice for these infections. The *Rickettsia*-like organism found in heartworms, *Wolbachia*, is susceptible to tetracyclines, which have been used as adjunctive treatment for heartworm disease. Many filarial nematodes, such as heartworm, have a symbiotic relationship with obligate intracellular bacteria belonging to the genus *Wolbachia (Rickettsiales*). Treatment with doxycycline reduces *Wolbachia* numbers in all stages of heartworms and improves outcomes and

decreased microfilaremia in dogs treated for heartworm disease. The American Heartworm Society recommends treatment with doxycycline in dogs diagnosed with heartworm disease. Preferably, it should be administered prior to treatment with an adulticide (melarsomine) at a dose of doxycycline of 10 mg/kg twice daily for 4 weeks. If doxycycline is not available, minocycline can be used as a substitute. In one assay, minocycline had better activity against *Wolbachia* than either doxycycline or rifampin (Townson et al., 2006).

Adverse Effects and Interactions

Interactions: Calcium-containing products or other di- or trivalent cations (Mg^{2+}, Fe^{2+}, Al^{+3}) will chelate with tetracyclines and interfere with oral absorption. Doxycycline is less susceptible to this interaction as calcium chelation is 19% for doxycycline but 40% and 36% for tetracycline and oxytetracycline, respectively (Barza et al., 1975). Interactions that affect oral absorption were discussed earlier in Section Absorption.

Gastrointestinal microflora changes: In horses the oral administration of oxytetracycline has been associated with proliferation of *Clostridium perfringens* or *Salmonella* in the colon, which has led to enteritis. This syndrome has been called *Colitis-X*. A more detailed discussion of the effects of tetracyclines in horses was reviewed by Papich (2003a, 2003b).

Esophageal lesions: Doxycycline entrapped in the esophagus from a broken tablet or incompletely dissolved capsule can cause injury to the esophagus and stricture. It has been demonstrated that, in cats, administration of a capsule or broken tablet can be lodged in the esophagus unless followed by some water. Therefore, one should be cautious about giving oral doxycycline medications to cats. This problem has been primarily associated with doxycycline hyclate (the form most common in the USA), rather than doxycycline monohydrate.

Problems in young animals: Tetracyclines bind to bone and teeth. They may produce teeth discoloration and inhibit growth of long bones in young animals or the offspring of pregnant animals treated with tetracyclines. The true incidence of this problem is not known in veterinary medicine, but in human medicine, tetracyclines are avoided in children less than 7 years of age (before tooth eruption). Tooth discoloration is determined by the duration of treatment rather than the dose. The discoloration is related to the chelation of tetracyclines to the calcium deposits in the developing teeth in the dentin (where it is mostly visible) and to a lesser extent in the enamel (Hamp, 1967; Hennon, 1965; Finerman and Milch, 1963; Moffitt et al., 1974). Exposure to sunlight is also important for this reaction. Although the occurrence of this in animals has not been well documented, it is prudent to avoid tetracyclines in animals during time of teeth development. The effects on bones are probably only important with high doses.

Renal tubular necrosis: Acute kidney injury has been associated with high doses and prolonged administration of oxytetracycline to ruminants (Riond and Riviere, 1989a) and dogs (Stevenson, 1980). When high doses are administered, the drug vehicle (such as propylene glycol) has been suspected to contribute to kidney effects.

Using outdated formulations: It often is stated in publications that kidney injury may occur when outdated tetracyclines are administered. The degradation products of the tetracyclines have been found to be nephrotoxic and are formed in the presence of heat, low pH, and moisture (Cleveland et al., 1965; Teuscher et al., 1982; Lowe and Tapp, 1966; Riond and Riviere, 1989a). Although we do not advocate administering outdated products, this problem does not occur with currently available formulations because the citric acid excipient is no longer used.

Hepatic disease: Idiosyncratic toxic hepatitis is possible (Böcker et al., 1982; Hopf et al., 1985). Drug-induced hepatitis has been described in people, and pregnant women appear to be at the greatest risk. The significance of hepatic reactions in veterinary medicine is unknown, but may be important at high doses.

Allergy: Hypersensitivity and drug fever have been reported. Cats appear to be more prone to drug fever from tetracyclines than other animals.

Photosensitivity: This is a direct toxic effect that damages cutaneous membranes when exposed to light. This reaction is rare in animals, but rather common in people. The incidence appears to be highest with doxycycline and demeclocycline.

Risks from IV administration: Tetracyclines administered intravenously rapidly can cause hypotension and collapse (McPherson et al., 1974; Wivagg et al., 1976; Gyrd-Hansen et al., 1981). In one study, a fast IV administration (60 seconds or less) to cattle caused collapse in 50% of the animals. Affected animals had low blood pressure, low heart rate, and ECG abnormalities. Collapse from IV injection has been prevented when the cattle were premedicated with calcium borogluconate, indicating that tetracycline may decrease the amount of

Table 34.11 Clinical dosages used for tetracyclines in animals – most frequently cited on product labels, CLSI susceptibility tables (CLSI, 2015), or in reputable references based on a consensus of the literature or pharmacokinetic studies

Drug	Species	Dose
Doxycycline	Dogs and cats	5 mg/kg q 12 h oral
Doxycycline	Horses	10–20 mg/kg q 12 h oral (never administer IV) The higher dose of 20 mg/kg will more consistently reach therapeutic targets
Oxytetracycline	Calves, cattle, and pigs	22 mg/kg q 24 h added to drinking water or in feed
Oxytetracycline	Calves, cattle	6.6–11 mg/kg q 24 h, IM
Oxytetracycline	Calves, cattle	20 mg/kg q 24 h, IM or SC; extra-label doses have been as high as 40 mg/kg
Oxytetracycline	Pigs	6.6–11 mg/kg q 24 h IM; doses as high as 20 mg/kg IM, q 24 h are also used
Oxytetracycline	Horses	10 mg/kg q 24 h, IM, or IV (slowly) (IM injections can cause pain)
Oxytetracycline	Dogs, cats	20 mg/kg, q 12 h, oral
Oxytetracycline	Sea turtles	40 mg/kg IM, followed by 20 mg/kg q 72 h, IM
Tetracycline HCL	Calves	11 mg/kg q 12 h PO
Minocycline	Horses	4 mg/kg q 12 h, PO
Minocycline	Dogs	5 mg/kg, oral, q 12 h (10 mg/kg can be considered for some organisms, but is more likely to produce vomiting)
Minocycline	Cats	8.8 mg/kg, oral, once daily (or 50 mg per cat, once daily)

calcium available to the heart for its role in contraction to the point of producing collapse of the animals.

The vehicle (solvent) used to administer tetracyclines may be responsible for adverse events. In calves, Gross et al. (1981) studied the cardiovascular effects of both oxytetracycline and the different vehicles used for injection (propylene glycol, saline, polyvinylpyrrolidine). They determined that the cardiovascular adverse effects were caused by the vehicles used and not oxytetracycline. The propylene glycol vehicle studied resulted in increased pulmonary arterial pressures and a decrease in cardiac output and stroke volume. Aortic pressure and heart rates were also depressed in association with the vehicle. They concluded that the cardiovascular effects observed were caused by endogenous release of histamine after propylene glycol injection, and this histamine release was not dependent on the animal being sensitized prior to exposure. No discernible cardiovascular effects were observed after injection with the oxytetracycline–saline combination, while the polyvinylpyrrolidine preparation and vehicle resulted in higher aortic pressure, heart rate, and overall systemic resistance.

Tetracycline has been reported to induce anaphylactic shock in dogs after intravenous injection (Ward et al., 1982) as well as possibly increasing alanine transaminase activity in the cat (Kaufman and Greene, 1993). Although there are warnings about administration of minocycline intravenously in humans, it has been administered to dogs and cats over a 5-minute interval without complication (Tynan et al., 2015; Maaland et al., 2014).

The most serious concern is intravenous injection of doxycycline in horses, which can be fatal (Riond et al., 1989a, 1992). IV administration of doxycycline to horses has caused sudden death, most likely caused by a cardiac arrhythmia. Oral administration of doxycycline to horses has not produced this problem (Davis et al., 2006).

Commonly Used Tetracyclines

Chlortetracycline

Chlortetracycline was the first tetracycline discovered and was first introduced for clinical use in 1948 (Figure 34.1). The use of chlortetracycline has mostly been confined to administration in feed and water to livestock. It has low oral absorption and the effects are likely caused by local effects in the intestine for weight gain. As mentioned earlier, production uses of tetracyclines for weight gain and improved feed efficiency have been phased out by an FDA guidance taking effect in 2017. Chlortetracycline is not utilized to any significant degree in small-animal or equine medicine. Doses for other animals are listed in Table 34.11. Because the therapeutic use has declined, the value of chlortetracycline has diminished. The reader is referred to earlier editions of this book for more detailed information about its past use.

Chlortetracycline has been used in pigs as a feed additive for the treatment of *Salmonella typhimurium* (Jones et al., 1983; Williams et al., 1978), coccidiosis (Onawunmi and Todd, 1976), and many other porcine diseases. Similar infections have been treated with chlortetracycline in poultry (Fagerberg et al., 1978; Nivas et al., 1976; Quarles et al., 1977; Landgraf et al., 1981; Dawson et al., 1983). Chlortetracycline has been reported to decrease the breeding rate of sows, although it did increase conception and farrowing rates. Birth weights, overall litter weights of pigs born alive, and

weights of pigs at weaning were also significantly higher than unmedicated controls (Soma and Speer, 1975).

Tetracycline

The use of tetracycline (Figure 34.1) is more limited today because other forms are used in livestock (e.g., oxytetracycline) or in horses and small animals (minocycline, doxycycline). Relatively little has been published in recent years on tetracycline and previous editions of this book may be consulted for older information. Some pharmacokinetic information on tetracycline is available in Table 34.6.

There are still approved formulations of tetracycline for small animals. These forms are occasionally used to treat various diseases such as *Rickettsia rickettsii* (Rocky Mountain spotted fever) when doxycycline is not available. A study by Breitschwerdt et al. (1991) determined that tetracycline, chloramphenicol, and enrofloxacin were all equally effective in treating this disease in experimentally infected dogs. Tetracycline was also found to be efficacious in the treatment of canine ehrlichiosis (*E. canis*) (Amyx et al., 1971; Davidson et al., 1978). However, it was less effective at clearing ehrlichiosis in dogs compared to imidocarb dipropionate (Price and Dolan, 1980).

Oxytetracycline

The most commonly used tetracycline in food animals is oxytetracycline (Figure 34.1). The most complete pharmacokinetic analysis was performed by the Food Animal Residue Avoidance Databank (Craigmill et al., 2004) for oxytetracycline in cattle. This analysis was derived from 41 data sets and 25 published papers (489 data points). A metaanalysis of this data from a dose of 20 mg/kg IM of long-acting tetracycline yielded the following population data: half-life 21.6 hours, peak concentration (C_{max}) 5.61 µg/ml, clearance 0.115 l/kg/h, and volume of distribution per fraction absorbed (Vd/F) 3.34 l/kg. Other pharmacokinetic values are shown in Table 34.7. Doses are listed in Table 34.11.

The clinical usefulness of oxytetracycline has been documented in most domestic species of animals, and previous editions of this textbook should be consulted for historic work on oxytetracycline. Oxytetracycline has been a common treatment for lung infections associated with bovine respiratory disease (BRD). Although the in vitro susceptibility may not always be favorable (Table 34.2), oxytetracycline may accumulate in pneumonic lung preferentially over normal lung, and an increased volume of distribution has been shown in diseased animals, which may improve treatment outcome (Ames et al., 1983, 1985; Baxter and McKellar, 1990). Tissue levels are maintained for 24 hours after dosing. Other uses for cattle have included treating cases of *Moraxella bovis*/infectious bovine keratoconjunctivitis infections in calves (Smith and George, 1985; George and Smith, 1985; George et al., 1985, 1988), mastitis, and anaplasmosis. Although oxytetracycline has been used as an intrauterine infusion for cows with retained fetal membranes, this practice has been discouraged (Dinsmore et al., 1996). Intrauterine use in cows may not improve reproductive performance in cows with retained fetal membranes and may cause illegal residues in milk of dairy cows (Dinsmore et al., 1996; Stevens et al., 1995).

Oxytetracycline has been used to treat ehrlichiosis in dogs (Adawa et al., 1992) as an alternative to doxycycline. However, the use of oxytetracycline in small animals is limited because of a lack of convenient dose forms and greater use of doxycycline and minocycline.

In horses oxytetracycline is sometimes administered for treatment of Potomac horse fever (*Neorickettsia risticii*) (Palmer et al., 1992). Larson and Stowe (1981) reported high serum concentrations obtained in clinically normal horses given 10 mg/kg oxytetracycline intravenously, with serum concentrations peaking at 30 minutes postinjection (16.85 µg/ml) and high concentrations persisting through at least 240 minutes (4.67 µg/ml). Oxytetracycline penetrated well into pulmonary and renal tissue, as well as into bronchial fluid. In another study of oxytetracycline in horses, Brown et al. (1981) used a dose of 5 mg/kg intravenously and found a peak concentration of oxytetracycline in the serum at 0.5 hours after dose, with a steady decline in serum levels through 36 hours. Similar fluid concentration versus time profiles were also demonstrated for oxytetracycline detected in the synovial fluid, peritoneal fluid, and urine after intravenous injection, suggesting that oxytetracycline crosses those membranes easily and that the concentrations obtained would be adequate for combating such infections as *Corynebacterium equi, Streptococcus zooepidemicus,* and *Actinobacillus* spp., but with limited efficacy in treating some *Staphylococcus aureus, Escherichia coli,* and *Salmonella* spp., and no efficacy in treating common *Pseudomonas aeruginosa* pathogens.

The pharmacokinetic features of oxytetracycline for some species are shown in Table 34.7. Data on the pharmacokinetics of oxytetracycline is available for dogs (Baggot et al., 1977; Cooke et al., 1981), calves (Burrows et al., 1987; Banting et al., 1985; Banting and Baggot, 1996; Schifferli et al., 1982; Meijer et al., 1993a, 1993c; TerHune and Upson, 1989; Toutain and Raynaud, 1983), ponies and donkeys (Horspool and McKellar, 1990), horses (Larson and Stowe, 1981; Brown et al., 1981; Teske et al., 1973), foals (Papich et al., 1995), chickens (Black, 1977), swine (Nielsen and Gyrd-Hansen, 1996; Hall et al., 1989; Pijpers et al., 1990; Mevius et al., 1986b), sheep (Immelman and Dreyer, 1986), elephants

(Bush et al., 2000), fish (Black et al., 1991; Grondel et al., 1989), and other species (Teare et al., 1985; Martinsen et al., 1992; McElroy et al., 1987; Kirkwood et al., 1988). Oxytetracycline is relatively well behaved from a pharmacokinetic perspective, which allows easy extrapolation across species. A physiological based pharmacokinetic model was developed, which allowed extrapolation of oxytetracycline from dogs to humans after intravenous or oral administration (Lin et al., 2015).

Oxytetracycline is the most common injectable tetracycline for reptiles. The elimination of oxytetracycline from reptiles is slow, which allows for infrequent dose intervals. Harms et al. (2004) showed that the half-life in sea turtles was over 60 hours, which would allow for extended-interval dosing (41 mg/kg once IM, followed by 21 mg/kg every 72 hours IM). In another study, Helmick et al. (2004), showed that in alligators the half-life of oxytetracycline was 74 hours, which allows for long intervals between doses (for example every 5 days).

Oxytetracycline solution in propylene glycol is 50–100 mg/ml (Oxy-Mycin®, Terramycin, Oxy-Tet®), and is available as a solution in povidone (IM use only). A "long acting" preparation is available with the viscosity excipient 2-pyrrolidone (200 mg/ml) in formulations such as Liquamycin® LA-200. Absorption of oxytetracycline is known to vary with injection site in calves. A report by Nouws and Vree (1983) found that site-to-site intramuscular injection bioavailability varied widely at 52 hours postinjection, with bioavailability being 79% in the buttock, 86% in the neck, and 98% in the shoulder.

Administration of the long-acting formulation, particularly in food animals, is intended to prolong serum and tissue concentrations for long periods of time – usually every 48 hours, but may be up to 3–5 days for some pathogens. Several studies have described the pharmacokinetic patterns of the conventional and long-acting formulations in dogs, sheep, cattle, and pigs. Toutain and Raynaud (1983) examined the pharmacokinetic parameters of oxytetracycline with the 2-pyrrolidone carrier (long-acting formulation) injected intramuscularly in young beef cattle. This intramuscular formulation resulted in rapid development of serum concentrations of 4 μg/ml within 60–90 minutes, followed by persistence of these levels for approximately 12 hours. Serum half-life was calculated to be 21.8 hours, and bioavailability was 51.5%. Serum concentrations exceeding 0.5 μg/ml were found to persist for approximately 87 hours, in contrast to approximately 52 hours for the conventional formulation in another study using cattle (Mevius et al., 1986a). Davey et al. (1985) injected cattle with the conventional oxytetracycline hydrochloride or the long-acting formulation, both at a standard 20 mg/kg dose, and found that although the long-acting formulation had lower peak serum concentrations when compared to the conventional formulation, the long-acting formulation had a longer serum half-life (36.9 hours) than the conventional formulation (11.1 hours). In addition, the time it took for serum concentrations to drop below 0.5 μg/ml was 86.8 hours for the long-acting formulation and 51.5 hours for the conventional formulation. Similar findings have been reported for dairy cows (Nouws et al., 1985b), calves (Nouws and Vree, 1983), pigs (Nouws et al., 1990; Xia et al., 1983; Nouws, 1984; Banting and Baggot, 1996), dogs (Immelman and Dreyer, 1981), and sheep (Nouws et al., 1990).

Despite the advantages of the long-acting oxytetracycline formulation cited above, there are also studies that cast doubt on the value of a long-acting formulation. In one such study, the long-acting oxytetracycline (in 2-pyrrolidone) was compared to a conventional formulation in pigs at a dose of 20 mg/kg of each formulation (Hall et al., 1989). There was no difference in area-under-the-curve (AUC) or disappearance rate constant from either formulation. The authors concluded that the long-acting formulation did not provide an advantage for pigs.

Doxycycline

The most popular drug in this class for small animals and birds is doxycycline (Figure 34.1). It is available in two forms, doxycycline hyclate and doxycycline monohydrate. Doxycycline hyclate (a dimer of two molecules) has been used more commonly but the monohydrate also is available. Doxycycline hyclate (Vibra-Tabs, and Vibramycin) is available in tablets and capsules. There is also a flavored doxycycline calcium suspension and monohydrate suspension for people. Doxycycline hyclate tablets (Ronaxan) is approved for dogs and cats in some countries, and doxycycline monohydrate (VibraVet) approved in other countries. There are no reported differences between these two formulations with respect to oral absorption, but the hyclate form is associated with more injury to the esophagus (see Section Adverse Effects and Interactions). Doxycycline hyclate (Vibramycin IV) also can be administered IV to patients (except horses) that cannot tolerate oral medications. The IV formulation is reconstituted before use and is stable for only 12 hours following reconstitution (72 hours in refrigerator, 8 weeks in the freezer).

Doxycycline and minocycline (discussed in Section Minocycline) differ from tetracycline, oxytetracycline, and chlortetracycline in that they are more lipophilic (five- to tenfold increase), resulting in higher tissue penetration, higher intracellular penetration, larger volumes of distribution, and better overall antimicrobial properties (Barza et al., 1975).

The pharmacokinetics of doxycycline has been studied in dogs and cats (Wilson et al., 1988; Riond et al., 1990; Bidgood and Papich, 2003), pigs (Riond and Riviere, 1990a, 1990b; Prats et al., 2005), calves (Meijer et al.,

1993b; Riond et al., 1989b), goats (Jha et al., 1989), rhesus monkeys (Kelly et al., 1992), horses and foals (Davis et al., 2006; Papich et al., 1995; Winther et al., 2011), and birds (Flammer et al., 2001, 2003; Powers et al., 2000; Prus et al., 1992; Greth et al., 1993). Some of the pharmacokinetic data for doxycycline for commonly encountered species of animals are listed in Table 34.8. Oral absorption was reported for various species (Table 34.4) and shown to be higher than for other tetracyclines. Doses are listed in Table 34.11.

High intracellular drug concentrations produce good activity against intracellular pathogens. Doxycycline is the first drug of choice for treatment of tick-borne infections caused by *Ehrlichia canis* and *Rickettsia,* as well as *Mycoplasma haemofelis* (formerly called *Haemobartonella felis).* Efficacy of doxycycline for rickettsial disease in animals was demonstrated by Breitschwerdt et al. (1997, 1999). The most common dose for dogs and cats is 5 mg/kg q12 h orally (25 mg/cat q12 h). It also has been considered one of the treatments of choice, in addition to azithromycin, for treatment of infections caused by *Bartonella* (Kordick et al., 1997; Brunt et al., 2006), although the most appropriate drug for *Bartonella* is still unknown (Brunt et al., 2006). The role of doxycycline for treatment of canine heartworm disease was discussed in Section Antimicrobial Spectrum and Clinical Uses. The effectiveness is attributed to the activity against the symbiont *Wolbachia.*

Doxycycline has also been used for infections in other species, including respiratory tract disease and systemic colibacillosis in poultry (Migaki and Babcock, 1977; George et al., 1977) and anaplasmosis in splenectomized calves (Kutter and Simpson, 1978).

An important use of doxycycline is in birds. Doxycycline has become a treatment of choice for psittacosis caused by *Chlamydophila psittaci* (formerly called *Chlamydia psittaci*) in birds because of its good oral absorption, tolerance, and efficacy (Flammer et al., 2001, 2003; Powers et al., 2000). The oral route is preferred for doxycycline because IM injections cause pain and tissue irritation and did not maintain therapeutic concentrations. Oral doxycycline can be administered to pet birds by simply adding doxycycline hyclate to drinking water. When doxycycline hyclate was added to drinking water at concentrations of 0.28 mg/ml and 0.83 mg/ml (280 and 830 mg/l), plasma concentrations in treated birds were maintained high enough for susceptible organisms during a 45-day treatment (Powers et al., 2000). Another study confirmed that when added to drinking water at a concentration of 0.8 mg/ml (800 mg/l) it produced effective concentrations in psittacine birds for a treatment duration of 42 days (Flammer et al., 2001). Lower water concentrations of 400 mg/l also may produce effective concentrations in some birds.

Minocycline

The administration of minocycline (Figure 34.1) is considered when doxycycline is not available, or an alternative is needed. Like doxycycline, it is more lipophilic than other tetracyclines (Barza et al., 1975) and generally has good oral absorption. Pharmacokinetics have been studied in dogs, cats, and horses and presented in Table 34.9. Doses are listed in Table 34.11.

Minocycline has been well tolerated, but as the dose is increased in dogs from 5 mg/kg to 10 mg/kg, more vomiting is expected. Intravenous administration has been tolerated in cats and dogs if administered slowly over 5 minutes (Tynan et al., 2015; Maaland et al., 2014). A toxicological study performed by Noble et al. (1967) examined the use of minocycline in Beagles administered a daily dose of 5, 10, 20, or 40 mg/kg intravenously for 1 month. Adverse effects occurred only in the high dose groups. Minocycline produced erythema of the skin and mucous membranes, characterized by papules around the eyes, muzzle, ears, and abdomen; the intensity of these lesions was directly proportional to the dose administered. Decreases in red blood cell packed cell volumes, hemoglobin concentrations, and red cell counts were noted in dogs receiving 10 mg/kg or more of minocycline intravenously. Similar adverse effects were noted by Wilson et al. (1985). Other toxicological studies with minocycline have been performed in dogs, rats, mice, and monkeys (Benitz et al., 1967).

Tissue distribution studies in dogs were reported by Maaland et al. (2014). After oral administration distribution to tissue fluids is approximately 50% of the plasma drug concentration. After a dose of 4 mg/kg oral to horses there was good penetration to equine joints (Schnabel et al., 2012). Minocycline appears to be minimally metabolized (Wilson and Green, 1986), but metabolism data are not available for all species. Systemic clearance values in dogs and cats suggest that glomerular filtration plays an important role in elimination (Tynan et al., 2015; Maaland et al., 2014).

Other Nonantimicrobial Uses of Tetracyclines

Tetracyclines also have been used as immunomodulating drugs and antiinflammatory drugs. This use of tetracyclines has focused on treatment of osteoarthritis, vasculitis, and dermatitis.

Dermatology

A review is available of the uses of tetracyclines in dermatology by Tsankov et al. (2003). The action of tetracyclines appears to be via inhibition of inflammatory

cell infiltration. Tetracyclines also may affect cyclooxygenase (COX-2) mediated prostaglandin (PGE-2) synthesis during inflammation. The antiinflammatory activity was reviewed by Suomalainen et al. (1992). The combination of tetracycline and niacinamide has been used in dogs for the treatment of discoid lupus erythematosus, pemphigus foliaceus, ulcerative dermatosis of Collies and Shetland Sheepdogs (vesicular cutaneous lupus erythematosus), lupoid onychodystrophy, and sterile pyogranulomatous disease (including sterile nodular panniculitis) (Auxilia et al., 2001; Rothstein et al., 1997; White et al., 1992). The exact mechanism to explain the efficacy of this combination is uncertain, but some inflammatory mechanisms are probably important. However, as an antipruritic treatment, this combination is not impressive (Beningo et al., 1999).

Alone, tetracyclines have been used for conditions in which an antiinflammatory mechanism may play a role (Suomalainen et al., 1992). Doxycycline was used in one study of plasmacytic pododermatitis in cats (Bettenay et al., 2001). Remission of signs occurred in 26% of cats.

Angular Limb Deformities in Foals

Another use of oxytetracycline has been the administration of high doses to newborn foals for the purpose of correcting angular limb deformities (Madison et al., 1994; Kasper et al., 1995). The doses have been as high as 50–70 mg/kg, IV, q 48 h. The explanation for this effect of oxytetracycline in horses may be explained by the relaxation of tendons. In rats, oxytetracycline is known to decrease viscoelastic properties of tail tendons in young animals (Wintz et al., 2012). The pharmacokinetics in foals at this dose and adverse effects were explored by Papich et al. (1995) and no adverse effects were reported.

Arthritis

Both in vivo and in vitro studies have documented antiinflammatory, chondroprotective, and antiarthritic effects of tetracyclines, particularly doxycycline and minocycline. These studies have been summarized (Schnabel et al., 2010, 2012; Maher et al., 2014; Haerdi-Landerer et al., 2007). The effects on arthritis may be caused by decreased inflammatory mediators, such as prostaglandins, and the reduced matrix metalloproteinases (MMP). In calves, the predominant effect was lower activity of MMP. In horses, these effects may be possible even after administration of a low dose that produces concentrations below the MIC of bacteria (Maher et al., 2014).

Yu et al. (1992) indicated that doxycycline administered prophylactically markedly reduced the severity of osteoarthritis in dogs with surgically induced transactions of the anterior cruciate ligament. Inhibition of classical lesions in that model was felt to be due to doxycycline's ability to inhibit (chelate) metalloproteases (collagenase, gelatinase, stromelysin) in the degenerating cartilage of the canine knee.

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35

Aminoglycoside Antibiotics

Mark G. Papich and Jim E. Riviere

Aminoglycoside antibiotics have been used in veterinary and human medicine for many years and have retained their importance for treating serious and routine infections. They are particularly valuable for treating infections caused by gram-negative bacilli, including bacteria that may be resistant to other agents. Their therapeutic importance derives from the rapid bactericidal effects, pharmacokinetics derived from a large variety of animal species, and relatively low rate of resistance. These advantages must be weighed against their potentially toxicity, requirement for administration by injection for systemic use, and high potential to produce chemical residues in food-producing animals.

Pharmacology of Aminoglycosides

General Properties

Aminoglycosides include the familiar drugs gentamicin, amikacin, kanamycin, and tobramycin. They also include less familiar drugs such as neomycin, dihydrostreptomycin, and paromomycin. Spectinomycin has been included with aminoglycosides in some textbooks, but we have instead included it with the miscellaneous antibiotics in Chapter 36. Aminoglycosides are a class of antimicrobial compounds produced from strains of *Streptomyces* spp. or *Micromonospora* spp. fungi. Those produced from *Streptomyces* are spelled with "mycin" and those produced from *Micromonospora* are spelled with "micin". Chemically, they are aminocyclitols: hydroxyl and amino or guanidine substituted cyclohexane with amino sugars joined by glycosidic linkages to one or more of the hydroxyl groups. These molecules have excellent solubility in water but poor lipid solubility, and are thermodynamically stable over a wide range of pH values and temperatures (Lancini and Parenti, 1982; Leitner and Price, 1982; Nagabhusban et al., 1982; Pechere and Dugal, 1979). They are large molecules with molecular weights ranging from 450 to 585. The aminoglycosides are basic polycations with pK_a values that range from 7.2 to 8.8 (Ziv and Sulman, 1974; Katzung, 1984; Prescott and Baggot, 1988).

The chemical structure of gentamicin is shown in Figure 35.1. A search for gentamicin reveals several products that have been identified (e.g., gentamicin C_1, C_2, C_{1A}, A_2, and A_3). The commercially available form contains a complex of gentamicin C_1, C_2, and C_{1A} as sulfate salts in a mixture. The proportion of each compound in a gentamicin complex can vary among commercial products. The other commonly used aminoglycosides are shown in Figure 35.2 Amikacin is a semisynthetic form synthesized from kanamycin to increase antimicrobial activity. The various mechanisms of nephrotoxicity (binding to proximal tubule brush-border vesicles and phospholipids, inhibition of mitochondrial function, etc.) may be associated with the number of free amino groups on the aminoglycoside molecule. In general, the most ionized aminoglycosides (i.e., neomycin, with six groups) are more toxic and show greater binding affinity than the least ionized aminoglycosides of the class (i.e., streptomycin, with three groups) (Bendirdjian et al., 1982; Cronin, 1979; Feldman et al., 1981; Humes et al., 1982; Just and Habermann, 1977; Kunin, 1970; Lipsky and Lietman, 1982; Luft and Evan, 1980a, 1980b; Weinberg et al., 1980). Other structural characteristics may account for differences in toxicity within groups of drugs with similar total ionization potentials (i.e., netilmicin, tobramycin, amikacin, and gentamicin, all with five ionizable groups).

Mechanism of Action

Aminoglycosides exert their antibacterial action by irreversibly binding to one or more receptor proteins on the 30S subunit of the bacterial ribosome and thereby interfering with several mechanisms in the mRNA translation process. These include disrupting an initiation complex between the mRNA and the 30S subunit, blocking further translation and thereby causing premature chain termination, or causing incorporation of an incorrect amino acid in the protein product. Although most antimicrobials that interfere with ribosomal protein synthesis are

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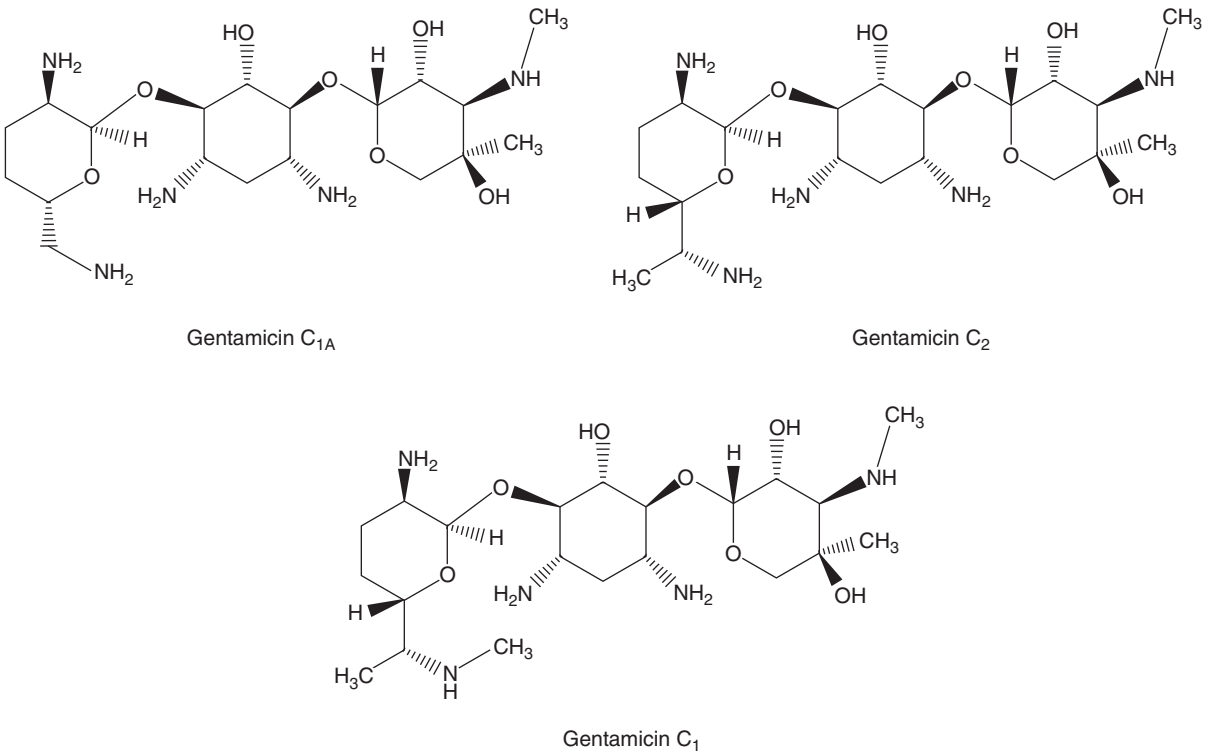

Figure 35.1 Structures of gentamicin.

bacteriostatic, aminoglycosides are bactericidal. Because of irreversible binding, significant postantibiotic effects can be observed.

The mechanism of bacterial penetration by the aminoglycoside through the cell membrane is biphasic. Aminoglycosides diffuse through the outer membrane of gram-negative bacteria through aqueous channels formed by the porin proteins. Once in the periplasmic space, an oxygen-requiring process transports the drug into the cell, where it interacts with the ribosome. Anaerobic bacteria are therefore resistant to the antibacterial effects of aminoglycosides. The oxygen-dependent transport is linked to an electron transport system, which causes the bacterial cytoplasm to be negatively charged with respect to the periplasm and external environment.

An additional mechanism is independent of ribosomal binding. These agents are positively charged by virtue of their amino groups (Figures 35.1 and 35.2). These agents disrupt the cell surface biofilm, particularly on gram-negative bacteria, to produce disruption, loss of cell wall integrity, and a rapid bactericidal effect. Magnesium and calcium are important to cross-bridge adjacent lipopolysaccharide molecules. Aminoglycosides competitively displace Ca^{++} and Mg^{++} and destabilize the bacteria outer membrane. Therefore, rapid death of the bacteria can be caused by a cell surface effect rather than inhibition of the ribosome. This helps explain the concentration-dependent effect and rapid bactericidal action that is a feature of aminoglycosides. This property is not as prominent for gram-positive bacteria unless administered with a cell-wall disrupting agent such as vancomycin or a β-lactam antibiotic.

The positively charged aminoglycosides also affect the accumulation in bacteria. Because of the positive charge, they are attracted electrostatically into the bacterial cytoplasm. Some divalent cations (such as Ca^{++} and Mg^{++}) are competitive inhibitors of this transport system. This proton-motive force also functions in the lysosomes and mitochondria in which aminoglycosides accumulate and may also be a factor in the intralysosomal accumulation of the aminoglycosides.

A characteristic of aminoglycoside activity is that bacterial killing is concentration-dependent, and a postantibiotic effect (PAE) is evident. The PAE is a persistent suppression of bacterial growth following the removal of an antimicrobial agent. Bactericidal action persists after serum concentrations fall below minimum inhibitory concentrations (MICs). This has ramifications for the design of clinical dosage regimens.

Spectrum of Activity

Aminoglycosides are effective against most gram-negative bacteria, including gram-negative bacteria of the Enterobacteriaceae and *Pseudomonas aeruginosa*. They are effective against staphylococci, although

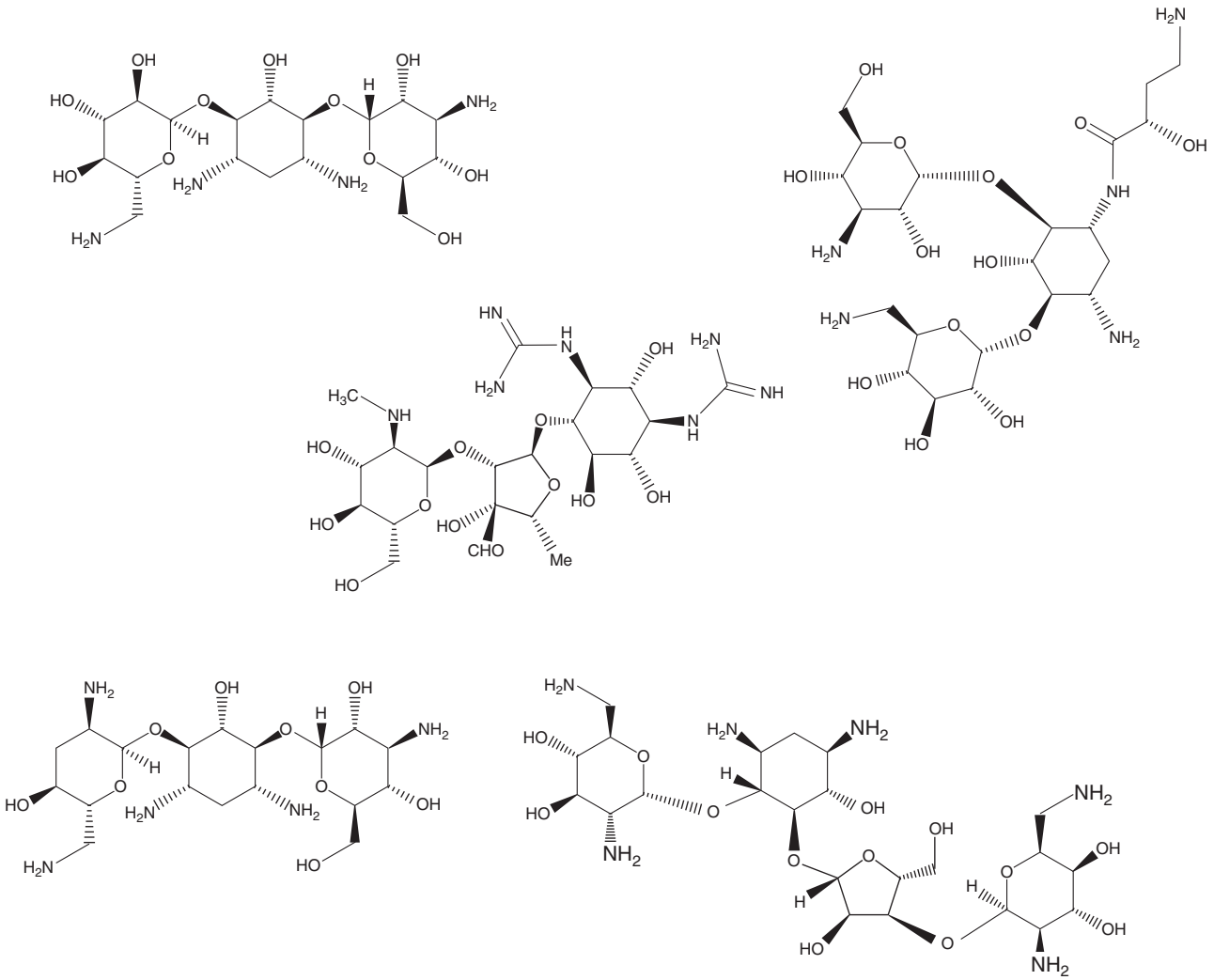

Figure 35.2 Structure of kanamycin, streptomycin, tobramycin, neomycin, and amikacin.

resistance can occur if used as monotherapy. Their action against streptococci and enterococci is limited unless they are combined with a β-lactam antibiotic. They have poor activity against *Pasteurella multocida*. Anaerobic bacteria are inherently resistant because drug transport into bacteria is oxygen dependent. Breakpoints for susceptibility testing have been established by the Clinical Laboratory Standards Institute (CLSI, 2015) and are shown in Table 35.1.

Comparison among drugs

Compared to other drugs in this group, amikacin usually has greater activity against gram-negative bacteria because it resists degradation by bacterial enzymes. This difference is observed with *E. coli* and particularly *Pseudomonas aeruginosa*. It is common for isolates obtained from dogs, cats, and horses to be resistant to gentamicin, yet still susceptible to amikacin. Gentamicin is approximately equal to tobramycin in activity, but tobramycin can be more active against some strains of *E. coli* and *Pseudomonas aeruginosa*. Kanamycin is least active compared to the others in this class, except for streptomycin.

Effect of tissue environment and other drugs on activity

pH effect: The action of aminoglycosides is pH dependent. The activity is less at low pH because high cation concentrations inhibit activity. The optimum pH for antibacterial activity is between 6 and 8. For example, gentamicin is 30 to 100-fold less active in an acidic (pH of 5.5 to 6.0) environment than at a pH of 7.4. Consequently, in some tissues and fluids (e.g., urine and abscesses) drug activity may be less because of lower pH.

Cellular debris: Aminoglycosides are bound to, and inactivated by, cellular debris and nucleic acid material that is released by decaying white blood cells. Therefore,

Table 35.1 Once-daily dosages for selected aminoglycosides

Species	Dose (in most cases the dose can be administered IV, IM, or SC)
Gentamicin	
Dog	9–14 mg/kg q 24 h
Cat	5–8 mg/kg q 24 h
Horse	Adult: 4–6.8 mg/kg q 24 h Foal (<2 weeks): 12–14 mg/kg q 24 h
Cattle	Adult: 5–6 mg/kg q24h Calf (<2 weeks): 12–15 mg/kg q 24 h
Sheep	Same as cattle
Amikacin	
Dog	15–30 mg/kg q 24 h
Cat	10–15 mg/kg q 24 h
Horse	Adult: 10 mg/kg q 24 h, IV, IM Foal (<weeks): 20–25 mg/kg q 24 h, IV

the activity in an abscess is poor. (One ml of purulent material can inactivate 700 μg of gentamicin.)

Oxygen tension: Low oxygen tension, such as that found in anaerobic tissue or decaying tissue, decreases the activity of aminoglycosides.

Cations: Because the uptake into bacteria is dependent on the drug's positive charge, divalent cations (e.g., Ca^{++}, Mg^{++}) can interfere with uptake of aminoglycosides into bacteria. Monovalent cations also may have some nonspecific inhibitory effect. The effects of cations on activity are discussed in Section Aminoglycoside Toxicity.

Other drugs: Aminoglycosides are inactivated if combined in vitro (for example in a vial or syringe) with other drugs, especially penicillins. This inactivation does not occur in vivo because concentrations in serum are not high enough to interact when two drugs are administered concurrently at the usual recommended doses. Aminoglycosides are synergistic with β-lactams against some bacteria in vitro, but this may not translate to improved clinical efficacy when the drugs are used simultaneously.

Resistance mechanisms

Anaerobic bacteria are intrinsically resistant because oxygen is necessary for aminoglycosides to enter bacteria. Resistance can occur by way of multiple effects. Some bacteria have an altered cell surface receptor, which is necessary to transport the drug into the bacteria. Bacteria can have a mutation in the target (ribosome) that resists binding, but this is uncommon.

A significant mechanism of resistance is degradation by bacterial enzymes. Several enzymes can be produced by bacteria that inactivate aminoglycosides. These enzymes can phosphorylate, adenylate, or acetylate groups on the molecule to render the drug inactive. The inactive drug can compete with the active drug for transport. Most drugs in this class are susceptible to many of the enzymes, but amikacin is susceptible to only one of the acetylase enzymes, which may account for amikacin's increased activity against some resistant bacterial strains in comparison to other aminoglycosides.

Pharmacokinetic–Pharmacodynamic Properties

The aminoglycosides are concentration-dependent bactericidal agents; therefore the higher the drug concentration, the greater the bactericidal effect. An optimal bactericidal effect occurs with peak drug concentration of 8–10 times the MIC, with little added benefit for concentrations above 10 times MIC. This target can be accomplished by administering a single dose once daily. This regimen is at least as effective, and perhaps less nephrotoxic, than lower doses administered more frequently (Freeman et al., 1997; Maglio et al., 2002; Drusano et al., 2007). Currently accepted dose regimens in small animals and horses employ this strategy. An additional benefit may be decreased resistance. According to Freeman et al. (1997), "Peak/MIC ratio of at least 10/1 may prevent the emergence of aminoglycoside-resistant pathogens". The single daily dose is usually calculated from the drug's volume of distribution. The peak may be achieved from IV, IM, or SC dosing. Total plasma concentration can be used because these drugs are essentially unbound (protein binding less than 10%).

Clinical Uses

The drugs used most often to any extent in veterinary medicine are amikacin, gentamicin, kanamycin, and neomycin (neomycin is used topically only). Netilmicin, sisomicin, and dibekacin are newer compounds but there are no reports of their use in veterinary medicine. Many streptomycin products have either been removed from the human market or are used only for certain infections (e.g., tuberculosis in people). Penicillin–dihydrostreptomycin combinations have been discontinued in the USA for use in animals.

Aminoglycosides are still considered to be important drugs of choice for treating serious aerobic gram-negative infections in veterinary medicine, although newer and less toxic antimicrobials (i.e., third-generation cephalosporins and fluoroquinolones) have replaced the use of aminoglycosides for some bacterial infections.

Neomycin is too toxic to be used systemically but is still used topically or oral for treating diarrhea. Kanamycin, was first introduced in the late 1950s, but many organisms are now resistant to this aminoglycoside and its use has subsequently declined. Gentamicin, introduced in the 1960s, has a broader spectrum and is associated with

Table 35.2 Susceptibility testing guidelines for aminoglycosides. Source: Data from CLSI. (2015). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Third Informational Supplement.* CLSI document VET01-S3. Wayne, PA: Clinical and Laboratory Standards Institute.

		MIC interpretive category (µg/ml)			
Drug	**Species**	**S**	**I**	**R**	**Comments**
Gentamicin	Dogs	≤ 2	4	≥ 8	Once-daily dose of 10 mg/kg
	Horses	≤ 2	4	≥ 8	Once-daily dose of 6.6 mg/kg
	Dogs	≤ 4	8	≥ 16	Once-daily dose of 15 mg/kg
Amikacin	Horses adult	≤ 4	8	≥ 16	Once-daily dose of 10 mg/kg
	Foals	≤ 2	4	≥ 8	For foals less than 11 days of age, 20 mg/kg, q 24 h, IV
Spectinomycin	Bovine	≤ 32	64	≥ 128	Respiratory pathogens

S, susceptible; I, intermediate; R, resistant.

less resistance than kanamycin. Amikacin, a semisynthetic derivative of kanamycin, was introduced clinically in the 1970s, has the broadest spectrum of activity of all the aminoglycoside antibiotics used clinically to date, and is the preferred antibiotic in severe gram-negative infections that are resistant to gentamicin or tobramycin.

Table 35.2 lists the dosage regimens for some of the aminoglycosides. It is important to note that these doses can be modified proportionately to correct for age, clinical or subclinical disease processes, renal insufficiency, or any of the other factors that may predispose the patient to aminoglycoside toxicosis (see Section Aminoglycoside Toxicity). Alterations in the dose can be best determined by monitoring serum creatinine concentrations or optimally by monitoring aminoglycoside serum concentrations at predetermined time points after dosing.

Single Daily Dose Administration

Because of the PK-PD properties, discussed in Section Pharmacokinetic–Pharmacodynamic Properties, single daily dosing of aminoglycosides may be as efficacious as administering the same dose divided over 24 hours. The concept of single daily dosing of aminoglycosides has been utilized and generally accepted within the human medical community (Bass et al., 1998; Christensen et al., 1997; Freeman et al., 1997; Karachalios et al., 1998; Rodvold et al., 1997). The efficacy of single dose administration is attributed to the rapid bactericidal action and the PAE, discussed in Section Mechanism of Action. Once-daily aminoglycoside dosage regimens that produce high peak and low trough concentrations also have less propensity to induce renal toxicity than multiple-dose regimens, which produce lower peak but higher trough concentrations. The clinical doses listed in Table 35.2 are derived from studies in these species that show that once-daily administration can achieve the targeted PK-PD value (Albarellos et al., 2004; Godber et al., 1995; Tudor et al., 1999; Martin et al., 1998; Magdesian et al., 1998; Bauquier et al., 2015; Tudor et al., 1999).

Local Administration

Intraarticular administration of aminoglycosides achieves higher concentrations in joint fluid compared to systemic therapy. This mode of administration may not be practical in all cases and is used most often in horses compared to other animals. In studies performed in experimental horses with septic arthritis, intraarticular administration of gentamicin (150 mg/joint) produced a much higher concentration in synovial fluid than IV administration. Twenty-four hours later, horses that received intraarticular gentamicin also had fewer bacteria in the synovial fluid. Amikacin and other drugs also have been administered via this route.

Aminoglycosides can be implanted directly using antibiotic-impregnated polymethylmethacrylate (AIPMMA) in the infection site. This material is a type of bone cement that hardens at the site once mixed and prepared. Antibiotics impregnated in this matrix results in high local concentrations that are released for a long period of time – sometimes for as long as 80 days. This technique avoids high systemic levels of drugs, reduces drug costs, and the need for frequent systemic administration. Aminoglycosides (tobramycin, amikacin, and gentamicin) are often used for this technique (Streppa et al., 2001).

Regional perfusion of antibiotics involves intravenous or interosseous administration of antibiotic in the limb of an animal while a tourniquet is applied proximal to the site of drug administration. This technique was reviewed by Rubio-Martinez and Cruz (2006). It has been performed in horses, cattle, and large zoo animals (e.g., elephants). It produces high concentrations of antibiotic in the distal limb (joint fluid and bone) of an animal (Murphey et al., 1999). High bactericidal concentrations are achieved during the interval when the tourniquet is applied. Then, concentrations quickly dissipate after tourniquet release. The high concentrations enter adjacent tissue via perfusion and can penetrate ischemic tissue and exudate via gradient diffusion. This technique

reduces the total amount of drug used and maintains high concentrations in bone and joint fluid distal to the tourniquet. The advantage of regional limb perfusion has been that it confines the drug to the lower limb, preventing systemic exposure, and avoids the need for high systemic doses (Anderson et al., 1995). Drugs used in this technique have usually been amikacin, gentamicin, or tobramycin. For example, gentamicin has been used to treat infections in horses using regional limb perfusion, particularly for lower limb and joint infections (Whitehair et al., 1992a, 1992b).

Susceptibility Testing

Clinical breakpoints have been established by the Clinical and Laboratory Standards Institute (CLSI) through an analysis of pharmacokinetics, PK-PD criteria, and MIC distributions. These breakpoints are listed in Table 35.1.

Regulatory Status

Although they are not banned by the US FDA, much of the use in food-producing animals is considered extralabel, except for a few oral products used to treat diarrhea. Under the animal medical drug use clarification act (AMDUCA) of 1994, extralabel use is not allowed if there are other approved animal products effective for the condition being treated. More information is available in Chapters 52 and 61 of this book. These drugs have very long withdrawal times for slaughter. As long as 18 months for withdrawal prior to slaughter in cattle is suggested by the food animal residue avoidance data bank (FARAD). The American Association of Bovine Practitioners (AABP) and Academy of Veterinary Consultants (AVC) have recommended that, until further scientific information becomes available, aminoglycosides should not be used in cattle.

Pharmacokinetics of Aminoglycosides

General

A comprehensive review of aminoglycoside pharmacokinetics has been reported by Brown and Riviere (1991) and is found in earlier editions of this book. The pharmacokinetics of the aminoglycosides is similar across species lines, but the variability within each animal population is large, indicating a significant amount of heterogeneity in aminoglycoside disposition in both diseased and normal animals (Sojka and Brown, 1986; Frazier et al., 1988). Although there is variability in aminoglycoside pharmacokinetic parameters, the therapeutic range for all of the aminoglycosides is relatively narrow, and the potential for toxicosis is greater than for most other classes of antimicrobials. Altered physiological or pathological states such as pregnancy (Lelievre-Pegorier et al., 1985), obesity (Sketris et al., 1981), subnormal body weight (Tointon et al., 1987), kidney disease (Frazier and Riviere, 1987; Martin et al., 1998; Martin-Jimenez and Riviere, 2001), dehydration (LeCompte et al., 1981; Brown et al., 1985a), immaturity (Sojka and Brown, 1986), sepsis (Mann et al., 1987), dietary protein (Grauer et al., 1994; Behrend et al., 1994), endotoxemia (Wilson et al., 1984; Jernigan et al., 1988c), and intraindividual variability (Mann et al., 1987), among many others, may alter the distribution, clearance, and half-life of aminoglycosides by as much as 1000-fold between individuals in a single study (Zaske et al., 1982).

Absorption

Aminoglycosides are not appreciably absorbed from the gastrointestinal tract because of their highly polar and cationic nature. However, if there is significant disruption of the intestinal mucosa from enteritis (Gemer et al., 1983; Miranda et al., 1984; Gookin et al., 1999), some absorption may occur. For example, neomycin administered orally to calves with enteritis could increase the risk of residues at slaughter. The aminoglycosides are not inactivated in the intestine and are eliminated in the feces unchanged after oral administration to normal animals. This lack of significant absorption through the gastrointestinal tract requires that all aminoglycosides be given by parenteral routes if therapeutic plasma concentrations are desired. Aminoglycoside absorption is practically complete after IM or SC injection. The peak serum concentrations after extravascular injection occur 14–120 minutes after the dose (Blaser et al., 1983; Ristuccia, 1984). Absorption is extremely rapid and complete if aminoglycosides are instilled into body cavities that contain serosal surfaces; administration by this route closely mimics parenteral administration (Jawetz, 1984; Sande and Mandell, 1985). Absorption from topical administration in open wounds also is possible and may increase the risk of nephrotoxicosis if high doses are used (Mealey and Boothe, 1994).

Distribution

The aminoglycoside antibiotics are highly hydrophilic and distribute rapidly in extracellular body fluids. Because of their polycationic nature, the penetration of aminoglycosides across membranous barriers by simple diffusion is limited; therefore, low concentrations of aminoglycosides are found in cerebrospinal fluid or in respiratory secretions (Riviere and Coppoc, 1981b; Strausbaugh and Brinker, 1983). Aerosol or intratracheal administration of aminoglycosides produces negligible serum concentrations in animals and this has been used for in-hospital treatment of bronchitis. Through this

route, substantial bronchial and pulmonary concentrations can be achieved (Riviere et al., 1981b; Wilson et al., 1981). Delivery with devices that nebulize these agents for delivery to the airways has been employed in hospitalized patients with gentamicin, amikacin, and tobramycin.

Plasma protein binding is negligible for all drugs in this group. These drugs easily pass from the capillaries through fenestrations in capillaries to achieve concentrations in interstitial fluids that are equivalent to plasma drug concentrations. The volume of distribution is approximately equal to the volume of extracellular fluid (typically in the range of 20–25% for most adult animals).

Physiological changes can alter the distribution. Decreases in body water (dehydration) can decrease the volume of distribution and increase plasma drug concentrations. Increases in body water caused by pregnancy, third-compartment fluid accumulation (e.g., ascites), and young age (neonate) will increase the volume of distribution and lower plasma drug concentrations. In studies performed in calves, foals, and puppies, the high body water – particularly extracellular water – produces a high volume of distribution for aminoglycosides. Because the plasma concentration is proportional to the volume of distribution: the larger the volume of distribution, the higher the dose that is needed to attain a targeted peak plasma concentration (C_{max}). For example, the volume of distribution for gentamicin or amikacin in foals is more than double the value for adult horses. Subsequently the dose needed to maintain the same blood concentration should be increased, at least by twofold.

Metabolism and Excretion

Several studies in animals (Black et al., 1963; Chiu et al., 1976; Chung et al., 1980; Gyselynck et al., 1971; Schentag and Jusko, 1977; Silverman and Mahon, 1979) have clearly demonstrated that aminoglycosides are eliminated nonmetabolized from the body in all animal primarily by renal glomerular filtration. Some degree of proximal tubular reabsorption occurs and results in an intracellular sequestration or storage in the tubule cells without a significant transepithelial flux from the intraluminal to peritubular space. Net aminoglycoside secretion along more distal nephron segments may also occur. Proximal tubule luminal absorption of aminoglycoside appears quantitatively to be the primary mechanism of intracellular uptake; however, selective peritubular or basolateral reabsorption, evident in isolated tissue slice studies, does occur and may be of toxicological significance in specific situations. Reabsorption requires metabolic energy and occurs along the midconvoluted and straight portions of the proximal tubule (Barza et al., 1980; Bennett et al., 1982; Hsu et al., 1977; Kaloyanides and Pastoriza-Munoz, 1980; Kluwe and Hook, 1978a, 1978b; Kuhar et al., 1979; Pastoriza-Munoz et al., 1979; Senckjian et al., 1981; Silverblatt, 1982; Silverblatt and Kuehn, 1979; Silverman and Mahon, 1979; Tulkens and Trouet, 1978; Vandewalle et al., 1981; Williams et al., 1981a, 1981b; Zaske, 1980). Renal cortical uptake of the aminoglycosides is dose-dependent up to a threshold concentration; then, cortical accumulation increases at a progressively slower rate as the dose is increased. Cumulative uptake of aminoglycosides in tissues indicates that the kidney is the major site of drug sequestration.

A typical plasma vs time profile for IV administration of an aminoglycoside antibiotic to animals shows three phases. The α (distribution) phase occurs within the first hour after IV dosing, the β phase (elimination) occurs between 1 and 24 hours after IV dosing (and probably the most useful in determining dose adjustments in clinical situations), and the γ phase occurs 24 hours after dosing and is the most important part of the elimination curve of aminoglycosides when considering drug residues in food-producing animals. Values for the beta- and gamma-phases are shown in Table 35.4 for gentamicin. The primary difference in pharmacokinetics among species is related to the glomerular filtration rate (GFR). The GFR is lower for larger animals because of allometric scaling; therefore, larger animals tend to have slower clearance and the half-lives are longer (Riviere, 1985; Riviere et al., 1997). Reptiles have lower GFR and lower renal clearance of aminoglycosides. This produces longer half-lives in reptile species.

The prolonged terminal elimination phase of aminoglycosides has major implication for veterinary therapeutics in food-producing animals. As discussed in Section Metabolism and Excretion, aminoglycosides accumulate in the renal cortex for prolonged periods of time, resulting in violative tissue residues even after short periods of administration. In some cases, aminoglycosides such as gentamicin may be detected for a year after parenteral administration! A withdrawal time of 18 months for cattle treated with gentamicin has been recommended by FARAD (see Chapter 61), but it is best to simply avoid use in these species altogether. Piglets may be treated up to 3 days of age with oral products, but even in this case the withdrawal time is 40 days.

Pharmacokinetics in Nonmammals

Veterinarians involved in nonmammal practice should be aware of variations in elimination in some animals. In birds the elimination half-life is usually 2–3 hours and dosing intervals are similar to what has been used in mammals. For amphibians and reptiles, however, elimination rates are much slower. Half-lives range from 38 to 72 hours in alligators and dose intervals of 72 to 96 hours have been used. In snakes half-lives can be as long as 80–121 hours. In turtles and tortoises, the half-life of

Table 35.3 Risk factors that predispose to aminoglycoside toxicosis

Age
Volume contraction (shock)
Acidosis
Sodium or potassium depletion
Sepsis
Renal transplantation
Prior renal insufficiency
Prior aminoglycoside exposure
Cumulative dose of aminoglycoside
Peak and trough serum concentrations
Hepatic disease
Total dose of drug administered
Duration of treatment
Concurrent administration of loop diuretics
Methoxyflurane anesthesia
Cephalosporin antibiotics
Nephrotoxic drugs

aminoglycosides has been in the range of 20–70 hours, with dose intervals usually every 48 hours to every 96 hours. Kidney injury may be greater because of the slower elimination in reptiles. Therefore, use these drugs cautiously in animals with slow clearance.

Aminoglycoside Toxicity

Aminoglycoside toxicity in domestic and laboratory animals was reviewed extensively by Riviere (1985). The possible risk factors that may predispose a patient to aminoglycoside toxicity are shown in Table 35.3.

Aminoglycosides can induce ototoxicity and nephrotoxicity because both organs have higher-than-normal concentrations of phospholipid (in particular, phosphatidylinositol) (Sastrasinh et al., 1982a, 1982b) in their cellular matrixes. Cationic aminoglycosides are chemically attracted to anionic membrane phospholipids. The tissues into which gentamicin preferentially accumulates (renal cortex and cochlear tissue) have disproportionately high amounts of phosphatidylinositol in their membranes compared with other tissues of the body (Hauser and Eichberg, 1973). Basolateral membranes of the renal proximal tubular epithelium also have a higher capacity for binding aminoglycosides than brush-border membranes because of their higher phosphatidylinositol content (Josepovitz et al., 1985).

Ototoxicity studies in a variety of species have shown injury from aminoglycosides that may affect both auditory and vestibular function due to destruction of the sensory hair cells in the cochlea and vestibular labyrinth. The mechanism of ototoxicity was described in a review (Lanvers-Kaminsky et al., 2017). Initially the outer hair cells of the cochlea are affected, which impars hearing at high frequencies. With continued exposure the inner hair cells are injured, which causes additional hearing imparirment and deafness. Injury may be caused by oxidative stress and inhibition of mitochondrial protein synthesis. Aminoglycosides enter the inner ear by active transport mechanisms. Once in the ear, they are cleared slowly with half-lives of 10–13 days after a single dose, but up to 30 days after multiple doses. Ototoxicity may be irreversible in some cases (Johnson and Hardin, 1992). Of pertinence to veterinary medicine, dogs tend to present with auditory toxicity, and cats tend to present with vestibular toxicity, although both usually occur after nephrotoxicity has ensued.

The interaction between the cationic aminoglycosides and the kidney anionic phospholipids appears to be electrostatic and proportional to the cationic charge of the drug. This interaction is saturable and is competitively inhibited by divalent cations (magnesium and calcium), spermine, poly-L-lysine, and other aminoglycosides. For example, diets high in calcium, or calcium supplementation may decrease the risk of aminoglycoside nephrotoxicity (Schumacher et al., 1991; Brashier et al., 1998). After binding, the aminoglycoside is internalized into the cell by pinocytosis (Bennett et al., 1982; Elliott et al., 1982; Feldman et al., 1981; Humes et al., 1982; Lipsky et al., 1980; Lipsky and Lietman, 1982; Pastoriza-Munoz et al., 1979; Schacht, 1978), where concentrations of the aminoglycoside can reach as high as 50 times the concentrations achieved in serum or plasma. The uptake of aminoglycosides into lysosomes is competitive and is dependent in part upon the charge density of the aminoglycoside molecule, which is a function of the number of amino groups. For example, neomycin (valence + 4.37 at pH 7.40) accumulates in the renal cortex more than gentamicin (valence + 3.46 at pH 7.40) due to a higher cationic charge.

There are several mechanisms that may explain the mechanism by which aminoglycosides initially damage the proximal renal tubule cells (Swann et al., 1990; Schumacher et al., 1991; Beauchamp et al., 1992). Lysosomal dysfunction is a component of the early phase of renal injury (Carbon et al., 1978; Feldman et al., 1982; Hull et al., 1981; Kaloyanides and Pastoriza-Munoz, 1980; Laurent et al., 1982; Lipsky and Lietman, 1982; Mazze, 1981; Meisner, 1981; Morin et al., 1980, 1981; Tulkens and Trouet, 1978). This view is consistent with the idea that lysosomes are the primary locus of aminoglycoside sequestration in proximal tubule cells. Lysosomes are also the first organelle to demonstrate morphological changes (myeloid body or cytosegresome formation) after exposure to the drugs (Riviere et al., 1981a). Decreased lysosomal function may also result in a decreased ability to degrade endogenous intracellular proteins and exogenous low-molecular-weight proteins reabsorbed from the tubular filtrate, events that would perturb nephron function (Cojocel et al., 1983; Cojocel

and Hook, 1983). The increase in lysosomal permeability could result in proximal tubule cell dysfunction, although this event is probably a late change in aminoglycoside-induced toxic nephropathy occurring after cell necrosis has been initiated by another factor (Humes et al., 1982). The appearance of lysosomal enzymes (for example, urinary γ-glutamyl transferase, GGT) in the urine of aminoglycoside-induced toxic nephropathy patients is secondary to proximal tubule cell necrosis, apical plasma membrane damage, or lysosome exocytosis.

Mitochondria are a second possible target of aminoglycosides because, both in vitro and in vivo, aminoglycosides decrease mitochondrial respiration, thereby impairing the tubule cell's bioenergetic profile (Appel and Neu, 1977; Cuppage et al., 1977; Kaloyanides and Pastoriza-Munoz, 1980; Kluwe and Hook, 1978a; Sastrasinh et al., 1982b; Simmons et al., 1980; Weinberg et al., 1980, 1990; Weinberg and Humes, 1980). This could selectively produce tubule dysfunction, which would initially be detectable biochemically but not morphologically. The mechanism of this toxicity may be secondary to a direct aminoglycoside interaction with mitochondrial membrane phospholipids, to a competitive interaction with the divalent cations magnesium or calcium, or to an alteration in the intracellular milieu that would indirectly affect mitochondrial function. The magnitude of aminoglycoside effects on mitochondrial respiration is associated with the net positive charge of the specific drug.

The third possible site of initial intracellular aminoglycoside is an interaction with the proximal tubule cell plasma membrane's phospholipids and enzymes (Feldman et al., 1981; Humes et al., 1982; Knauss et al., 1983; Lullmann and Vollmer, 1982; Sastrasinh et al., 1982a, 1982b; Schacht, 1979; Silverman and Mahon, 1979; Williams et al., 1981a, 1981b). Binding of aminoglycosides to membrane polyphosphoinositides could perturb the regulation of membrane permeability, thereby promoting cellular dysfunction. The enzyme interactions at the basolateral membrane could result in significant cellular dysfunction by altering intracellular electrolyte balance or osmolality.

An additional site of aminoglycoside interaction with the nephron is at the level of the glomerulus, where gentamicin has been demonstrated to reduce the glomerular ultrafiltration coefficient and to reduce the number and size of glomerular endothelial fenestrae (Avasthi et al., 1981; Huang et al., 1979; Luft and Evan, 1980a, 1980b; Luft et al., 1978). These effects may be mediated by a charge interaction between the cationic aminoglycosides and the anionic endothelial cell surfaces or could be a feedback response to a primary tubular injury (known as tubuloglomerular feedback).

The relative contributions of the lysosomal, mitochondrial, and membrane tubular mechanisms and glomerular injury to clinical aminoglycoside-induced toxic nephropathy is not known. It is possible that cellular dysfunction is a result of a combination of the above processes.

Dogs

Aminoglycoside-induced kidney injury in dogs follows a progression that consists of an initial subclinical (subazotemic) phase marked by a urinary concentrating defect followed by a clinical (azotemic) phase. It also serves as the basis for simple noninvasive clinical monitoring (for example, monitoring urine specific gravity and proteinuria) for toxicosis since urinary changes preceded the more irreversible systemic changes. If identified early, aminoglycoside-induced kidney injury can recover.

Urine GGT (γ-glutamyl transferase):creatinine and NAG (*N*-acetyl-β-D-glucosaminidase):creatinine ratios and 24-hour urinary excretions of NAG and GGT have been used as markers of aminoglycoside-induced kidney injury. Elevated GGT:creatinine ratio precedes clinically significant elevations in serum creatinine, urine specific gravity, and urine protein:creatinine ratios.

Risk factors in dogs (Brown et al., 1985a) were identified that contributed to nephrotoxicosis in 10 dogs. Risk factors included dehydration, fever, old age, and preexisting renal disease. In addition, low protein and electrolyte abnormalities were documented in these dogs. Other risk factors are shown in Table 35.3.

Ototoxicity in dogs, manifested as either vestibulotoxic and/or ototoxic effects, can occur after systemic aminoglycoside therapy, but toxicity after topical use of aminoglycosides is apparently rare (Strain et al., 1995). Although it is sometimes recommended among dermatologists to avoid topical gentamicin in animals with a ruptured tympanum (ear drum) this apparently is not a risk. In a study designed to detect ototoxicity in dogs treated with topically administered gentamicin using brain stem auditory evoked potential (BAEP), dogs underwent a unilateral myringotomy, followed by instillation of 7 drops of the 3 mg/ml buffered aqueous solution of gentamicin instilled into one ear twice a day for 3 weeks. There was no evidence in any treated dogs of drug-induced detectable changes in cochlear or vestibular function.

Cats

Cats have a relatively more concentrated urine and retain the ability to produce concentrated urine even when the GFR is significantly reduced (Ross and Finco, 1981), making urine monitoring less successful than in dogs. Consistent with the studies cited in dogs, studies in cats have shown that high doses, prolonged administration,

or both, can produce kidney injury, with histological changes and increases in serum urea nitrogen and creatinine (Welles et al., 1973; Waitz et al., 1971).

Nephrotoxicosis associated with the topical use of gentamicin has been reported in cats (Mealey and Boothe, 1994). A cat was administered 10 ml of an undiluted gentamicin injectable solution (50 mg/ml) to lavage an open wound twice. The cat eventually progressed to an azotemic state and was euthanized. Histologically, the kidneys showed severe acute proximal tubular necrosis compatible with aminoglycoside toxicosis. Elevated serum levels of gentamicin were noted as late as 96 hours after administration. Although a number of factors may have contributed to the death of this cat, the topical administration of such large quantities of gentamicin was most likely the major determinant.

Horses

As in other animals, aminoglycoside-induced kidney and otic injury has been documented in horses (Nostrandt et al., 1991). Clinically, aminoglycoside-induced toxic nephropathy is more common in young animals, with toxicity rarely reported in adults (Riviere et al., 1982; Tobin, 1979). As in other animals the injury is marked by elevations in serum creatinine and serum urea nitrogen (Tobin, 1979; Riviere, 1982). The shift in dosing regimens from multiple times per day, to once per day has apparently decreased the risk of aminoglycoside-induced kidney injury in recent years and is now the accepted protocol used clinically (Tudor et al., 1999; Geor and Papich, 2003; Godber et al., 1995).

Examples of Drugs

Gentamicin

Gentamicin is available in solutions of 5, 50, and 100 mg/ml (Garasol, Gentocin, and generic), as well as oral solution for pigs (4.35 or 5 mg/ml) and powder for oral solution (66.7 or 333.3 mg per gram of powder) Gentamicin has been the most commonly administered drug in this class used in veterinary medicine. The common clinical approach is to rely on gentamicin for IV, IM, or SC administration when routine use of an aminoglycoside is indicated. In some instances (for example, to broaden the spectrum) it may be administered with a β-lactam antibiotic (e.g., penicillin, ampicillin, or a cephalosporin). Examples of gentamicin dosages are listed in Table 35.2. Representative pharmacokinetic data for gentamicin in animals are shown in Table 35.4.

Intramuscular injection is a reliable route of delivery as the absorption (bioavailability, F) of gentamicin from IM sites is high, usually approaching 90% or higher in most species (Jernigan et al., 1988e; Haddad et al., 1985b, 1986; Wilson et al., 1989; Pedersoli et al., 1989, 1990; Bird et al., 1983) and bioavailability from SC sites is similar to IM bioavailability (Gilman et al., 1987; Jernigan et al., 1988e; Wilson et al., 1989). Subcutaneous administration is acceptable but the maximum concentration after SC administration is usually lower and occurs later after injection than that observed after an equivalent IM dose (Jernigan et al., 1988a; Wilson et al., 1989), which is most likely due to less blood flow to the SC injection sites than to the IM injection sites, resulting in a slower rate of absorption but not altering the extent of absorption. Systemic availability from intrauterine (IU) administration is 30% in normal cows, with maximum plasma concentrations of 3.70 μg/ml and 17.5 μg/ml being observed 30 minutes after IU doses of 2 and 4 mg/kg, respectively (al-Guedawy et al., 1983).

Effect of Age on Disposition of Gentamicin

As discussed in the disposition section earlier, neonatal animals have a larger proportion of their body weight as extracellular fluid; therefore, gentamicin volume of distribution (Vd) is larger in immature animals than in adults. The difference is usually at least twofold higher in young animals compared to adults (Riviere and Coppoc, 1981a; Sojka and Brown, 1986; Riviere et al., 1983; Cummings et al., 1990; Clarke et al., 1992).

Because systemic clearance of gentamicin relies on kidney function, this also is affected by age and is typically lower in neonates (Sojka and Brown, 1986; Sweeney et al., 1992; Martin et al., 1998; Frazier et al., 1988; Riond et al., 1986). Clearance is correlated with the plasma creatinine concentration (Sweeney et al., 1992; Martin et al., 1998).

Effect of Body Condition and Disease on Gentamicin Disposition

Because gentamicin and other drugs from this class are water soluble, dehydration reduces the apparent volume of distribution (Hunter et al., 1991; LeCompte et al., 1981). Likewise, gentamicin does not distribute to fat and obese animals will have a lower apparent volume of distribution than lean animals (Wright et al., 1991). Dose adjustments should be considered when administering gentamicin to obese animals, extremely lean animals, and animals with fluid accumulations (e.g., ascites).

In endotoxemic animals, there was decreased plasma gentamicin concentrations in dogs and cats by approximately 20–30% (Pennington et al., 1975; Jernigan et al., 1988c). However, differences between healthy and febrile goats were not significant (Ahmad et al., 1994). In a population pharmacokinetic model across species, the covariate of fever seemed to influence gentamicin volume of distribution (Martin-Jimenez and Riviere, 2001). Other

Table 35.4 Single-dose intravenous serum or plasma pharmacokinetics of gentamicin in various species. Source: Adapted from Brown and Riviere, 1991.

Species	Dose (mg/kg)	Volume of Distribution (l/kg)	Clearance (ml/min/kg)	Half-life (β) (hour)	Half-life (γ) (hour)	Reference
Dogs (juvenile)	10	0.354 (0.036)	4.08 (0.62)	1.01 (0.12)	N/A	Riviere and Coppoc, 1981a
Dogs	10	0.38 (0.029)	4.20 (0.70)	1.05 (0.13)	N/A	Riviere et al., 1981a; Riviere et al., 1981b
Dogs	10	0.30 (0.06)	3.44 (0.38)	1.01 (0.08)	N/A	Rivierie et al., 1981a; Riviere et al., 1981b
Dogs	10	0.335 (0.094)	2.94 (0.67)	1.36 (0.09)	N/A	Baggot, 1977
Dogs	4.4	0.227 (0.076)	2.27 (0.41)	1.09[a]	N/A	Brown et al., 1991
Dogs	4	0.255	3.33	1.06	N/A	Batra et al., 1983
Dogs	3	NR	2.29 (0.48)	0.91 (0.25)	N/A	Wilson et al., 1989
Cats	4.4	0.190	1.61	1.36	N/A	Short et al., 1986
Cats	5	ND	1.38 (0.35)	1.25 (0.30)	86[a]	Jernigan et al., 1988e
Cows	5	0.19 (0.04)	1.32 (0.17)	1.83 (0.18)	N/A	Haddad et al., 1986
Cattle (1 day old)	4.4	0.393 (0.040)	1.92 (0.43)	2.49 (0.73)	N/A	Clarke et al., 1985
Cattle (5 days old)	4.4	0.413 (0.050)	2.44 (0.34)	1.99 (0.33)	N/A	Clarke et al., 1985
Cattle (10 days old)	4.4	0.341 (0.021)	2.02 (0.27)	1.97 (0.21)	N/A	Clarke et al., 1985
Cattle (15 days old)	4.4	0.334 (0.039)	2.10 (0.32)	1.85 (0.13)	N/A	Clarke et al., 1985
Cattle (4–5 weeks old)	3	1.95 (1.24)	4.9 (1.9)	3.96 (1.67)	N/A	Ziv et al., 1982
Cattle (adult)	4.4	0.140 (0.020)	1.29 (0.26)	1.26 (0.19)	N/A	Clarke et al., 1985
Horse (mare)	6.6	0.21	1.1	2.2	ND	Santschi and Papich, 2000.
Horse (clinical)	4.4	0.17	1.2	1.61	ND	Tudor et al., 1999
Horse (clinical)	6.6	0.17	1.3	1.47	ND	Tudor et al., 1999
Horse	2.2	0.46	ND	0.83	ND	Godber et al., 1995
Horse	6.6	0.115	ND	0.78	ND	Godber et al., 1995
Horse (foal)	4.0	0.32–0.38	1.7–3.7	1–2.1	ND	Cummings et al., 1990
Horse (adult)	4.0	0.17	1.7	1.1	ND	Cummings et al., 1990
Horse	2.2	0.18	1.1	1.82–1.96	ND	Jones et al., 1998
Horse	2.2	0.48	1.2	4.4	ND	Whittem et al., 1996
Horse	6.6	0.19	0.95	2.3	ND	Magdesian et al., 1998
Horses	5	0.254 (0.031)	2.54 (0.33)	2.54 (0.33)	N/A	Pedersoli et al., 1980
Horses (2–3 months old)	4.5	ND	1.65 (0.79)	3.23 (0.62)	N/A	Riviere et al., 1983
Horses	2.2	ND	0.87 (0.05)	3.85 (0.40)	N/A	Bowman et al., 1986
Horses	2.2	ND	0.68 (0.17)	3.51 (0.59)	142 (31)	Bowman et al., 1986
Horses	3	0.202 (0.028)	1.41 (0.19)	1.66 (0.06)	N/A	Wilson et al., 1983
Ponies	5	0.20 (0.01)	1.27 (0.18)	1.82 (0.22)	N/A	Haddad et al., 1985b
Mammoth asses	2.2	0.12 (0.025)	1.22 (0.18)	2.07	ND	Miller et al., 1994
Sheep	2.2	0.194 (0.059)	1.56 (0.40)	1.44 (0.085)	N/A	Wilson et al., 1981
Sheep	3	ND	0.660 (0.256)	1.33[a]	41.9 (18.5)	Brown et al., 1986b
Sheep	10	ND	1.03 (0.015)	2.4 (0.5)	30.4 (18.9)	Brown et al., 1985b
Sheep	10	ND	0.805 (0.317)	1.72[a]	88.9 (19.8)	Brown et al., 1986b
Sheep	20	ND	0.882 (0.342)	1.77[a]	167.2 (42.7)	Brown et al., 1986b
Sheep (Desert)	3	0.27	0.07	4.20	ND	Elsheikh et al., 1997
Goat	3	0.22	0.08	1.041	ND	Elsheikh et al., 1997
Pigs	2	0.32 (0.032)	1.66 (0.12)	1.9 (1.47–4.89)	20.2 (13.9–34.6)	Riond and Riviere, 1988
Pigs (newborn)	5	ND	ND	5.19	ND	Giroux et al., 1995
Pigs (42 days)	5	ND	ND	3.50	ND	Giroux et al., 1995
Rabbits	20	ND	2.90–4.0	0.98–1.15	11.4–15.1	Huang et al., 1979
Rabbits	3.5	ND	2.82 (0.97)	0.74	ND	Ogden et al., 1995

(*continued*)

Table 35.4 (*Continued*)

Species	Dose (mg/kg)	Volume of Distribution (l/kg)	Clearance (ml/min/kg)	Half-life (β) (hour)	Half-life (γ) (hour)	Reference
Hawks	10	0.24 (0.03)	2.09 (0.16)	1.35 (0.18)	N/A	Bird et al., 1983
Owls	10	0.23 (0.02)	1.41 (0.10)	1.93 (0.24)	N/A	Bird et al., 1983
Eagles	10	0.21 (0.01)	1.01 (0.06)	2.46 (0.32)	N/A	Bird et al., 1983
Catfish	1	0.156	0.126	12.2	N/A	Setzer, 1985
Catfish	10	0.176	0.215	11.87	N/A	Rolf et al., 1986
Guinea pigs	40	ND	3.4	1.01	1.01	Chung et al., 1982
Buffalo calves	5	0.43	54.61	5.69	ND	Garg et al., 1991a, 1991b
Turkeys	5	0.190	49.8	2.570	ND	Pedersoli et al., 1989
Roosters	5	0.228 (0.019)	0.775 (0.132)	3.38 (0.62)	N/A	Pedersoli et al., 1990
Turtles	3	ND	ND	40–44	ND	Beck et al., 1995

Values reported as arithmetic mean followed by SD or SEM in parentheses. N/A, not applicable (inappropriate term for the model used); ND, not determined; NR, not reported.
[a]Harmonic mean; data are IV and IM data pooled together.

conditions that have been shown to alter gentamicin disposition include endocrinopathies, pregnancy, and other concurrent drug administrations.

Amikacin

Amikacin is approved for animals as a 50 mg/ml injectable solution (Amikin, Amiglyde) as well as an intrauterine infusion (250 mg/ml intrauterine solution) for horses. Tables 35.5, 35.6, and 35.7 list some selected pharmacokinetic parameters for amikacin in various species of animals. It is common to initially rely on gentamicin for routine aminoglycoside treatment, but when resistance is observed or suspected, amikacin should be considered because resistance is uncommon for amikacin. Amikacin is particularly important for treating infections caused by *E. coli* and *Klebsiella pneumoniae* that have acquired multidrug resistance from extended spectrum β-lactamase (ESBL). Amikacin is often one of the few agents, other than a carbapenem, that is active against these bacteria.

Amikacin shows in vitro activity against *Staphylococcus* spp., although it is not a common agent for treatment of staphylococcal infections. Many guidelines recommend addition of a β-lactam antibiotic for treating infections caused by *Staphylococcus* spp. Amikacin is considered for treating methicillin-reisistant *Staphylococcus pseudintermedius* infections of the skin and soft tissue. However, there may be an association between amikacin resistance and methicillin resistance in these isolates (Gold et al., 2014).

Like gentamicin, amikacin is hydrophilic and is rapidly taken up by either IM or SC administration with bioavailability of approximately 90% or higher (Gronwall et al.,

Table 35.5 Pharmacokinetic data for amikacin in horses, foals, and dogs

		Half-life (hour)	Volume of Distribution (l/kg)	Clearance (ml/kg/min)	Mean residence time (hour)
Compilation of 10 data sets from 44 adult horses; average dose 8.3 mg/kg	Mean	1.83	0.214	1.45	2.50
	Std. Dev	0.75	0.076	0.45	0.565
Compilation of 6 data sets from 37 foals, 1–11 days of age; average dose 20 mg/kg	Mean	4.43	0.68	1.83	5.4
	Std. Dev	1.09	0.19	0.40	0.22
Compilation of 9 data sets from 42 dogs; average dose 13.6 mg/kg	Mean	1.0	0.22	2.40	1.50
	Std. Dev	0.24	0.11	0.77	0.92

Data from: Pinto et al., Equine Vet J. 43, 112–116, 2011; Orsini JA, et al., Can Vet J 37, 157–160, 1996; Brown MP et al., Am J Vet Res. 45: 1610–1613, 1984; Horspool et al., J Vet Pharmacol Therap. 17: 291–298, 1994; Orsini et al., J Vet Pharmacol Therap. 8: 194–201, 1985; Magdesian et al., Am J Vet Res. 65: 473–479; 2004; Golenz MR et al., Equine Vet J. 26: 367–373, 1994; Bucki et al., J Vet Intern Med. 18: 728–733, 2004; KuKanch and Coetzee, J Vet Pharmacol Therap. 31:102–107, 2008; Cabana and Taggart, Antim Agent Chemo. 3: 478–483, 1973; Baggot et al., Am J Vet Res. 46: 1793–1796, 1985.

Table 35.6 Single-dose intravenous pharmacokinetics of amikacin in various species (horses and dogs see Table 35.5). Source: Adapted from Brown and Riviere 1991.

Species	Dose (mg/kg)	Volume of distribution (l/kg)	Clearance (ml/min/kg)	Half-life (hour)	Reference
Cats	5	0.134 (0.008)	110 (15)	NR	Shille et al., 1985
Cats	10	0.14 (0.008)	121 (22)	NR	Shille et al., 1985
Cats	20	0.18 (0.022)	138 (2.6)	NR	Shille et al., 1985
Cats	5	NR	1.46 (0.26)	79[a] (19)	Jernigan et al., 1988c
Calves	7.5	350	1.5	150.5	Carli et al., 1990
Sheep	7.5	200	0.7	115.5	Carli et al., 1990
African grey parrots	5	289	188	1.06	Gronwall et al., 1989
African grey parrots	10	184	142	0.90	Gronwall et al., 1989
African grey parrots	20	444	229	1.34	Gronwall et al., 1989

NR, not reported.
[a]Harmonic mean (±SD).

1989; Bloomfield et al., 1997; Jernigan et al., 1988d; Cabana and Taggart, 1973; Ziv, 1977; Baggot et al., 1985; Carli et al., 1990).

Amikacin is also used in reptiles (snakes, turtles) and has a rapid absorption, but slow renal clearance. In these animals, clearance of amikacin (like many other drugs in cold-blooded animals) is temperature dependent.

Pharmacokinetics have been studied in adult horses and foals. Pharmacokinetic data from various studies are summarized in Table 35.5. As for gentamicin, the volume of distribution in neonatal foals is much higher than adults, necessitating higher clinical doses (by at least two times) compared to adults.

Kanamycin

Kanamycin is among the least active aminoglycosides in comparison to gentamicin and amikacin. Subsequently, the clinical use of kanamycin has fallen out of popularity in veterinary medicine in recent years. For example, against clinical isolates of *Pseudomonas aeruginosa* the highest rate of resistance was for kanamycin (90% resistant) compared to gentamicin (7%) and amikacin (3%) (Rubin et al., 2008).

Because kanamycin has chemical properties that are similar to other aminoglycosides (amikacin is synthesized from kanamycin), pharmacokinetic properties are

Table 35.7 Nonintravenous disposition values for amikacin in various species (means with standard deviations in parentheses). Source: Adapted from Brown and Riviere 1991.

Species	Dose (mg/kg)	Route	Half-life (hour)	F (%)	Reference
Horses	4.4	IM	NR	100	Orsini et al., 1985
Horses	6.6	IM	NR	100	Orsini et al., 1985
Horses	11	IM	NR	100	Orsini et al., 1985
Cats	5	IM	NR	NR	Shille et al., 1985
Cats	10	IM	NR	NR	Shille et al., 1985
Cats	20	IM	NR	NR	Shille et al., 1985
Cats	5	SC	NR	NR	Shille et al., 1985
Cats	10	SC	NR	NR	Shille et al., 1985
Cats	20	SC	NR	NR	Shille et al., 1985
Cats	5	IM	119	90 (36)	Jernigan et al., 1988d
Cats	5	SC	118	100 (19)	Jernigan et al., 1988d
Sheep	7.5	IM	1.96	87	Carli et al., 1990
Calves	7.5	IM	1.94	99	Carli et al., 1990
African grey parrot	5	IM	1.08	98	Gronwall et al., 1989
African grey parrot	10	IM	1.04	61	Gronwall et al., 1989
African grey parrot	15	IM	0.97	106	Gronwall et al., 1989
Gopher snake (25° C)	5	IM	1.2 (0.17)	ND	Mader et al., 1985
Gopher snake (37° C)	5	IM	1.25 0.5)	ND	Mader et al., 1985

ND, not determined; NR, not reported.

also similar. Absorption from IM or SC injection is complete and rapid, volume of distribution resembles the volume of extracellular fluid, and excretion from the kidneys is close to the glomerular filtration rate for that species.

Apramycin

Apramycin, an aminoglycoside derived from *Streptomyces tenebrarius* (Ryden and Moore, 1977), is available for veterinary use, but it has limited use. The only formulations currently approved in the US of this drug are powder (Apralan) to be added to feed (Type A medicated feed article) at 150 grams per ton of feed, and the soluble powder to be added to water (100 mg per liter) to deliver 12.5 mg/kg for 12 days of treatment in medicated water. For each formulation, the indication is to treat porcine colibacillosis (pig scours) caused by *Escherichi coli*. More details on apramycin in selected species are presented in Table 35.8.

Tobramycin

Tobramycin is produced by *Streptomyces tenebrarius* and is structurally similar to kanamycin. Tobramycin is not extensively used in veterinary medicine, although it is used occasionally in dogs and cats because of its good activity against most *Pseudomonas aeruginosa* organisms. Typically, amikacin is used more often when it is necessary to treat resistant infections. However, occasionally amikacin has been unavailable because of manufacturing shortages and tobramycin has been used as a substitute.

Pharmacokinetic properties (absorption characteristics, volume of distribution, clearance, and half-life) are similar to other aminoglycosides. In cats, tobramycin systemic clearance was 2.21 ± 0.59 and 1.69 ± 0.36 ml/min/kg after doses of 5 mg/kg and 3 mg/kg IV, respectively, and a Vd(ss) of 0.19 ± 0.03 and 0.18 ± 0.03 l/kg, respectively (Jernigan et al., 1988b). Bioavailability after IM and SC tobramycin administration in cats was rapid and complete (Jernigan et al., 1988d). Urine tobramycin concentrations following 2.2 mg/kg three times a day were 66 ± 39 µg/ml when urine was obtained as a 6-hour collection in dogs (Ling et al., 1981). In horses (Newman et al., 2013), after a dose of 4 mg/kg it had a volume of distribution of 0.18 l/kg, and elimination half-life of 4.6 hours, with a clearance of 1.2 ml/kg/min. It was over 80% absorbed from IM injection.

After IV administration to camels (Hadi et al., 1994), tobramycin (1.3 mg/kg) elimination half-life was 189 minutes. The apparent Vd (area method) was 245 ml/kg and Vd(ss) was 228 ml/kg. Clearance was measured at 0.9 ml/min/kg. After a 1.0 mg/kg IM dose of tobramycin, bioavailability was almost 91%, with an elimination half-life of 201 minutes.

Neomycin

Most neomycin is used topically or administered orally (e.g., Biosol used for enteritis caused by *Escherichia coli*) to achieve a local effect in the intestine. Approved formulations are powder for addition to feed at 715 grams per kg of feed (Neomix), or neomycin oral solution (200 mg/ml) to be added to drinking water, both the oral solution and feed additive are designed to deliver 22 mg/kg up to 14 days. The oral solution also may be administered directly to individual animals.

Pharmacokinetic information about neomycin's use in human and veterinary medicine is limited because systemic use is practically nonexistent, but some details are available in previous editions of this book.

Dihydrostreptomycin and Streptomycin

The clinical use of dihydrostreptomycin and streptomycin has declined substantially in veterinary medicine. An old formulation of penicillin–dihydrostreptomycin (Pen-Strep) is off the market in North America. There is still a formulation containing dihydrostreptomycin sulfate (500 mg/ml) registered for treatment of *Leptospira*

Table 35.8 Selected pharmacokinetic parameters of apramycin

Species	Volume of distribution (l/kg)	Clearance (l/kg/h)	Half-life (hour)	Reference
Sheep	0.167	0.078	90.96	Lashev et al., 1992
Cow (lactating)	1.263	12.164[a]	2.10	Ziv et al., 1995
Ewe (lactating)	1.446	14.142[a]	1.85	Ziv et al., 1995
Goat (lactating)	1.357	11.68[a]	2.14	Ziv et al., 1995
Rabbits	0.284	0.258	48.06	Lashev et al., 1992
Adult chickens	0.182	0.078	100.54	Lashev et al., 1992
18-day-old chicks	0.254	0.218	48.0	Lashev et al., 1992
Japanese quail	0.133[b]	0.186	0.50	Lashev and Mihailov, 1994
Pigeons	0.077	0.210	15.24	Lashev et al., 1992

[a]Value in ml/kg/min.
[b]V_α is area, not steady state.

in dogs, horses, pigs, and ruminants. Although dihydrostreptomycin has been used in the treatment of cows infected with *Leptospira interrogans* serovar *hardjo* subtype *hardjobovis* (Gerritsen et al., 1994), this use is not common and the product is not marketed.

There is an oral formulation of streptomycin (250 mg/ml) still registered for oral treatment of bacterial enteritis in pigs, cattle, and poultry. It has been administered directly or added to drinking water. Because the use of these products is uncommon, previous editions of this book should be consulted for more detailed information on their use.

After IM doses of 5.5 mg/kg dihydrostreptomycin, maximum concentrations ranged from 5.1 to 17.0 µg/ml, with peak concentrations occurring earlier and more variable from the commercial preparation containing procaine penicillin G, dihydrostreptomycin, dexamethasone, and chlorpheniramine than from the commercial product containing only dihydrostreptomycin and procaine penicillin G (Rollins et al., 1972). Half-lives range from 2.35 to 4.50 hours. Because streptomycin and dihydrostreptomycin are chemically very similar, their dispositions also may be nearly identical.

Paromomycin

Paromomycin is a wide-spectrum aminoglycoside antibiotic produced by *Streptomyces rimosus* var. *paromomycinus* and, unlike others in this class, has both gram-positive and gram-negative activity. Paromomycin is poorly absorbed from the gastrointestinal tract, which is clearly an advantage if used to treat certain bacterial or protozoal gastrointestinal infections. The pharmacokinetics of paromomycin in the dog has been described by Belloli et al. (1996); see Table 35.9.

Table 35.9 Pharmacokinetic parameters of paromomycin in the dog. Source: Belloli et al., 1996.

Parameter	IV	IM	SC
Half-life (hour)	91.03	114.22	120.86
VD (l/kg)	0.51	ND	ND
VD_{ss} (l/kg)	0.33	ND	ND
Clearance (l/min/kg)	0.0037	ND	ND
C_{max} (µg/ml)	ND	32.1	36.3
F	ND	>0.99	>0.99
MRT (min)	98.7	204.8	203.8
K_{el} (min^{-1})	0.0186	0.0061	0.0057

ND, not determined.

Giardia, Leishmania, Entamoeba histolytica, and *Balantidium coli* are susceptible to paromomycin (Barr et al., 1994; Belloli et al., 1996). Paromomycin has been used to treat cryptosporidiosis in a cat (Barr et al., 1994) and leishmaniasis (*Leishmania infantum*) in dogs (Poli et al., 1997). However, a retrospective case study in cats treated with high-dose oral paromomycin (165 mg/kg) suggested that 4 of 31 individuals developed acute nephrotoxicity, deafness, and/or possible cataract formation (Gookin et al., 1999), implying that enough oral absorption occurred for this large and highly charged aminoglycoside to exert an adverse effect. Therefore, use of this drug at these high doses should be approached with caution until further data are available.

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Chloramphenicol and Derivatives, Macrolides, Lincosamides, and Miscellaneous Antimicrobials

Mark G. Papich

Drugs listed in this chapter do not warrant a separate chapter and are included together, as they comprise narrow uses in veterinary medicine. They have some features in common – for example they inhibit protein synthesis in bacteria (with macrolides, lincosamides, and chloramphenicol acting at a similar site), and have some similar pharmacokinetic features.

Some of these drugs are not as common or available as in previous years. Some older drugs have given way to newer derivatives and their discussion has been greatly abbreviated in this edition of the book. Older editions may be consulted for more detailed and historical information.

Chloramphenicol

Chemical Features

Chloramphenicol chemically is D-(-)-threo-1-*p*-nitrolphenyl-2-dichloroacetamido 1,3-propanediol (Figure 36.1), has a pK_a of 5.5, and was first isolated from the soil organism *Streptomyces venezuelae* in 1947. The chloramphenicol used today is manufactured synthetically. Chloramphenicol is slightly soluble in water and freely soluble in propylene glycol and organic solvents. Chloramphenicol is a broad-spectrum antibiotic, inhibiting gram-positive and gram-negative organisms, aerobic and anaerobic bacteria, and many intracellular organisms. Chloramphenicol has three functional groups that largely determine its biological activity: the *p*-nitrophenol group, the dichloroacetyl group, and the alcoholic group at the third carbon of the propanediol chain (Yunis, 1988). Replacement of the *p*-NO_2 group by a methylsulfonyl (HC_3-SO_2) moiety produces thiamphenicol and a substantial change in biological activity, while modification of the propanediol group by the addition of a fluorine atom produces florfenicol. Both of these are discussed in more detail in Sections Thiamphenicol and Florfenicol. Loss of the dichloroacetyl group altogether results in loss of biological activity (Yunis, 1988; Hird and Knifton, 1986).

After the discovery of chloramphenicol in 1947 it was in popular use decades ago, but has been gradually replaced by safer alternatives. The small animal formulation is approved by the FDA (Chloromycetin) but is not actively marketed. The use of chloramphenicol diminished in the 1970s and 80s because other active and safer drugs became available. Today, chloramphenicol has experienced a bit of a resurgence in companion animal medicine. Multidrug resistant bacteria, particularly methicillin-resistant *Staphylococcus* spp., are usually susceptible to chloramphenicol and this is one of the most common drugs selected for use by small animal veterinarians (Papich, 2012; Bryan et al., 2012; Frank and Loeffler, 2012). Antibiotic-resistant *Enterococcus* spp. are also often susceptible. Chloramphenicol has the disadvantage of a narrow margin of safety in dogs and cats, and necessity of frequent administration in dogs to maintain adequate concentrations (three or four times daily oral administration). These disadvantages still exist, but the activity of chloramphenicol against bacteria (e.g., staphylococci) that are resistant to other oral drugs has created increased use of chloramphenicol in recent years.

Drug Formulations

Many formulations have been removed from the commercial market because chloramphenicol no longer is in wide use for humans. Chloramphenicol has FDA approval for use in dogs, and is available in 100, 250, and 500 mg tablets (Chloromycetin). The oral suspension of chloramphenicol palmitate is rarely available. Chloramphenicol is not soluble and injectable formulations include esters such as succinate and palmitate, glycinate, or undecylenate. There also has been a propylene glycol solution. None of the injectable formulations are used today. Although chloramphenicol is poorly soluble (<5 mg/ml), the poor solubility does not interfere with oral absorption. Chloramphenicol is absorbed orally

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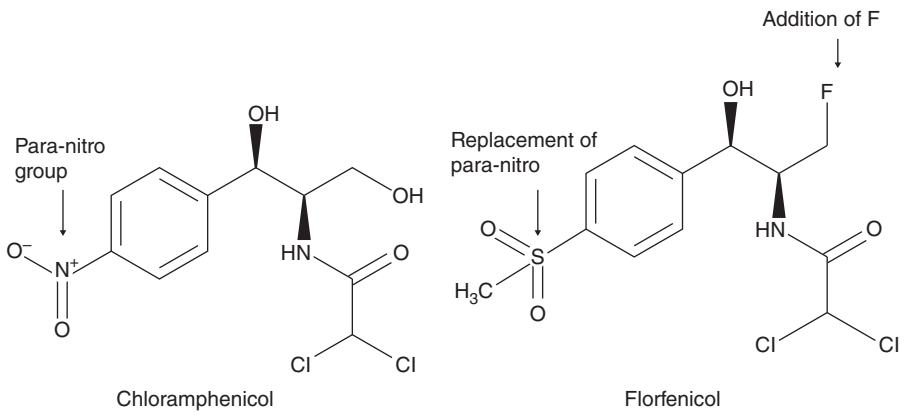

Figure 36.1 The chemical structure of chloramphenicol and modifications to form florfenicol.

with or without food (except some formulations in cats). Tablets and capsules have similar oral absorption in dogs. Topical formulations of chloramphenicol have been used for otic and ophthalmic use, but the otic formulations have been replaced by newer forms containing florfenicol.

Mechanism of Action

Chloramphenicol inhibits protein synthesis. Its biological activity is due to interference with peptidyltransferase activity at the 50S ribosomal subunit, which is near the site of action of macrolide antibiotics and for which there can be competition (Yunis, 1988). Because of the interaction with peptidyltransferase, binding with the amino acid substrate cannot occur, and peptide bond formation is inhibited. Chloramphenicol affects mammalian protein synthesis to some degree, especially mitochondrial protein synthesis. Mammalian mitochondrial ribosomes have a strong resemblance to bacterial ribosomes (both are 70S), with the mitochondria of the bone marrow especially susceptible. Prolonged administration to animals has been associated with a dose-related bone marrow suppression, especially in cats (Watson, 1980).

The action of chloramphenicol (and florfenicol) is regarded as bacteriostatic, rather than bactericidal (Maaland et al., 2015). There are isolated examples in which bactericidal effects have been observed, but chloramphenicol and similar drugs in this class usually behave as bacteriostatic agents and the drug concentration in animals should be maintained above the MIC for as long as possible during the dose interval.

Spectrum of Activity

Chloramphenicol has a broad spectrum of activity. It is active against *Staphylococcus pseudintermedius, S. aureus,* streptococci, and some gram-negative bacteria, such as *Pasteurella multocida*, *Mannheimia haemolytica*, and *Histophilus somni. Escherichia coli, Proteus* spp., and *Salmonella* spp. may be susceptible, but resistance can occur with many gram-negative bacteria, especially the Enterobacteraceae. One reason for the increased use of chloramphenicol, especially in dogs, is that it has retained activity against *Staphylococcus pseudintermedius,* including methicillin-resistant strains (Perreten et al., 2010). However, resistance by staphylococci may occur from repeated administration. Anaerobic bacteria, *Mycoplasma* spp., and many Rickettsiae also are susceptible. The Clinical Laboratory Standards Institute (CLSI, 2015) approved break point for susceptibility is ≤4 μg/ml for streptococci and ≤8 μg/ml for other organisms (Watts et al., 1999).

Bacterial Resistance

Four mechanisms of resistance to chloramphenicol have been described (Yunis, 1988; Schwarz et al., 2004). The most important is plasmid mediated due to the presence of the chloramphenicol acetyltransferase enzyme, which catalyzes a reaction that causes enzymatic inactivation by acetylation of the drug. This can occur through different types of chloramphenicol acetyltransferases (Schwarz et al., 2004). The acetyltransferases that cause resistance to chloramphenicol are less likely to affect florfenicol, making florfenicol more active against some pathogens (discussed in Section Florfenicol). Other mechanisms of resistance include efflux systems, inactivation by phosphotransferases, decreased bacterial cell wall permeability, altered binding capabilities at the 50S ribosomal subunit, and inactivation by nitroreductases.

Pharmacokinetics

Absorption and Distribution

The pharmacokinetic parameters of chloramphenicol have been studied in several animal species and are summarized in Tables 36.1 and 36.2. Chloramphenicol in animals is well absorbed via both oral and parenteral routes, with a few notable species exceptions. Plasma half-lives vary, ranging from 0.9 hours in ponies to 5.1 hours in the

Table 36.1 Chloramphenicol pharmacokinetics in dogs (compilation of nine studies)

		Data set										
		1	2	3	4	5	6	7	8	9	Mean	SD
Breed		Mixed	Beagle	Ghound	large dog	small dog	Ghound	Ghound	Ghound	Ghound		
n		4	6	6	6	6	4	5	4	6		
Formulation (50 mg/kg)		capsule	capsule	capsule	capsule	capsule	capsule	capsule	tablet	capsule		
Elimination rate	1/hour	0.42	0.52	0.40	0.18	0.54	0.14	0.23	0.22	0.40	0.34	0.15
Half-life	hour	1.64	1.35	1.75	3.85	1.29	4.82	2.99	3.19	1.75	2.51	1.25
T_{max}	hour	4.00	2.00	3.00	3.00	1.50	2.00	3.00	1.50	3.00	2.56	0.85
C_{max}	µg/ml	16.70	19.65	18.60	27.50	20.00	16.50	18.50	23.80	18.60	19.98	3.54
AUC	h*µg/ml	97.79	69.85	109.91	191.15	82.44	89.52	114.95	110.62	109.91	108.46	34.51
V/F	ml/kg	1212.94	1389.17	1149.22	1454.03	1124.29	3884.62	1874.17	2081.52	1149.22	1702.13	886.07
Cl/F	ml/h/kg	511.33	715.87	454.90	261.57	606.47	558.53	434.95	451.99	454.90	494.50	126.82
MRT	h	4.63	3.00	4.28	5.94	3.42	5.73	5.75	5.43	4.28	4.72	1.07

Ghound, Greyhound; T_{max}, time to peak concentration; C_{max}, peak concentration; AUC, area under the curve; V/F, volume of distribution per fraction absorbed orally; CL/F, clearance per fraction absorbed; MRT, mean residence time, SD, standard deviation.
Data set sources: (1) Eads, 1952; (2) Mercer, 1971; (3, 4, 5) Watson, 1974; (6) Watson, 1972a; (7) Watson, 1972b; (8) Watson and McDonald, 1976; (9) Watson, 1973.

cat (Davis et al., 1972). Fasted cats showed differences in absorption between the chloramphenicol tablets and the chloramphenicol palmitate suspension (Watson 1992). The liquid formulation showed a lower systemic drug availability, indicating that hydrolysis of the palmitate form is necessary and that there is a higher risk of drug failure when the palmitate suspension is used to treat sick cats that are also not eating. In ruminants, microflora present in the ruminant forestomach tend to metabolize chloramphenicol faster than it can be absorbed, making chloramphenicol administered orally of little use in ruminant animals. This point is rather moot since administration of chloramphenicol to food animals in the United States is currently illegal (discussed in more detail in Chapter 55). In most animals, 30–46% of chloramphenicol is bound to plasma proteins, leaving much of the drug in the free and active form. Chloramphenicol is widely distributed to many tissues of the body due to its nonionized state and high lipophilicity, enabling it to cross lipid bilayers quite easily. The volume of distribution (Vd) is typically greater than 1.0 l/kg and has been measured at 1–2.5 l/kg (Tables 36.1 and 36.2). Chloramphenicol reaches sufficient concentrations in most tissues of the body, including the eye, central nervous system (CNS), heart, lung, prostate, saliva, liver, and spleen, among others (Ambrose, 1984; Hird and Knifton, 1986). Chloramphenicol concentrations in cerebrospinal fluid (CSF) are approximately 50% of corresponding plasma concentrations. Chloramphenicol can also cross the placental barrier in pregnant animals and can diffuse into the milk of nursing animals.

Metabolism and Excretion

Chloramphenicol is metabolized by the liver after absorption into the systemic circulation. One of the largest drawbacks to chloramphenicol administration is the rapid metabolic clearance, producing short half-lives in many species and necessity for frequent administration. As shown in Table 36.1 for dogs, the short half-life translates to a need to be administered three times daily. In horses, because of rapid elimination rates, tissue fluid concentrations persisted for only 3 hours after IV administration of chloramphenicol sodium succinate (Brown et al., 1984). Phase II glucuronidation is the principal pathway for the hepatic biotransformation of chloramphenicol, with the principal metabolite being chloramphenicol glucuronide. A few hydrolysis products have also been identified. Cats excrete chloramphenicol more slowly than other animals, perhaps owing to the cat's deficiency in some glucuronidase enzymes. One report notes that 25% of the total dose of chloramphenicol is excreted in the urine in the active form in cats compared to 6% in dogs (Hird and Knifton, 1986). Most of the absorbed chloramphenicol (approximately 80%) is excreted into the urine as inactive metabolites via tubular secretion.

The effect of age on clearance of chloramphenicol is inconsistent. Calves showed differences compared to older cattle, but this is probably irrelevant because it should not be used in food animals (Burrows et al., 1984). Brumbaugh et al. (1983) found that in neonatal horses, elimination and Vd did not differ from adults. Bioavailability in foals was 83%, with an oral half-life of 2.54 hours.

Adverse Effects and Precautions

Bone marrow suppression has been the most important adverse effect associated with chloramphenicol administration to people. Bone marrow injury from chloramphenicol takes two forms (Yunis, 1988). The first type

Table 36.2 Selected serum pharmacokinetic parameters of chloramphenicol in animals

Species	Dose (mg/kg)	Route	Formulation	Half-life (hr)	Volume of distribution (l/kg)	Comments	Reference
Cats	22	IV	Base	5.1	2.36	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
Sheep	30	IV	Base	1.702	0.691		Dagorn et al., 1990
	30	SC	Base	17.93	NA		Dagorn et al., 1990
	30	IM	Base	2.71	NA		Dagorn et al., 1990
Adult swine	22	IV	Base	1.3	1.05	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
Piglets	25	IV	Base	12.7	0.9411	Normal piglets	Martin and Wiese, 1988
	25	IV	Base	17.2	0.9549	Colostrum-deprived piglets	Martin and Wiese, 1988
Goats	25	IV	Succinate	1.22	1.683	Nonfebrile animals	Kume and Garg, 1986
	25	IV	Succinate	1.29	1.962	Febrile animals	Kume and Garg, 1986
	25	IM	Succinate	1.46	3.019	Nonfebrile animals	Kume and Garg, 1986
	25	IM	Succinate	1.45	2.769	Febrile animals	Kume and Garg, 1986
	22	IV	Base	2.0	1.33	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
	10	IV	Succinate	1.47	0.312	Normal animals	Abdullah and Baggot, 1986
Goats	10	IV	Succinate	3.97	0.287	Starved animals	Abdullah and Baggot, 1986
	22	IV	Base	2.0	1.33	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
	10	IV	Succinate	1.47	0.312	Normal animals	Abdullah and Baggot, 1986
	10	IV	Succinate	3.97	0.287	Starved animals	Abdullah and Baggot, 1986
Cattle	40	IV	Base	2.81	0.351		Sanders et al., 1988
	90	IM	Base	1.345	NA	2 doses 48 hours apart	Sanders et al., 1988
	90	SC	Base	1.153	NA	2 doses 48 hours apart	Sanders et al., 1988
Calves	30	IV	Base	3.98	1.208	Age not reported; average weight 73 kg	Guillot and Sanders, 1991
1-day-old calves	25	IV	Base in PG vehicle	7.56	1.031		Burrows et al., 1983
7-day-old calves	25	IV	Base in PG vehicle	5.96	0.808		Burrows et al., 1983
14-day-old calves	25	IV	Base in PG vehicle	4.0	0.903		Burrows et al., 1983
28-day-old calves	25	IV	Base in PG vehicle	3.69	0.69		Burrows et al., 1983
9-month-old calves	25	IV	Base in PG vehicle	2.47	1.38		Burrows et al., 1983
Horses	22	IV	Base in PG vehicle	0.51–0.78	0.86–1.26		Varma et al., 1987
Ponies	22	IV	Base	0.9	1.02	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
Foals							
1 day old	25	IV	Succinate	5.29	1.1		Adamson et al., 1991
3 days old	25	IV	Succinate	1.35	0.759		Adamson et al., 1991
7 days old	25	IV	Succinate	0.61	0.491		Adamson et al., 1991
14 days old	25	IV	Succinate	0.51	0.426		Adamson et al., 1991
42 days old	25	IV	Succinate	0.34	0.362		Adamson et al., 1991
1–9 days old	50	IV	Succinate	0.95	1.6	After oral suspension administered oral, availability was 83% and half-life of 2.54 hours	Brumbaugh et al., 1983
Rabbits	100	IV	Succinate	1.1575	NA		Mayers et al., 1991
Chickens	20	IV	Succinate	8.32	0.24	Normal animals	Atef et al., 1991a
	20	IV	Succinate	26.21	0.3	*E. coli*-infected animals	Atef et al., 1991a
	20	IM	Succinate	7.84	0.44		Atef et al., 1991a
	20	PO	Succinate	8.26	0.41		Atef et al., 1991a

NA, data not available; PG, propylene glycol.

is the most common and involves a dose-related suppression of the bone marrow precursor erythroid series. This toxicosis is reversible. The evidence suggests that this bone marrow suppression is the result of suppression of mitochondrial protein synthesis in bone marrow cells. In bone marrow cells there is vacuolation of the myeloid and erythroid series precursor cells, and inhibition of erythroid and granulocytic colony forming units (IARC, 1976, 1990). In both the dog and the cat, dose-related bone marrow suppression is possible. However, signs of toxicity reverse when chloramphenicol therapy is discontinued.

The second type of bone marrow toxicity, aplastic anemia, has been described in people but not in animals. In people, it is rare and independent of dose and treatment duration. This toxicity results in bone marrow aplasia, chiefly characterized by a profound and persistent pancytopenia. Aplastic anemia occurs in approximately 1 : 10,000 to 1 : 45,000 humans who receive chloramphenicol. It appears that the para-nitro group of the chloramphenicol molecule is responsible for this more serious form of bone marrow toxicity (Figure 36.1). The para-nitro group undergoes nitroreduction, leading to the production of nitrosochloramphenicol and other toxic intermediates, which trigger the stem cell damage in humans (IARC, 1976, 1990; Yunis, 1988). Modification of the molecule to eliminate the para-nitro group to produce either thiamfenicol or florfenicol reduces the risk of chloramphenicol-associated aplastic anemia (Figure 36.1).

Chloramphenicol-induced aplastic anemia in humans is important from a food-animal residue standpoint. If chloramphenicol is used to treat infections in food animals, it is possible that low concentrations of chloramphenicol in milk, meat, and other edible tissues from the animals will be consumed by people and cause aplastic anemia in susceptible individuals. Chloramphenicol residues have been known to persist for prolonged periods in food animals (Korsrud et al., 1987). Even though the amount consumed may be small, reaction that may occur in people are not dependent on dose. Thus, there is a public health risk for individuals consuming these products. For this and other reasons, the use of chloramphenicol in food-producing animals has been banned in the United States. The hazards of using chloramphenicol in food animals have also been reviewed by others (Settepani, 1984; Lacey, 1984).

Other adverse effects caused by chloramphenicol in animals have been observed since the drug is used more in recent years to treat drug-resistant bacteria. Young animals and cats are sensitive to intoxication due to impaired glucuronidation pathways. Cats given 60 and 120 mg/kg/day PO every 8 hours for 21 and 14 days (respectively) showed clinical signs of depression, dehydration, reduced fluid intake, weight loss, emesis, and diarrhea. Bone marrow hypoplasia was also documented in addition to pancytopenia (Watson, 1980). Other investigators (Penny et al., 1967, 1970) administered to cats 50 mg/kg/day IM, with the cats showing marked depression and inappetence by day 7 of administration, severe bone marrow changes by day 14, and becoming extremely ill by day 21.

Gastrointestinal (GI) disturbances are among the most common in dogs (Short et al., 2014; Bryan et al., 2012). Dogs may exhibit events such as vomiting, diarrhea, anorexia, drooling, gagging or any combination of these clinical signs. They tend to resolve when the medication is discontinued. These effects may be related to GI injury as oral administration resulted in intestinal mucosal damage and diarrhea in calves and reduced glucose absorption (Rollin et al., 1986). Another issue that has emerged now that chloramphenicol is used more often is peripheral neuropathy. In one report, this adverse effect was almost as common as the gastrointestinal problems (Short et al., 2014). Signs that may be observed are ataxia, rear limb weakness, trouble standing, and/or jumping, or trembling on the back legs. This is believed to be caused by peripheral neuropathy, but the cellular mechanism is unknown. One microscopic study in three dogs (Kuroda et al., 1974) identified degenerative changes in peripheral nerves. Larger-breed dogs may be at higher risk for the neuropathy based on anecdotal accounts. Most dogs recover when the medication is discontinued.

Drug Interactions

Chloramphenicol is an inhibitor of the cytochrome P450 (CYP) drug-metabolizing enzymes. Enzyme specificity has not been fully characterized, but there is evidence that one of the enzymes inhibited is canine CYP2B11 (Martinez et al., 2013). Among the drugs substrates that may be affected by inhibition from chloramphenicol are anticonvulsants (e.g., phenobarbital), propofol, benzodiazepines, and other anesthetics. For example, chloramphenicol significantly affected metabolism of methadone in dogs (KuKanich and KuKanich, 2015).

Clinical Use

The FDA-approved dose for dogs is 55.5 mg/kg oral, every 6 hours. This dose is likely to increase the risk of adverse effects and the most common clinical dose, based on pharmacokinetic studies and evidence of efficacy is 50 mg/kg every 8 hours oral. Chloramphenicol has been used for treatment of a wide range of susceptible microbial infections, including those caused by salmonellae, intracellular and extracellular bacteria, rickettsiae, and mycoplasmata; infections of the eyes and CNS; and infections due to anaerobic organisms (IARC,

1976, 1990). One of the reasons for its popularity has been the high lipophilicity. Chloramphenicol readily penetrates cells, making it active against intracellular bacteria. It can penetrate tissues that otherwise are difficult to treat, such as the CNS, which is further discussed below. Chloramphenicol was shown in one study to be equally effective for treatment of Rocky Mountain spotted fever in dogs as enrofloxacin and tetracyclines (Breitschwerdt et al., 1990). Chloramphenicol has been used to treat infections caused by *Staphylococcus* spp., streptococci, *Brucella* spp., *Pasteurella* spp., *E. coli, Proteus* spp., *Salmonella* spp., *Bacillus anthracis, Arcanobacterium pyogenes, Erysipelothrix rhusiopathiae,* and *Klebsiella pneumoniae*. It is consistently active against anaerobic bacteria.

Chloramphenicol has been suggested for treatment of infections of the CNS (encephalitis, meningitis) because it is able to cross the inflamed or uninflamed blood–brain barrier and attain therapeutic concentrations in the CSF and the brain. Despite the rationale for this use, some experts have suggested that since chloramphenicol is merely bacteriostatic against gram-negative pathogens, and there is a lack of phagocytes or immunoglobulins in CSF, chloramphenicol is not well suited to treat serious infections of the CNS (Rahal and Simberkoff, 1979).

Chloramphenicol attains high concentrations in the eye when given systemically or after topical application on the cornea and is useful in treating susceptible bacterial conjunctivitis, panophthalmitis, endophthalmitis, and bacterial diseases of the cornea (Conner and Gupta, 1973). Topical formulations are not as readily available owing to the risk of aplastic anemia (discussed in Section Adverse Effects and Precautions), which can be caused by topical exposure.

Chloramphenicol has been used to treat bacterial infections of the respiratory tract because it may have better penetration across the blood–bronchus barrier into respiratory secretions and respiratory lining fluid than more polar or less lipophilic antibiotics. Respiratory infections in horses, dogs, cats, and exotic animals are among the uses of oral chloramphenicol.

Chloramphenicol is among the few drugs that can be administered orally to horses with safety. It achieves moderate systemic absorption of 21–40% (Gronwall et al., 1986) and has no serious adverse effects on the equine digestive system. For treatment in horses, tablets or capsules are mixed with vehicles such as molasses or corn syrup to facilitate oral administration. Chloramphenicol has been administered to horses for respiratory infections, pleuritis, CNS infections, and joint infections. Because there are other active drugs available, it is most often considered as an option when bacteria are resistant to other antibiotics. The recommended doses are based on specific pharmacokinetic studies in adults and foals (Gronwall et al., 1986; Brumbaugh et al., 1983).

Chloramphenicol has been administered to exotic animals, especially reptiles and amphibians, to treat a variety of infections (Clark et al., 1985); although florfenicol (see Section Florfenicol) has taken over some of these uses. Chloramphenicol administration in 15 species of birds was examined, and the investigators concluded that after IM injections of 50 mg/kg, chloramphenicol would produce adequate concentrations to treat susceptible bacteria for 8–12 hours, except in pigeons, macaws, and conures because effective concentrations could not be achieved in these birds (Clark et al., 1982). However, oral absorption was poor, and this route of administration was discouraged for all birds.

Chloramphenicol Derivatives

The ban on the use chloramphenicol in food-producing animals in the mid-1980s left a gap in the veterinarian's armamentarium of effective antimicrobial drugs. Because the idiosyncratic aplastic anemia is associated with the presence of the para-nitro group on the chloramphenicol molecule, attempts were made to modify the chloramphenicol structure to simultaneously retain chloramphenicol's broad spectrum of antimicrobial activity and eliminate the induction of aplastic anemia in people. Compounds synthesized in attempts to accomplish this goal are thiamphenicol and florfenicol. Thiamfenicol is not approved in the United States and will be discussed here only briefly. However, florfenicol has been approved for use in pigs, cattle, and fish (in some countries) and has been effective for treatment of various infections, especially bovine respiratory disease in cattle and swine respiratory disease in pigs intended for human consumption.

Thiamphenicol

Thiamphenicol is a semisynthetic structural analog of chloramphenicol. It is not available in North America; therefore, all of the experience has been learned from research studies or use in other countries. The major structural difference between chloramphenicol and thiamphenicol is that the para-nitrophenol group has been replaced by the methyl sulfonyl moiety (Figure 36.1). The mechanism of action and spectrum are similar to that of chloramphenicol. However, its structural differences result in different pharmacokinetic properties and decreased potency. Thiamphenicol is more water soluble and less lipid soluble and therefore diffuses more slowly through lipid membranes. It is not metabolized to a

significant extent in the liver (Ferrari et al., 1974) and most of the dose is excreted in the urine as the unchanged active compound (Yunis, 1988; Lavy et al., 1991a; Gamez et al., 1992). Resistance to thiamphenicol is also similar to that of chloramphenicol, with bacterial acetylation of the thiamphenicol molecule, but at a rate approximately 50% less than that of chloramphenicol.

Few pharmacokinetic studies have been performed on food-producing animals, but thiamphenicol pharmacokinetics has been studied in veal calves (Gamez et al., 1992) and lactating goats (Lavy et al., 1991a). Both studies found thiamphenicol to have a large volume of distribution and rapidly eliminated in the urine. In dogs, thiamfenicol had a half-life of 1.7 hours and a volume of distribution of 0.66 l/kg (Castells et al., 1998). In dogs the injection of thiamfenicol was well absorbed, with availability of 97%, but the terminal half-life was longer (5.6 hours), suggesting slow release from the injection site.

Thiamphenicol is considered to be less toxic than chloramphenicol, yet a reversible bone marrow suppression has been reported in humans. However, millions of people have been treated with thiamphenicol in countries in which it is approved, with no reports linking its use to aplastic anemia (Adams et al., 1987). In a thiamphenicol toxicity study in rabbits (Kaltwasser et al., 1974), no changes attributed to thiamphenicol in erythrocyte, reticulocyte, or plasma iron parameters were noted after long-term treatments of up to 90 mg/kg/day.

Florfenicol

Florfenicol is structurally related to thiamphenicol; however, florfenicol contains fluorine at the 3′ carbon position (Figure 36.1). The fluorine molecule substitution at this position also reduces the number of sites available for bacterial acetylation reactions to occur, possibly making the antibiotic more resistant to bacterial inactivation. Florfenicol is as potent, or more potent, than either chloramphenicol or thiamphenicol against many organisms in vitro. The study by Maaland et al. (2015) using *Staphylococcus pseudintermedius* and *E. coli* isolates, showed that there were fewer nonwild-type isolates for florfenicol than chloramphenicol. There were no nonwild-type isolates of *Staph. pseudintermedius* for florfenicol. These results agree with previous studies that show that resistance mechanisms may be less likely for florfenicol compared to chloramphenicol (Schwarz et al., 2004).

The list of susceptible bacteria for florfenicol is the same as listed previously for chloramphenicol. However, as mentioned earlier, some bacteria resistant to chloramphenicol because of inactivation by acetylation may be susceptible to florfenicol. The CLSI (CLSI, 2015) quality control ranges of MIC for florfenicol are 2–8 μg/ml (Marshall et al., 1996). *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* are several-fold more susceptible in vitro than bacteria of the Enterobacteriaceae, with MIC_{90} for *Pasteurella* and *Histophilus somni* in the range of 0.5–1.0 μg/ml. The CLSI breakpoints are ≤2 μg/ml (susceptible) 4 μg/ml (intermediate), and ≥8 μg/ml (resistant) for isolates of bovine and swine respiratory disease (CLSI, 2015). By comparison, the susceptible breakpoint for chloramphenicol is ≤8 μg/ml for organisms other than streptococci, and ≤4 μg/ml for *Streptococcus* spp. Florfenicol breakpoints for other animals and other bacteria have not been determined.

The advantage of florfenicol for administration to food animals is that it lacks the para-nitro group (Figure 36.1) that could contribute to the induction of aplastic anemia associated with chloramphenicol use in humans. Therefore, if residues were to occur in animals treated with florfenicol, no dangerous public health risk would ensue. However, it is possible that florfenicol can still produce a dose-related form of reversible bone marrow suppression with prolonged use or high doses. Such reactions have not been reported from routine use of florfenicol in animals, probably because it is rarely used for a long time. However, in one clinical account in a zoo animal, high doses induced bone marrow suppression (Tuttle et al., 2006).

Pharmacokinetics

The pharmacokinetics of florfenicol are summarized in Table 36.3.

Absorption: Studies in calves and other species listed in Table 36.3 show that absorption from all routes after either IM or SC injection is generally high. Oral absorption in horses and dogs was also high. After IM or SC injection, the absorption is slow and often prolonged in animals because of the vehicle in the solution. Therefore, the IM or SC injection produces a *flip-flop* effect, in which the terminal half-life is determined by the slow absorption. This can be seen in Table 36.3 in which the IM and SC half-life is generally much longer than the IV half-life. This effect prolongs the duration of effective concentrations.

Distribution: Like chloramphenicol, florfenicol has a wide distribution in most tissues of the body (Adams et al., 1987), with a volume of distribution approaching 1 l/kg (0.7–0.9 l/kg in most cattle studies) (Table 36.3). Protein binding is low in cattle with values of 13–19% reported (Bretzlaff et al., 1987; Lobell et al., 1994), but in other studies it was 5% at high concentrations and negligible at low concentrations in cattle plasma (Foster et al., 2016). Protein binding has not been reported for other animals. High concentrations are detected in the kidney,

Table 36.3 Selected pharmacokinetic parameters of florfenicol in animals

Species	Dose mg/kg	Half-life (h)	Absorption (%) (%)	Volume of distribution (l/kg)	C_{max} (µg/ml)	Reference
Cats	22 (all routes)	4 (IV)	–	0.61	57 (IV)	Papich, 1999
		7.8 (oral)	>100 (oral)	–	28 (oral)	
		5.6 (IM)	>100 (IM)	–	20 (IM)	
Dogs	20 mg (all routes)	2 (IV)	28 (SC)	1.2	44 (IV)	
		18 (SC)	16 (IM)	–	0.93 (SC)	
		9 (IM)	–	–	1.64 (IM)	
		3 (oral)	>100 (oral)	–	17 (oral)	
	20 IV	1.11	–	1.45	–	Park et al., 2008
	20 oral	1.24	95.43	–	6.18	Park et al., 2008
Sea turtles	20 IM, IV	2–7.8 h (IM)	67 (IM)	10–60	0.5–0.8 (IM)	Stamper et al., 2003
Sharks	40 IM	269	ND	2.9	10.5	Zimmerman et al., 2006
Fish (red pacu)	10 IM	4.25	ND	5.69	1.09	Lewbart et al., 2005
Horses	22 IV	1.83	81 (IM)	0.72	4 (IM)	McKellar and Varma, 1996
	22 oral	ND	83 (oral)	ND	13 (oral)	
Cattle	50 IV	3.2	ND	0.67	157.7	Bretzlaff et al., 1987
Feeder calves	20 IV	2.65	ND	0.88	73	Lobell et al., 1994
	20 IM	18.3	78.5	ND	3.07	Lobell et al., 1994
Veal calves	22 oral	ND	88	ND	11.3	Varma et al., 1986
	22 IV	2.87	ND	0.78	66	Varma et al., 1986
	11 IV	3.71	ND	0.91	26.35	Adams et al., 1987
	11 oral	3.7	89	ND	5.7	Adams et al., 1987
Angus calves	40 SC	27.5	ND	ND	6.04	Sidhu et al., 2014
Dairy calves	40 SC	28.44	ND	ND	3.42	Foster et al., 2016
Lactating cows	20 IV	2.9	–	0.35	12.4	Soback et al., 1995
	20 IM	12	38	ND	3.6	
Alpaca	20 IM	17.59	ND	11.07 (Vd/F)	4.31	Holmes et al., 2012
	40 SC	99.67	ND	55.74 (Vd/F)	1.95	
Llama	20 IV	2.2	63	0.96	–	Pentecost et al., 2013
	20 IM	11.6	–	–	3.2	
Camels	20 IV	1.49	69.2	0.89	–	Ali et al., 2003
	20 IM	2.52	–	–	0.84	
Sheep	20 IV	1.31	65.8	0.69	–	
	20 IM	2.28	–	–	1.04	
Goats	20 IV	1.19	60.9	0.57		
	20 IM	2.12	–	–	1.21	

Route of administration used is listed with dose. C_{max} is the maximal concentration after administration. ND, not determined. Empty cells indicate that the parameter is not relevant for the route administered.

urine, bile, and small intestine, but less penetration in the CSF, brain, and aqueous humor of the eye than that attained with chloramphenicol. Concentrations in brain and CSF are one-quarter and one half the corresponding concentrations in plasma, respectively. Although in one study the distribution into CSF was only 46% relative to plasma, these levels were high enough to produce concentrations in CSF of cattle to inhibit *Histophilus somni* for over 20 hours (De Craene et al., 1997). Florfenicol reached high concentrations in the synovial fluid of cattle following regional limb perfusion (Gilliam et al., 2008). Florfenicol also penetrated well into the milk of lactating goats after IV and IM administration; therefore, it could be used to treat microbial infections in the udder of lactating animals (Lavy et al., 1991b) if appropriate milk withdrawal times are available. The penetration into interstitial fluid was almost 98%, a reflection of the low protein binding. In the same study, the penetration into the pulmonary epithelial lining fluid of calves was over 200% and produced high concentrations for treating respiratory infections (Foster et al., 2016).

Metabolism and Elimination: The elimination half-life in various species and for different routes is shown in Table 36.3. Most of the dose administered to cattle is excreted as the parent drug (64%) in the urine, with the remaining excreted as urinary metabolites. Florfenicol amine is the metabolite that persists longest in tissues of cattle and is used as the marker residue for withdrawal determination.

Pharmacokinetic studies in other species: As seen in Table 36.3, there have been pharmacokinetic studies in small animals and some exotic and zoo animals. As in cattle, it has rapid clearance when injected IV, but more prolonged terminal half-life if administered by other routes.

Pharmacokinetic–pharmacodynamic properties: The Pharmacokinetic–pharmacodynamic (PK-PD) properties for florfenicol may be dependent on the organism studied. There is evidence for a bactericidal effect against some bacteria but not others (Maaland et al., 2015; Sidhu et al., 2014). Florfenicol may be bactericidal against isolates of *Staph. pseudintermedius but not E. coli* (Maaland et al., 2015). Against bovine isolates of *Mannheimia haemolytica* and Pasteurella multocida, florfenicol appears to have bactericidal activity. It is not established if the parameter for predicting efficacy is time above MIC (T>MIC) or area-under-the-curve (AUC) / MIC. It is likely that as for most other protein synthesis inhibiting agents with little or no postantibiotic effect, the AUC/MIC would be the best parameter to predict clinical efficacy. In a study in calves (Sidhu et al., 2014) a AUC/MIC ratio of approximately 18–27 was identified from modeling experiments.

Clinical Use

Florfenicol is available in three injectable solutions: 300 mg/ml solution for injection (Nuflor, or Nuflor gold), and a solution combined with flunixin meglumine (Resflor Gold, 300 mg/ml florfenicol plus 16.5 mg/ml flunixin). There is also a solution to be added to drinking water for swine (23 mg/ml, to be added as 400 mg per gallon) and a Type A medicated feed. For fish there is a 500 gram per kilogram premix for fish (Aqua-Flor).

Cattle and pigs: Several studies in cattle have been conducted to support the use of florfenicol for treating bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*. Florfenicol penetrates well into the epithelial lining fluid of the airways of cattle (Foster et al., 2016), and has been effective for treating undifferentiated bovine respiratory disease (Hoar et al., 1998; Jim et al., 1999). There are two doses approved for cattle: 20 mg/kg SC or IM, given every 48 hours and injected in the neck, or a single dose for cattle at 40 mg/kg SC in the neck. Florfenicol is also approved for treatment of bovine interdigital phlegmon (foot rot, acute interdigital necrobacillosis, infectious pododermatitis) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*.

Florfenicol has been effective in calves for treating experimentally induced infections and naturally occurring infectious bovine keratoconjunctivitis (Dueger et al., 1999; Angelos et al., 2000). In the naturally occurring case, florfenicol was administered one dose SC at 40 mg/kg or IM two doses 48 hours apart at 20 mg/kg. Concentrations persist in CSF for a long enough period after administration of 20 mg/kg in cattle that concentrations are above MIC of susceptible pathogens for at least 20 hours.

The swine dose is 15 mg/kg IM in the neck, every 48 hours. For pigs, florfenicol can also be added to the feed (182 g per ton of feed), or drinking water (400 mg per gallon) for 5 days for the control of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis,* and *Bordetella bronchiseptica*.

Small animals: Although some pharmacokinetic studies have been conducted in small animals and exotic animals, there are no reports of efficacy. Pharmacokinetic studies in reptiles and dogs suggest that frequent dosing with high doses would be necessary to maintain plasma concentrations above the MIC for susceptible organisms throughout the dosing interval. By contrast, florfenicol solution in cats was absorbed well from both routes, with peak concentrations of 20 μg/ml and 27 μg/ml after IM and oral dose, respectively. Absorption was high from both routes (greater than 100% from IM and oral). The half-life was 5.6 hours and 7.8 hours for IM and oral dose, respectively. In cats, florfenicol produced inhibitory concentrations for 12 hours. These studies indicate that a dose of 22 mg/kg administered every 12 hours orally or parenterally would be adequate to produce sustained plasma concentrations for treatment of susceptible bacteria. Safety of these doses for small animals have not been established.

Topical forms: Two topical formulations were approved by the FDA in 2014 and 2015. The product Osurnia® contains 10 mg florfenicol, 10 mg terbinafine, and 1 mg betamethasone acetate per ml in a gel for topical administration. The product Claro™ contains florfenicol 15 mg/ml, terbinafine 13.3 mg/ml, and mometasone 2 mg/ml. The indication for each product is for the treatment of otitis externa in dogs associated with susceptible strains of bacteria (*Staphylococcus pseudintermedius*) and yeast (*Malassezia pachydermatis*).

Use in fish: Florfenicol has been administered orally for treatment of infections in captive fish and is approved in some countries for this use (Aqua-Flor®). Florfenicol is effective for treating bacterial infections in fish, such as trout and salmon (Fukui et al., 1987). Florfenicol premix is approved in some countries for treatment of furunculosis in salmon caused by *Aeromonas salmonicida*. Florfenicol has been administered orally for treatment of furunculosis caused by susceptible strains of *Aeromonas*

salmonicida in captive fish and is approved in other countries. The premix (Aqua-Flor®) is approved for use in catfish and salmonids at a dose of 10 mg/kg for 10 days to treat susceptible fish pathogens.

The typical dose for fish is 10 mg/kg. At this dose, the half-life is 12–16 hours in most fish, with a peak (C_{max}) concentration of approximately 3–10 µg/ml (Pinault et al., 1997; Martinsen et al., 1993; Horsberg et al., 1994). Red pacu had a shorter half-life and lower C_{max} after 10 mg/kg IM (Lewbart et al., 2005). In sharks at a dose of 40 mg/kg IM, florfenicol produced effective levels for 120 hours (Zimmerman et al., 2006). Admnistration to sharks every 3–5 days will produce concentrations in a therapeutic range.

Other species: Pharmacokinetic studies in horses show that florfenicol has longer half-life than chloramphenicol, good distribution, and good absorption. However, in experimental horses there were consistent loose stools and elevated bilirubin (McKellar and Varma, 1996). Until additional studies are available to establish safe doses, florfenicol cannot be recommended for horses. Adverse effects were observed in alpaca at repeated doses of 40 mg/kg SC, but not in llamas administered a single dose of 20 mg/kg (Holmes et al., 2012; Pentecost et al., 2013). The authors recommended a dose in llamas of 20 mg/kg once daily, IM. After a single injection of 20 mg/kg (IM and IV) there were no adverse effects identified clinically or in blood tests in camels, sheep, or goats (Ali et al., 2003). However, the low concentrations achieved and the short half-life in the study by Ali et al., raises questions about whether or not it would be clinically effective at this dose.

In snakes (boas), the half-life was 28 hours from IM injection. It was estimated that 50 mg/kg once daily for boas is the best dose to produce therapeutic plasma concentrations, even though efficacy studies are not available. In sea turtles the clearance was rapid (60–100 ml/kg/h) and the half-life was short (Stamper et al., 2003). It was concluded that florfenicol was not a practical drug for treatment of infections in sea turtles.

Adverse Effects

Effect of florfenicol on bovine pregnancy, reproduction, and lactation have not been determined. Mild diarrhea and elevated bilirubin have been reported from administration to horses (McKellar and Varma, 1996). Reversible, dose-related bone marrow suppression is possible but not reported, except for a reaction reported in a zoo animal that was mentioned previously (Tuttle et al., 2006). In cattle, diarrhea and decreased feed consumption have been observed, which are transient. A local tissue reaction from IM or SC injection is possible. When toxic overdoses were administered to calves (200 mg/kg) there was marked anorexia, decrease in body weight, ketosis, and elevated liver enzymes. In dogs administered high doses for prolonged periods there was CNS vacuolation, hematopoietic toxicity, and renal tubule dilation. Adverse effects were detected in alpacas after a dose of 40 mg/kg SC that may be related to the prolonged concentrations at this dose (Table 36.3) (Holmes et al., 2012). These effects included significant hematological abnormalities and protein decrease. Caution should be used if administering to these animals for repeated doses.

Regulatory Information

The tolerance for florfenicol is 3.7 ppm for florfenicol amine (the marker residue) in liver and 0.3 ppm in muscle. Withdrawal time for use in salmon is 12 days. Withdrawal time for oral administration to pigs in feed is 13 days and for administration in water 16 days. After injection to cattle, the withdrawal time for slaughter is 28 days if injected at a dose of 20 mg/kg IM (36 days in Canada). If injected at a dose of 40 mg/kg SC, the withdrawal time for slaughter is 38 days. A formulation with different excipients (Nuflor Gold) has a withdrawal time of 44 days in cattle when injected at 40 mg/kg SC, once. More than 10 ml should not be injected at each site to avoid tissue reactions and injections should be administered in the neck (both SC and IM). Do not administer to dairy cows older than 20 months, to calves under 1 month of age, or to calves on an all-milk diet.

Macrolide Antibiotics

Source and Chemistry

The macrolide antibiotics are a group of structurally similar compounds, most of which are derived from various species of *Streptomyces* soil-borne bacteria. Chemically, all the drugs in this group are classified as macrocyclic lactones, with members containing 12–20 atoms of carbon in the lactone ring structure (Table 36.4). Attached to this lactone ring are various combinations of deoxy sugars held to the lactone ring

Table 36.4 Macrolides used in animals

Drug	Structure	Brand Name
Erythromycin	14 member ring	Gallimycin (and generic)
Tilmicosin	16 member ring	Micotil, Pulmotil
Azithromycin	15 member ring	Zithromax (human drug)
Gamithromycin	15 member ring	Zactran
Tylosin	16 member ring	Tylan
Tildipirosin	16 member ring	Zuprevo
Tulathromycin	15 member ring	Draxxin

by glycosidic linkages. Since erythromycin's discovery in the early 1950s from the soil organism *Streptomyces erythreus,* numerous other macrolides have been isolated or synthesized from the parent molecule erythromycin, including tylosin, roxithromycin, erythromycylamine, tilmicosin, dirithromycin, azithromycin, tulathromycin, clarithromycin, spiramycin, and flurithromycin (Kirst and Sides, 1989). The most common agents used clinically in veterinary medicine are listed in Table 36.4.

Erythromycin and tylosin (Figure 36.2) are traditional macrolides. The newer drugs (Table 36.4 and Figure 36.3) were either developed for use in people (azithromycin) or specifically for use in cattle and/or pigs. These newer drugs differ from erythromycin in that they have a prolonged action and can be administered intermittently, or for just a single injection. Other macrolides such as oleandomycin and carbomycin have been used as feed additives for growth promotion in food animals and will not be discussed in detail here.

Macrolides are composed of a macrolactone ring of 12, 14, 15, or 16 carbon atoms, substituted with sugar moieties. Erythromycin, the prototype of this class, consists of a 14-atom polyhydroxylactone erythronolide ring and the two sugars clandinose and desosamine. Similarly, tylosin is composed of a 16-atom lactone ring (a tylonolide) to which three sugars – mycinose, mycaminose, and mycarose – are attached (Wilson, 1984; Kirst et al., 1982). Azithromycin is the first drug in the group of azalides, which are semisynthetic derivatives of erythromycin (Lode et al., 1996). Azithromycin has a 15-member ring structure. Tulathromycin, resembles azithromycin (Figure 36.3) and also has a 15-member ring structure. The structure of the newer agents includes basic nitrogen groups. All macrolides are weak bases, with pK_a ranges

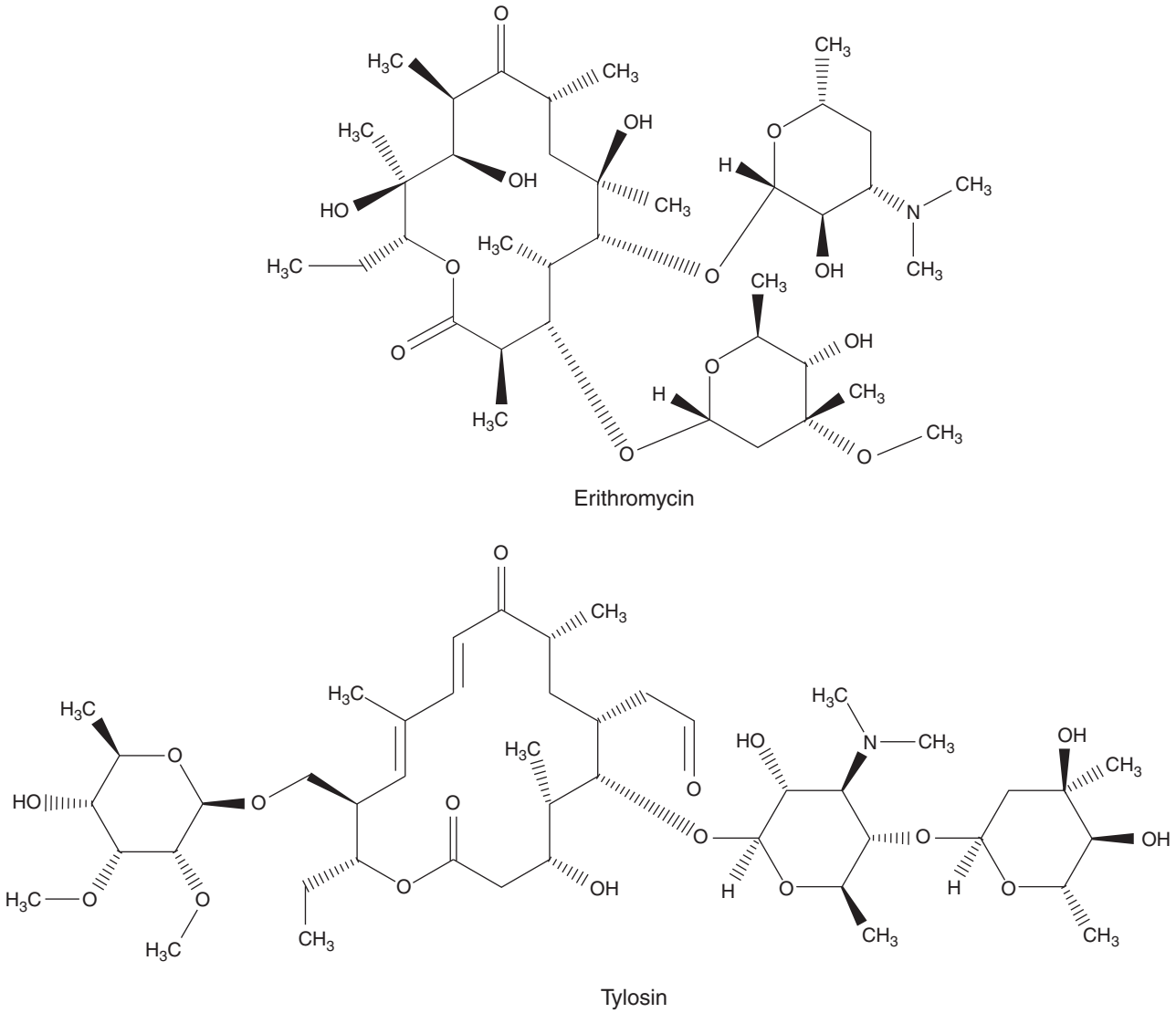

Figure 36.2 The chemical structures of erythromycin (top) and tylosin (bottom).

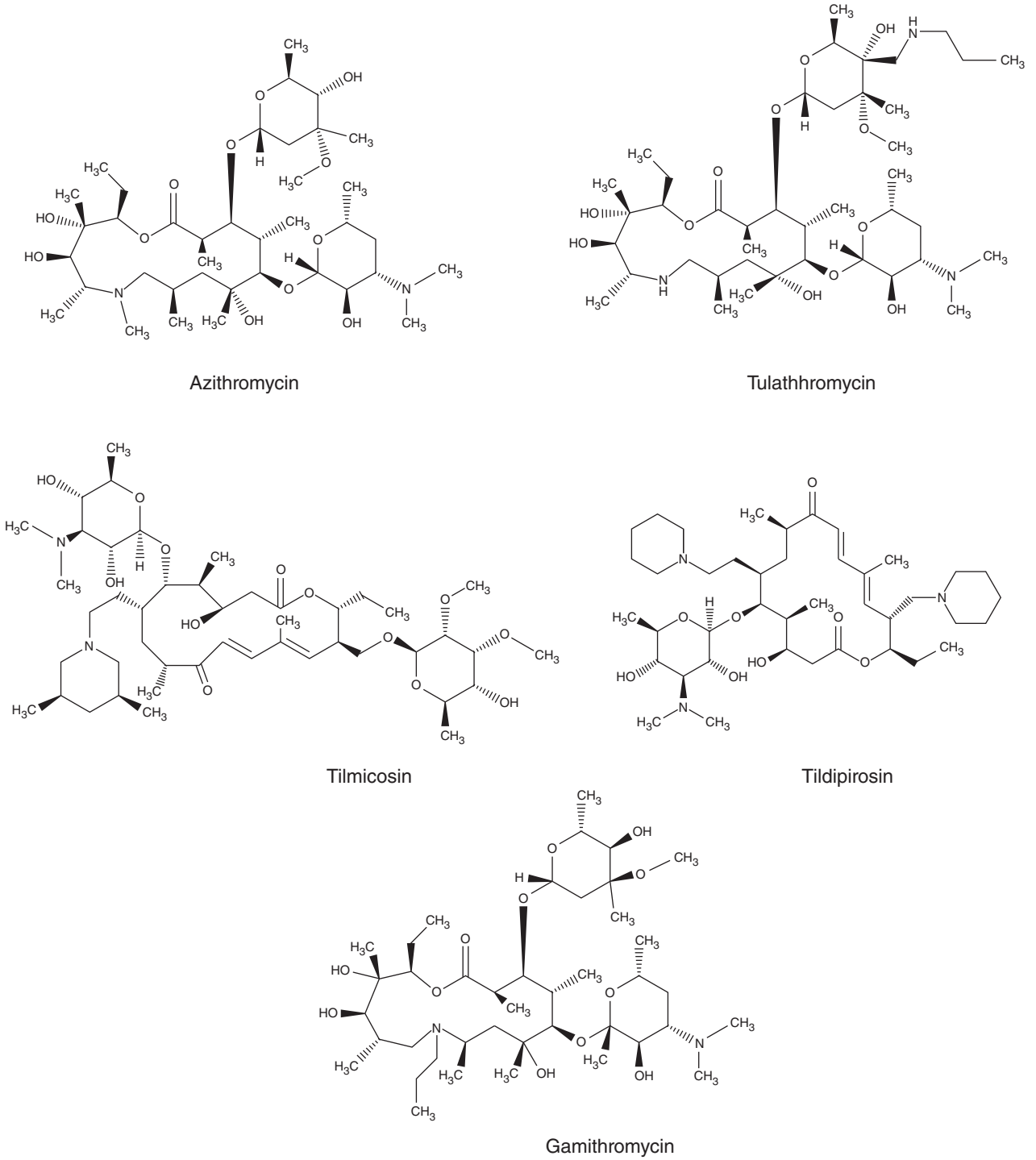

Figure 36.3 The chemical structures of new macrolide derivatives: azithromycin, tulathromycin, tildipirosin, tilmicosin, and gamithromycin. Azithromycin is a human drug and the others are approved for use in cattle and/or pigs.

from 6 to 9. They can have either two (di-basic) or three (tri-basic) nitrogen groups. For example, tulathromycin has three (tribasic) and has been referred to as a "trimilide". Tildipirosin also has three basic groups. The basic nitrogen groups on these newer agents (Figure 36.3) produces a positive charge in an acidic environment below their pKa. The positive charge increases the affinity for intracellular sites caused by ion trapping. It is this property that gives these agents such large intracellular distribution and a high volume of distribution.

Effect on Antibacterial Activity: The basic nature of the macrolides also influences the antibacterial activity. The in vitro antibacterial activity of macrolides varies according to the pH of the culture medium and the pH at the site of infection. Subsequently, antibacterial activity decreases in acid pH and increases in alkaline conditions. A change in pH of only 0.2 units has been known to change the MIC by a full Log-2 dilution step. These changes become important when CO_2 is used during culture incubation because it lowers the pH of the medium.

Mechanism of Action

The antibacterial action of macrolides is produced by an inhibition of protein synthesis by binding to the 50S ribosomal subunit at the 23S rRNA site of prokaryote organisms. The binding site on the ribosome is near, but not identical to, that of chloramphenicol, and antagonism of effect is possible if macrolides are administered with chloramphenicol. By binding to the 50S ribosomal site, macrolides cause dissociation of peptidyl-tRNA from the ribosomes during the elongation phase, which disrupts addition of new peptide bonds and thus prevents synthesis of new proteins. Although macrolides can bind to mitochondrial ribosomes, they are unable to cross the mitochondrial membrane (in contrast to chloramphenicol) and do not produce bone marrow suppression in mammals. Macrolides do not bind to mammalian ribosomes, making them a relatively safe group of drugs for veterinary use.

Although most authors have listed macrolides as bacteriostatic at therapeutic concentrations (Wilson, 1984), this effect may be both bacterial species, concentration, and drug dependent. For example, the agents developed for pigs and cattle (Table 36.4) can have bactericidal activity against bovine and swine respiratory pathogens, including *Mannheimia haemolytica, Pasteurella multocida*, *Histophilus somni,* and *Actinobacillus pleuropneumoniae*. As discussed above, the antimicrobial action of macrolides is enhanced by a high pH (Sabath et al., 1968), with the optimum antibacterial effect at a pH of 8. Therefore, in an acidic environment, such as in an abscess, necrotic tissue, or urine, the antibacterial activity is suppressed.

Resistance Mechanisms

Resistance to macrolides is usually plasmid mediated, but modification of ribosomes may occur through chromosomal mutation. Resistance can occur from: (i) decreased entry into bacteria (most common with the gram-negative organisms), and also mediated by *mef*-efflux genes, (ii) synthesis of bacterial enzymes that hydrolyze the drug, and (iii) modification of target (the ribosome in this instance) by RNA methylation or RNA sequence changes through mutation. The ribosomal attenuation (most common mechanism) involves methylation of the 50S drug receptor site. This resistance, coded by *erm* genes (e.g., *erm*A, *erm*B, *erm*C) may also lead to cross-resistance with other antibiotics that preferentially bind to these sites, such as other macrolides and lincosamides (Wilson, 1984). Resistance to erythromycin in animals in several microorganisms has been discussed in more detail elsewhere (Maguire et al., 1989; Dutta and Devriese, 1981, 1982a, 1982b; Leclercq and Courvalin, 1991; Devriese and Dutta, 1984). In small animals with staphylococcal infections, resistance was more likely if antibiotics had previously been prescribed, especially in cases of recurrent pyoderma (Lloyd et al., 1996; Medleau et al., 1986; Noble and Kent, 1992). Seven to 22% of small animal isolates of *Staphylococcus* spp. can exhibit resistance (depending on region and use), and in some countries this has remained relatively stable at around 22–24%.

Spectrum of Activity

Erythromycin is mainly effective against gram-positive organisms such as streptococci, staphylococci, including staphylococci that may be resistant to β-lactams because of β-lactamase synthesis or modification of the penicillin-binding protein target. Other organisms that show in vitro susceptibility to macrolides include *Mycoplasma, Arcanobacterium, Erysipelothrix, Bordetella,* and *Bartonella*. Although the spectrum favors the gram-positive group, a few gram-negative bacteria are susceptible, especially *Pasteurella* spp. Activity against anaerobic bacteria is only moderate. Most other gram-negative bacteria, such as those of the Enterobacteriaceae or *Pseudomonas* spp., are resistant. Azithromycin is an exception among the macrolides and can exhibit more activity against gram-negative bacteria. In addition to better activity against Enterobacteriaceae, it also has activity against other enteric pathogens, such as *Campylobacter* spp. (Gordillo et al., 1993).

The activity of newer derivatives (Table 36.4 and Figure 36.3) is similar to that of erythromycin, but these agents have better activity against respiratory pathogens, including *Pasteurella, Mannheimia haemolytica,* and *Histophilus somni,* which corresponds to their use for treating respiratory tract infections in pigs and cattle. These macrolides also have activity against *Mycoplasma* spp. The activity of macrolides against *Rhodococus equi* is important for treating lung infections caused by this organism in horses, particularly foals (Jacks et al., 2003). The MIC values for 32 antimicrobials against *Rhodococcus equi* were compared by Riesenberg et al. (2014). In decreasing order of activity, the MIC_{90} values for clarithromycin, erythromycin, azithromycin, tilmicosin, tylosin, tulathromycin were 0.06, 0.5, 1, 32, 32, and 64 μg/ml, respectively. In a separate study,

Table 36.5 Interpretive criteria for macrolides and lincosamides used in animals. Source: Data from CLSI, 2015.

Drug	Species	MIC Interpretive Category (µg/ml) S	I	R	Comments / Pathogens
Erythromycin	Humans	≤ 0.5	1-4	≥ 8	Human *Staphylococcus*. No criteria available for animals.
	Humans	≤ 0.25	0.5	≥ 1	Human *Streptococcus*. No criteria available for animals.
Azithromycin	Humans	≤ 0.5	1	≥ 2	Human only. No criteria available for animals.
Tilmicosin	Bovine	≤ 8	16	≥ 32	Bovine respiratory pathogens.
	Swine	≤ 16	–	≥ 32	Swine respiratory pathogens
Tulathromycin	Bovine	≤ 16	32	≥ 64	Bovine respiratory pathogens (*Mannheimia, Pasteurella*, Histophilus)
	Swine	≤ 16	32	≥ 64	*Pasteurella multocida, Bordetella bronchiseptica*
		≤ 64	–	–	*Actinobacillus pleuropneumoniae*
Tildipirosin	Bovine	≤ 8	16	≥ 32	Bovine respiratory pathogens (*Histophilus, Pasteurella*)
		≤ 4	8	≥ 16	Bovine respiratory pathogens (*Mannheimia*)
	Swine	≤ 8	–	–	*Bordetella bronchiseptica*
		≤ 4	–	–	*Pasteurella multocida*
		≤ 16	–	–	*Actinobacillus pleuropneumoniae*
Gamithromycin	Bovine	≤ 4	8	≥ 16	Bovine respiratory pathogens. (*Mannheimia, Pasteurella*, Histophilus)
Clindamycin	Canine	≤ 0.5	1-2	≥ 4	*Staphylococcus* spp. *Streptococcus* spp.

S, susceptible; R, resistant; I, intermediate.

gamithromycin had a MIC_{90} of 1 µg/ml (Berghaus et al., 2012). Therefore, all macrolides are not alike with respect to their activity against this equine pathogen.

The CLSI (CLSI, 2015) standards and interpretive categories are shown in Table 36.5 for susceptibility testing. As shown in this table, drugs in this class vary in their potency and activity against various pathogens. Because of their targeted use, most of this data were generated for respiratory pathogens (Watts, 1999).

Pharmacokinetic–Pharmacodynamic Properties

The PK/PD properties of macrolides have been more difficult to define compared to other antimicrobials. Plasma concentrations, especially for the newer, long-acting agents (Table 36.6) are often below the MIC of pathogens for most, or all of the dose interval. Therefore, parameters such as peak above MIC (C_{max}/MC) or time above MIC (T>MIC) cannot be used to predict efficacy. Efficacy is probably best attributed to the concentrations at the site of infection – the pulmonary epithelial lining fluid (PELF). Although concentrations in the PELF have been reported from many studies in research animals (Giguère and Tessman, 2011; Villarino et al., 2013), this fluid is difficult to sample routinely in clinical cases. Therefore, the plasma drug concentration has been examined as a surrogate marker for efficacy from administration of macrolides and their derivatives. The parameter that is best suited to predict efficacy is the AUC of the plasma drug concentration to MIC (AUC/MIC) ratio (Drusano, 2005; Toutain et al., 2017). It has been suggested that the high concentrations in inflammatory cells deliver high concentrations to infected site and this effect is responsible for the efficacy in infected tissues. However, as summarized in the review by Villarino et al. (2013), citing studies by their laboratory and others, the concentrations contained in these cells are not likely high enough to contribute significantly to the PK/PD properties of the macrolides. This view was supported by the analysis by Toutain and associates (Toutain et al., 2017).

The magnitude of the AUC/MIC target has emerged from laboratory animal studies and analysis of clinical results. The azithromycin free serum AUC/ MIC for a 24-hour interval (AUC_{24}) ratio of >25 has been suggested from a mouse thigh infection model reported by Craig et al. (2002). However, this ratio is likely lower in non-neutropenic animals and Rodvold et al. (2003) suggested plasma AUC_{24}/ MIC ratios of at least 10 in nonneutropenic hosts with pneumonia, and higher AUC_{24}/ MIC ratios of 25–30 for worst case scenarios with experimental neutropenia. The study by Sevillano et al. (2006) showed that a serum azithromycin AUC_{24}/MIC ratio of approximately 27 was adequate for sustained bactericidal activity against susceptible strains. The analysis by Toutain and colleagues cited earlier supported a AUC_{24}/MIC value of approximately 24 for tulathromycin treatment of pneumonia in calves (Toutain et al., 2017).

Because most of the newer macrolides have long half-lives and produce concentrations for much longer than 24 hours, some investigators have considered the AUC values for the duration of treatment, rather than limited to 24 hours. In the study by Muto et al. (2011), using this approach, the AUC/MIC ratio >5 for azithromycin was associated with successful clinical outcome. If one examines the pharmacokinetics from studies of the

Table 36.6 Pharmacokinetics of macrolide derivatives, including azalides, in animals

Drug	Species	Dose (mg/kg)	Half-life (hour)[a]	Volume of distribution (Vd) (l/kg)[b]	Peak Concentration (C_{max}) (µg/ml)	Reference
Azithromycin	Foals	10 IV	20.3	18.6	–	Jacks et al., 2003
	Foals	10 oral	44 (MRT)	–	0.57	
	Foals	5 IV	16	12.4	–	Davis et al., 2002
	Foals	10 oral	18.32	–	0.72	
	Dogs	20 oral	35	–	4.2	Shepard and Falkner, 1990
	Dogs	20 IV	29	12	–	
	Cats	5 IV	35	23	–	Hunter et al., 1995
	Cats	10 oral	30 (MRT)	–	0.97	
Clarithromycin	Foals	10 oral	4.81	–	0.92	Jacks et al., 2002
	Foals	7.5 IV	5.4	10.4	–	Womble et al., 2006
	Foals	7.5 oral	7.1 (MRT)	–	0.52	
	Foals[c]	7.5 oral	6.11	–	0.614	Peters et al., 2011
	Foals[c]	7.5 oral	7.17	–	0.61	Peters et al., 2012
	Foals[c]	7.5 oral	5.62	–	0.27	Berlin et al., 2016
	Foals[c]	7.5 IV	5.91	–	1.71	Berlin et al., 2016
	Dogs	10 IV	3.9	1.4	–	Vilmànyi et al., 1996
	Dogs	10 (oral tablet)	4.6-5.9	–	3.3-3.5	
Gamithromycin	Calves	6 SC	62	–	0.43	Giguére et al., 2011
	Calves	6 SC	50.8	24.9	0.75	Huang et al., 2010
	Feeder cattle	6 SC	52.8 (MRT)	97.4 (V/F)	0.13	DeDonder et al., 2016
	Foals	6 IM	39.1	–	0.33	Berghaus et al., 2012
	Sheep	6 SC	34.5	35.5 (V/F)	0.573	Kellermann et al., 2014
Tildipirosin	Calves	4 SC	210	–	0.71	Menge et al., 2012
	Calves	6 IV	204	49.4	0.64	
	Pigs	4 IM	106	–	0.895	Rose et al., 2013
Tulathromycin	Calves	2.5 SC	81.24	–	1.82	Foster et al., 2016
	Calves	2.5	79.5	11	0.39	Villarino et al., 2013[d]
	Pigs	2.5	73.95	28.9	0.75	
	Horses	2.5	122	–	0.57	
	Goats	2.5	76.7	29.3	0.94	
	Goats	2.5 SC	45.7	7.0 (V/F)	1.0	Romanet et al., 2012
	Sheep	2.5 SC	118.4	–	3.6	Washburn et al., 2014
Tilmicosin	Calves	20 SC	33.34	5.5 (V/F)	3.48	Foster et al., 2016
	Dairy cow	10 IV bolus	0.76	2.14	–	Ziv et al., 1995
	Dairy cow	10 SC	4.18	–	0.13	
	Beef cattle	10 IV	28	28.2	1.56	Lombardi et al., 2011
	Beef cattle, light	10 SC	31.15	–	0.71	
	Beef cattle, light	20 SC	31.13	–	1.06	
	Beef cattle, heavy	10 SC	30.83	–	0.55	
	Beef cattle, heavy	20 SC	30.98	–	1.07	
	Pigs	20 oral	25.3	–	1.19	Shen et al., 2005
	Pigs	40 oral	20.7	–	2.03	

[a]For some studies, half-life was not reported and mean residence time (MRT) is listed in the table.
[b]For some studies, the volume of distribution was from a nonintravenous route and is listed as Vd/F.
[c]Data listed for clarithromycin by Peters et al., (2011, 2012), and Berlin et al., (2016) is without coadministration of rifampin. Administration of rifampin with clarithromycin lowers the concentration by 70% to over 90%. These values are provided in detail in those papers.
[d]Data referenced for Villarino et al. (2013) are an average of multiple studies reported in their paper.

long-acting macrolides listed in Table 36.6, AUC/MIC ratios of 5–10 for gamithromycin, azithromycin, and tildipirosin have been associated with clinical success. Tildipirosin, which has a longer half-life, produces a ratio of approximately 24. The study by DeDonder et al. (2016) showed that for gamithromycin administration to feeder cattle with bovine respiratory disease associated with *Mannheimia haemolytica* or *Pasteurella multocida*, the AUC infinity/MIC ratio associated with clinical success in these cases was 3.49 (*Mannheimia haemolytica*) and 3.21 (*Pasteurella multocida*).

Immunomodulatory Effects

Virulence properties of some bacteria may be inhibited by macrolides at concentrations that are less than the MIC required for inhibition or killing. This property, along with the effects on immunomodulation described in more detail below, may explain many of the benefits

of macrolides for treating pneumonia (Kovaleva et al., 2012).

The macrolides, particularly the ones that concentrate in immune cells (Figure 36.3) have multiple immunomodulatory effects that likely contribute to the therapeutic response in respiratory infections, and perhaps other diseases. Beneficial effects may be produced by enhanced degranulation and apoptosis of neutrophils and inhibition of inflammatory cytokine production. Enhanced macrophage functions may also may help clear infections faster. These properties have been studied for azithromycin (Parnham et al., 2014) and for the veterinary drugs tilmicosin and tulathromycin (Chin et al., 2000; Duquette et al., 2015). As these reviews and studies point out, there is likely an immunomodulatory effect of these agents that contributes to the therapeutic benefits that is independent of the direct effect on bacteria. These drugs have been known to produce therapeutic benefits in patients even when the bacteria have MIC values in the range that is considered resistant, and above achievable concentrations in plasma or the epithelial lining fluid of the respiratory tract. The authors of these studies are careful to point out that the effect of macrolides is best termed *immunomodulatory* rather than immunosuppressive, which implies that it may modify or regulate functions of the immune system without impairing normal responses to combat bacterial infection (Kanoh and Rubin, 2010). According to Kanoh and Rubin (2010) the 14- and 15- membered macrolides exert these immunomodulatory effects, but not 16-membered macrolides. However, tilmicosin, a 16-membered ring macrolide (Figure 36.3, Table 36.4) also exhibits some of these properties (Chin et al., 2000).

As reviewed by others (Parnham et al., 2014; Kanoh and Rubin, 2010; Giamarellos-Bourboulis, 2008) macrolide antibiotics have shown inhibition in models of inflammation. These mechanisms include inhibition of inflammatory cells, improved epithelial function, and attenuated expression of inflammatory mediators. These properties have led to the recommended use of macrolides to treat some inflammatory diseases in people (Giamarellos-Bourboulis, 2008; Kanoh and Rubin, 2010). Because macrolides attain high concentrations in leukocytes and remain for a long time, primarily in lysosomes, there may be a biphasic response whereby initially the macrolides activate neutrophils and produce an initial burst that increases antibacterial activity, followed by suppression of inflammatory mediators and increased neutrophil apoptosis.

Pharmacokinetics

Absorption and Distribution

Erythromycin pharmacokinetics has been studied in most animals and in humans; some of these parameters are shown in Table 36.7. Tylosin pharmacokinetics in some animals is shown in Table 36.8. Oral erythromycin is discussed in more detail below in the section on erythromycin and summarized in Table 36.7. Tylosin has good absorption from the gastrointestinal tract, and no enteric coating is required to maintain the stability of the compound in the stomach. It is widely distributed to basically the same tissues as described for erythromycin, metabolized by the liver, and excreted via the bile and feces.

Oral absorption of most of the newer macrolides (Table 36.4) is not an issue because these are injected. For the others, oral absorption is moderate, but depends on the species. Azithromycin was absorbed 56% (Jacks et al., 2003) or 39% (Davis et al., 2002) in foals. It was absorbed 52% in cats (Hunter et al., 1995) and 97% in dogs (Shepard and Falkner, 1990). Clarithromycin was absorbed 57% in foals (Womble et al., 2006) and 71% in foals (Vilmànyi et al., 1996).

SC or IM injections of erythromycin can be painful and irritating; therefore, the PO route is preferred whenever possible. The only formulations that can be given IV are the glucoptate and lactobionate forms, because these are the only forms soluble in aqueous solution.

Pharmacokinetics of the newer macrolides are shown in Table 36.6. These properties have been extensively studied in many domestic animals. They have also been examined in several exotic and zoo animals (not shown in the table). These macrolides are characterized by much longer terminal half-lives compared to erythromycin. These long half-lives allow for intermittent administration (for example every-other-day in foals), and a single administration for gamithromycin, tildipirosin, tilmicosin, and tulathromycin in pigs and cattle. The volumes of distribution are very large, often in excess of 10 l/kg, and as high as 49 l/kg. The high volume of distribution is attributed to the extensive distribution to intracellular sites in tissues. Many of the studies referenced in Table 36.6 also reported tissue concentrations.

Macrolides tend to concentrate in some cells because the basic drug is trapped in cells that are more acidic than plasma. Tissue concentrations for macrolides, especially the newer azalides (Figure 36.3) are higher than serum concentrations. High concentrations have been documented in the respiratory tract, where PELF, bronchoalveolar fluid (BAL), leukocycte, and alveolar macrophages are many fold higher than plasma drug concentrations, often exceeding 100 times plasma concentrations. It is likely that the high concentrations in the epithelial lining fluid of the airways contribute significantly to the clinical efficacy for preventing and treating pneumonia.

Protein binding for macrolides is low to moderate, with values of 18–30% for most species. Protein binding in

Table 36.7 Selected serum pharmacokinetic parameters of erythromycin in animals

Species	Dose (mg/kg)	Route	Formulation	Half-life (hour)	Volume of distribution (l/kg)	Reference
Cows	12.5	IV	Base	3.16	0.789	Baggot and Gingerich, 1976
Calves	15	IV	Base in PG vehicle	2.91	0.835	Burrows et al., 1989
	15	IM	Base in PG vehicle	5.81	NA	Burrows et al., 1989
	15	SC	Base in PG vehicle	26.87	NA	Burrows et al., 1989
	30	IV	Base in PG vehicle	4.09	1.596	Burrows et al., 1989
	30	IM	Base in PG vehicle	11.85	NA	Burrows et al., 1989
	30	SC	Base in PG vehicle	18.3	NA	Burrows et al., 1989
Mice	10	IV	Base	0.65	3.6	Duthu, 1985
Rats	25	IV	Base	1.27	9.3	Duthu, 1985
Rabbits	10	IV	Base	1.4	6.8	Duthu, 1985
Dogs	10	IV	Base	1.72	2.7	Duthu, 1985
Dogs	25	Oral	Extolate	2.92	ND	Albarellos et al., 2008
Dogs	10	IV	Lactobionate	1.35	4.8	Albarellos et al., 2008
Pigs (1 day)	10	IV	Base	3.0	0.68	Kinoshita et al., 1995
Pigs (3 day)	10	IV	Base	1.43	3.28	Kinoshita et al., 1995
Foal	25	Oral	Ethylsuccinate	1.52	ND	Lakritz et al., 2002
	25	Oral	Base	1.8, 1.3	ND	Lakritz et al., 2000a
	25	Oral	Estolate	0.52	ND	Lakritz et al., 2000b
	25	Oral	Phosphate	0.81	ND	Lakritz et al., 2000b
	10	IV	Lactiobionate	1.18	0.91	Lakritz et al., 2000a
	10	IV	Lactiobionate	0.97	3.52	Lakritz et al., 1999
	25	Oral	Base	17.6 (MRT)	ND	Lakritz et al., 1999
	5	IV	Gluceptate	1.0	3.7	Prescott et al., 1983
	20	IV	Gluceptate	1.1	7.2	Prescott et al., 1983
Horse (Mares)	5	IV	Gluceptate	1.0	2.3	Prescott et al., 1983
Horse	25	Oral	Estolate	2.42	ND	Ewing et al., 1994
	37.5	Oral	Estolate	6.2	ND	Ewing et al., 1994
	25	Oral	Phosphate	2.49	ND	Ewing et al., 1994
	37.5	Oral	Phosphate	1.68	ND	Ewing et al., 1994
	25	Oral	Stearate	1.84	ND	Ewing et al., 1994
	25	Oral	Ethylsuccinate	4.76	ND	Ewing et al., 1994
Cats	15	PO	Ethylsuccinate	Not detectable concentrations		Albarellos et al., 2011
Cats	4	IV	Lactobionate	0.75	2.34	Albarellos et al., 2011

NA, data not available; PG, propylene glycol, MRT, mean residence time.

Table 36.8 Selected serum pharmacokinetic parameters of tylosin in animals

Species	Dose (mg/kg)	Route	Half-life ($t_{1/2\beta}$) (hour)	Vd (l/kg)	Reference
Dogs (Beagle)	10	IV	0.9	1.7	Weisel et al., 1977
Ewes	20	IV	2.05	NA	Ziv and Sulman, 1973b
Goats	15	IV	3.04	1.7	Atef et al., 1991b
Cows	12.5	IV	1.62	1.1	Gingerich et al., 1977
Cows	20	IV	2.14	NA	Gingerich et al., 1977
Calves					
2 days old	10	IV	2.32	7	Burrows et al., 1983
1 week old	10	IV	1.26	7.2	Burrows et al., 1983
2 week old	10	IV	0.95	11.1	Burrows et al., 1983
4 week old	10	IV	1.53	9	Burrows et al., 1983
>6 week old	10	IV	1.07	11.1	Burrows et al., 1983
Avians (emus)	15	IV	4.7	NA	Locke et al., 1982
Avians (quail, pigeons, cranes)	15	IM	1.2	NA	Locke et al., 1982

NA, data not available.

some species may be predominantly to the α-1-acid glycoprotein, rather than albumin (Kinoshita et al., 1995).

Metabolism and Excretion

Metabolism of erythromycin is by hepatic microsomal enzymes. For the other drugs, the metabolic pathway has been less well characterized. In people, most of azithromycin is excreted in the feces. Low concentrations are anticipated in the urine and kidney dysfunction is not expected to produce an appreciable effect on the elimination half-life in the body. Because of the low urine concentrations, lower activity at acidic pH, and spectrum of activity that does not favor Enterobacteriaceae, these agents are not a good choice for treating urinary tract infections (Sabath et al., 1968).

Adverse Effects and Precautions

When humans are treated with erythromycin, many adverse effects are reported, which include nausea and vomiting (oral forms), fever, skin eruptions, cholestatic hepatitis, elevated serum aspartate aminotransferase, epigastric distress, and transient auditory impairment, among many other side effects. Cholestatic hepatitis is associated with the estolate ester, with the symptoms starting 10–20 days after beginning therapy and ending a few days after the cessation of therapy. Cholestasis associated with erythromycin use in humans is considered to be a hypersensitivity reaction (Sande and Mandell, 1990a). In animals, these effects are less common. However, regurgitation and/or vomiting has been commonly reported in small animals, especially dogs after oral administration of erythromycin. In one report, erythromycin was the drug that most frequently caused side effects after oral dosing in dogs (Kunkle et al., 1995). Stimulation of gastrointestinal motility may play a role in small-animal vomiting (discussed in Section Clinical Use of Erythromycin to Modify Gastrointestinal Motility). Erythromycin has been associated with producing diarrhea in horses (Papich, 2003). Although these reactions in the horse may limit its use in some patients, it is still frequently used to treat infections in horses, especially in the foal. Hyperthermia (febrile syndrome) has been observed in foals associated with erythromycin treatment (Stratton-Phelps et al., 2000), which was accompanied in some foals by diarrhea and respiratory distress. Other adverse effects are discussed for specific agents in each section.

Drug Interactions

Erythromycin is a well-known hepatic microsomal enzyme inhibitor. Erythromycin is both a substrate and an inhibitor for the cytochrome P450 enzymes, which is the enzyme system that is most often involved in drug metabolism. As an inhibitor of the cytochrome P450 enzymes, it may inhibit metabolism of drugs such as theophylline, cyclosporine, digoxin, and warfarin. Concentrations of these drugs may increase when animals receive erythromycin, resulting in a potentiation of the pharmacological effect or toxicity.

Effects of the other macrolides on animal drug metabolism has not been investigated in much detail. Azithromycin can also be an enzyme inhibitor in people, but less so than for erythromycin. Nevertheless, concurrent use of any of the drugs in this class with other drugs that have a narrow therapeutic index should be monitored.

Erythromycin

Erythromycin is inactivated in the stomach due to gastric acidity, which is the reason that other formulations, such as erythromycin estolate or stearate forms or enteric-coated formulations, are used. These modified forms have better bioavailability owing to decreased destruction of erythromycin in the acidic environment of the stomach. Crushed tablets of enteric-coated preparations are substantially degraded in the stomach or are metabolized in the intestine wall and are not recommended for oral administration to animals. The presence of food in the stomach also tends to decrease absorption of erythromycin in most species, including the dog (Wilson, 1984; Eriksson et al., 1990). Erythromycin salts (erythromycin-stearate and erythromycin-phosphate) dissociate in the intestine and are absorbed as the active drug. Erythromycin esters (erythromycin-ethylsuccinate and erythromycin-estolate) are absorbed as the esters and hydrolyzed in the body to release active drug. There is no proven difference among these formulations as to which is the most favorable in most animals. However, in horses, it was shown that the salt forms (erythromycin phosphate or erythromycin stearate) are preferred for oral administration (Ewing et al., 1994) because they provided the most favorable blood concentrations. A series of studies by Lakritz et al. (1999, 2000a, 2000b, 2002) examined the absorption of various oral formulations in foals. The ethylsuccinate form was poorly absorbed, but the phosphate, estolate, and microencapsulated forms were better absorbed (16%, 14.7%, and 26%, respectively). Absorption was better in foals when food was withheld.

There are also oral formulations intended to be added to the feed or drinking water to treat infections for poultry. Examples of these preparations are erythromycin thiocynate premix and erythromycin phosphate powder (Ery-Mycin). Veterinary forms of erythromycin injectable (e.g., Erythro-100 and Gallimycin-100) are 100 mg/ml formulations intended for IM injection only; they should not be administered SC or IV. Doses of

erythromycin are listed in Table 36.7. Erythromycin and other macrolide antibiotics are sometimes used as a penicillin alternative when penicillins have either failed or when there is allergy to penicillins. Infections treated by erythromycin include those caused by *Staphylococcus* spp., *Streptococcus* spp., *Arcanobacterium* spp., *Clostridium* spp., *Listeria* spp., *Bacillus* spp., *Erysipelothrix* spp., *Histophilus*, *Brucella* spp., *Fusobacterium* spp., *Pasteurella* spp., *Borrelia* spp., and *Mycoplasma* spp. Erythromycin has also been used as a treatment for undifferentiated bovine respiratory disease and for pig infections caused by *Erysipelothrix* and for pig respiratory infections caused by *Streptococcus* and *Pasteurella*. In poultry, erythromycin is used for treatment of respiratory infections caused by *Mycoplasma*. In foals, erythromycin is used, in combination with rifampin for treatment of pneumonia caused by *Rhodococcus equi*. However, there is some evidence that erythromycin administered alone may be equally efficacious. For this use in horses, azithromycin or clarithromycin have become more common (see Sections Azithromycin and Clarithromycin).

In small animals, erythromycin has been used to treat pyoderma caused by staphylococci (Noli and Boothe, 1999), respiratory infections caused by *Mycoplasma,* and diarrhea caused by *Campylobacter* organisms. However, because of pharmacokinetic studies in dogs and cats, inadequate oral absorption and need for frequent IV administration limits the practical use. When treating *Campylobacter,* erythromycin stopped the shedding but did not eliminate the organism. Respiratory infections have sometimes been treated with erythromycin, but other drugs (e.g., azithromycin) have become more common because of better spectrum, longer half-life, and fewer adverse gastrointestinal effects. Experience in cats has been very limited. Based on a pharmacokinetic study (Albarellos et al., 2011), the IV administration to cats had a very short half-life and effective concentrations sustained for only 1.5 hours. Intramuscular injections in cats produced pain at the injection site and would not be a practical route for repeated injections. Erythromycin in the ethylsuccinate form as tablets or oral suspension did not produce measureable serum concentrations (15 mg/kg) after oral administration to cats (Albarellos et al., 2011). In dogs the ethylsuccinate and estolate oral formulations were poorly absorbed (Albarellos et al., 2008). The half-life was also short and would require frequent administration. These finding raises doubt about the oral use of these formulations for treatment in dogs or cats.

Clinical Use of Erythromycin to Modify Gastrointestinal Motility

Although some nausea from oral erythromycin is possible, most of this effect is believed to be related to a drug-induced increase in gastrointestinal motility. This mechanism appears to be related to an increase in activation of motilin receptors, via release of endogenous motilin, or via cholinergic mechanisms in the upper gastrointestinal tract (Hall and Washabau, 1997; Lester et al., 1998). At small doses (1 mg/kg) erythromycin has been considered for use as a motility-stimulating drug in animals. In calves, administration of erythromycin, tylosin, or tilmicosin increased the rate of abomasal emptying, with erythromycin (8.8 mg/kg IM) producing the most significant effect (Nouri and Constable, 2007; Nouri et al., 2008; Wittek and Constable, 2005). In calves, these drugs increase the abomasal emptying rate and erythromycin (10 mg/kg IM) has been used to improve postoperative abomasal rate in dairy cows undergoing surgical correction of left abomasal displacement (Wittek et al., 2008). These properties of erythromycin are discussed in more detail in Chapter 46. Although 14-membered macrolides appear to have the most profound effect on the gastrointestinal tract (Table 36.4), there is also an effect from 16-membered macrolides such as tylosin and tilmicosin (Nouri and Constable, 2007).

Regulatory Considerations

Erythromycin has a 6-day withdrawal time when used according to label in cattle in the United States. Erythromycin added to feed or water for poultry has a withdrawal time of 1–2 days; the specific product label should be consulted for the exact withdrawal time. In the United States, erythromycin should not be administered to lactating dairy cattle because macrolides concentrate in the milk for a long time after treatment. However, Canadian labeling lists a milk withholding time of 72 hours after a dose of 2.2–4.4 mg/kg.

Tylosin

Pharmacokinetic data for tylosin are listed in Table 36.8. Tylosin has been used therapeutically to treat "pinkeye" (*Moraxella bovis*) in cattle; respiratory tract infections; swine dysentery; pleuropneumonia due to *Haemophilus parahemolyticus;* and other infections in cats, chickens (Ose and Tonkinson, 1985), quail (Jones et al., 1976), and turkeys (Wilson, 1984). Tylosin has been used more extensively as a feed additive in food-producing animals, such as swine, cattle, and chickens, among others (Wilson, 1984). Tylosin phosphate premix has been added to feed for cattle, pigs, or poultry, and tylosin tartrate (Tylan soluble) for the drinking water of poultry.

Residues from tylosin have been discussed in other papers (Knothe, 1977a, 1977b; Anderson et al., 1966). After administration to cattle there is a 21- and 14-day withdrawal time for slaughter for cattle and pigs, respectively. Tylosin concentrates in milk for a long time after administration and should not be administered to lactating dairy cattle. Specific product information should be

consulted for withholding times when tylosin is administered in feed or water to pigs or poultry because withdrawal times can vary from 0 to 5 days, depending on the use.

Tylosin has also been used to treat diarrhea in dogs, which is discussed in Chapter 46 in more detail. This type of diarrhea has been characterized as "tylosin-responsive chronic diarrhea in dogs" (Westermarck et al., 2005). In these animals, tylosin has been effective at improving clinical signs that occur with or without organisms being identified.

Tilmicosin

Tilmicosin (Micotil) 300 mg/ml for SC injection (10–20 mg/kg) to cattle and sheep (10 mg/kg). Tilmicosin phosphate (Micotil 300) has been effective for treating bovine respiratory disease (Musser et al., 1996; Hoar et al., 1998; Jim et al., 1999). One study (Ose and Tonkinson, 1988) reports that 90% of the *Mannheimia haemolytica* and *Pasteurella multocida* isolates tested were susceptible to tilmicosin at concentrations of ≤6.25 μg/ml, and the drug was also active against *Mycoplasma,* including those from bovine isolates. Other organisms with in vitro susceptibility to tilmicosin include staphylococci and streptococci. Most gram-negative organisms other than those causing bovine respiratory disease are resistant.

Tilmicosin administered to calves with pneumonia were found to respond better when treated with 10 mg/kg SC tilmicosin than with a 20 mg/kg IM dose of oxytetracycline (Laven and Andrews, 1991). Like other macrolides, tilmicosin reaches high concentrations in lung tissues and this may account for efficacy treating bovine pneumonia (Gourlay et al. 1989). Resistance among cattle respiratory pathogens has been recognized (Musser et al., 1996), but treatment response in cattle with bovine respiratory disease was not associated with the MIC of the pathogens, and there was treatment success even when bacteria recovered had MIC values in the resistant range (McClary et al., 2011).

Tilmicosin also has been used as a prophylactic antibiotic (metaphylaxis) for administration to calves entering a feedlot situation. Tilmicosin reduced the incidence of pneumonia in susceptible calves when administered prophylactically as a single 10 mg/kg SC injection (Morck et al., 1993; Schumann et al., 1990). Tilmicosin used as a metaphylactic treatment in newly arrived feedlot calves reduced prevalence of bovine respiratory disease and improved growth of calves (Vogel et al., 1998).

The CLSI breakpoint (Table 36.5) for tilmicosin susceptibility is ≤8 μg/ml for cattle respiratory pathogens (*Mannheimia haemolytica*) and ≤16 μg/ml for swine respiratory disease pathogens. The currently approved dose is 10–20 mg/kg SC as a single treatment in cattle, and 10 mg/kg in sheep. After treatment with tilmicosin phosphate in cattle, there is a 28-day withdrawal time. Tilmicosin should not be administered to lactating dairy cattle because residues may persist in milk for more than 30 days.

Tilmicosin phosphate is approved for treatment of swine respiratory disease caused by *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*. This form (Pulmotil) is administered as a feed additive and has been shown to be effective for controlling pneumonia in swine (Moore et al., 1996). There is a 7-day withdrawal time for slaughter when administered to swine.

Tilmicosin has also been used for treatment of pasteurellosis in rabbits (McKay et al., 1996). Single doses of 25 mg/kg SC were an effective treatment for pasteurellosis in rabbits.

Adverse Reactions to Tilmicosin

Injections of tilmicosin to horses, goats, swine, or nonhuman primates can be fatal. The heart is the target of toxicity in animals, perhaps mediated via depletion of cardiac intracellular calcium, resulting in a negative inotropic effect (Main et al., 1996). Epinephrine worsens the cardiac toxicity in pigs, but dobutamine has alleviated the cardiac depression in dogs (Main et al., 1996). The effects of toxicity are increased heart rate, arrhythmia, and depressed contractility. Injected doses of 20 and 30 mg/kg to pigs caused death, but oral tilmicosin in pigs produces no toxic effects. In cattle, injected SC doses of 50 mg/kg caused myocardial toxicity; 150 mg/kg was lethal. Doses as low as 10 mg/kg administered by the IV route have caused cardiac toxicity as well (Ziv et al., 1995).

The risk of cardiac toxicity is particularly important for humans. There are warnings on the tilmicosin label that accidental injection into humans has caused death. Published reports (Veenhuizen et al., 2006) indicate that several people have died as a result of tilmicosin administration.

Tulathromycin

The injectable formulation of tulathromycin (Draxxin) is 100 mg/ml for use as a single SC injection at 2.5 mg/kg. Tulathromycin is an azalide derivative of erythromycin, with three charged nitrogen groups; therefore it has been called a *triamilide* (Evans, 2005). These charged groups may be important to increase the intracellular concentrations compared to other macrolides. It is approved for use in cattle and pigs and has occasionally been used in other species. In cattle and pigs it is used for treating respiratory infections (bovine respiratory disease and swine respiratory disease), for which the pathogens have been discussed earlier in this chapter and CLSI breakpoints are listed in Table 36.5. In

addition to these pathogens the label includes *Mycoplasma bovis* in this drug's indications. It is administered once (e.g., 2.5 mg/kg SC in cattle and IM in swine) and produces sustained drug concentrations in lung tissue for several days. It also is used to prevent bovine respiratory disease when administered to cattle (metaphylaxis) that are at risk for developing respiratory disease (Booker et al., 2007). Withdrawal times are 18 days for cattle and 5 days for swine. It has also been administered to other species and a dose of 2.5 mg/kg as a single injection has been used in other domestic food animals and zoo hoof stock.

There is limited evidence that tulathromycin may be useful for treating pulmonary infections in foals (Venner et al., 2007; Rutenberg et al., 2017). At 2.5 mg/kg IM once per week, it resolved pulmonary lesions and, except for diarrhea in some foals, was well tolerated. In a study of 240 foals endemic for infections caused by *Rhodococcus equi,* treated with 2.5 mg/kg IM, once per week, it was effective, but not as effective as the combination of azithromycin–rifampin (Rutenberg et al., 2017). However, it is less active than other agents against *Rhodococcus equi* and is not recommended (Giguère et al., 2011).

Clarithromycin

Clarithromycin (Biaxin®) is semisynthetically derived from erythromycin. It is primarily used in people because it is tolerated better than erythromycin, has a broader spectrum, and concentrates in leukocytes. Clarithromycin in combination with ranitidine and bismuth (Tritec®) is also used to treat *Helicobacter pylori* infections in people. In dogs, clarithromycin does not have pharmacokinetic features that are as favorable as those of azithromycin (the half-life is not as long) and the use is rare.

Most veterinary experience has been in foals, where clarithromycin has been investigated as a potential treatment for respiratory infections. It has more activity against *Rhodococcus equi* isolated from foals than other macrolides (Jacks et al., 2003; Riesenberg et al., 2014; Berghaus et al., 2013). In foals, clarithromycin is absorbed orally and has a half-life of 4–6 hours, depending on the study (Table 36.6). Not shown in Table 36.6 is the effect of coadministration of rifampin on clarithromycin concentrations in foals. Because of induction of enzymes and transporters, coadministration with rifampin decreases plasma drug concentrations by over 90%, which is discussed in more detail in Section Rifampin (Rifampicin).

The concentrations in the respiratory ELF, bronchial/alveolar epithelial cells, and BAL cells of foals is many fold higher than plasma drug concentrations – reaching levels that are over 30–40 times higher in the ELF and over 300–1800 times higher in BAL cells (Peters et al., 2011, 2012). However, the concentrations do not persist in tissues for as long as azithromycin (Suarez-Mier et al., 2007). Oral absorption in foals was 57% (Womble et al., 2006) and 41.5% (Berlin et al., 2016), compared to 70–75% in dogs. In foals, oral clarithromycin at a dose of 7.5 mg/kg every 12 hours produces concentrations sufficient for treatment of *Rhodococcus equi* infections (Jacks et al., 2002; Giguère et al., 2011). It has been more successful at this dose than azithromycin (Giguère et al., 2004). It is also metabolized in horses to 14-hydroxyclarithromycin, which is microbiologically active and contributes to the activity (Peters et al., 2011, 2012; Berlin et al., 2016).

Gamithromycin

Gamithromycin (Zactran) is a 15-membered ring (like azithromycin and tulathromycin). The mechanism of action is the same as other macrolides. Gamthromycin has a spectrum of activity that is limited to gram-positive bacteria and some gram-negative bacteria that cause respiratory diseases in cattle (e.g., *Mannheimia haemolytica, Mycoplasma,* and *Pasteurella multocida*). Susceptibility information and pharmacokinetics are listed in Tables 36.5 and 36.6. Like other long-acting macrolides, the half-life is long (Giguère et al., 2011, Table 36.6) with long persistence in lungs, which prolongs the drug concentration at the site of infection.

In cattle, several studies have established the efficacy of gamithromycin for treatment of bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* (Torres et al., 2013a, 2013b; Lechtenberg et al., 2011a, 2011b, 2011c, 2011d). In two studies it had a higher morbidity rate and retreatment rate than cattle treated with tulathromycin (Torres et al., 2013a, 2013b), but was otherwise equivalent. It is also effective for treating infections caused by *Mycoplasma bovis*. It also may be used for control of respiratory disease in beef and nonlactating dairy cattle at high risk of developing bovine respiratory disease (metaphylaxis) associated with *Mannheimia haemolytica* and *Pasteurella multocida*.

The MIC values for *Rhodococcus equi* are low and a IM dose of 6 mg/kg has been investigated for treatment of horses (Berghaus et al., 2012; Hildebrand et al., 2015). Even though it was effective in foals with bronchopneumonia, it had a higher incidence of adverse effects in foals that included colic and hind limb lameness. Almost 60% of treated foals showed reactions to the administration of gamithromycin.

Tildipirosin

Tildipirosin (Zuprevo) is a 16-membered ring (like tilmicosin) macrolide antimicrobial with three charged

nitrogen atoms (like tulathromycin), which is currently limited to the treatment and control/prevention of bovine respiratory disease and the treatment of swine respiratory disease, although it has also been used for the control/prevention of swine respiratory disease. The mechanism of action is the same as for other macrolides. Pharmacokinetics and other properties were reported by Menge et al. (2012) and Rose et al. (2013). Tildipirosin has a spectrum of activity that is limited to gram-positive bacteria and some gram-negative bacteria that cause respiratory diseases in cattle and pigs (e.g., *Mannheimia haemolytica, Mycoplasma, Pasteurella multocida, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica,* and *Haemophilus parasuis*). *Escherichia coli* and *Pseudomonas aeruginosa* are resistant. Some *Staphylococcus* spp. and *Streptococcus* spp. may be susceptible. There is evidence for bactericidal activity against *Mannheimia haemolytica*, bovine *Pasteurella multocida, Histophilus somni, Haemophilus parasuis*, and *Actinobacillus pleuropneumoniae*, but bacteriostatic activity against *Bordetella bronchiseptica*.

Pharmacokinetics in cattle (Table 36.6) shows that the half-life is long and bioavailability from injection in cattle is 79%. The volume of distribution is larger than other macrolide antibiotics, with a volume of distribution in cattle of 49 l/kg. The lung concentrations in cattle are over 150 times the plasma drug concentrations, with a half-life of 10 days. Bronchial fluid concentrations are approximately 40 times the plasma drug concentrations, with a half-life of 11 days.

In pigs, the plasma half-life is 106 hours (4.4 days), with a peak concentration of 0.9 μg/ml after IM injection of 4 mg/kg. The lung concentrations in pigs were approximately 80 times higher than plasma concentrations, with a half-life of 6.8 days. The bronchial fluid concentrations were 680 times higher than plasma drug concentrations at 5 days after injection. Tildipirosin, like other macrolides, exerts therapeutic benefits not solely explainable by antibacterial activity and may have immunomodulatory effects.

Tildipirosin has been approved for the treatment and control/prevention of bovine respiratory disease associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* and in some European countries for the treatment of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Bordetella bronchiseptica,* and *Haemophilus parasuis*.

Azithromycin

Azithromycin (Zithromax®) is the first drug in the class of azalides approved for people, but it also is administered frequently to small animals, exotic species, and horses. Azithromycin has better oral absorption, is better tolerated, has a much longer half-life (especially in tissues), and has a broader spectrum of activity than erythromycin.

Azithromycin is active against gram-positive aerobic bacteria (staphylococci and streptococci) and anaerobes. However, the activity against staphylococci is not as good as erythromycin. It has some activity against gram-negative bacteria such as *Haemophilus* but limited activity against enteric gram-negative bacteria, and ineffective against *Pseudomonas aeruginosa*. It has activity against many intracellular organisms, including *Chlamydophilia* (formerly called *Chlamydia*) and *Toxoplasma.* It is also active against mycobacteria and *Mycoplasma* (Lode et al., 1996).

The primary pharmacokinetic difference between azithromycin and erythromycin is the long half-life and high concentration in tissues. Pharmacokinetic properties are shown in Table 36.6. Azithromycin reaches high concentrations in tissues, particularly leukocytes, macrophages, and fibroblasts. The tissue concentration can be as much as 100 times serum concentrations and concentrations in leukocytes can be at least 200–300 times the concentrations in serum (Panteix et al., 1993). In cats, the serum half-life is 35 hours, tissue half-lives vary from 13 to 72 hours, and the volume of distribution is 23 l/kg (Hunter et al., 1995). In dogs, it also exhibits rapid uptake and persistent concentrations in tissues; the volume of distribution is 12 l/kg, and plasma and tissue half-lives are 29 and 90 hours, respectively (Shepard and Falkner, 1990). Oral absorption is high, with bioavailability values of 58% in cats (Hunter et al., 1995) and 97% in dogs (Shepard and Falkner, 1990). In people, azithromycin is absorbed much better on an empty stomach (Lode et al., 1996) but the effect of feeding on oral absorption has not been explored in cats or dogs.

There also is interest in administering azithromycin to horses (Davis et al., 2002; Jacks et al., 2002, 2003; Suarez-Mier et al., 2007). Davis et al. (2002) showed that oral absorption was 39% and had a plasma half-life of 18 hours in foals. More importantly, the drug persisted in leukocytes and alveolar macrophages for at least 120 hours after a single dose at concentrations greater than 5.0 μg/ml, with a half-life in leukocytes of over 49 hours. As in other species, concentrations in PMNs were over 200 times the plasma concentrations. It had a volume of distribution in horses of 12 l/kg, which probably accounts for the long persistence in inflammatory cells.

Like other long-acting macrolide antibiotics, azithromycin produce high concentrations in tissues and leukocytes, even after the plasma concentrations have declined below detectable levels (Girard et al., 1990). Intracellular stores of azithromycin in leukocytes also can serve as a mode of delivery of azithromycin to infected tissues, especially early abscesses, since the

leukocytes are attracted to these sites via chemotaxis (Girard et al., 1993). The immunomodulatory effects of azithromycin have been studied extensively and discussed previously in this chapter (Parnham et al., 2014). Beneficial effects of azithromycin are attributed, in part, to these effects on inflammatory cells and immune function.

Clinical Use of Azithromycin

Azithromycin has become popular for treating infections in dogs, cats, exotic animals, and birds. Results of treatment of intracellular infections caused by *Toxoplasma* spp. and *Mycobacterium* spp. have been conflicting in people and are not yet reported for animals. Because of the long half-life and persistence of drug in tissues, the regimen employed in people is to administer a dose once daily for 3–5 days. Thereafter, effective drug concentrations are expected in tissues for up to 10 days. In dogs, doses of 5–10 mg/kg once daily orally for 1–5 days have been suggested. In cats, doses of 5–10 mg/kg once daily or every other day or one dose two to three times a week orally have been used.

Despite the popularity of azithromycin for treatment of infections in dogs and cats, there is little clinical evidence published to demonstrate benefits over other drugs. In shelter cats with upper respiratory infections, it was no better than amoxicillin for treatment (Ruch-Gallie et al., 2008). In cats with chlamydophilosis (*Chlamydophila felis*) it was ineffective for clearing the infection. In dogs it has been effective for some skin infections based on limited reports, but was not as effective as other agents for treatment of Rocky Mountain spotted fever (*Rickettsia rickettsii*) (Breitschwerdt et al., 1999).

There are several reports of azithromycin clinical use in foals with pulmonary infections, such as those caused by *Rhodococcus equi.* Because of favorable pharmacokinetics, cited above, plasma, leukocyte, and alveolar macrophage concentrations persist long enough to allow for every-other-day administration. Based on this work the dose for foals is 10 mg/kg every 24 hours initially, followed by treatment every 48 hours orally. When azithromycin was administered orally to foals (10 mg/kg every 48 hours) it effectively reduced the pneumonia attributed to *Rhodococcus equi* (Chaffin et al., 2008). The use of azithromycin and other macrolides for treatment of *Rhodococcus equi* infections in foals was summarized in an ACVIM Consensus Statement (Giguère et al., 2011).

Safety of Azithromycin

Azithromycin is generally well tolerated. In people, gastrointestinal disturbances are the most common side effects (nausea, vomiting, diarrhea, abdominal pain). In dogs, high doses may cause vomiting. From the clinical reports, it appears to have been well tolerated in foals but transient diarrhea is possible. Adult horses may be more prone to developing diarrhea and more caution is urged with clinical use in these animals.

Erythromycin is well known to decrease the activity of drug-metabolizing enzymes in the liver. This can increase the toxicity of some drugs administered concurrently. Although azithromycin is reported to have less effect on the hepatic enzymes, some caution is needed when combining azithromycin with other drugs.

Lincosamide Antibiotics

Lincosamides are a group of monoglycoside antibiotics containing an amino acid-like side chain. There are two antibiotics within this group: lincomycin and clindamycin. Lincomycin and clindamycin are structurally similar. Lincomycin has a hydroxyl moiety at the 7 position of the molecule, and clindamycin contains a chlorine at this position (Figure 36.4), making

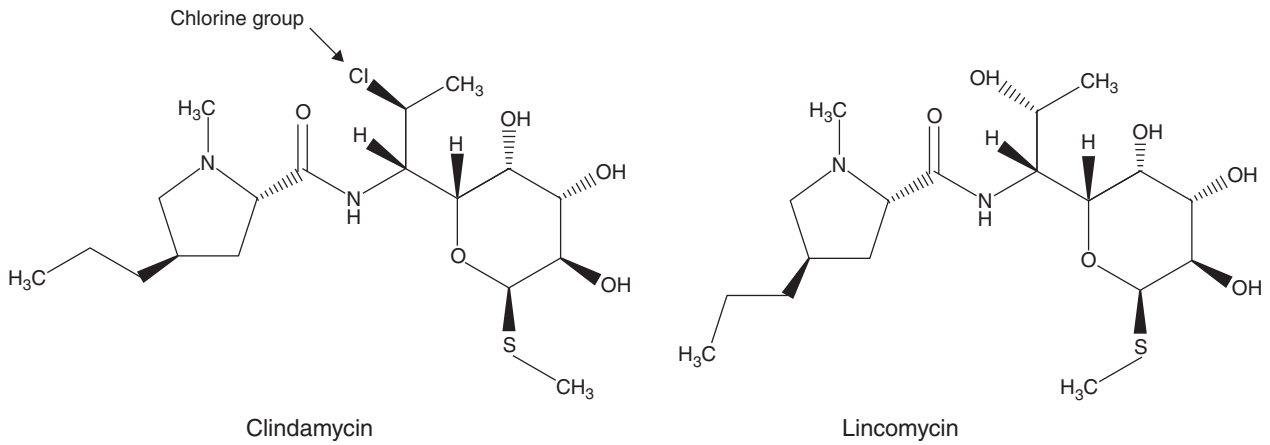

Figure 36.4 The chemical structures of clindamycin (left) and lincomycin (right). Structural difference is in the chlorine group on clindamycin.

clindamycin a more active molecule against bacteria than its parent molecule, lincomycin, and better absorbed orally. The lincosamides, like the macrolides, are used primarily to treat gram-positive infections in cases where there is resistance or intolerance to penicillins. Clindamycin also is a common drug for treatment of anaerobic infections. Common infections treated with lincosamides include infections involving *Staphylococcus* spp. and *Streptococcus* spp. (Burrows, 1980).

Lincomycin

Source and Chemistry

Lincomycin is the antibiotic produced by *Streptococcus lincolnensis* var. *lincolnensis,* discovered in the 1950s; its name comes from cultures of soil that originated in Lincoln, Nebraska. Veterinary formulations were first developed in the 1960s. Lincomycin is a weak base with a pK_a of 7.6 (Riviere et al., 1991).

Formulations

Lincomycin is available as an oral premix for pigs and chickens (Lincomix) and a soluble powder for drinking water (Lincomix). Lincomycin hydrochloride oral syrup and tablets have been used for dogs and cats (Lincocin), as well as lincomycin hydrochloride injection, but use in small animals is not as common as it once was. Ruminants and horses should not be exposed to lincomycin-supplemented feed. The toxicity is described in Section Adverse Effects and Precautions. There has been combination products of lincomycin and spectinomycin available in the past (for example Linco-Spectam), but this product has been discontinued in many countries. Veterinarians should consult local availability in each country of use.

Mechanism of Action and Spectrum

Lincomycin inhibits protein synthesis in the microbial cell by binding to the 50S ribosomal subunit in much the same way described for macrolides. Other antibiotics, such as erythromycin and clindamycin, function similarly by binding at different sites to the same ribosomal subunit. The spectrum of activity is similar for macrolides and lincosamides, with exceptions listed for individual drugs in this chapter. Bacteria with resistance to macrolides usually show cross-resistance to lincosamides. Macrolides and lincosamides should not be used together because this may produce a decrease in the overall efficacy against the microbe due to one bound antibiotic physically overlapping the binding site of another (Burrows, 1980).

Absorption and Distribution

Lincomycin is rapidly but incompletely absorbed when administered orally to animals, with one report stating that lincomycin oral absorption in swine given 10 mg/kg is in the range 20–50% (Hornish et al., 1987). Peak serum levels in most animals are reached within 60 minutes after an oral dose and within 2–4 hours after IM injection. Lincomycin is well distributed in the body, with highest tissue concentrations in the liver and kidneys, while very low levels are obtained in the CSF (Burrows, 1980; Ford and Aronson, 1985; Kleckner, 1984). The Vd in animals ranges from 1 to 1.3 l/kg.

Metabolism and Excretion

The half-life after oral, IM, or IV administration is approximately 2–4 hours. Most of the oral dose, measured as ^{14}C-labeled lincomycin, was recovered in the feces and 14% in the urine after a single oral administration to the dog (Kleckner, 1984); thus, biliary secretion of lincomycin appears to be an important route of elimination. After a single IM injection, 38% of the dose was found in the feces and 49% in the urine of the dog. Urine excretion of the radiolabeled drug was complete in 24 hours and fecal excretion was complete within 48 hours for both dosing routes. It is not known whether this radioactivity was associated with an unchanged/unmetabolized lincomycin or with the metabolites of this compound. An unpublished report cited by Hornish et al. (1987) stated the parent drug was the primary form present in the urine of dogs and humans.

Because of the potential for residues in meat, the metabolism and excretion of lincomycin have been studied more extensively in swine and chickens (Hornish et al., 1987). Lincomycin concentrations are highest in the liver and kidney, with low, albeit detectable, levels in muscle and skin. Lincomycin can pass unchanged from the body via the bile and feces or urine or can be metabolized to the glucuronide, *N*-demethyl lincomycin, or lincomycin sulfoxide forms by the liver. Swine given oral doses of lincomycin showed that 11–21% was excreted into the urine: 50% unchanged lincomycin, trace amounts of *N*-demethyl lincomycin, no lincomycin sulfoxide or glucuronide forms, and the rest labeled "unidentified substances." The feces contained the remainder of the excreted lincomycin: 17% unchanged lincomycin, possible trace amounts of lincomycin sulfoxide, and 83% uncharacterized metabolites (Hornish et al., 1987). Similarly conducted studies in chickens treated orally for 7 days with lincomycin showed that the excreta contained ≈80% lincomycin, ≤10% lincomycin sulfoxide, ≤5% *N*-demethyl lincomycin.

Adverse Effects and Precautions

Dogs and cats have few adverse reactions to lincomycin. Loose stools in the dog and vomiting in the cat have been the major side effects reported (Kleckner, 1984). Pigs may occasionally develop diarrhea and/or swelling of the anus

within the first 2 days of treatment and will self-correct within a week after withdrawal from the antibiotic.

The most serious adverse effect from lincomycin reported in people is that of pseudomembranous colitis. This is a serious disease in people caused by an overgrowth and production of toxin from *Clostridium difficile,* which may be fatal. In animals with fermenting gastrointestinal tracts (horses, ruminants, rabbits, hamsters, chinchillas, and guinea pigs) there also is a high risk of gastrointestinal bacterial overgrowth with *Clostridium* spp. from lincomycin treatment. Severe enteritis, enterocolitis, may lead to diarrhea and death. Other bacteria also have been implicated in this reaction, such as *Salmonella* spp. or *E. coli* (Burrows, 1980; Plenderleith, 1988). Lincomycin-induced enterocolitis has been reported for rabbits (Maiers and Mason, 1984; Thilsted et al., 1981; Rehg and Pakes, 1982), horses (Raisbeck et al., 1981; Plenderleith, 1988), sheep (Bulgin, 1988), and large ruminants (Plenderleith, 1988). Lincomycin has been reported to produce ketosis in dairy cows (Rice and McMurray, 1983).

Clinical Use

There are 85 products listed on the FDA list of approved drugs for animals. These products are in oral and injectable form for pigs, dogs, cats, and poultry. Lincomycin is used to treat gram-positive aerobic and anaerobic infections in patients for many of the same indications for which one would use erythromycin or other macrolides. In dogs and cats, lincomycin has been used to treat penicillin-resistant or suspected penicillin-resistant strains of *Staphylococcus* spp. and *Streptococcus* spp. bacteria found in bone, the upper respiratory tract, and the skin. Although it has been used for skin infections, it is not as popular as it once was (Noli and Boothe, 1999). Oral doses in dogs and cats generally are 22 mg/kg every 12 hours orally. The use of lincomycin to treat bacterial infections in dogs and cats has been largely replaced by clindamycin therapy (see Section Clindamycin).

Lincomycin has been utilized to treat bacterial arthritis in swine caused by *Staphylococcus* spp., *Streptococcus* spp., *Erysipelothrix* spp., and *Mycoplasma* spp., and pneumonia caused by *Mycoplasma* spp. Lincomycin has been added to the feed and water to control swine dysentery and *Mycoplasma* infections (Rainier et al., 1980; Hamdy, 1978; Hamdy and Kratzer, 1981). Injections have been administered to pigs for *Mycoplasma* infections (11 mg/kg every 24 hours IM). In cattle and sheep lincomycin has been injected IM for treatment of septic arthritis and mastitis and to control *Mycoplasma* infections. It should never be administered orally to these animals because of risk of inducing enteritis.

In broiler chickens, lincomycin has been used as a feed additive to increase the rate of weight gain and improve feed efficiency (this use has been phased out in the United States), in addition to treating necrotic enteritis in this species. The addition of 2 g/ton of lincomycin to the feed of broilers resulted in a significant decrease in the incidence of necrotic enteritis (Maxey and Page, 1977). Lincomycin has also been used with success in psittacines (Mandel, 1977). Lincomycin use in the eyes of rabbits has also been reported (Kleinberg et al., 1979). Topical corneal administration of 1% lincomycin in water to rabbits showed local therapeutic levels could be maintained from 30–45 minutes to 2 hours postdose in the cornea, aqueous humor, and iris-ciliary body and that deepithelialization of the corneal epithelium served to enhance the ocular topical absorption of this antibiotic.

Sheep, goats, and calves have been treated with parenteral lincomycin–spectinomycin antibiotic combinations for gram-positive and gram-negative respiratory tract infections. The lincomycin–spectinomycin combination (Linco-Spectam, 50 mg lincomycin with 100 mg spectinomycin per ml) at a dose of 1 ml/10 kg body weight IM has been used to treat foot rot in sheep caused by *Bacteroides nodosus* with better success than systemic penicillin–streptomycin therapy (Venning et al., 1990). However, in many countries the combination product of lincomycin–spectinomycin has been discontinued (see the discussion in section Spectinomycin).

Regulatory Considerations

When added to feed for poultry and pigs, the slaughter withdrawal time ranges from 0 to 6 days, depending on the preparation and dose. When injected in pigs, the withdrawal time for slaughter is 2 days. Because a large number of products are listed on the FDA approved drug list, consult the package insert for specific recommendations.

Clindamycin

Source and Chemistry

Clindamycin chemically is 7-chlorolincomycin, a derivative of lincomycin and an antibiotic produced by *Streptococcus lincolnensis* var. *lincolnensis*. The replacement of the hydroxyl group at the C7 position of the lincomycin molecule by a chloride results in a more active antibacterial effect when compared to lincomycin. The chemical structure of clindamycin is shown in Figure 36.4. It is a weak base with a pK_a of 7.6. Both clindamycin hydrochloride (HCl) and clindamycin palmitate are for oral administration. Clindamycin HCl is directly active when administered, whereas the palmitate form must be converted to clindamycin in the small intestine. Clindamycin palmitate is more palatable than clindamycin HCl. Clindamycin phosphate is the parenteral form of clindamycin and must undergo hydrolysis in the plasma for it to become active.

Mechanism of Action and Resistance

Clindamycin exerts its antibiotic activity by inhibiting protein synthesis at the 50S ribosomal subunit (Hedstrom, 1984) in a manner identical to that described for lincomycin. Resistance to clindamycin is most often caused by methylation of the 23S rRNA, which is the same mechanism that is most common for macrolide antibiotics. This mechanism of resistance is mediated by *erm* genes. Bacteria carrying these genes can be resistant to both macrolides and clindamycin. The other mechanism of resistance for macrolides is the efflux pump mediated by the *mef* gene. Clindamycin is not affected by this gene. Although modification of the 23S ribosome is the most common mechanism of resistance, if bacteria are resistant to macrolides because of the efflux pump mechanism, they may still be susceptible to clindamycin. The third mechanism of resistance, enzymes directed at the drug, is uncommon. Bacteria resistant to lincomycin are also resistant to clindamycin.

Spectrum of Activity

The chlorine substitution (Figure 36.4) produces higher activity against some bacteria than lincomycin. Clindamycin has been reported to be as much as 20 times more potent than lincomycin in the treatment of *Staphylococcus* and *Streptococcus* infections in humans (Harvey, 1985). Clindamycin is active against aerobic species of organisms, including *Staphylococcus, Streptococcus, Actinomyces, Nocardia, Mycoplasma,* and *Toxoplasma*. Because macrolide efflux is the predominant mechanism of macrolide resistance for *Streptococcus* spp., clindamycin remains active against most streptococci because they are not affected by this mechanism. The anaerobic bacterial spectrum of activity includes *Bacteroides fragilis, Fusobacterium* spp., *Peptostreptococcus* spp., and *Clostridium perfringens* (Harari and Lincoln, 1989). Clindamycin is not active against aerobic and facultatively anaerobic gram-negative bacilli such as the Enterobacteriaceae or *Pseudomonas* spp. *Pasteurealla* spp. (gram-negative aerobe) isolated from bite wounds of small animals are usually resistant to clindamycin.

Although most staphylococci are susceptible to clindamycin, approximately 25–36% of *Staphylococcus* spp. may be resistant to clindamycin, depending on the study and region from which the bacteria were isolated. Most methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolated from dogs are resistant to clindamycin. On the other hand, many community-acquired methicillin-resistant *Staphylococcus aureus* may be susceptible to clindamycin.

In small animals, anaerobic infections are one of the major uses of clindamycin. One report (Jang et al., 1997) indicated that 83% of *Bacteroides* from small animals were susceptible to clindamycin and 80% of the *Clostridium.* Clindamycin resistance among *Bacteroides* is mediated by the *erm* gene and increased rates of resistance among anaerobes may reflect the prevalence of this gene. Most *Fusobacterium* spp. are also susceptible to clindamycin. An additional organism for which there is activity is *Toxoplasma,* but the clinical use of clindamycin for treating toxoplasmosis in cats is controversial (described in more detail in Section Clinical Use).

The CLSI breakpoint for susceptibility testing is ≤0.5 μg/ml, 1–2 μg/ml, and ≥4 μg/ml for susceptible, intermediate, and resistant categories, respectively (CLSI, 2015). Clindamycin may be used to test for lincomycin susceptibility, although clindamycin may be more active against some staphylococci than lincomycin.

Pharmacokinetics – Pharmacodynamics

Clindamycin exerts a bacteriostatic rather than a bactericidal effect on bacteria; therefore, it is important to maintain the plasma drug concentration above the MIC throughout the dose interval. The area-under-the-curve (AUC) to MIC ratio (AUC/MIC) for the free drug concentration is the best predictive parameter for clindamycin efficacy. The AUC/MIC target for a bacteriostatic effect is approximately 25. Protein binding is 92–95% in dogs and 91.5–94.5% in cats (protein binding tends to be lower at high concentrations ranging from 0.5 μg/ml to 5 μg/ml). Therefore, PK/PD calculations should use the free drug fraction (fraction unbound).

Absorption and distribution: Clindamycin is better absorbed from the gastrointestinal tract than lincomycin, yielding higher plasma concentrations (Nichols and Keys, 1984). Unlike lincomycin, the presence of food does not appear to affect oral absorption of clindamycin. Oral absorption was 73% in dogs after capsule administration and highly absorbed from IM injection (87%). When injected SC, the absorption is slow, producing a "flip-flop" effect and a longer half-life. Other pharmacokinetics are shown in Table 36.9. At doses administered to cats (Brown et al., 1989, 1990) 5.5 and 11.0 mg/kg oral doses maintained a serum MIC above that necessary for most *Staphylococcus aureus* infections and that the 11.0 and 22.0 mg/kg doses gave serum concentrations above the MIC for many susceptible anaerobes. Cats may be reluctant to accept the oral liquid form of clindamycin because of poor palatability.

In one study, it was reported that clindamycin is too painful for IM administration (Budsberg et al., 1992), but in another study, IM administration of a buffered, more concentrated 20% solution was better tolerated. SC administration may be better tolerated than IM injections (Lavy et al., 1999).

Little is known in most exotic or zoo species, but in sea turtles clindamycin had extremely rapid clearance and a short half-life. There was very little oral absorption.

Table 36.9 Pharmacokinetics of clindamycin in animals

Species	Dose (mg/kg)	Half-life (hour)	Volume of distribution (Vd) (l/kg)[b]	AUC (µg h/ml)	Peak Concentration (C_{max}) (µg/ml)	Reference
Cats	11–33 oral	9.02	3.75	31-42 at 11 mg/kg	6.6-7.4 at 11 mg/kg	Boothe et al., 1996; Brown et al., 1989[a]
	5.5 oral	4.25	–	6.7	1.9	Saridomichelakis et al., 2011
	11 oral	9.92	–	18.35	3.3	Saridomichelakis et al., 2011
	11 IV	3.24	1.5	34.99	–	Budsberg et al., 1992
	11 IM	3.91	–	35.7	5.3	Budsberg et al., 1992
	10 IV	2.1	1.23	24.28	–	Lavy et al., 1999
	10 IM	7.1	–	30.1	4.4	Lavy et al., 1999
Dogs	10 SC	5.2	–	87.63	20.8	Lavy et al., 1999
	11 IV	4.37	3.08	22.5	–	Batzias et al., 2005
	11 oral	4.37	2.84	16.2	3.25	Batzias et al., 2005

[a]Data represents a mean of the published values.
[b]Values for Vd represent Vd/F for oral doses.

These results in sea turtles suggest that it would be impractical to use in these animals (Harms et al., 2011).

In dogs and cats the volume of distribution is over 1 l/kg and there is good penetration into respiratory secretions, pleural fluid, the prostate, bones, and joints, but with low concentrations in the CSF. The concentrations of clindamycin in phagocytes are 10- to 20-fold (and as high as 40) times the plasma concentrations (Harari and Lincoln, 1989). Despite the high intracellular concentrations of clindamycin in phagocytes, intracellular killing is poor (Yancy et al., 1991), perhaps because the drug is sequestered in subcellular sites. Macrophages take up clindamycin by an active transport mechanism and concentrate clindamycin up to 50 times the extracellular concentration (Dhawan and Thadepalli, 1982). Because phagocytes are the cells most likely to enter infected tissues, such as abscesses, it is possible for clindamycin to be transported to an abscess to produce high concentrations in these sites (Yancy et al., 1991). Clindamycin also crosses the placental barrier, but its safety during pregnancy has not been determined for animals.

Metabolism and Excretion

Clindamycin HCl requires no metabolism to be active once administered orally. Clindamycin phosphate requires hydrolysis to occur in the plasma to be active; similarly, clindamycin palmitate requires the removal of the palmitate moiety in the small intestine to be active. The commercial form for small animals (Antirobe) is clindamycin HCl; other formulations are available for people. Elimination half-lives are shown in Table 36.9. For dogs and cats the half-life varies among studies, dose, and formulation. Generally, the half-life is long enough after administration of the oral formulation that once- or twice-daily administration is sufficient.

Clindamycin metabolites are much like those described for lincomycin. In dogs, 36% of the administered dose of clindamycin is excreted unchanged by the bile and urine. The balance of the dose appears to be active or inactive metabolites, 28% excreted by the liver in the glucuronide form (no antimicrobial activity), 28% as clindamycin sulfoxide (25% of the antimicrobial activity of the parent antibiotic), and 9% as *N*-demethyl clindamycin, which has four to eight times the antimicrobial activity of the parent compound (Dhawan and Thadepalli, 1982). The bile is the major excretion route. The presence in the colon of people administered clindamycin suppressed microbial activity for as long as 2 weeks after the discontinuation of therapy.

Adverse Effects and Precautions

Like lincomycin, the most serious adverse effect in humans is pseudomembranous colitis, from overgrowth of *Clostridium difficile*. This has not been a reported problem in animals. In dogs and cats, vomiting and diarrhea are possible but they are transient and not serious. However, gastrointestinal problems such as those discussed for lincomycin in ruminants, horses, rabbits, and rodents, are possible, and the same precautions apply that were discussed for lincomycin. Although pseudomembranous colitis from *Clostridium difficile* has not been described in animals, bacterial overgrowth and diarrhea are still possible with oral administration of clindamycin to dogs and cats.

Greene et al. (1992) reported that administration of 25 and 50 mg/kg clindamycin HCl to cats, divided in two doses, produced diarrhea and vomiting as the most common clinical signs associated with oral therapy. The highest frequency for both of these clinical signs occurred in the 50 mg/kg treatment group and was thought to be related to either a direct irritant effect on the gastrointestinal tract or some effect on intestinal water absorption. As reported for other oral drugs in cats (for example, doxycycline hyclate) oral administration of clincamycin hydrochloride has been associated with esophageal injury (Beatty et al., 2006).

A study was performed in cats to ascertain the effect of prolonged clindamycin therapy on vitamin K-dependent blood clotting times (Jacobs et al., 1989). The study showed that factor VII levels did not significantly change in cats treated with a total daily dose of 25 mg/kg orally once daily for 6 weeks compared to controls.

Clinical Use

Clindamycin possesses an antimicrobial spectrum similar to that of lincomycin, but it is much more extensively used clinically than lincomycin because of higher activity against anaerobes, increased potency, and more complete oral absorption. Clindamycin has been used to treat wounds, abscesses, osteomyelitis, and periodontal diseases caused by susceptible organisms in dogs and cats. Clindamycin is found in high concentrations in the prostate, making it an acceptable choice for treating bacterial prostatitis when caused by gram-positive organisms.

The use of clindamycin for treating toxoplasmosis is controversial. Lappin et al. (1989) performed a retrospective study of cats diagnosed with *Toxoplasma gondii* infections and found that those cats treated with clindamycin resolved all clinical signs of the disease except those lesions involving the eyes. Clindamycin alone or in combination with a corticosteroid helped to resolve the active retinochoroiditis and the anterior uveitis associated with this disease. Even though clindamycin may help clinical signs associated with toxoplasmosis, it may not help to clear organisms from the CNS or the eye. In experimentally infected cats, there was a paradoxical effect in that cats with toxoplasmosis treated with clindamycin had a worsening of clinical signs. As discussed in more detail by Davidson et al. (1996), this paradoxical effect may be due to an inhibition of intracellular killing of organisms by clindamycin.

Clindamycin has been effective in dogs with experimentally induced posttraumatic osteomyelitis caused by *Staphylococcus* spp. (Braden et al., 1987, 1988). An oral dose of 11 mg/kg twice daily for 28 days was found to be efficacious in treatment of these infected dogs, resulting in a 94% recovery rate in the clindamycin-treated dogs. Clindamycin also has been shown to be effective for treatment of superficial and deep pyoderma in dogs and is a common choice as an alternative to β-lactam antibiotics (Harvey et al., 1993; Noli and Boothe, 1999; Scott et al., 1998). Although 11 mg/kg every 24 hours has been used to treat staphylococcal infections (pyoderma) in dogs, dosing of 11 mg/kg every 12 hours is used by many veterinarians for treating most *Staphylococcus* spp. infections. Once per day at 11 mg/kg may be sufficient for bacteria with MIC values ≤0.5 μg/ml; twice-daily administration should be used if the bacteria have MIC values in the intermediate range of 1–2 μg/ml.

Miscellaneous Antibiotics

Bacitracin

Bacitracin is a complex labile polypeptide consisting of five to ten separate chemical components first isolated from a *Bacillus subtillus* contaminated wound in 1943 (Teske, 1984). Bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$) is the major component of this mixture and accounts for most of the antibiotic activities. Bacitracin inhibits peptidoglycan synthesis in bacteria by nonspecifically blocking phosphorylase reactions, some of which occur during cell wall synthesis (Lancini and Parenti, 1982). Development of resistance to bacitracin is rare.

Bacitracin is not absorbed from the gastrointestinal tract when given orally. Systemic administration has resulted in a high incidence kidney injury (albuminuria, cylindruria, azotemia), in addition to pain, induration, and petechiae at the site of injection. In contrast, bacitracin is nonirritating and rarely induces allergic reactions when used topically. Bacitracin (bacitracin, bacitracin methylenedisalicylate, bacitracin manganese, zinc bacitracin) has been used as a feed additive in livestock, but its most common use today is in topical applications to treat susceptible skin, ear, and eye infections. Bacitracin inhibits many organisms found on skin, such as hemolytic and nonhemolytic *Streptococcus* spp., coagulase-positive *Staphylococcus* spp., and some *Clostridium* spp., and it is often combined with other antibiotics that have a gram-negative spectrum of activity (polymyxin B, neomycin). Zinc bacitracin administered topically may increase the activity of bacitracin due to zinc's astringent properties, which decrease inflammation (Harvey, 1985).

Novobiocin

Novobiocin is a dibasic acid (pK_a = 4.3 and 9.1) derived from coumarin and is utilized clinically as a mono-(Na^+) or dibasic- (Ca^{++}) salt form. Novobiocin possesses activity against both gram-positive and gram-negative bacteria but is more active against the gram–positive bacteria, in particular *Staphylococcus* species. Other susceptible organisms include *Neisseria* spp., *Haemophilus* spp., *Brucella* spp., and some strains of *Proteus* spp. It may be used as an alternative to penicillins in cases involving penicillin-resistant *Staphylococcus* spp., although other penicillin substitutes (cephalosporins, macrolides, clindamycin) are better clinical choices.

Novobiocin has several toxic effects on bacteria, but its exact mechanism and site of action are unknown. Novobiocin has been shown to cause nonspecific inhibition of cell wall synthesis by inhibiting formation of alternating *N*-acetylmuramic acid pentapeptide and *N*-acetylglucosamine residues; it also inhibits teichuronic

acid in some species of bacteria. The concentrations needed to inhibit these cell wall components are greater than the minimal concentration needed to inhibit growth, suggesting these effects on bacteria are secondary effects. DNA and RNA synthesis, protein synthesis (β-galactosidase), respiration, and oxidative phosphorylation are also inhibited in some species of bacteria and in rat liver homogenates (Morris and Russell, 1971), with none seemingly being the primary antibiotic effect. Novobiocin is also known to induce an intracellular magnesium deficiency, but there is no direct convincing evidence that this is the mechanism responsible for novobiocin's antimicrobial activity.

Novobiocin is initially active against *Staphylococcus* spp. infections, but resistance to this antibiotic develops quickly (Morris and Russell, 1971; Harvey, 1985). Novobiocin has been combined with tetracycline to produce synergistic activity, broaden the spectrum of activity and to decrease the resistance to novobiocin, but these older combinations are used infrequently today. Novobiocin and tetracycline have been reported to be efficacious in cases of canine upper respiratory diseases such as "kennel cough" and tonsillitis (Maxey, 1980), but the use of antibiotics for this problem in dogs has declined substantially. Toxic side effects in animals and humans given novobiocin systemically have been reported and include skin rashes, leucopenia, pancytopenia, anemia, agranulocytosis, thrombocytopenia, nausea, vomiting, and diarrhea. Few side effects have been reported for this antibiotic used in its topical form in domestic animals.

Thiostrepton

Thiostrepton is a polypeptide antibiotic produced by *Streptomyces aureus* and has a predominately gram-positive spectrum, although some gram-negative organisms are also affected. Thiostrepton is not absorbed from the gastrointestinal tract and is used primarily for topical local therapy, usually combined with other antibiotics and/or glucocorticosteroids for dermatological therapy.

Rifampin (Rifampicin)

Rifampin is an antibiotic, available for many years, that has been used in people to treat tuberculosis. Equine practitioners have been familiar with rifampin for many years because of its use for treating lung infections caused by *Rhodococcus equi*. Small animal veterinarians are becoming familiar with this antibiotic because it has appeared on susceptibility reports as being active against methicillin-resistant *Staphylococcus* spp.

This antibiotic was originally discovered in the pine forests of France in the 1950s and was introduced into clinical medicine in the 1960s. It is a complex macrocyclic high-molecular-weight semisynthetic antibiotic derived from rifamycin B, produced by *Nocardia mediterrea*. Rifamycin B is chemically modified to produce rifampin. Rifampin is the United States Pharmacopeia (USP) official name, and rifampicin is the International Nonproprietary Name (INN) and British Approved Name (BAN) name; both names are synonymous. Rifamycin and rifabutin are structurally similar antibiotics – all in the group of rifamycins – but are not identical.

Mechanism of Action and Spectrum

Rifampin is a bactericidal antibiotic that acts by inhibiting bacterial RNA polymerase. Rifampin enters the microbial cell and forms stable complexes with the β subunit of DNA-dependent RNA polymerases of microorganisms. This binding results in inactive enzymes and inhibition of RNA synthesis by preventing chain initiation. This inhibition can also occur in mammalian cells, but much higher concentrations are needed. MICs for gram-positive organisms generally occur at 0.1 μg/ml, while gram negative bacteria have MIC values ranging from 8 to 32 μg/ml. This large disparity in MIC values is attributed to rifampin's ability to more easily permeate the gram-positive organism cell wall than the gram-negative organism cell wall, rather than differences in bacterial RNA polymerases. The PK/PD parameter that best predicts rifampin efficacy is the AUC/MIC ratio.

Rifampin is highly lipophilic and the intracellular penetration has made this drug valuable for treating intracellular bacteria in people and animals, including *Mycobacterium* (tuberculosis), *Staphylococcus* spp., and *Rhodococcus equi*. Rifampin has activity against gram-positive bacteria (*Staphylococcus* spp.), *Mycobacterium* spp., *Haemophilus* spp., *Neisseria* spp., and *Chlamydia* spp., but more limited activity against the gram-negative bacteria. Rifampin is active against most strains of methicillin-resistant *Staphylococcus pseudintermedius* (Perreten et al., 2010) although resistance among canine isolates has been identified (Kadlec et al., 2011).

A single mutation of the amino acid sequence of the β subunit of the DNA-dependent RNA polymerase enzyme produces resistance. Mutations result in rifampin having less affinity for the RNA polymerase enzyme. After mutations arise, clonal spread of a resistant strain may occur.

For some infections, resistance can be minimized if other antibiotics are used concurrently that will kill the mutant strains of bacteria produced in response to rifampin. The study by Berghaus et al. (2013) showed that the mutant prevention concentration (MPC) is lower when rifampin is combined with macrolide antibiotics against *Rhodococcus equi.* To reduce the rate of mutation, combination therapy with other agents has been recommended in human guidelines (Liu et al., 2011) and was the recommendation from a veterinary study for treatment of *Staphylococcus* infections in dogs (Kadlec et al.,

2011). For other bacterial infections, the combination of rifampin with other antibiotics may not produce a synergistic effect (Forrest and Tamura, 2010). Whether or not combination therapy is needed for veterinary use is discussed in more detail in Section Clinical Use. There is some evidence for a synergistic effect between amphotericin B and rifampin against some fungi, particularly *Saccharomyces cerevisiae, Histoplasma capsulatum,* several species of *Aspergillus,* and *Blastomyces dermatitidis* (Medoff, 1983). However, rifampin is rarely considered for treatment of a fungal infection because other effective antifungal drugs have emerged (see Chapter 38).

Pharmacokinetics

Pharmacokinetic features of rifampin in several species are presented in Table 36.10. Rifampin is lipophilic with a large volume of distribution and good absorption in practically all animal species studied. The oral absorption is moderate to high, ranging from 38–48% in foals to 70% in adult horses (Table 36.10). In sheep the oral absorption is 16–37%. It has even been absorbed from oral and rectal administration to elephants (Egelund et al., 2015). Rifampin absorption is highest in an acidic environment, although feeding has decreased oral absorption in foals and ruminants. Rifampin is approximately 80–85% bound to plasma proteins in people and 94% in foals. Despite high protein binding, it is widely distributed to all tissues of the body, with particularly high concentrations of the drug found in the lungs, pulmonary epithelial lining fluid, liver, bile, and urine. After oral absorption or parenteral administration, rifampin is primarily metabolized to the bioactive metabolite 25-desacetyl rifampin (25-*O*-desacetyl rifampin), which is active microbiologically. There are also some minor glucuronidation products formed in the liver. Both parent and metabolite compounds are excreted in the bile. Both forms are passively filtered through the kidneys, with renal clearance being approximately 12% of total glomerular filtration rate.

Multiple dosing of rifampin often results in decreased, rather than increased, peak serum concentrations. This phenomenon is due to autoinduction of liver enzymes and is known to occur in humans, swine, dogs, calves, horses, and rodents (Frank, 1990; Berlin et al., 2017). Hepatic enzyme induction and induction of efflux mechanisms by rifampin will also alter the disposition of other drugs (discussed in more detail in Section Interactions).

The half-life ranges from 11 to 14 hours in foals and the peak concentrations vary widely (Table 36.10). The rate of excretion in the foal is lower than in the adult horse, mainly due to biliary excretion mechanisms being less developed in the foal. In dogs the half-life is approximately 8 hours, with a peak concentration of 40 µg/ml.

Interactions

Rifampin is a potent activator of a transcription factor that increases the levels of many drug metabolizing proteins, including P-glycoprotein (P-gp), and cytochrome

Table 36.10 Pharmacokinetics of rifampin/ rifampicin in animals[a]

Species	Dose (mg/kg)	Half-life (hour)	Volume of distribution (Vd) (l/kg)[b]	AUC (µg h/ml)	Peak Concentration (C_{max}) (µg/ml)	Reference
Dogs	10 oral	8	–	–	35	–
	10 oral	5.84	–	42.3	7.4	Burrows et al., 1985[c]
	10 IV	6.05	0.635	120.2	2.9	Burrows et al., 1985[c]
	10 IV	7.27	0.932	118.57	–	Wilson et al., 1988[c]
Horses	20 oral	11.5	–	246.19	13.35	Wilson et al., 1988[c]
Foals	10 oral	14.7	–	160 (0–12 hours)	18.1	Peters et al., 2012
	10 oral	6.79	–	72.3	5.50	Berlin et al., 2017[d]
	20 oral	7.61	–	161	12.3	Berlin et al., 2017[d]
	10 IV	11.0	0.85	193	16.8	Berlin et al., 2017
	10 oral	11.5	–	77.0	8.2	Berlin et al., 2016
	10 IV	8.1	0.782	127.33	–	Kohn et al., 1993
	10 oral	–	–	67.65	3.86	Kohn et al., 1993
Calves[c]	10 oral	11.4	–	310.9	11.7–24.6	Sweeney et al., 1988
Calves[c]	10 IV					Sweeney et al., 1988
Sheep[c]	10 oral	4.3		11.7	0.6-2.4	Sweeney et al., 1988
Sheep[c]	10 IV	2.9	1.32	32	–	Sweeney et al., 1988
Sheep	20 oral	6.42	–		3.27	Jernigan et al., 1986
Sheep	20 IV	4.56	0.46	–	–	Jernigan et al., 1986

[a]Data represents a mean of the published values.
[b]Values for Vd represent Vd/F for oral doses.
[c]Concentrations measured using a microbiological assay, which can over-estimate the concentration because the metabolite is active.
[d]Data from Berlin et al., 2017; oral dose in foals was after repeated dosing for 10 days.

P-450 enzymes CYP3A, and CYP2C. The activation of occurs through an up-regulation of gene expression of intestinal and hepatic cytochrome P450 enzymes and transporters through the nuclear pregname X receptor (PXR) pathway (Reitman et al., 2011). The list of drugs identified in people that are affected by rifampin is long and summarized in other papers (Frank, 1990; Barriere et al., 1989; Lee et al., 1993; Reitman et al., 2011; Forrest and Tamura, 2010). The consequence of induction is a diminished effect of the coadministered drug and may require a higher dose or more frequent administration. When clarithromycin and rifampin were administered together in foals, it decreased the plasma drug concentration of clarithromycin by over 90% (Peters et al., 2011; Berlin et al., 2016). But in the same study, the concentration of rifampin was not affected by administration of clarithromycin. In people 4 weeks are required for full recovery of the rifampin effect after discontinuation (Reitman et al., 2011). Rifamin may have dual effects in which it can be an inhibitor of intestinal transport, as well as an inducer of other proteins.

Adverse Effects

Adverse effects have been associated with high doses and include liver injury and gastrointestinal disturbances. In a study of 344 dogs (Bajwa et al., 2013) adverse effects occurred in 16% of treated dogs. Adverse effects included vomiting, anorexia, lethargy, and weight loss. Gastrointestinal effects were the most common. There were 27% of dogs with increases in liver enzymes and this occurred between days 19 and 27 days of initiating treatment. Liver injury from rifampin is more common in dogs than in people or horses. It has been estimated that 20% or more of dogs receiving 5–10 mg/kg may develop increases in liver enzymes and some may develop hepatitis. It is advised to monitor hepatic enzymes during treatment in dogs and not to exceed a dose of 10 mg/kg per day. In the Bajwa study (Bajwa et al., 2013) cited above, a reduction in dose resolved adverse effects in many dogs. Rifampin has an unpalatable taste. It also may produce a reversible discoloration (orange–red color) to the urine, tears, and sclera. Pet owners should be warned of this possibility. Rifampin is teratogenic in laboratory animals, so its use in pregnant animals should be restricted.

Clinical Use

Susceptible organisms of interest to veterinarians include *Staphylococcus* species (including methicillin-resistant strains), *Streptococcus* spp., including *Streptococcus zooepidemicus*, *Rhodococcus equi*, *Corynebacterium pseudotuberculosis*, and most strains of *Bacteroides* spp., *Clostridium* spp., *Neisseria* spp., and *Listeria* spp. Organisms known to be resistant to rifampin are *Pseudomonas aeruginosa, E. coli, Enterobacter* spp., *Klebsiella pneumoniae*, *Proteus* spp., and *Salmonella* spp. Some gram-negative organisms may be susceptible, but it may require higher concentrations. Breakpoints for susceptibility testing of isolates from animals have not been established and the human breakpoint of ≤1, 2, and ≥4 μg/ml for susceptible, intermediate, and resistant, respectively, can be used until veterinary breakpoints are established by CLSI.

Rifampin has been used for treatment of gram-positive cocci infections in dogs (and occasionally cats), particularly methicillin-resistant *Staphylococcus* spp. that are resistant to other drugs. Rifampin has been effective for treatment of canine pyoderma caused by *Staphylococcus pseudintermedius* at a dose of 5 mg/kg once daily for 10 days (Sentürk et al., 2005).

Veterinary surgeons have recommended the addition of rifampin to treatment when biofilms are suspected to occur from surgical implants or chronic infections. Rifampin achieves high concentrations within neutrophils, endothelial cells, macrophages, and biofilms and, in people, has improved activity in combination with another antibiotic compared to that if it is used alone (Forrest and Tamura, 2010). No studies on biofilm infections have been reported in veterinary medicine.

There is a long history of rifampin use in horses. Rifampin is one of the first choices for treatment of infections in foals caused by *Rhodococcus equi* (Giguère et al., 2011). The dose for foals is typically 5 mg/kg oral, every 12 hours, but 10 mg/kg once per day also has been shown to attain effective concentrations (Berlin et al., 2017). It is routinely administered with one of the macrolide antibiotics – erythromycin, azithromycin, or clarithromycin (these drugs are discussed in Section Macrolide Antibiotics). In one uncontrolled retrospective study (Giguère et al., 2004) rifampin–clarithromycin combination was more effective than either rifampin–azithromycin or rifampin–erythromycin. In another study rifampin–azithromycin was more effective in foals than injections of tulathromycin (Rutenberg et al., 2017). Nevertheless, azithromycin is often used instead of clarithromycin because it is more convenient to administer.

Although not used frequently in ruminants, the pharmacokinetics have been studied (Table 36.10) and the recommended dose was 20 mg/kg orally once a day. The most common use in ruminants is for treatment of *Mycobacterium paratuberculosis* in cattle and sheep. It may cause remission of the infection, but does not eradicate the organism. It has also been used to treat *Mycobacterium tuberculosis* in elephants (10 mg/kg per day) (Egelund et al., 2015).

Monotherapy or combination therapy? Rifampin has been combined with other antimicrobials for treatment of *Staphylococcus* infections in dogs, and for treatment of *Rhodococcus equi* infections in foals in some protocols.

The reason for combination treatment for *Staphylococcus* infections in dogs is ostensibly to reduce emergence of resistance. However, there are no clinical studies in veterinary medicine that have demonstrated greater emergence of resistance from monotherapy with rifampin compared to combination therapy in dogs. The study by Sentürk et al. (2005) showed that monotherapy for *Staphylococcus* treatment in dogs was successful. In experimental infections rifampin monotherapy successfully eradicated staphylococci from pus in vitro and from abscesses in experimental infections (Lobo and Mandell, 1972).

The study by Kadlec et al. (Kadlec et al., 2011; Perreten et al., 2010) showed that rifampin resistance among staphylococci is infrequent. When treating staphylococcal infections in people (Falagas et al., 2007) addition of a second antibiotic did not confer additional effectiveness compared to rifampicin monotherapy for eradication of methicillin-resistant *Staphylococcus*. The study by Achermann et al. (2013) identified risk factors that contributed to resistance in staphylococcal joint infections. Rifampin monotherapy was not a statistical risk factor for development of resistance. This suggests that resistance to rifampin is possible with or without combination therapy.

The recommendation to use rifampin in combination with other antimicrobials to decrease emergence of resistance has been mainly validated in clinical situations in which long-term therapy with rifampicin was necessary (e.g., tuberculosis) and may not be the same for short-term treatment of *Staphylococcus.* Forrest and Tamura (2010) provided an extensive review of the use of rifampin in nonmycobacterial infections. They concluded that combinations of other drugs with rifampin results in indifference or antagonism and there are few examples showing synergism. For example in the treatment of staphylococcal infections, this analysis indicated that, "With a review period covering several decades, the in vitro data for rifampin combination therapy against staphylococci appear to frequently show antagonism or indifference, with synergy being found inconsistently." This questions the benefit of adding other antibiotics to rifampin therapy for nonmycobacterial infections.

For the treatment of foals with *Rhodococcus equi* infection, rifampin has been combined with macrolide antibiotics – erythromycin, azithromycin, or clarithromycin most commonly. The recommendation of combination treatment of foals comes from consensus statements from experts (Giguère et al., 2011) and pharmacokinetic studies (Berlin et al., 2017). In pharmacokinetic studies, the concentrations in foal pulmonary epithelial lining fluid and bronchoalveolar lavage cells produced concentrations slightly lower than plasma concentrations but above the MIC_{90} for *Rhodococcus equi* (Berlin et al., 2017). It is possible that single-agent treatment is also effective, but this has not been compared with randomized, controlled studies. There is no evidence that combination treatments are synergistic using in vitro time-kill kinetic methods at achievable serum concentrations (Nordmann and Ronco, 1992), although combination with rifampin lowered the mutant prevention concentration (MPC) in vitro for some macrolides (Berghaus et al., 2012). The effects of rifampin on the pharmacokinetics of other antibiotics was discussed above in the Interactions section.

Nitrofurans

Nitrofurans comprise several synthetic compounds derived from 5-nitrofuran and possess antimicrobial activity, the 5-nitro group being required for this activity. Over 3,500 nitrofurans have been synthesized to date, with only a handful being useful in animal chemotherapy. Nitrofurans and furazolidone are banned from use in food-producing animals.

Nitrofurantoin is the main drug in this group administered orally. The mechanism of action is not well understood. After penetrating bacteria intracellular nitroreductases convert the drug to an active form through reduction of the nitro group. This action produces intermediate metabolites that bind to bacterial ribosomes and inhibit bacterial enzymes responsible for DNA and RNA synthesis.

The spectrum includes *E coli, Staphylococcus* spp., and *Enterococcus* spp. Resistance among bacteria is unusual, although *Proteus* and *Pseudomonas aeruginosa* are inherently resistant. Nitrofurans can be administered orally or topically. Oral absorption is 80% in people, but unknown in animals. Absorption is enhanced when administered with food. Serum concentrations are almost undetectable, or very low and therapeutically active concentrations are achieved only in urine. Effective concentrations cannot be achieved in the prostate or kidneys for treating upper urinary tract infection. An acid environment is required for the nitrofurans to diffuse across the cell membranes. Acidification of the urine promotes tubular reabsorption, which decreases the overall urine concentration of the drug.

Adverse Effects

The toxicology of furazolidone (*N*-5-nitro-2-furfurylidene amino-2-oxazolidinone) has been investigated extensively in laboratory, food, and companion animals as well as in humans, and has been reviewed by Ali (1989). The effects of feeding furazolidone to poultry have been reported (Ali, 1989; Mustafa et al., 1975; Czarnecki et al., 1974a, 1974b; Jankus et al., 1972).

Furazolidone has been demonstrated to be carcinogenic when used at a 0.15% w/w concentration in feed for 1 year, inducing mammary tumors in a dose-related

manner. Mice fed a 0.03% w/w concentration in feed for life developed bronchial adenocarcinomas in both sexes (Ali, 1983). DNA is the principal target of furazolidone in some cells in vivo, causing cuts and mutations in DNA and binding to DNA, hence blocking the replication and transcription processes. Mutagenesis by nitrofurans in general also occurs and has been extensively reviewed by McCalla (1983), who notes several possible metabolic pathways by which nitrofurans can cause mutagenesis in mammalian cells. This potential for mutagenesis and carcinogenesis has caused the ban from use in food-producing animals.

The major disadvantage of nitrofurans to treat systemic infections is that the concentrations needed to reach the MIC also induce systemic toxicity. There are early reports in the veterinary literature on the toxicities induced when the nitrofurans are used systemically (Ali, 1983). Oral nitrofurantoin adverse effects include nausea, vomiting, and diarrhea. It turns urine rust-yellow brown color. In people, respiratory problems (pneumonitis) and peripheral neuropathy have been reported. The polyneuropathies in people are caused by a demyelination and are more common from long-term use or in patients with renal compromise. The respiratory problems have not been reported in animals, but neuropathy has been observed in dogs; the risk may be higher if there is renal insufficiency.

Clinical Use

Except for topical use, the most frequent use of nitrofurantoin in veterinary medicine is for treatment and prevention of urinary tract infections in dogs and cats that are resistant to other antimicrobials. The oral forms (Macrodantin, Furalan, Furatoin, Furadantin, and generic brands) have been administered to dogs for urinary tract infections when there are other options are limited. Although this use has been incorporated into some urinary tract infection protocols for dogs and cats, the efficacy is undetermined. Typical doses are 10 mg/kg/day divided into four daily treatments, then reduced to 1 mg/kg per day. It does not attain high enough concentrations for other infections. Although oral absorption has not been studied in dogs, in people the macrocrystalline form is slowly absorbed and less likely to cause gastric upset. The microcrystalline form is rapidly absorbed in intestine.

Although efficacy has not been examined in animals, a metaanalysis of human studies showed efficacy for short-term treatment of uncomplicated urinary tract infections (Huttner et al., 2015). Their analysis included a review of clinical trials from 1946 to 2014. They concluded that there was overall equivalence between nitrofurantoin when given for 5 or 7 days and trimethoprim–sulfamethoxazole, fluoroquinolones, and amoxicillin. Adverse effects in people were uncommon if treatment duration was kept short. The most serious adverse effects of pulmonary fibrosis and liver injury are the result of administration for several months or years.

Virginiamycin

Virginiamycin is a combination of two chemicals produced by *Streptomyces virginiae,* isolated from soil samples in Belgium in the early 1960s. Virginiamycin is classified as a peptolide antibiotic composed of the predominate M fraction ($C_{28}H_{35}N_3O_7$) and the lesser S fraction ($C_{43}H_{49}NO_{10}$) (Crawford, 1984). The optimum ratio of M : S is 4 : 1 (Gottschall et al., 1988). Administered separately, both M and S fractions have a reversible bacteriostatic action on susceptible bacterial populations; used together, their activity is synergistic, bactericidal, and approximately 100 times that found when used separately. Virginiamycin is not known to be synergistic with other classes of antibiotics. Virginiamycin is primarily active against gram-positive organisms, *Haemophilus* spp., and *Neisseria* spp., and has mild activity against the protozoan *Toxoplasma* spp. It works by inhibiting protein synthesis at the 23S ribosomal subunit, blocking translation but not transcription in susceptible bacteria. Virginiamycin is rapidly absorbed when administered orally, is excreted by the bile with no enterohepatic circulation, and has an affinity for dermal tissues (Crawford, 1984). Gottschall et al. (1988) reported that ^{14}C-virginiamycin, specifically the M fraction, was extensively metabolized in the rumen. The S fraction underwent no detectable metabolism in the rumen, and the M fraction metabolites had considerably less antimicrobial activity than the parent compound.

All virginiamycin-like antibiotics fall into one of two groups. Group A consists of polyunsaturated cyclic peptolides that have a molecular weight of approximately 500 and that contain substituted aminodecanoic acid and an oxanzole system. Group B consists of cyclic hexadepsipeptides with an approximate molecular weight of 800, and most members contain one molecule of pipecolic acid or its derivative. Both groups have low solubilities in aqueous solvents and are more soluble in organic solvents. All strongly absorb ultraviolet radiation and are therefore degraded in its presence. Virginiamycin-like antibiotics tend to affect gram-positive bacteria more than the gram negative, with *Mycobacteria* spp. being relatively resistant and *Haemophilus* spp. and *Neisseria* spp. being very sensitive. Differences in bacterial sensitivity to different virginiamycin-like antibiotics are caused by each antibiotic's particular ability to permeate that bacteria's cell wall to gain access to the ribosomes (Cocito, 1979).

Virginiamycin and virginiamycin-like antibiotics are not commonly used to treat clinical bacterial disease in domestic animals, despite their rather broad spectrum

of activity. They have been used to treat swine dysentery (*Treponema hyodysenteriae*) (Olsen and Rodabaugh, 1977), but other antibiotics have proven to be more efficacious. Its main use has been as a feed additive for growth promotion in food animals such as swine (Ravindran and Kornegay, 1984; Moser et al., 1985), being approved for this use since 1975, but use of antibiotics for growth promotion is being phased out in most countries. Virginiamycin has also been studied in turkeys (Salmon and Stevens, 1990), broilers (Miles et al., 1984), and laying hens (Miles et al., 1985) as a growth promotant, all of which experienced either increased weight gain or increased egg production. The regulation and use of antibiotics used in feed for food producing animals is discussed more in other chapters (Chapters 52 and 59).

Carbadox

Carbadox (methyl 3-(2-quinoxalinylmethylene) carbazate N_1,N_4 dioxide) is a synthetic antibacterial agent primarily active against the gram-positive bacteria, although some gram-negative bacteria are affected as well. Carbadox is an antibiotic used in swine (hogs and pigs) for production purposes (e.g., increased rate of weight gain and improved feed efficiency) and therapeutic purposes (e.g., to control swine dysentery and bacterial swine enteritis). While carbadox is an antimicrobial, it does not pose the same resistance issues as other antimicrobials and is not considered important to human medicine.

Available in 1973, carbadox was marketed as a growth promotant in swine and also for the control of swine dysentery (*Treponema hyodysenteriae*), bacterial enteritis (in particular, *Salmonella cholerasuis*), and nasal infections of *Bordetella bronchiseptica* in swine (Farrington and Shively, 1979). Carbadox was shown to be better than lincomycin for the treatment of swine dysentery (Anonymous, 1980; Rainier et al., 1980). Resistance to carbadox has been reported in *E. coli* via R-plasmids (Ohmae et al., 1981). There are three approved New Animal Drug Applications (NADAs) for animal drug products containing carbadox: a premix with carbadox alone, carbadox plus pyrantel tartrate, and carbadox plus oxytetracycline.

The daily feeding of carbadox in feed concentrations of more than 100 ppm for growth promotion in pigs has resulted in toxicities in some weaned pigs, which include growth retardation, dry feces, wasting, dehydration, urine drinking, and a strong interest in salt-containing products (van der Molen et al., 1989a). It is now known that carbadox suppresses aldosterone production, leading to hypoaldosteronism, which then leads to decreased plasma sodium and increased plasma potassium concentrations. These ion alterations are due to stimulation of the renin–angiotensin system with subsequent morphological changes in the zona glomerulosa of the adrenal cortex (van der Molen et al., 1989a, 1989b, 1989c).

Regulatory actions: The United States FDA Center for Veterinary Medicine (CVM) has questions about the safety of carbadox. On April 8, 2016, the FDA announced a proposal to withdraw the approval of the drug applications for products containing carbadox. In addition, the use of antimicrobial agents for growth production in farm animals is being phased out in most countries. Regulation of food-animal antibiotics is discussed in greater detail in other chapters (Chapters 52 and 59).

Glycopeptides (Vancomycin)

Of the glycopeptides, vancomycin is the only one used in veterinary medicine. Teicoplanin has been used in Europe but is not available in the United States. Use of vancomycin in veterinary medicine is limited, but has been necessary in some cases for resistant enterococcal or staphylococcal infections. In August 20, 1997 the US Food and Drug Administration prohibited the extralabel use of glycopeptides in food-producing animals. The reason for this action is because of a fear of glycopeptide-resistant bacteria transmitted to people from treated animals (Bates et al., 1994). This action emerged from the association between resistant enterococci and the use of glycopeptides such as avoparcin and ardacin in animal feed in Europe (Bates et al., 1994). Use of these glycopeptides in animal feeds has been discontinued.

Over 80 *n*-alkyl vancomycins have been synthesized by reductive alkylation of vancomycin, with some forms being five times more active than vancomycin and with some having longer elimination half-lives (Nagarajan et al., 1989). There are new drugs related to vancomycin that have been added to human treatment, but their use of these has not been reported in animals. These drugs include dalbavancin, oritavancin, and telavancin. Telavancin (Vibativ) is a lipoglycopeptide, for once-daily administration. Dalbavancin (Dalvance) is a long-acting IV lipoglycopeptide, similar to telavancin (Vibativ). The half-life in people is 14 days, which allows for once/week treatment. Oritavancin (Orbativ) is a long-acting IV lipoglycopeptide, also similar to telavancin. The half-life is approximately 14 days, which allows for a single treatment.

Vancomycin was discovered in the 1950s. In the 1960s and 1970s it was not used much because the penicillins and cephalosporins were active against most gram-positive bacteria. But in the last 10–15 years drug-resistant enterococcal and staphylococcal infections have generated more reliance on vancomycin in human medicine. Vancomycin is a tricyclic glycopeptide having an approximate molecular weight of 1500.

It is produced by the soil-borne actinomycete *Streptomyces orientalis*. It is freely soluble in water, odorless, and slightly bitter to the taste. Vancomycin inhibits the transpeptidase and transglycosylase steps in bacterial cell-wall synthesis of gram-positive bacteria by binding to the terminal D-alanyl-D-alanine of the stem pentapeptide (cell wall precursor) of the nascent peptidoglycan. Vancomycin is bactericidal for most organisms and bacteriostatic for enterococci. The bactericidal action occurs by activating bacterial cell wall autolysins. This action occurs slowly and there is a gradual loss of cell wall integrity that may not have full effects until 24 hours. The action is time dependent, but the PK-PD that best predicts clinical results is the area-under-the-curve (AUC) to MIC ratio (AUC : MIC) with a target of > 400 considered ideal (Vandecasteele et al., 2013).

Vancomycin is highly active against gram-positive cocci (in particular, *Staphylococcus* spp. and streptococci), enterococci (*Enterococcus faecium* and *E. faecalis*), as well as *Neisseria* spp. Because it has activity against methicillin-resistant *Staphylococcus* species, including *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) and β-lactam resistant *Enterococcus* species, it is valuable for the treatment of these infections. It also is active against gram-positive anaerobic cocci (but not anaerobic gram-negative bacteria) and has been administered to people for diarrhea caused by *Clostridium* spp.

Adverse Effects

Toxicity studies on vancomycin have been performed in many species of laboratory animals (Wold and Turnipseed, 1981). The LD_{50} for the canine was 292 mg/kg, but death did not occur until several days after dosing. Dogs died because of kidney injury, with death due to acute nephrotoxic renal failure.

If vancomycin is administered according to the recommended dosing rates, adverse reactions described earlier are uncommon. A slow infusion is recommended to minimize histamine release. To avoid other toxic reactions, dose recommendations are designed to avoid high plasma concentrations. In people, therapeutic drug monitoring is often performed to ensure that peak concentrations are below 50 μg/ml. The current recommendations are for trough serum vancomycin concentrations of 15–20 μg/ml for intermittent dosing and plateau serum vancomycin concentrations of 20–25 μg/ml for continuous infusions. If animals have renal disease or unique physiological changes (e.g., pregnant or a neonatal animal), drug disposition may change, and peak and trough plasma concentrations should be monitored to adjust the dose appropriately.

Early formulations of vancomycin were associated with a high incidence of adverse effects. Most of these effects were associated with rapid IV administration, which induced flushing of the skin, pruritus, tachycardia, and other signs attributed to histamine release. Ototoxicity also was reported. Kidney injury risk is greater with high doses and longer exposure. Vancomycin toxicity acts as an oxidative stressor in the renal proximal tubule and can produce interstitial nephritis. The incidence of nephrotoxicity and ototoxicity may be partially caused by the common practice of simultaneously administering vancomycin with aminoglycosides. Newer and higher quality formulations of vancomycin have avoided some of the most serious adverse events, but histamine release still is possible from IV administration.

Clinical Use and Administration Guidelines

Clinical use of vancomycin has been limited in veterinary medicine and most of our clinical recommendations for use are derived from pharmacokinetic studies performed in dogs and horses and recommendations of effective blood concentrations for people. Vancomycin must be administered via IV infusion, although in rare instances intraperitoneal administration has been used. Vancomycin is poorly absorbed orally and this route is not used except to treat intestinal infections. IM administration is painful and irritating.

In dogs the half-life is somewhat shorter and the volume of distribution smaller than in humans (Zaghlol and Brown, 1988). In people, the suggested trough concentration is 15–20 μg/ml, but it is difficult to maintain these concentrations in dogs because of the short half-life. A dose rate of 15 mg/kg q 6–8 h IV actually produces peaks and troughs of approximately 40 and 5 μg/ml, respectively, but it is the most convenient dose that can be used because of the short half-life in dogs. This dose should be infused slowly over 30–60 minutes, or at a rate of approximately 10 mg/min. The total dose to be administered can be diluted in 0.9% saline or 5% dextrose solution, but not alkalinizing solutions. Vancomycin is available in vials of 500 mg to 5 g (Vancocin, other brands, and generic). If vancomycin is used to treat enterococcal infections, it is strongly recommended to coadminister an aminoglycoside (e.g., amikacin or gentamicin) because when used alone, vancomycin is not bactericidal.

Vancomycin is used infrequently in horses, but has been necessary occasionally for treatment of methicillin-resistant *Staphylococcus* infections and drug-resistant infections caused by *Enterococcus*. The guidelines for treatment were developed by Orsini et al. (2005) from their pharmacokinetic studies. A dose of 7.5 mg/kg is infused over at least 30 minutes every 8 hours in horses. Adverse reactions with this protocol have been minimal. To treat distal limb infections in horses regional limb perfusion or interosseous regional infusions have been used according to a protocol developed by Rubio-Martinez et al. (2005, 2006), in which 300 mg total dose is diluted in 60 ml saline solution and infused in the distal limb.

Methenamine

Methenamine (hexamethylenetetramine) is a urinary antiseptic most commonly used to treat urinary tract infections in small animals. It may be used in conjunction with an antibiotic or occasionally by itself in some cases of bacterial urinary tract infections that have become refractory to conventional antibiotic therapies. Methenamine is activated by a hydrolysis reaction to form formaldehyde and ammonia in acidic urine. It may be effective against a wide variety of gram-positive and gram-negative organisms. It can be either bacteriostatic or bactericidal depending on the pH of the urine (Harvey, 1985).

Methenamine is quickly absorbed when given orally, but absorption is not complete because some is hydrolyzed in the stomach. It is excreted via the urine, and is associated with a low systemic toxicity.

The urine must be at an acidic pH in order to liberate free formaldehyde; therefore, methenamine is most effective when the urine pH is 6 or below. One of the forms of methenamine used is methenamine hippurate, which is available in human tablet form and is administered to dogs at a dose extrapolated from people of 500 mg per dog, oral, every 12 hours. Another form, methenamine mandelate is no longer available commercially. Methenamine has also been administered with urinary acidifiers to lower the urine pH. Concurrent use of other urinary acidifiers, ascorbic acid, arginine HCl, methionine, cranberry juice, and ammonium chloride, may enhance the antibacterial action of methenamine because acidic urine also exerts some independent weak antibacterial activity. Sulfonamides should not be administered with methenamine due to the formation of insoluble formaldehyde–sulfonamide precipitates. Since methenamine is largely eliminated via the kidney, its use should be restricted or closely monitored in cases of renal insufficiency (Harvey, 1985). Methenamine is less effective for treating infections caused by urea-splitting organisms, which increase the urine pH (e.g., *Proteus mirabilis*).

Methenamine mandelate has been used experimentally in the treatment of burn wounds in rats. Topical doses of 5% and 10% were highly efficacious against experimentally induced burns infected with a virulent strain of *Pseudomonas* spp. (Taylor et al., 1970).

Polymyxins

Polymyxins are a group of *N*-monoacetylated decapeptides discovered in 1947 and are produced by *Bacillus polymyxa*. They contain seven amino acids in a cyclic configuration and have a molecular weight of approximately 1000. Several polymyxins have been isolated and have been named A, B, C, D, E, and M. Of these six antibiotics, B and E in their sulfate salt forms are the only ones used clinically. Polymyxin B1 has a pK_a ranging from 8 to 9. The largest use of polymyxin has been in topical ointment preparations. Polymyxin B sulfate is a mixture of polymyxin B1 ($C_{56}H_{98}N_{16}O_3$) and polymyxin B2 ($C_{55}H_{96}N_{16}O_{13}$); polymyxin E is more commonly known as colistin (Harvey, 1985). Colistin has seen a resurgence for treatment of carbapenem-resistant Enterobacteriaceae (CRE) in people for which few other treatment options exist. This use has not been examined in veterinary medicine.

Polymyxins are basic surface-active cationic detergents that interact with the phospholipid within the cell membrane, penetrate that membrane, and then disrupt its structure. This action subsequently induces permeability changes within the cell that result in cell death, giving polymyxins bactericidal properties.

Polymyxins are not absorbed to any extent from the gastrointestinal tract when administered orally. Administration is by injection, usually IV. After administration polymyxin B is 70–90% plasma protein binding and distributed to the heart, lungs, liver, kidney, and skeletal muscle, with excretion mainly via the urine (Sande and Mandell, 1990a). The pharmacokinetics of some of the polymyxins in calves, ewes, rabbits, and dogs is reviewed in greater detail elsewhere (Ziv and Sulman, 1973a; Ziv et al., 1982; Craig and Kunin, 1973; al-Khayyat and Aronson, 1973a, 1973b). Ziv and Sulman (1973a) reported that an IV administration of 5 mg/kg polymyxin B in ewes resulted in a serum half-life of 2.7–4.3 hours and a Vd of 1.29 l/kg.

Polymyxins have a gram-negative antibacterial spectrum, which includes species of *Aerobacter, Escherichia, Histophilus, Klebsiella, Pasteurella, Pseudomonas, Salmonella,* and *Shigella. Proteus* spp. and most strains of *Serratia* spp. are not affected by polymyxins, and all gram-positive bacteria are resistant. If bacteria are sensitive to the polymyxins, they rarely acquire resistance.

Since the polymyxins are not absorbed into the body when given orally, polymyxin B has been used for "bowel sterilization" prior to abdominal surgeries and in irrigation solutions to flush the peritoneal cavities during those procedures. Polymyxins used to be the major drugs for treatment of *Pseudomonas* infections in humans, but since the advent of better penicillins, aminoglycosides, and cephalosporins, their use has declined over time. Nephrotoxicity occurs due to glomerulus and tubular epithelium damage. In addition, respiratory paralysis (usually caused by a rapid IV injection, too much peritoneal lavage, or a preexisting renal condition) and CNS dysfunction, including depression, pyrexia, and anorexia, also occur.

Polymyxins are mainly used in topical skin, mucous membrane, eye, and ear preparations. One of the most common "first-aid" antibiotics is the *Triple Antibiotic*

containing bacitracin zinc, neomycin sulfate, and polymyxin B sulfate. Typically, one gram of ointment contains 400 units bacitracin zinc, 3.5 mg neomycin sulfate, and 5,000 units polymyxin B sulfate 5,000. No adverse systemic effects have been reported when they are applied to intact or denuded skin surfaces. Polymyxin antibacterial activity is markedly decreased in the presence of pus, in tissues containing acidic phospholipids, divalent cations, unsaturated fatty acids, debris, purulent exudate, quaternary ammonium compounds, and in the presence of anionic detergents or other chemicals that antagonize cationic detergents (Harvey, 1985).

In addition to its narrow-spectrum antimicrobial properties, polymyxin B has demonstrated a protective effect against the adverse effects of endotoxin produced by gram-negative bacteria. Polymyxin is capable of acting as a chelating agent to bind the lipid A portion of endotoxin in a 1 : 1 ratio to neutralize lipopolysaccharide. This effectively renders the endotoxin inactive, thereby preventing most of the adverse effects of gram-negative endotoxin. This property has been studied more in horses than in other species and has been part of an antiendotoxin protocol in equine medicine (Morresey and Mackay, 2006). Infusions ranging from 1,000 to 10,000 units per kg (a common dose is 6,000 units/kg, equivalent to 1 mg/kg) administered every 8 hours have been shown to be safe and effective for treating endotoxemia in horses.

Polymyxin B is available in vials containing 500,000 polymyxin B units. In some dosage protocols, the dose is listed in milligrams instead of units. One milligram of polymyxin B base is equivalent to 10,000 units of polymyxin B, and each microgram of pure polymyxin B base is equivalent to 10 units of polymyxin B.

Spectinomycin

Spectinomycin (Spectam) resembles aminoglycosides in some properties. It is highly water soluble and is easily mixed in aqueous solutions. Compared to aminoglycosides, it does not contain amino sugars or glycosidic bonds, but it has an aminocyclitol nucleus like the aminoglycosides. An important difference between spectinomycin and the aminoglycosides is in the adverse effects and spectrum of activity. Spectinomycin lacks the toxic effects of aminoglycoside antibiotics and can be used without concerns about kidney injury. Most formulations of spectinomycin have been removed from the commercial market. The combination products for livestock (for example, lincymycin–spectinomycin) have been discontinued in some countries.

Antimicrobial Activity

Spectinomycin, like aminoglycosides inhibits protein synthesis via a 30S ribosomal target. It is a broad-spectrum drug with activity against gram-positive and some gram-negative bacteria, but little anaerobic activity. It also has good activity against *Mycoplasma*. It is used in cattle because it has activity against *Mannheimia (Pasteurella) haemolytica, Pasteurella multocida,* and *Histophilus somni* (formerly *Haemohilus somnus).*

Pharmacokinetics

Like the aminoglycosides, spectinomycin has a small volume of distribution. It is poorly absorbed after oral administration (10% or less), but some systemic levels are achieved after oral administration in monogastric animals. Most of an oral dose is eliminated in the feces, but after an injection the primary route of elimination is the kidneys. Oral administration has been used for a local effect for treatment of diarrhea.

The half-life of spectinomycin is approximately 2 hours in cattle at a dose of 10 mg/kg IM or IV, and 1.2 hours after a dose of 20 mg/kg IV. After oral dosing in dogs of 100 or 500 mg/kg, the plasma concentrations (peak) were 22 and 80 µg/ml, respectively. The half-life in dogs is 2.72 hours at the 100 mg/kg dose. After IM injection in dogs, the half-life was 1.1 hours. In pigs, the half-life was 1 hour after IM administration.

Clinical Use

Older formulations have been used that contain both lincomycin and spectinomycin. Formulations containing only spectinomycin (Spectam) include spectinomycin oral solution, spectinomycin powder for drinking water, and spectinomycin hydrochloride injection for poultry. Spectinomycin sulfate has been used for injection in cattle (Adspec®), which is more highly absorbed than the hydrochloride salt. Spectinomycin tablets for dogs are an old formulation that is not currently used. (The sponsor of Adspec®, Zoetis, has announced discontinuation of the manufacture of this drug.) There are no small animal formulations currently available but the old formulation for small animals was spectinomycin hydrochloride pentahydrate administered as tablets (22 mg/kg twice daily) or by injection (5–10 mg/kg) for treatment of nonspecific infections.

Clinical use is primarily confined to food animals. Administration to horses has not been reported. In pigs, spectinomycin has been used orally as spectinomycin hydrochloride oral solution. It has in vitro activity against some gram-negative bacteria and has been used as a feed additive for pigs because of its activity against *Mycoplasma*. It has also been administered orally to pigs for treatment of bacterial enteritis caused by *E. coli* (50–100 mg/animal, oral) and as an injection for treatment of respiratory infections. In poultry, spectinomycin has been used as injection and added to drinking water for prevention and treatment of respiratory disease and other infections.

In cattle, spectinomycin has been used as an IM injection for treatment of respiratory infections (10–15 mg/kg SC in the neck, q 24 h × 3–5 days). Poultry formulations were used in cattle off-label prior to approval of Adspec®, when it was a past practice to mix the water soluble powder intended for drinking water as an IV solution for administration to cattle. This use is discouraged because it may result in severe pulmonary edema and death.

Oxazolidinones

The oxazolidinones are infrequently used in veterinary medicine. The most common one used from this class is linezolid (Zyvox®), which was the first one introduced to human medicine. It is valuable for use in people to treat resistant gram-positive infections caused by enterococci and methicillin-resistant *Staphylococcus* spp. Because there are so few drugs active against methicillin-resistant *Staphylococcus* (MRSA), it is the only oral drug that is consistently effective for this infection in people and also used for drug-resistant *Enterococcus*. Because of the increase in the incidence of methicillin-resistant *Staphylococcus* in dogs and cats (methicillin-resistant *Staphylococcus pseudintermedius,* MRSP), the use of linezolid has been increasing in these animals. The brand name of this drug (Zyvox®) was almost prohibitively expensive, but the newer generic forms are one-tenth of the cost, or less, which has increased the use. There is a new oxazolidinone available for people, tedizolid (Sivextro®) which is available in tablets (200 mg) and IV. It has properties similar to linezolid and clinical uses similar in people.

Linezolid inhibits protein synthesis by binding to the bacterial ribosome. It is bacteriostatic by binding to a site on the bacterial 23S ribosomal RNA of the 50S subunit. This prevents formation of the 70S ribosomal unit, and therefore protein synthesis is inhibited. It has activity primarily against gram-positive bacteria, particularly staphylococci and enterococci. Linezolid has good penetration into cells and extracellular fluid. Urine concentrations are high enough to inhibit urinary tract pathogens. In dogs the pharmacokinetics are similar to humans. The oral absorption is almost 100%, with or without food, and the half-life is slightly faster than humans. Linezolid does not undergo hepatic P450 metabolism, and one-third of the total clearance relies on the kidneys.

Adverse effects

Adverse effects have not been reported from use in dogs or cats, but clinical use has been rare enough that this may not be detected. In people, nausea and diarrhea can occur. Long-term use can cause bone marrow suppression (e.g., thrombocytopenia) in people, but this has not been reported in dogs or cats. Rarely, anemia and leukopenia have been observed in people. The risk of bone marrow suppression is most evident after 2 weeks of treatment. Long treatment courses in animals should be accompanied by periodic CBC measurements until the risk of bone marrow suppression is better understood in these animals.

Drug Interactions

Linezolid is a Type-A monoamine oxidase inhibitor (MAOI). Possible interactions occur with serotonin reuptake inhibitors such as fluoxetine and selegiline to produce serotonin syndrome. Interactions are also possible if administered with adrenergic drugs such as phenylpropanolamine. However, this effect has not been reported for animals. Linezolid is not expected to affect metabolism of other drugs. Rifampin may decrease plasma concentrations. Intravenous formulation is physically incompatible with other drugs in IV line. If administered with other drugs IV, flush out the administration line first.

Clinical Use

The use of linezolid should be selected on the basis of susceptibility monitoring and it is not recommended for routine use in animals unless other options are not available because of drug resistance. CLSI lists the break point for susceptibility as less than or equal to 4.0 µg/ml for *Staphylococcus* spp. and less than or equal to 2.0 µg/ml for *Enterococcus*. Linezolid is available in 400- and 600-mg tablets, 20-mg/ml oral suspension powder, and 2-mg/ml injection. The tablets have been expensive, but recent introduction of generic forms has lowered this cost. A typical dose for dogs and cats is 10 mg/kg q 12 h oral or IV.

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37

Fluoroquinolone Antimicrobial Drugs

Mark G. Papich

The fluoroquinolone antibacterial agents are among the most important antimicrobials used in veterinary medicine. They are used in practically all species and have a broad spectrum of activity that includes most of the important veterinary bacterial pathogens. Since their first introduction in the late 1980s (discussed in previous editions of this book), the use of fluoroquinolones has expanded tremendously.

The fluoroquinolones are synthetic antibacterial agents introduced in veterinary medicine first as enrofloxacin. Since then, there has been a great deal of research on this class of drugs to better understand their mechanism of action, antimicrobial spectrum, pharmacokinetics in a wide variety of animal species, and clinical uses. In addition, pharmaceutical companies have developed new agents in this class to increase the number of drug choices available to veterinarians. The advantages of the fluoroquinolones are that they are rapidly bactericidal against a wide variety of clinically important bacterial organisms, are potent, are well tolerated by animals, and have been administered via a variety of routes (orally via tablets and drinking water, subcutaneously, intravenously, intramuscularly, and topically).

Fluoroquinolones approved for use in veterinary medicine for small animals are shown in Table 37.1. Fluoroquinolones that are approved for humans and are of potential interest for veterinary medicine include ciprofloxacin. The newest generation of fluoroquinolones, with increased activity against gram-positive cocci and anaerobic bacteria, includes levofloxacin, moxifloxacin, gatifloxacin, and the veterinary drug pradofloxacin.

Chemical Features

The currently available fluoroquinolones have the same quinolone structure (Figure 37.1). The addition of the fluorine group at position 6 gives these the characteristics of a fluoroquinolone, and various other chemical substitutions and side groups account for the different physical characteristics of each drug. Enrofloxacin has one fluorine substitution, difloxacin has two fluorine substitutions, and orbifloxacin has a three-fluorine substitution, but the presence of more than one fluorine does not increase antibacterial effects (Asuquo and Piddock, 1993). When lipid solubility is expressed as the octanol: water partition coefficient, enrofloxacin has among the highest lipophilicity. By comparison, ciprofloxacin has a partition coefficient that is approximately 100-fold less than that of enrofloxacin; the corresponding partition coefficients of orbifloxacin and marbofloxacin are slightly higher than that of ciprofloxacin (Asuquo and Piddock, 1993; Takács-Novák et al., 1992). But there are no studies available to show that these chemical differences among the drugs can account for differences in clinical response. However, chemical differences may account for some variation in absorption and distribution. For example, ciprofloxacin oral absorption is approximately one-half that of enrofloxacin in dogs; ciprofloxacin absorption in horses is less than 10%, compared to 60% for enrofloxacin. The less lipid-soluble fluoroquinolones (marbofloxacin, orbifloxacin) have a lower volume of distribution (Vd) than the ones with higher lipid solubility (enrofloxacin) (Table 37.2). One explanation for this observation is that the more lipid-soluble drugs have higher intracellular concentrations, but higher tissue binding also could explain the differences in volume of distribution.

The most important structural difference in recent years is the addition of a substitution at position 8. These are usually referred to as *third-generation* fluoroquinolones to distinguish them from the *second-generation* drugs such as ciprofloxacin and enrofloxacin. For some human drugs, the addition is a methoxy (moxifloxacin), and for one of the veterinary drugs, it is a cyano group at position 8 that confers increased spectrum of activity.

Quinolones are amphoteric molecules that can be protonated at the carboxyl and the tertiary amine portion of the molecule (Martinez et al., 2006). The pK_a varies

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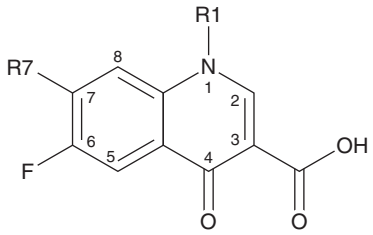
Companion Website: www.wiley.com/go/riviere/pharmacology

Table 37.1 Examples of fluoroquinolones used in veterinary medicine

Drug	Brand name(s)	Approved species
Enrofloxacin	Baytril, and generic	Dogs, Cats, Cattle, Pigs
Danofloxacin	Advocin	Cattle
Orbifloxacin	Orbax	Dogs, Cats
Pradofloxacin	Veraflox	Cats, (also dogs in some countries)
Marbofloxacin	Zeniquin, Marbocyl	Dogs, Cats (other species outside the U.S.)
Ciprofloxacin	Cipro and generic	Not approved for animals; human drug
Ibafloxacin	Ibaflin	No longer available (formerly dogs, cats)
Difloxacin	Dicural	No longer marketed (formerly dogs, and in some countries, cattle, and poultry)



Fluoroquinolone Structure

Figure 37.1 Structure of the fluoroquinolones. Features necessary for antibacterial activity are fluorine at position 6, ketone at position 4, and carboxyl at position 3. (Figure 37.2 shows other additions.) Addition of cyclopropyl, ethyl, or fluorophenyl at position 1 and of piperazine at position 7 increases the spectrum of antibacterial activity. Substitutions at position 8 broaden the spectrum of activity.

among the drugs slightly, but generally the pK_a for the carboxyl group is 6.0–6.4 (5.5–6.3 in some references) and the pK_a for the nitrogen of the piperazine group is 7.5–8 (Nikaido and Thanassi, 1993) (as high as 7.6–9.3 in some references). For two common drugs, enrofloxacin and ciprofloxacin, the pK_a for the carboxyl group is 6.0 and 6.1, respectively, and 8.8 and 8.7 for the amine, respectively (Martinez et al., 2006). The isoelectric point is midway between the pK_a for each ionizable group. Therefore, at physiological pH fluoroquinolones exist as zwitterions, in which both of the respective anionic and cationic groups are charged. It is at the isoelectric point (near the physiological pH) that fluoroquinolones are the most lipophilic and capable of diffusing across lipid membranes (Takács-Novák et al., 1992; Martinez et al., 2006).

Structure–Activity Relationships

Figure 37.1 shows the basic quinolone structure. The carboxyl group at position 3 and the ketone at position 4 are necessary for the antibacterial activity. The fluorine at position 6 differentiates the quinolones from the fluoroquinolones and accounts for the improved gram-negative and gram-positive activity over the nonfluorinated quinolones, increased potency, and increased entry into bacteria. At position 1, addition of a cyclopropyl (as for enrofloxacin and ciprofloxacin in Figure 37.2), an ethyl or a fluorophenyl improve the spectrum of activity against gram-positive and gram-negative bacteria. Addition of a piperazine at position 7, as demonstrated for ciprofloxacin, marbofloxacin, and enrofloxacin (Figure 37.2), improves the spectrum of activity to include pseudomonads, among other gram-negative bacteria. The change from a nitrogen (nalidixic acid) to a carbon at position 8 decreased some of the adverse central nervous system effects and increased activity against staphylococci.

Table 37.2 Interpretive categories for susceptibility break points (CLSI, 2015)

Drug	Species	Susceptible (μg/ml)	Intermediate (μg/ml)	Resistant (μg/ml)
Enrofloxacin	Dogs, cats	≤0.5	1–2	≥ 4
Orbifloxacin	Dogs, cats	≤1.0	2–4	≥ 8
Ciprofloxacin*	People	≤1.0	2	≥ 4
Marbofloxacin	Dogs, cats	≤1.0	2	≥ 4
Enrofloxacin	Pigs (gram negative)	≤0.25	0.5	≥ 1
Enrofloxacin	Pigs (gram positive)	≤0.5	1	≥ 2
Enrofloxacin	Horses	≤0.25	0.5	≥ 0.5
Enrofloxacin	Cattle (BRD)	≤0.25	0.5–1	≥ 2
Pradofloxacin	Dogs, Cats	≤0.25	0.5–1	≥ 2
Danofloxacin	Cattle (BRD)	≤0.25	0.5	≥ 1

* The breakpoint listed for ciprofloxacin is based on human use, not veterinary use.

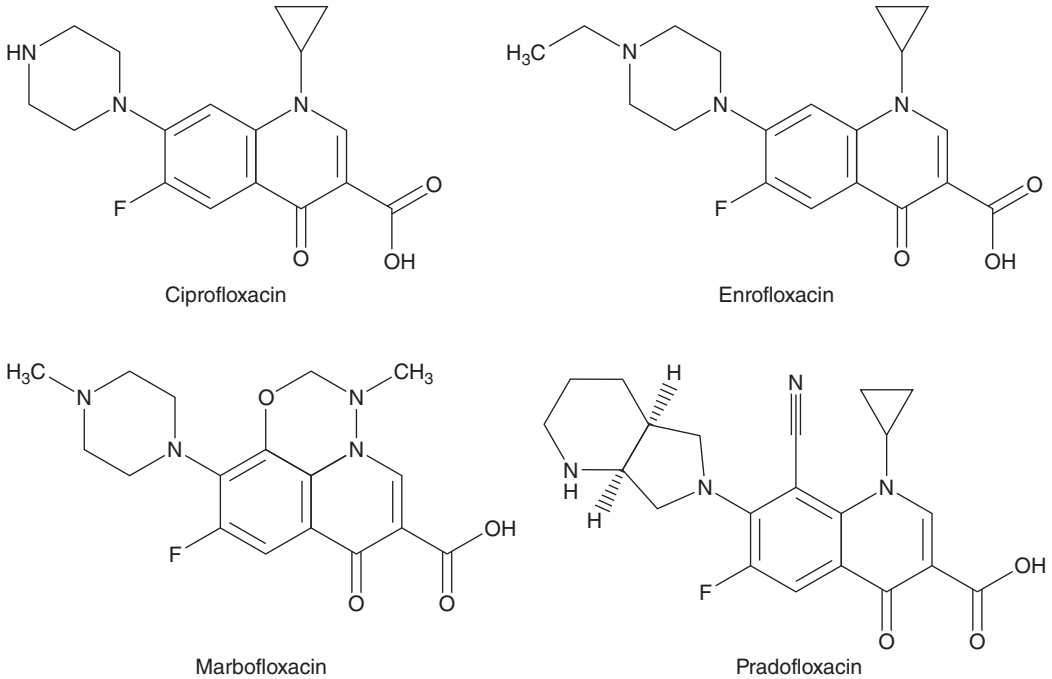


Figure 37.2 Structure of the fluoroquinolones. These representative structures show modifications from the basic structure presented in Figure 32.1. Features necessary for antibacterial activity are fluorine at position 6, ketone at position 4, and carboxyl at position 3. Addition of cyclopropyl, ethyl, or fluorophenyl at position 1 and of piperazine at position 7 increases the spectrum of antibacterial activity.

The *third-generation* fluoroquinolones have a bicyclic substitution at position 7, instead of a piperazine. This increased the activity to include a wider range of bacteria. In addition, a substitution at the 8 position on the ring enhances the bactericidal effects and improves the spectrum of activity to include more gram-positive and anaerobic bacteria. For example, the 8-methoxy substitution produces the drug moxifloxacin, a human quinolone with improved activity against gram-positive bacteria (Behra-Miellet et al., 2002; Aktaç et al., 2002; Pestova et al., 2000). Pradofloxacin, a veterinary quinolone with similar activity has a cyano substitution at position 8, which improves the activity against gram-positive and anaerobic activity compared to enrofloxacin and ciprofloxacin (Silley et al., 2007). This drug is discussed in more detail in Section Pradofloxacin Use in Dogs and Cats. A comparison of the activities of the veterinary fluoroquinolones against gram-positive and gram-negative bacteria can be illustrated in Figure 37.3 (using data from Wetzstein, 2005).

Mechanism of Action

Quinolones are bactericidal by inhibiting bacterial DNA replication and transcription. Two-stranded DNA is tightly coiled in the cell and must be separated for transcription and translation. To facilitate coiling, winding, and unwinding, the enzyme DNA gyrase allows the strands to be cut and reconnected. This allows coiling because negative supercoils can be introduced. DNA gyrase, a topoisomerase, consists of A and B subunits. The most common target site for quinolones is the A subunit of DNA gyrase coded by the gene *gyrA*. Mammals are resistant to the killing effects of quinolone antimicrobials because topoisomerase II in mammalian cells is not inhibited until the drug concentration reaches 100–1000 µg/ml. Bacteria are inhibited by concentrations of 0.1–10 µg/ml or less. Concentrations achieved in animals and people are much lower as seen by the Clinical Laboratory Standards Institute (CLSI, 2015) breakpoints (Table 37.2). Another target is the topoisomerase IV enzyme composed of subunits *parC* and *parE*. This site of action is less important for gram-negative bacteria but is a target of fluoroquinolones in some gram-positive bacteria such as streptococci and staphylococci (Ferrero et al., 1995). Among the newer generation fluoroquinolones – for example the veterinary drug pradofloxacin, and the human drugs moxifloxacin and gatifloxacin – the primary target for gram-positive bacteria may be the DNA-gyrase rather than topoisomerase IV, or these drugs also may be dual inhibitors against both targets (Intorre et al., 2007). Therefore, older fluoroquinolones have high activity against DNA-gyrase in gram-negative bacteria, but less against the topoisomerase IV of gram-positive bacteria. But the broader activity of newer fluoroquinolones can be attributed to higher affinity for both the DNA-gyrase in gram-negative and gram-positive bacteria,

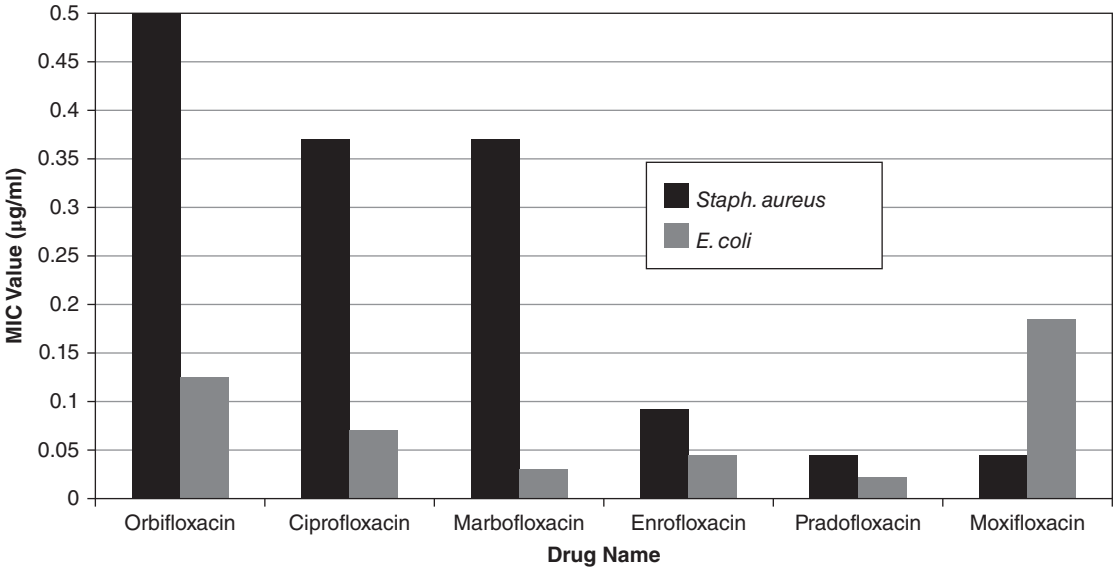


Figure 37.3 Comparison of fluoroquinolone MIC values for veterinary drugs, and compared to two human drugs, moxifloxacin and ciprofloxacin. Source: Data from Wetzstein, 2005.

as well as activity for the topoisomerase IV of gram-positive bacteria (Drlica and Zhao, 1997; Blondeau et al., 2004).

Antibacterial Spectrum

Fluoroquinolones in general exhibit good activity against most gram-negative bacteria, especially those of the Enterobacteriaceae. Representative minimum inhibitory concentration (MIC) values are shown in Table 37.3. *Escherichia coli, Klebsiella* spp., *Proteus* spp., *Salmonella* spp., and *Enterobacter* spp. are usually susceptible. *Pseudomonas aeruginosa* is variably susceptible and, when it is susceptible, usually has a higher MIC than other susceptible organisms. Against *P. aeruginosa,* ciprofloxacin is the most active (Rubin et al., 2008). Other bacteria susceptible to fluoroquinolones include intracellular organisms (*Rickettsia* spp., *Chlamydia,* and *Mycobacterium* spp.) and *Mycoplasma* spp.

Gram-positive bacteria are variably susceptible. *Staphylococcus aureus, Staphylococcus pseudintermedius,* and other *Staphylococcus* species usually are susceptible. However, the MIC values for staphylococci typically are higher than for gram-negative bacteria, and staphylococcal resistance to fluoroquinolones can be common.

In general, ciprofloxacin is more active than enrofloxacin against gram-negative bacilli, and enrofloxacin is more active against *Staphylococcus* species (Grobbel et al., 2007; Blondeau et al., 2012). Marbofloxacin activity usually falls between enrofloxacin and ciprofloxacin. The newer fluoroquinolones, such as moxifloxacin, gatifloxacin, and the veterinary drug pradofloxacin, have increased activity against gram-positive cocci and anaerobic bacteria (Behra-Miellet et al., 2002; Aktaç et al., 2002; Pestova et al., 2000; Silley, 2007) (Figure 37.3).

Factors that may affect activity are cations at the site of infection and low pH. Cations such as Al^{+3}, Mg^{+3}, Fe^{+2},

Table 37.3 Comparative microbiological data for common pathogens. Sources: Pirro et al., 1997, 1999; Asuquo and Piddock, 1993; Stegemann et al., 1996; Spreng et al., 1995; pradofloxacin FOI summary, and manufacturer data.

	MIC of bacteria (µg/ml)			
Drug	***Pasteurella multocida***	***Escherichia coli***	***Staphylococcus pseudintermedius***	***Pseudomonas aeruginosa***
Ciprofloxacin	0.015	0.03	0.25	0.5
Difloxacin	<0.05	0.11–0.23	0.25–0.91	0.92
Enrofloxacin	0.03	0.03–0.06	0.125	2.0
Marbofloxacin	0.04	0.125–0.25	0.23–0.25	0.94
Orbifloxacin	0.05	0.125–0.39	0.25–0.39	6.25–12.5
Pradofloxacin	0.008	0.03	0.06	

MIC values listed are MIC_{90} and represent an average from available published literature or manufacturer technical information.

and Ca^{+2} can bind a carboxyl group on the drug and significantly decrease activity. Low pH at the site of action also can affect the MIC (Ross and Riley, 1994), especially for drugs that have a piperazine at position 7 (Figures 37.1 and 37.2). For example, in urine, the MIC for fluoroquinolones may increase due to the presence of cations in the urine and low pH (Fernandes, 1988). This activity in urine may increase the MIC from fourfold to 64-fold. Fluoroquinolone activity in an abscess is not diminished despite the observation that in pus there is cellular material that can bind drugs, a low pH, and slow-growing bacteria (Bryant and Mazza, 1989). The presence of a foreign body, albumin, globulin, pus, anaerobic conditions, and dead bacteria did not affect the activity of fluoroquinolones (Rubinstein et al., 2000). The activity of fluoroquinolones in this milieu may explain its efficacy for treating infections associated with abscessation.

Susceptibility Testing

Susceptibility testing is performed either by the agar-disk-diffusion (ADD) method or broth dilutions (MIC test). The CLSI (CLSI, 2015) has published interpretive categories for breakpoints for most fluoroquinolones (Table 37.2). For isolates in the "intermediate" range, this is a category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used. Therefore, the dose can be increased in these instances, or successful treatment might be possible of the drug is applied topically or for a urinary tract infection. The MIC quality control ranges for wild-type organisms are also available from CLSI.

Resistance

There are multiple resistance mechanisms that have been identified for fluoroquinolones. These include: decreased drug permeability, increased drug efflux (efflux pumps), gyrase protecting enzymes, altered targets, and plasmid-mediated resistance. Of all the mechanisms listed, resistance most frequently develops via the *gyrA* mutation that codes for the A subunit of the DNA gyrase enzyme. A mutation at the serine-83 residue has been one of the most common, but at least 10 additional mutations at the *gyrA* gene have been identified to confer resistance (Ferrero et al., 1995). A mutation in the *parC* gene that codes for topoisomerase IV enzyme also is important (*grlA* mutation in staphylococci). A mutation in *parC* coding for resistant topoisomerase IV causes resistance when detected with mutations of *gyrA,* thus presence of both mutations usually produces high-level resistance. The multidrug resistance membrane efflux mechanisms reduce the accumulation of antibiotics in the bacteria. These efflux mechanisms are known to produce a high-level resistance to fluoroquinolones when they are present with other target site mutations (Zhanel et al., 2004). Because the efflux membrane pumps may affect other drugs, this may produce cross-resistance to other antimicrobials. Generally, there is cross-resistance among the fluoroquinolones. That is, although MIC values among susceptible strains can vary by a few dilutions, a fully resistant strain will typically be resistant to all the fluoroquinolones.

Resistance can occur through a multistep process (Everett et al., 1996). A single mutation can increase the MIC slightly (perhaps one dilution), and each subsequent mutation produces a progressively higher level resistance in a stepwise fashion. Unlike plasmid-mediated bacterial resistance, in which resistance may disappear after selective antibiotic pressure is removed, chromosomal (mutational) resistance exhibited by fluoroquinolone-resistant bacteria can be maintained in bacteria after drug administration is discontinued. Plasmid-mediated resistance has been found in *E. coli* and *Klebsiella* organisms, but the clinical significance of plasmid-mediated resistance has not been identified (Martinez-Martinez et al., 1998).

Clinical Resistance Problems

Resistance to fluoroquinolones has become a problem in human medicine and some investigators have attributed this to over-prescribing of these drugs. Resistance to fluoroquinolones by *E. coli, Staphylococcus aureus,* and *Streptococcus pneumoniae* has been well documented (Chen et al., 1999; Murphy et al., 1997; Sanders et al., 1995; Neu, 1992; Peña et al., 1995; Perea et al., 1999; Everett et al., 1996). Clinical resistance in human hospitals among staphylococci appeared relatively quickly after introduction of ciprofloxacin (Neu, 1992; Sanders et al., 1995; Hedin and Hambreus, 1991).

Resistant bacteria also have been identified in practically all animal species. Resistance in small animals has been documented for *E. coli, P. aeruginosa, Enterobacter, Proteus,* and other gram-negative bacteria. Resistance among staphylococci isolated from small animals has also been documented, particularly with methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (Couto et al., 2014; Bemis et al., 2009; Jones et al., 2007; Cole et al., 1998). The acquisition of specific *gryA/grlA* genes associated with phenotypic resistance to fluoroquinolones is strongly correlated with multidrug resistant methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (McCarthy et al., 2015).

Pseudomonas organisms have been particularly troublesome because single-step mutations are common for this bacteria, which can easily progress to high-level

resistance. This is an important problem in companion animal medicine because, except for the fluoroquinolones, there are no other oral drugs with which to treat infections caused by *Pseudomonas aeruginosa.* Factors leading to resistant *P. aeruginosa* are an inadequate dosage and extended treatment at low doses. Various studies have confirmed increased frequency of resistance among *Pseudomonas aeruginosa* isolated from chronic infections in dogs (Petersen et al., 2002; Martin Barrasa et al., 2000; Cole et al., 1998).

Human Health Risks from Drug Resistant Bacteria

Infectious disease experts have warned that administration of fluoroquinolones to animals may be a public health concern. Transfer of fluoroquinolone resistance from animals to people has been suggested to occur for *Campylobacter* species (Endtz et al., 1991) and *Salmonella typhimurium* type DT-104 (Threlfall et al., 1995, 1997; Griggs et al., 1994). An increase in the incidence of resistant *Campylobacter jejuni* infecting people was linked to consumption of *Campylobacter*-contaminated chicken. The increased resistance occurred primarily after 1995, which coincides with the time that fluoroquinolones were approved for use in poultry as an additive to drinking water (Smith et al., 1999). Investigators have also associated resistance in salmonellae with veterinary use of fluoroquinolones in livestock (Piddock et al., 1998). However, resistant strains of *Salmonella typhimurium* may have occurred spontaneously because some of the resistant salmonellae have come from farms in which fluoroquinolones were not administered to animals (Griggs et al., 1994). Nevertheless, some scientists have warned that use of fluoroquinolones in livestock is a public health risk because it can potentially lead to resistant mutants of salmonellae being passed on to humans through the food chain. Because of these concerns, there have been limited approvals of fluoroquinolones for food-producing animals, and the extralabel use of fluoroquinolones is prohibited in food-producing animals in the United States. Because of the risk of *Campylobacter* resistance, all fluoroquinolone formulations for poultry have been removed in the USA.

In order to provide continued monitoring of fluoroquinolone resistance bacteria from food animals, the National Antimicrobial Resistance Monitoring System (NARMS) performs routine surveillance of antibiotic resistance data from human clinical samples, slaughter samples and retail meat samples. In their most recent report (available at: http://www.cdc.gov/narms/), NARMS concluded that "Overall, ciprofloxacin resistance has been consistently low among *Salmonella* isolated from all sources. Similarly, ciprofloxacin resistance in *E. coli* was absent or very low (0–1.7%) in isolates from retail meats, chicken and cecal samples." They also found that for *Campylobacter jejuni* in retail chicken samples, resistance to ciprofloxacin was at its lowest level to date (11%), while ciprofloxacin resistance in *C. jejuni* isolated from chicken slaughter samples has not declined (22% in 2013).

Pharmacokinetics

Extensive pharmacokinetic data is provided in Table 37.4. These are presented as examples because there are far more pharmacokinetic studies available in the literature than can be presented in this table. A literature search of pharmacokinetic studies in animals will reveal many available studies in the entire array of species treated by veterinarians – ranging from large animal species of elephants and whales, to mice and small invertebrate species.

Mammals are relatively consistent in elimination half-life and volume of distribution. Reptiles with lower renal clearance generally demonstrate longer half-lives – as long as 55 and 36 hours for enrofloxacin in alligators and monitor lizards, respectively (Papich, 1999). There may be an allometric relationship in pharmacokinetic parameters among mammals ranging in size from mice to cattle (Bregante et al., 1999; Cox et al., 2004). The allometric relationship was improved considerably when the pharmacokinetic parameters were corrected for the percentage of protein-unbound enrofloxacin in the plasma (Table 37.5).

Because most of these drugs are administered either once daily or as a one-time administration, the elimination half-lives shown in Table 37.4 may have little effect on clinical outcome. For example, it does not appear that differences in half-life can account for different clinical results for skin infection treatment in dogs because enrofloxacin, which has the shortest half-life, and marbofloxacin, which has a long half-life, have both been reported to be effective when administered once daily (Paradis et al., 1990; Carlotti et al., 1995; Carlotti, 1996; Gruet et al., 1997; Koch and Peters, 1996; Ihrke, 1996, 1998; Cester et al., 1996). In cattle and pigs, enrofloxacin is approved for either a one-time dose of 7.5–12.5 mg/kg, or 2.5–5 mg/kg once daily for three to five treatments. With either regimen, the outcome is the same. Likewise, danofloxacin is approved for use in cattle at a dose of 6 mg/kg administered twice, or a single dose of 8 mg/kg, with similar outcomes.

Although the volume of distribution varies among animals (and among studies), these differences have not translated into differences in clinical efficacy. Marbofloxacin and orbifloxacin, which have a volume of distribution in the range of 1–2– l/kg, achieve effective skin concentrations and appear as clinically effective as drugs

Table 37.4 Pharmacokinetic comparison of fluoroquinolones

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (C_{max}) (μg/ml)	AUC[a] (μg · hr/ml)	%*F*	Assay[b]	Reference
Dogs									
Enrofloxacin	5	5	4.61	ND	1.75	11.65	Nd	HPLC	Frazier et al., 2000
Enrofloxacin	5	5	2.23	3.64	1.24	4.46	63.22	HPLC	Bidgood and Papich, 2005
Enrofloxacin	5.0 IV	5.0	2.7–3	5.0–5.6	—	4.05–4.34	—	HPLC	Intorre et al., 1995
Enrofloxacin	5.0	5.0	2.52	2.5	1.12 (oral)	7.27	72.3	HPLC	Cester et al., 1996
Enrofloxacin	5.8	5.0	4.4 (IV) 2.7 (oral)	4.5	1.44 (oral)	8.2	83.0	HPLC	Monlouis et al., 1997
Enrofloxacin	5.5	2.75–11.0	4.0 (oral)	nd	2.45 (oral)	16.32	Nd	Bioassay	Walker et al., 1992
Enrofloxacin	5.0	5.0	2.4	4.5	1.16 (oral)	3.9	100	HPLC	Küng et al., 1993a
Enrofloxacin	5.0	5.0–20.0[c]	4.8	4.2	1.6	8.15	—	HPLC	Stegemann et al., 1996
Ciprofloxacin	5.0	nd	3.17	2.23	0.35	4.18	43.0	HPLC	Cester et al., 1996
Ciprofloxacin	5.8[f]	nd	5.2	nd	0.34	7.2	Nd	HPLC	Monlouis et al., 1997
Ciprofloxacin	10.0	10.0–20.0	2.4	3.0	—	12.93	—	HPLC	Abadia et al., 1994
Ciprofloxacin	10.0	10.0–20.0	7.5	—	1.18 (oral)[d]	9.58 (oral)[d]	46[e]	Bioassay	Walker et al., 1990
Ciprofloxacin	25 (oral)	25–30 once daily	2.59	Na	4.44	22.5	58.36	HPLC	Papich, 2012
Ciprofloxacin	10 IV	15 IV once daily	3.72	2.39	Na	16.67	Na	HPLC	Papich, 2012
Difloxacin	5.0	5.0–10.0[c]	9.3	4.63	1.8 (oral)	12.93	96.0	nd	Manufacturer's data
Orbifloxacin	2.5	2.5–7.5[c]	5.6	1.5	2.33 (oral)	14.3	97–100	HPLC	Manufacturer's data
Marbofloxacin	5	5	7.63	1.54	3.63	42.08	104.6	HPLC	Bidgood and Papich, 2005
Marbofloxacin	2 SC	2 SC	13	Na	1.52	19.76	98.7	HPLC	Schneider et al., 1996
Marbofloxacin	4 SC	4 SC	13.4	Na	3.04	41.54		HPLC	Schneider et al., 1996
Marbofloxacin	4 oral	4 oral	12.5	Na	2.93	45.60	107	HPLC	Schneider et al., 1996
Marbofloxacin (2 studies)	2 IV	2 IV	12.4, 13.9	1.90, 2.25	Na	20.95, 18.60	Na	HPLC	Schneider et al., 1996
Marbofloxacin	2 oral	2 oral	14.0	Na	1.38	22.01	105	HPLC	Schneider et al., 1996
Marbofloxacin	2.0	2.0	9.8	1.4	1.35 (oral)	23.31	99.8	HPLC	Cester et al., 1996
Marbofloxacin	5.55 and 2.8 mg/kg	2.75–5.55[c]	9.5 (IV) 11 (oral)	1.27	2.0 at 2.8 mg/kg oral; 4.2 at 5.55 mg/kg oral	59.0	94.0	HPLC	Manufacturer's data
Marbofloxacin	5	5	4.07	Nd	1.41	8.74	Nd	Bioassay	Heinen, 2002
Marbofloxacin	2	2	9.07	Nd	1.47	13.07	Nd	Bioassay	Heinen, 2002
Marbofloxacin	2.75	2.75	10.9	Nd	2.53	35.44	Nd	HPLC	Frazier et al., 2000
Orbifloxacin	2.5	2.5	2.42	Nd	1.37	12.72	Nd	Bioassay	Heinen, 2002
Difloxacin	5	5	6.9	Nd	1.1	9.34	Nd	Bioassay	Heinen, 2002
Pradofloxacin	3	3–5	7.2–8	2.22	1.6	13.7	100	HPLC	Manufacturer's data

(*continued*)

Table 37.4 (*Continued*)

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (C_{max}) (μg/ml)	AUC[a] (μg · hr/ml)	%*F*	Assay[b]	Reference
Cats									
Levofloxacin	10	10	8.37	1.57	4.38	57.5	71.2	Bioassay	Albarellos et al., 2004
Enrofloxacin	5	5	6.7	2.5	1.0	20.3	107	HPLC	Seguin et al., 2004
Enrofloxacin (kitten)	5	5	3.7–6.3	1.8–3.9	0.5–1.1	5–12.7	33.7–72.8	HPLC	Sequin et al., 2004
Enrofloxacin	4.7	5[c]	6.7	6.3	1.66 (oral)	7.2	100	HPLC	Richez et al., 1997b
Orbifloxacin	2.5	2.5–7.5[c]	5.5	1.4	2.06	10.82	100	HPLC	Manufacturer's data
Ciprofloxacin	10 oral	10 mg/kg q12h oral	2.37	12.52	0.73	3.17	33	Bioassay	Albarellos et al., 2004
Ciprofloxacin	10 IV	10 mg/kg q12h	4.53	3.85	Na	17.20	Na	Bioassay	Albarellos et al., 2004
Pradofloxacin	5, 7.5 oral	5–7.5	7.3	4.45	2.1 at 5 mg/kg, 2.9 at 7.5 mg/kg	9.4 at 5 mg/kg; 13.3 at 7.5 mg/kg	69 tablets, 58% suspension	HPLC	Manufacturer's data
Horses									
Moxifloxacin	5.8	NR	34	5.3	3.12	50.9	Nd	HPLC	Gardner et al., 2004
Ciprofloxacin	5	NR	5.8	4.9 (Vdss)	0.6 (PO)	8.1	10.5	HPLC	Yamarik et al., 2010
Enrofloxacin	7.5	7.5 (oral)	5.9 (IV)	4.2	2.22	14.37	65.6	HPLC	Boeckh et al., 2001
Enrofloxacin	7.5	7.5 (oral)	5.3 (IV)	2.9	1.85	21.0	78.3	Bioassay	Haines et al., 2000
Enrofloxacin	5	5 (IV)	6.7	1.9	12.4 (IV)	25.3	Nd	HPLC	Papich et al., 2002
Enrofloxacin	2.5 and 5.0	5.0 (IV, IM) 5.0–7.5 (oral)	5.9–6.1	0.78 (5 mg/kg)	5.44	58.3	62.5	Bioassay	Giguère et al., 1996
Enrofloxacin (foals)	5.0	2.5–5.0	16.5	2.31	2.12 (10 mg/kg oral)	48.54	42.0	HPLC	Bermingham et al., 2000
Enrofloxacin	5	Nd	6.15 (IV)	2.32 (Vdss)	1.0 (approx)	13.8	55	HPLC	Peyrou et al., 2006
Marbofloxacin	2	Nd	7.42 (IV)	1.6 (Vdss)	0.8 (approx)	7.36	59	HPLC	Peyrou et al., 2006
Marbofloxacin	2	2	4.74 (IV)	1.17 (Vdss)	1.42 (IM)	11.27	87.9 (IM)	HPLC	Carretero et al., 2002
Marbofloxacin	2	2	7.56 (IV)	2.83	0.89 (PO)	8.26	62 (PO), 98 (IM)	HPLC	Bousquet-Melou et al., 2002
Ciprofloxacin	3.0	Not recommended	4.9 (IV) 10.7 (IM)	0.147	0.77 (IM)	6.97	96.0 (IM)	HPLC	Yun et al., 1994
Ciprofloxacin	5.0	Not recommended	2.6 (IV)	3.88	Na	4.83	6.8	Bioassay	Dowling et al., 1995
Ciprofloxacin	5 IV	Not recommended	5.80	4.93	Na	8.13	Na	HPLC	Yamarik et al., 2010
Ciprofloxacin	20 oral	Not recommended	3.63	38.075	0.63	3.30	10.47	HPLC	Yamarik et al., 2010
Orbifloxacin	2.5	2.5–5.0 (oral)	5.1	2.4	1.25 (oral)	9.06	68.3 (oral)	HPLC	Davis et al., 2006

Cattle									
Enrofloxacin	12.5	12.5	6.79	ND	0.96	14.95	ND	HPLC	Davis et al., 2007
Enrofloxacin	7.5	7.5	9.23	–	0.89	17.79	ND	HPLC	Foster et al., 2016b
Enrofloxacin	7.5	7.5	4.53	–	1.07	13.22	ND	HPLC	Foster et al., 2016b
Enrofloxacin	12.5	12.5	6.79	–	1.82	31.13	ND	HPLC	Foster et al., 2017)
Enrofloxacin	8	8	7.28	NS	0.81	7.51	ND	HPLC	TerHune et al., 2005
Enrofloxacin (1-day-old calves)	2.5	2.5–5 per day or 7.5–12.5 SC once	6.61	1.70	nd	13.94	nd	HPLC	Kaartinen et al., 1997
Enrofloxacin (1-week-old calves)	2.5	2.5–5 per day or 7.5–12.5 SC once	4.87	2.61	nd	6.73	nd	HPLC	Kaartinen et al., 1997
Enrofloxacin (lactating cows)	5.0	nd	1.68 (IV) 5.9 (IM)5.55 (SC)	1.63	0.73 (IM) 0.98 (SC)	7.42	82.0 (IM) 137.0 (SC)	HPLC	Kaartinen et al., 1995
Enrofloxacin (cows)	2.5	nd	2.82	2.98	nd	5.28	nd	HPLC	Bregante et al., 1999
Enrofloxacin (adult cattle)	5.0	2.5–5 per day or 7.5–12.5 SC once[c]	2.3	1.65	0.73 (SC)	10.08	88.0 (SC)	HPLC	Richez et al., 1997a
Enrofloxacin (calves)	5.0	2.5–5 per day or 7.5–12.5 SC once[c]	2.2	1.98	0.87 (SC)	7.99	97.0 (SC)	HPLC	Richez et al., 1997a
Enrofloxacin (beef steers)	5 IV	Na	5.15	1.59	Na	7.18	Na	HPLC	Idowu et al., 2010
Enrofloxacin (dairy cows)	5 IV	Na	3.69	1.56	Na	3.62	Na	HPLC	Idowu et al., 2010
Ciprofloxacin (calves)	2.8	nd	2.4	2.5	0.27	nd	53.0 (oral)	HPLC	Nouws et al., 1988
Danofloxacin	6	6	4.21	ND	1.7	9.72	ND	HPLC	TerHune et al., 2005
Danofloxacin	1.25	Nd	2.25	2.04	0.28	132.85	289	Bioassay	Shem-Tov et al., 1998
Danofloxacin	5	Nd	2.9	ND	0.82	4.7	78 (IM)	HPLC	Mann and Frame, 1992
Danofloxacin	1.25	Nd	6.26	4.0	0.18	2.9	91	HPLC	Apley and Upson, 1993
Marbofloxacin	10 IM	10 IM, once	17.5	Nd	7.92	52.66	Na	HPLC	Vallé et al., 2012
Marbofloxacin (Buffalo calves)	4 SC	4 SC x 3 days	7.37	Nd	1.63	14.17	Na	HPLC	Baroni et al., 2014
Goats									
Danofloxacin	1.25	Nd	4.67	3.8	0.33	2.29	110 (SC)	HPLC	Aliabadi and Lees, 2001
Enrofloxacin	5	5	1.1	1.3	6.7	6.7	ND	HPLC	Rao et al., 2000
Marbofloxacin	2	2	7.2	1.3 (Vdss)	1.9	8.44	100.7 (IM)	HPLC	Waxman et al., 2001

(*continued*)

Table 37.4 (*Continued*)

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (C_{max}) (µg/ml)	AUC[a] (µg · hr/ml)	%*F*	Assay[b]	Reference
Sheep									
Enrofloxacin	5	10	4.8	1.15	2.69	57.5	98.07	HPLC	Bermingham and Papich, 2002
Enrofloxacin	2.5	5.0 mg/kg/day SC	3.73	2.18	0.78 (IM)	5.47	85.0 (IM)	HPLC	Mengozzi et al., 1996
Enrofloxacin	2.5	5.0 mg/kg/day SC	3.8	1.3	0.6 (oral)	10.4	60.6 (oral)	Bioassay	Pozzin et al., 1997
Enrofloxacin	2.5	nd	2.5	1.53	nd	8.98	nd	HPLC	Bregante et al., 1999
Alpacas									
Enrofloxacin	5 and 10	5–10	13.0	1.6	7.8 (SC), 15.3 (PO)	58.4	90 (SC), 29 (PO)	HPLC	Gandolf et al., 2005
Elephants									
Enrofloxacin	2.5 (PO)	2.5 (PO)	18.4	Nd	1.31	34.93	Nd	HPLC	Sanchez et al., 2005
Mice									
Enrofloxacin	10.0	nd	1.48	10.5	nd	2.45	na	HPLC	Bregante et al., 1999
Rats									
Enrofloxacin	7.5	nd	1.8	4.78	nd	5.65	nc	HPLC	Bregante et al., 1999
Rabbits									
Enrofloxacin	7.5	nd	2.2	4.94	nd	5.52	nd	HPLC	Bregante et al., 1999
Enrofloxacin	7.5 (IV)	nd	1.87	3.97	na	5.38	na	HPLC	Aramayona et al., 1996
Enrofloxacin	5.0 (IV)	5.0	2.18 (IV)	4.4	na	3.89	na	HPLC	Cabanes et al., 1992
Enrofloxacin	5.0 (IM)	5.0	1.8	na	3.04	3.84	92.0	HPLC	Cabanes et al., 1992
Enrofloxacin	5.0 (IV)	5.0	2.5	2.12	na	8.6	na	HPLC	Broome et al., 1991
Enrofloxacin	5.0 (oral)	5.0	2.4	na	0.45	5.4	61.0	HPLC	Broome et al., 1991
Moxifloxacin	5	nd	1.84	2.12	1.3 (PO)	6.28	75.5 (PO)	HPLC	Fernández-Varón et al., 2005
Marbofloxacin	2 (IM)	2	4.33	1.65	1.8	12.44	100	HPLC	Abo-El-Sooud and Goudah, 2010
Marbofloxacin	5 oral	5 oral	8	Na	1.73	10.5	Na	LC-MS	Carpenter et al., 2009
Chickens									
Ciprofloxacin	5.0	5.0–15.0 IM, SC, oral	9.01	2.02	4.67	78.04	70.0	Bioassay	Atta and Sharif, 1997
Enrofloxacin	10.0	10.0	5.6 (IV)	5.0	1.88 (oral)	16.17	89.2	HPLC	Knoll et al., 1999
Ciprofloxacin	5.0	5.0	9.0	2.0	4.67	78.04	70.0 (oral)	Bioassay	Atta and Sharif, 1997
Enrofloxacin	10.0	10.0	10.3 (IV)	4.3	2.44 (oral)	34.51	64.0	HPLC	Anadón et al., 1995
Enrofloxacin	10 (PO)	NR	14	Nd	1.5	35	Nd	HPLC	Da Silva et al., 2006
Camels									
Enrofloxacin	2.5	2.5 IM, SC	3.58	1.4	1.44 (IM)	18.95	85.0 (IM)	Bioassay	Gavrielli et al., 1995
Harbor Seals									
Marbofloxacin	5	5	4.96	1.29	3.44	30.91	Nd	HPLC	KuKanich et al., 2007

Invertebrates									
Enrofloxacin Cuttlefish	10	10	1.0	Nd	10.95	26.71	Nd	HPLC	Gore et al., 2005
Sea Urchin	10 inject	10 inject or 10 mg/L water bath	38.8	0.45	90.92	1,200	Na	HPLC	Phillips et al., 2016
Sea Stars	5 inject	5 inject or 5 mg/L water bath	42.76	0.713	18.9	345.85	Na	HPLC	Rosenberg et al., 2016
Pigs									
Enrofloxacin	2.5		345	3.34	1.17	5.97	150	HPLC	Zeng and Fung, 1997
Enrofloxacin	2.5 IM	2.5 IM	12.06	1.85	1.17	19.18	74.53	HPLC	Anadón et al., 1999
Enrofloxacin	2.5 IV	Nd	9.64	1.41	Na	25.69	Na	HPLC	Anadón et al., 1999
Enrofloxacin	2.5 IM	2.5 IM	9.3	3.22	0.695	8.903	Na	HPLC	Bimazubute et al., 2010
Enrofloxacin	2.5		7.73	3.5	0.61 (IM)	9.94	95.0 (IM)	HPLC	Richez et al., 1997a
Enrofloxacin	2.5		5.5	3.95	0.75 (IM)	5.03	101 (IM)	Bioassay	Pijpers et al., 1997
Enrofloxacin	10.0 oral		nd	nd	nd	27.0	73.0–80.0	HPLC	Gyrd-Hansen and Nielsen, 1994
Enrofloxacin	7.5 SC	7.5 SC	26.6	6.4	1.10	47.86	Na	HPLC	Messenger et al., 2012
Ciprofloxacin	3.06	nd	2.57	3.83	0.17	2.88	37.0	HPLC	Nouws et al., 1988
Danofloxacin	5	Nd	8.0	ND	0.8	6.0	76 (IM)	HPLC	Mann and Frame, 1992
Marbofloxacin (12 weeks)	8 IV	8 IM or IV	13.5	Nd	Nd	88.6	Na	HPLC	Schneider et al., 2014
Marbofloxacin (12 weeks)	8 IM	8 IM or IV	13.2	1.58	5.55	79.9	89.6	HPLC	Schneider et al., 2014
Marbofloxacin (16 weeks)	8 IV	8 IM or IV	18	Nd	Nd	102	Na	HPLC	Schneider et al., 2014
Marbofloxacin (16 weeks)	8 IM	8 IM or IV	16.7	1.75	5.86	106	105	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	8 IV	8 IM or IV	18.4	1.58	Nd	127	Nd	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	4 IM	8 IM or IV	15.4	Nd	3.38	56.9	90.0	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	8 IM	8 IM or IV	15.1	Nd	6.3	115	91.5	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	16 IM	8 IM or IV	15.2	Nd	15.5	228	90.1	HPLC	Schneider et al., 2014
Fish									
Enrofloxacin trout	5.0 and 10.0	5.0 mg/kg q24h	24.0 and 30.0	3.22 and 2.56	0.945 and 1.28	109.2 and 171.3	42 and 49	Bioassay	Bowser et al., 1992
Enrofloxacin red pacu	5.0	5.0 mg/kg q48h IM	29.0	nd	1.64 (IM) 0.8 (oral)	46.3 (IM)	57.0 (relative)	HPLC	Lewbart et al., 1997
Enrofloxacin Atlantic salmon	10.0	5.0 mg/kg q24h oral	131.0	22.4	0.29 (oral) 0.54 (oral)	84.3 1.3 (IP)	89.0 (IP) 46 (oral)	Bioassay 66 (IM)	Stoffregen et al., 1997

(*continued*)

Table 37.4 (*Continued*)

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (C_{max}) (µg/ml)	AUC[a] (µg · hr/ml)	%*F*	Assay[b]	Reference
Enrofloxacin Korean Catfish	10 IV	10	17.4	3.93	Na	60.34	Na	LC/MS	Kim et al., 2006
Enrofloxacin Korean Catfish	10 oral	10 oral	34.13	Na	1.02	38.43	64.6	LC/MS	Kim et al., 2006
Turtles									
Enrofloxacin Yellow-bellied sliders	5 IM	5 IM	17.6	Na	6.28	75	Na	HPLC	James et al., 2003
Enrofloxacin Yellow-bellied sliders	10 oral	10 oral	32.8	Na	3.44	53.2	93.6	HPLC	James et al., 2003
Enrofloxacin, Red-eared sliders	10 IP	10 IP	47.6	2.5	10.36	308.8	Na	HPLC	Giorgi et al., 2013
Enrofloxacin Loggerhead sea turtles	10 oral	Na	37.8	2.17	4.1	261	Na	Bioassay	Jacobson et al., 2005
Enrofloxacin Loggerhead sea turtles	20 oral	20 (once per week)	54.4	0.93	21.3	1799	Na	Bioassay	Jacobson et al., 2005
Frogs									
Enrofloxacin African Clawed Frog	10 IM	10 IM	5.32	0.84	10.85	57.59	Na	HPLC	Howard et al., 2010
Enrofloxacin African Clawed Frog	10 SC	10 SC	4.08	0.92	9.79	47.42	Na	HPLC	Howard et al., 2010

Na = Data not available or not applicable.
Nd = Not determined, NR = not recommended.
$t_{1/2}$ = Half-life of the terminal portion of the plasma concentration vs. time curve.
AUC = Area under the curve of the plasma concentration vs. time curve.
C_{max} = Maximum plasma concentration after administration of oral or IM dose.
%*F* = Percentage of oral or IM administered dose absorbed (determined from comparison of IV dose).
[a]Half-life, volume of distribution, and AUC are from an IV dose unless otherwise noted. Apparent volume of distribution is typically from the area method. Where the dose was administered by a non-IV route, the Vd reported is Vd/F.
[b]Assay type = Assay using HPLC is able to distinguish between enrofloxacin and ciprofloxacin, and values shown in table represent enrofloxacin. Assays performed by bioassay represent the parent drug and active metabolites. Bioassay may include concentrations of ciprofloxacin.
[c]Registered dose with the FDA in the United States. In some European countries doses may vary or may not include the flexible range. In most cases, when treating non-*Pseudomonas* infections, the lowest dose in the range listed is used.
[d]After multiple dosing with ciprofloxacin, the C_{max} was 1.18 mg/ml and the 12 hr AUC was 9.58 mg/hr/ml.
[e]Oral absorption of ciprofloxacin estimated from a comparison of independent oral and IV studies.
[f]These parameters determined after administration of 5.8 mg/kg of enrofloxacin.
Note that in enrofloxacin studies, ciprofloxacin also was detected at varying concentrations (depending on species and study). Ciprofloxacin pharmacokinetics were measured in those studies but is not reported in this table. The ciprofloxacin data can be found in the studies referenced.

Table 37.5 Plasma/serum protein binding of fluoroquinolones in animals (% bound)

Animal	Enrofloxacin	Ciprofloxacin	Marbofloxacin	Orbifloxacin	Pradofloxacin
Camel	17–24				
Cattle	56, 36–45, 60, 46	31, 70, 19, 49.6, 33.8			
Sheep	69				
Horse	22	37		21 (at 1 μg/ml)	
Pig	27	23.6, 35			
Dog	15–25, 27, 35, 72	44, 18.5	9.1, 22	13.24 (at 1 μg/ml)	30–35 28.8–31
Rabbit	53, 50, 35, 6.0	33, 28			
Chicken	21	30			
Mouse	42				
Rat	50				

Sources: Villa et al., 1997; Gavrielli et al., 1995; Nouws et al., 1988; Aramayona et al., 1994; Idowu et al., 2010; Davis et al., 2007; Bidgood and Papich, 2005, and author Papich's unpublished data.
When two or more values are listed, they represent results from independent studies.

such as enrofloxacin with a volume of distribution of 2.5–5 l/kg. Heinen (2002) demonstrated that even though approved doses may vary among enrofloxacin, marbofloxacin, and orbifloxacin in dogs, they are all capable of achieving pharmacokinetic–pharmacodynamic (PK-PD) targets (these targets are discussed in Section Pharmacokinetics / Pharmacodynamics). The data published (Bidgood and Papich, 2005, Walker et al., 1990, 1992; Messenger et al., 2012; Davis et al., 2007) or available from manufacturer technical information show that the fluoroquinolones, regardless of their volume of distribution and lipophilicity, achieve effective concentrations in tissues. Some exceptions are tissues not easily penetrated such as the central nervous system and eye.

Oral Absorption

Whether fluoroquinolones are administered with or without food has little effect on oral absorption. Feeding may delay the time to peak concentration (T_{max}), but this has little effect on clinical outcome because the extent of absorption, determined by the total AUC (area under the curve of the plasma concentration versus time curve) is not affected significantly. Administration with food has prolonged the terminal half-life when enrofloxacin was administered orally to reptiles (Papich, 1999), sheep, pigs (Gyrd-Hansen and Nielsen, 1994), and chickens (Anadón et al., 1995). This may be caused by a "flip-flop" pharmacokinetic effect introduced in Chapter 3 and discussed below in this section.

In clinical situations involving animals difficult to dose, fluoroquinolones may be added to a patient's food in order to provide a more convenient dosing form. For example, enrofloxacin tablets were placed in whole fish, which were fed to dolphins to produce good absorption (Linnehan et al., 1999), and enrofloxacin was injected into mice and fed to monitor lizards, also producing good absorption (Hungerford et al., 1997). Chewable tablets of enrofloxacin (Taste-Tabs) do not affect oral absorption (manufacturer's data).

In dogs, cats, and pigs, oral absorption of fluoroquinolones approaches 100% (Table 37.4), but in large animals, the extent of absorption has been less. Oral absorption of fluoroquinolones is variable in horses. Ciprofloxacin showed an oral absorption of only 6.8% in ponies (Dowling et al., 1995) and only 10% (mean) in adults (Yamarik et al., 2010). But enrofloxacin absorption is 63% (Giguère et al., 1996) in adult horses and 42% in foals (Bermingham et al., 2000). The value reported for adult horses is probably artificially high because the study used a bioassay that overestimates the concentration of enrofloxacin in plasma. With the exception of ciprofloxacin, other studies listed in Table 37.4 indicate relatively good oral absorption of these drugs in horses. Studies in ruminants produce conflicting results on oral absorption. In sheep, oral absorption was reported to be 61% (Pozzin et al., 1997), but absorption in ruminant calves was listed as less than 10% (Vancutsem et al., 1990). In sheep, absorption from oral administration was high (Bermingham et al., 2002). In addition, this study in sheep showed that when enrofloxacin was mixed with food and administered orally, absorption was high and half-life longer than after the IV dose. The long oral half-life was the result of slow release from the feed (possible adsorption) or slow emptying from the rumen ("flip-flop" effect). Absorption is also good in nondomestic ruminants such as the hoofstock kept in zoological parks (Gandolf et al., 2003; Gandolf, 2006). In camels (although not a true ruminant), oral absorption was reported to be negligible in one study (Gavrielli et al., 1995), but in another study absorption was 29% (mean) in alpacas and was capable of producing high enough concentrations to treat susceptible gram-negative pathogens at 10 mg/kg (Gandolf et al., 2005).

In birds, oral absorption of enrofloxacin is good, with effective levels being achieved by adding the drug to the bird's drinking water. This method of administration has been employed to treat sick pet birds (Flammer, 1998) and chickens (Knoll et al., 1999). However, as mentioned previously, administration to food-producing poultry is now prohibited in the United States. After continuous medication in the drinking water, steady-state plasma concentrations of enrofloxacin are 0.53 μg/ml (Knoll et al., 1999). In fish enrofloxacin absorption has been estimated to be 40–50% (Lewbart, 1998).

Intramuscular and Subcutaneous Injection

Absorption is virtually complete from intramuscular (IM) and subcutaneous (SC) injection. However, in some animals there was delayed absorption from IM or SC injections, which produced longer half-lives from these routes compared to IV administration (this is known as "flip-flop" absorption kinetics). Delayed absorption from injection is possibly due to a slow release from the injection site caused by tissue binding or tissue injury that disrupts blood flow. The large animal formulation of enrofloxacin (Baytril-100) is quite alkaline and can cause irritation at the injection site that can produce a delay in absorption.

In cattle, for example (Kaartinen et al., 1995), half-life was 1.68 hours from IV injection of enrofloxacin (5 mg/kg), but 5.9 and 5.55 hours from IM and SC injection, respectively, even though the extent of absorption was high. In the study by Davis et al. (2007) the half-life of enrofloxacin after a SC injection to calves at 12.5 mg/kg was 6.79 hours (mean), and 7.25 hours for the metabolite ciprofloxacin. For danofloxacin, the IV half-life in cattle was 0.9 hours and the IM half-life was 2.26 hours.

Metabolism

Metabolism of enrofloxacin to ciprofloxacin occurs via de-ethylation of the ethyl group on the piperazine ring. Activity against many bacteria is similar, but ciprofloxacin is somewhat more active than enrofloxacin against gram-negative bacilli, and enrofloxacin more active against *Staphylococcus* species (Riddle et al., 2000; Blondeau et al., 2012; Wetzstein, 2005; Grobbel et al., 2007). Together they produce an additive effect, and may broaden the spectrum compared to each drug alone. Other metabolites are produced from additional metabolism of ciprofloxacin, but these are minor and do not contribute to the antibacterial effects. There are also minor insignificant metabolites of some of the other drugs.

Examination of the extent of metabolism of enrofloxacin to ciprofloxacin in dogs and cats was reported in several studies (Küng et al., 1993a; Monlouis et al., 1997; Cester et al., 1996; Richez et al., 1997b; Kordick et al., 1997; Heinen, 1999; Seguin et al., 2004) in which high-pressure liquid chromatography (HPLC) was used to determine the specific concentrations of enrofloxacin and ciprofloxacin after enrofloxacin administration. The peak concentration (C_{max}) of ciprofloxacin accounts for approximately 20% in dogs and cats. Cattle produce more ciprofloxacin from enrofloxacin than most other species studied. The proportion of ciprofloxacin in plasma from administration of enrofloxacin to cattle has been measured as 25% (Richez et al., 1994), 31% (Foster et al., 2016a, 2016b), 37–39% (Idowu et al., 2010), and as high as 41% (Davis et al., 2007). The amount of ciprofloxacin produced in beef steers was slightly higher than dairy cows (ratio 64% and 59%, respectively) (Idowu et al., 2010). The studies by Davis et al. (2007), Foster et al. (2016a, 2016b), and Anadón et al. (1995) show that the ratio of ciprofloxacin: enrofloxacin concentrations in tissue can increase to greater than 1.0 after administration of enrofloxacin despite the low plasma concentrations of ciprofloxacin.

In pigs and foals there were only small traces of ciprofloxacin metabolized from enrofloxacin (Zeng and Fung, 1997; Bermingham et al., 2000; Richez et al., 1997a; Messenger et al., 2012). Older pigs may have greater metabolism to ciprofloxacin (Anadón et al., 1999).

In nonmammal species, the amount of ciprofloxacin produced from administration of enrofloxacin varies greatly. Studies in fish show that after administration of enrofloxacin, only 2% of the total fluoroquinolone concentration is comprised of ciprofloxacin (Lewbart et al., 1997). Reptiles and invertebrates tend to produce such small amounts of ciprofloxacin that it does not contribute to the plasma profile or AUC (Gore et al., 2005; Helmick et al., 1997; Howard et al., 2010; Hungerford et al., 1997; Papich, 1999; Raphael et al., 1994; Phillips et al., 2016; Rosenberg et al., 2016; Giorgi et al., 2013; James et al., 2003). In chickens, the concentrations of ciprofloxacin in plasma and tissues after administration of enrofloxacin are minimal (Knoll et al., 1999; Anadón et al., 1995).

If there are active metabolites produced, such as ciprofloxacin from enrofloxacin, this can cause errors in the interpretation of drug assays when a bioassay (microbiological assay) is used because a bioassay does not distinguish parent drug from active metabolite. Pharmacokinetic studies performed using bioassay techniques compared with studies using HPLC methods have demonstrated that bioassay can overestimate the combined enrofloxacin and ciprofloxacin concentrations in animals by as much as 70% for the AUC and 29% for the peak concentration (Cester et al., 1996). This finding agrees with another study in dogs: a microbiological assay overestimated the total enrofloxacin plus ciprofloxacin AUC determined by HPLC by as much as 30–70% (Küng et al., 1993b).

Excretion

The fluoroquinolones are primarily excreted via the kidneys by glomerular filtration and tubular excretion (Bregante et al., 1999). The role of tubular excretion has been demonstrated by showing that probenecid can decrease the clearance for some fluoroquinolones. For most of the drugs a major portion of the parent drug or metabolites can be recovered in the urine, with a smaller amount recovered in the feces. An exception is difloxacin (no longer marketed for animals), for which 80% of a dose was recovered in the feces and renal clearance accounted for less than 5% of the total systemic clearance.

Protein Binding

Protein binding is moderate for most of the fluoroquinolones (Table 37.5). The extent of protein binding has not limited distribution to tissues and has not produced a protein-binding interaction if displaced by another drug. Although protein binding is generally low, as shown in Table 37.5, there is a lack of consistency among studies, which is probably related to differences in technique used to measure protein binding. Protein binding can produce a reduction in antibacterial activity (Zeitlinger et al., 2008), but does not impair distribution to tissues. When in vivo ultrafiltration techniques were used in animals, the free drug concentration (protein unbound) in tissue fluid exceeded the fraction unbound in plasma (Davis et al., 2007; Bidgood and Papich, 2005; Foster et al., 2016a, 2016b; Messenger et al., 2012; Hauschild et al., 2013).

Tissue Distribution

Distribution to most tissues is listed for each drug in the manufacturer's package insert or Freedom of Information (FOI) summary. Specific studies for enrofloxacin have been conducted to show that it distributes to bone (Duval and Budsberg, 1995), prostate (Dorfman et al., 1995), and skin (DeManuelle et al., 1998). Specific studies using in vivo ultraviltration techniques show that distribution of the unbound drug (microbiologically active concentration) generally exceeds the value predicted by the unbound concentration in plasma (Davis et al., 2007; Bidgood and Papich, 2005; Foster et al., 2016a, 2016b; Messenger et al., 2012; Hauschild et al., 2013). Thus, extracellular tissue fluid concentration of the unbound fluoroquinolone will generally be in equilibrium with, or exceed, plasma drug concentrations.

In tissues in which the fluoroquinolones accumulate intracellularly, high tissue concentrations are reported because measurement of tissue concentrations is typically performed by homogenizing the tissue, which disrupts cells and releases intracellular concentrations. Tissue concentrations measured in this manner represent both intracellular and extracellular concentrations and can overestimate active drug concentrations. Some tissues, such as liver and kidney, may have fluoroquinolone concentrations several-fold higher than corresponding plasma concentrations.

Fluoroquinolones attain moderately high intracellular concentrations in macrophages and neutrophils. The cellular: extracellular (C : E) ratio is often greater than 4, but usually less than 10 compared to plasma concentrations (Pascual et al., 1990; Tulkens, 1990; Easmon and Crane, 1985; Drusano et al., 1998). The reason for intracellular concentration is unclear (Drusano et al., 1998; Van Bambeke et al., 2006). Fluoroquinolones are moderately lipophilic and also have a slower efflux from these cells. In humans, ciprofloxacin has an intracellular half-life of 6.7 hours in neutrophils versus 3.7 hours in serum (Easmon et al., 1986).

Concentrations in urine may be several times higher than plasma concentrations. Concentrations of enrofloxacin, marbofloxacin, and orbifloxacin in urine of dogs are listed by the manufacturer to be 43, 40, and 84.5 μg/ml, respectively. One exception to the high urine excretion is difloxacin (no longer marketed) for which less than 5% of the dose is cleared in the urine. Fluoroquinolones are among the few drugs that adequately penetrate the prostate gland in sufficient concentrations to treat prostatitis caused by infection. Enrofloxacin concentration (determined by bioassay) in the prostatic fluid and prostate tissue exceeded serum concentration at all times after administration (Dorfman et al., 1995). There were no differences in tissue concentrations when infected prostate was compared to healthy tissue. Concentrations of other fluoroquinolones in prostate tissue have been reported by the manufacturers to be 3.36, 5.6, and 1.35 μg/gram for difloxacin, marbofloxacin, and orbifloxacin, respectively.

Pharmacokinetics / Pharmacodynamics

Minimum inhibitory concentration values for bacteria are listed in Table 37.3 and breakpoints for susceptibility testing listed in Table 37.2. Even though there are differences in potency among the currently available fluoroquinolones, a pattern is apparent: *Pasteurella,* such as the strains found in skin wounds and cause of respiratory infections in livestock, are the most susceptible; wild-type strains of the gram-negative enteric bacilli (e.g., *E. coli* and *Klebsiella*) also have low MIC values. The gram-positive cocci such as *Staphylococcus aureus* and the common canine skin pathogen *Staphylococcus pseudintermedius* have MIC values at a somewhat higher range, and *P. aeruginosa* has MIC values that are among the highest for susceptible bacteria. Although not listed

in Table 37.3, streptococci, enterococci, and anaerobic bacteria typically have MIC values high enough that they are usually in the resistant category. Fluoroquinolones of the third generation, such as pradofloxacin (Figure 37.3) have lower MIC values for gram-positive bacteria than the second-generation drugs.

Fluoroquinolones are bactericidal and they act in a concentration-dependent manner rather than a time-dependent manner. The exposure to the bacteria has been measured by using the maximum peak concentration (C_{max}) in relation to the bacteria MIC and expressed as the C_{max} : MIC ratio. A C_{max} : MIC ratio that is at least 8–10 times (i.e., a peak concentration that is 8–10 times the MIC) is desirable. A high C_{max} : MIC ratio is optimal to decrease emergence of resistance (Drusano et al., 1998; Blaser et al., 1987), but the area-under-the-curve (AUC) to MIC ratio (AUC : MIC) may be used to predict efficacy. When low C_{max} : MIC ratios were achieved, the mutant strains that occur spontaneously were not suppressed, and resistance emerged because these mutant strains have MIC values that are at least four to eight times that of the parent (wild-type) strain (Drusano et al., 1993).

The area under the curve (AUC) for a 24-hour dose interval in relation to the MIC is expressed as the AUC_{24} : MIC ratio. It is understood that the AUC represents the protein-unbound concentration. An AUC : MIC ratio of 125–250 has been associated with the optimum antibacterial effect (Lode et al., 1998; Hyatt et al., 1995; Dudley, 1991; Nicolau et al., 1995; Wright et al., 2000). According to USCAST (2015, http://www.uscast.org/) the free-drug AUC : MIC targets for a 2-$\log_{10}$ reduction (99% reduction) is 140, 65, 187, and 34 for Enterobacteriaceae, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus*, respectively, based on experimental conditions.

These targeted C_{max} : MIC and AUC : MIC ratios were based on in vitro or in vivo studies performed with immunosuppressed laboratory animals or on clinical studies involving people with serious illness (Forrest et al., 1993; Blaser et al., 1987; Sullivan et al., 1993).

As reviewed by Wright et al., (2000), there is evidence that for some clinical situations, AUC : MIC ratios as low as 30–55 are necessary for a clinical cure, since the study in which 125 was cited involved critically ill human patients. The USCAST evaluation cited above (2015, http://www.uscast.org/) lists clinical AUC : MIC targets above 70 for Enterobacteriaceae and *Pseudomonas aeruginosa,* and 34 for *Streptococcus*. A lower AUC : MIC ratios for stereptococci than for gram-negative bacteria was also shown by Ambrose and Grasela (2000), in which they presented data and reviewed the relevant publications.

Some veterinary studies have shown that the ratios necessary for clinical results may not be as high as reported from studies in laboratory rodents or people (Lees and Aliabadi, 2002).Many veterinary patients treated with fluoroquinolones are not as immunosuppressed as laboratory animals or ill as people that participated in some clinical studies. Therefore the targeted ratios may be less in veterinary patients than cited above. For example, if one compares the AUC in Table 37.3 to representative MIC values from Table 37.4, lower AUC : MIC ratios in some patients appears adequate.

Dose Guidelines

Specific clinical uses are discussed in this chapter for the major species. Doses listed in Tables 37.4 and 37.6 cover a wide MIC range among susceptible bacteria, from as low as 0.03 μg/ml to as high as 1.0 μg/ml. The upper end of the dose is limited by safety (such as gastrointestinal or central nervous system effects); the lower dose is determined by efficacy. There is no advantage to frequent dosing (multiple times/day) as long as a sufficiently high C_{max} : MIC or the same AUC : MIC is achieved; therefore, the doses discussed for mammals and listed in Table 37.4 are intended for once-daily administration, or for some uses in cattle and pigs, a single high dose may be used for respiratory infections (Table 37.7). The single high dose regimen has not been tested for animals other than pigs and cattle.

Clinical Use

Dogs and Cats

The administration of fluoroquinolones to dogs and cats constitutes one of the largest applications of these drugs for veterinary medicine. They have been used for over 25 years for infections of the skin, soft tissue, oral cavity, urinary tract, prostate, ear, wounds, respiratory tract, and bone (Paradis et al., 1990; Ihrke and DeManuelle, 1999; Ihrke, 1996; Ihrke et al., 1999; Carlotti et al., 1999; Griffin, 1993; Hawkins et al., 1998; Dorfman et al., 1995; Cotard et al., 1995). The first veterinary quinolone was enrofloxacin, and veterinarians now have experience with marbofloxacin, orbifloxacin, and pradofloxacin (and occasionally others in different countries) (Table 37.1). The efficacy of the fluoroquinolones was established for indications listed on the labels approved by the US Food and Drug Administration (FDA), European authorities, and licensing in other countries.

At the approved doses, orbifloxacin, enrofloxacin, and marbofloxacin reach therapeutic targets in dogs against susceptible bacteria, even though the doses and pharmacokinetics vary among the drugs (Heinen, 2002). The efficacy of enrofloxacin and marbofloxacin has been

Table 37.6 Dose recommendations for enrofloxacin in exotic animals

Animal	Dose	Route	Interval	Reference
Alligator	5 mg/kg	IV, oral	Every 96 hr	Helmick et al., 1997
Savanna monitor	5 mg/kg (10 mg/kg for *Pseudomonas* spp.)	IM, oral	Every 96 hr	Hungerford et al., 1997
Burmese python	5 mg/kg (higher doses for *Pseudomonas* spp.)	IM	Every 48 hr	Young et al., 1997
Indian star tortoise	5 mg/kg	IM	Every 24 hr	Raphael et al., 1994
Red-eared slider	5 mg/kg	oral, IM	Every 72 hr (oral), every 48 hr (IM)	James et al., (unpublished data)
Gopher tortoise	5 mg/kg	IM	Every 24–48 hr	Prezant et al., 1994
Bottlenose dolphin	5 mg/kg	oral	Every 24 hr	Linnehan et al., 1999
Parrot and cockatoo	7.5–15 mg/kg	oral	Every 12 hr	Flammer, 1998
Fish (ornamental)	5 mg/kg	IM, oral, or IP	Every 48 hr	Lewbart, 1998

These recommendations are based on an analysis of pharmacokinetic data and limited clinical experience. There have been no well-controlled efficacy studies or safety studies in these animals.

demonstrated specifically for canine pyoderma, including deep pyoderma, through published reports (Ihrke and DeManuelle, 1999; Ihrke, 1996; Paradis et al., 1990; Carlotti et al., 1999; Ihrke et al., 1999; Koch and Peters, 1996). Although efficacy has been shown for treating pyoderma in animals, guidelines for treatment recommend that they not be used as a first-choice agent (designated as "second tier" or "second-line" antibiotics) (Beco et al., 2013; Hillier et al., 2014). Another common use of fluoroquinolones in small animals is for treatment of urinary tract infections (Weese et al., 2011). In some patients, a 3-day course of treatment is sufficient for a clinical cure (Westropp et al., 2012).

In addition to treatment of infections in these common sites, fluoroquinolones have been used to treat rickettsial infections (Breitschwerdt et al., 1990, 1999) and *Bartonella* infections in cats (Kordick et al., 1997). Against *Rickettsia rickettsii*, enrofloxacin is equally as effective as doxycycline or chloramphenicol (Breitschwerdt et al., 1990), but the success for eliminating *Bartonella* in cats has been equivocal (Kordick et al., 1997). Enrofloxacin has been used successfully to treat acute ehrlichiosis in dogs caused by *E. canis* and *E. platys* at a dosage of 5 mg/kg once daily for 15 days (Kontos and Athanasiou, 1998). However, success in treating chronic ehrlichiosis has not been demonstrated. Fluoroquinolones also have been used to treat infections caused by *Mycoplasma* and *Mycobacteria*. Although the activity against *Mycoplasma* can be variable (Hannan et al., 1997), it has been effective for some opportunistic mycobacterial infections in cats (Studdert and Hughes, 1992).

Ciprofloxacin Use in Dogs and Cats

Despite the availability of safe and effective veterinary-labeled fluoroquinolones (enrofloxacin, marbofloxacin, orbifloxacin), ciprofloxacin oral tablets, available in a generic formulation for people, are increasingly being used for treatment in dogs. Veterinarians in the USA can legally prescribe unapproved human-label drugs to non-food producing animals according to the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994. The oral absorption in dogs, according to published studies, is variable, inconsistent, and lower than in humans. Estimates from some studies (Abadia et al., 1994, 1995; Walker et al., 1990), indicate that oral absorption may approach 74 to 97%, but has been as low as 42%. In a more recent study (Papich, 2012) the average oral absorption was 58.4%, but with high variability (coefficient of variation, CV 45.4%). The range in oral absorption was from approximately 30% to 98%. The variable oral absorption appeared to be caused by the incomplete and inconsistent dissolution of the human generic oral tablet. A larger population pharmacokinetic study (Papich, 2017) showed a low and variable absorption with a C_{max} of 1.19 μg/ml, AUC of 13.8 μg h/ml, and half-life of 4.35 hours. Simulations using a dose of 25 mg/kg oral, once per day

Table 37.7 Single high dose for fluoroquinolones used in veterinary medicine

Drug	Brand Name(s)	Species	Dose
Enrofloxacin	Baytril, and generic	Cattle	7.5–12.5 mg/kg SC
Enrofloxacin	Baytril, and generic	Pigs	7.5 mg/kg, SC
Danofloxacin	Advocin	Cattle	8 mg/kg, SC

would reach therapeutic targets only for bacteria with MIC values of 0.06 µg/ml, or less, which is far less than the human breakpoint for susceptible bacteria (Table 37.2).

Studies with ciprofloxacin in cats (Albarellos et al., 2004) showed low oral absorption at 10 mg/kg (33%). The authors concluded that a dose of 10 mg/kg every 12 hours might be sufficient for susceptible gram-negative bacteria with low MIC values, but this dose would likely not meet therapeutic targets against other bacteria.

Pradofloxacin Use in Dogs and Cats

Pradofloxacin (Veraflox®) is one of the newest fluoroquinolones (sometimes referred to as a third-generation fluoroquinolone; see Section Structure–Activity Relationships for differences). Other drugs that meet this definition are the human drugs grepafloxacin, gatifloxacin, gemifloxacin, and moxifloxacin. Because the older, second-generation fluroroquinolones (e.g., enrofloxacin, marbofloxacin, orbifloxacin) have less activity against gram-positive cocci and anaerobic bacteria (Figure 37.3), prafofloxacin have improved activity against some organisms in veterinary medicine (Blondeau et al., 2004; Lees, 2013). Pradofloxacin MIC_{90} values are 0.06–0.125 µg/ml for *Staphylococcus* spp., 0.06 µg/ml for *E. coli*, and 0.015 µg/ml for *Pasteurella multocida*. Pradofloxacin has been evaluated in dogs and cats for skin, soft-tissue, oral, and urinary tract infections (deJong et al., 2004; Spindel et al., 2008; Hartmann et al., 2008b; Litster et al., 2007; Mueller and Stephan, 2007). Pharmacokinetic studies are also available (Hartmann et al., 2008a; Hauschild et al., 2013). Susceptibility data indicate that it is more active than other fluoroquinolones against bacterial isolates from dogs and cats (deJong et al., 2004; Silley et al., 2007; Stephan et al., 2007b) (Figure 37.3). Because it is active against two targets of fluoroquinolones (topoisomerase IV and DNA gyrase) development of resistant mutants may be less likely (Wetzstein, 2005; Stephan et al., 2007a).

At a dose of 3 mg/kg orally it was effective for treatment of urinary tract infections in dogs and at 5 mg/kg was effective for canine pyoderma (Mueller and Stephan, 2007). At a dose of 5 mg/kg in a 2.5% oral suspension it was effective for urinary tract infections in cats (Litster et al., 2007).

Pradofloxacin is approved in the USA for cats only, and in other countries for both dog s and cats. Pradofloxacin has been safe in cats with respect to ocular lesions (Messias et al., 2008). The European label and US label are slightly different. In Europe, pradofloxacin is indicated for the treatment of dogs and cats for infections caused by *Staphylococcus pseudintermedius*, superficial and deep pyoderma caused by susceptible strains of *S. pseudintermedius*, acute urinary tract infections caused by susceptible strains of *E. coli* and *S. pseudintermedius,* and adjunctive treatment to mechanical or surgical periodontal therapy in the treatment of infections of the gingiva and periodontal tissues caused by susceptible strains of anaerobic organisms (*Porphyromonas* spp. and *Prevotella* spp.) The registered dose is 3 mg/kg once daily for 7–35 days, depending on the indication. For cats, pradofloxacin 25 mg/ml oral suspension is indicated for the treatment of acute infections of the upper respiratory tract caused by *P. multocida, E. coli,* and *S. pseudintermedius* and for the treatment of wound infections and abscesses caused by *P. multocida* and *S. pseudintermedius* in cats. The US label is more limited and only applies to cats for treatment of skin infections (wounds and abscesses) in cats caused by susceptible strains of *P. multocida, Streptococcus canis, Staphylococcus aureus, Staphylococcus felis*, and *S. pseudintermedius*. The dose in cats according to US labeling is 7.5 mg/kg daily and 5 mg/kg once daily on European labels. Breakpoints are established for testing pathogens from dogs and cats (Table 37.2).

Small Mammals

Enrofloxacin and other fluoroquinolone antibiotics are used frequently in small mammals such as rabbits, mice, rats, and exotic species for skin and visceral infections (Göbel, 1999; Cabanes et al., 1992; Broome and Brooks, 1991). Fluoroquinolones are popular for treatment in small mammals because of potent activity against gram-negative pathogens affecting these animals and have good oral absorption. Oral tablets of fluoroquinolones have been administered directly or crushed to make a suspension that can be conveniently administered orally to the small mammals mixed with water, fruit, or some other palatable flavoring. When mixed in a compounded formulation with these vehicles, enrofloxacin was stable for 56 days (Petritz et al., 2013). Another study showed that marbofloxacin tablets could be crushed and mixed with flavored vehicles for rabbits and was stable for 14 days (Carpenter et al., 2009).

Small mammals such as rodents and rabbits are prone to gastrointestinal disturbances and enteritis caused by overgrowth of bacteria, especially *Clostridium* organisms after administration of β-lactam and macrolide antibiotics. Because fluoroquinolones are not active against the anaerobic bacteria that compete with *Clostridium* organisms, bacterial overgrowth of pathogenic opportunistic bacteria has not been a problem as it has with other drugs, such as penicillins or macrolides.

Of the available drugs, enrofloxacin has been the most extensively studied. The doses listed in textbooks and review articles for mice, gerbils, hamsters, rats, and guinea pigs are 2.5–5.0 mg/kg up to 10–20 mg/kg IM, SC, or orally administered twice daily. The pharmacokinetics has been reported (Table 37.4), and there is

some experience with the drug's efficacy. In rabbits oral enrofloxacin has been effective for improving clinical signs associated with pasteurellosis. The recommended dose of enrofloxacin for rabbits is 5 mg/kg IM, SC, or oral. Although it does not completely eradicate the bacteria in pasteurellosis in rabbits, it is considered the drug of choice (Göbel, 1999; Broome and Brooks, 1991). Marbofloxacin and moxifloxacin also have been studied in rabbits and pharmacokinetics were favorable (Abo-El-Sooud and Goudah, 2010; Fernández-Varón et al., 2005; Carpenter et al., 2009). The recommended dose of marbofloxacin for rabbits is 5 mg/kg oral, once every 24 hours (Carpenter et al., 2009).

Reptiles

The use of fluoroquinolones in reptiles has become popular because of their activity, safety, and convenience of administration (Papich, 1999; Jacobson, 1999; Rosenthal, 1999). Enrofloxacin has been studied more than any other drug in this class in reptiles. It is active against gram-negative organisms often implicated in serious infection of reptiles, including *Salmonella* spp., *Aeromonas hydrophilia, Klebsiella* spp., and *P. aeruginosa,* and its pharmacokinetics has been summarized in a review (Papich, 1999). It shows remarkable differences among the reptiles, but generally the elimination is much longer than in mammals or birds, which allows long dose intervals – as long as every 96 hours in some species (Table 37.4). The elimination rate of drugs in reptiles varies with the animal's body temperature, because body temperature affects metabolic rate. When enrofloxacin is administered, there is variable metabolism to the active metabolite ciprofloxacin among the reptiles. Elimination half-life ranged from 55 hours in alligators to 5.1 hours in tortoises (Young et al., 1997; Raphael et al., 1994; Helmick et al., 1997; Hungerford et al., 1997; Prezant et al., 1994). Monitor lizards, and pythons had half-lives of 36 and 17.6 hours, respectively. Turtles have long half-lives ranging from 18 hours to more than 50 hours that allow for infrequent administration of once per day to once per week (Table 37.4). Analysis of pharmacokinetic data and appraisal of clinical experience (Jacobson, 1999; Papich, 1999) suggest a range of doses (Table 37.6), but safety and efficacy studies have not been performed.

Pharmacokinetic studies have shown good absorption of enrofloxacin from IM administration, and this route may prolong the half-life, probably because of delayed absorption from the injection site. Although some authors have suggested that oral administration should be avoided in reptiles because of unreliable absorption, absorption was good after oral administration to alligators, lizards, and turtles (Helmick et al., 1997; Hungerford et al., 1997; James et al., 2003). Because of slow gastrointestinal transit time, oral absorption may prolong the half-life ("flip-flop" effect).

Birds

The fluoroquinolones are an important group of antibiotics for pet birds and exotics kept in zoo collections, but they are prohibited in poultry in the United States. Administration is via drinking water, oral gavage, or by injection. Fluoroquinolones have the advantage of good activity against bacterial pathogens important to birds, including *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., and for treatment of *Chlamydophila psittaci* (formerly called *Chlamydia psittaci*). Resistance is possible for *E. coli* and *Pseudomonas* spp., however, and activity against gram-positive cocci (e.g., streptococci and enterococci) is low. Although there is in vitro susceptibility of *Chlamydophila* to fluoroquinolones, experience suggests that enrofloxacin can decrease clinical signs but not eliminate the infections (Flammer, 1998). Therefore, fluoroquinolones are not recommended for mass medication of pet birds, and doxycycline is still the choice for this indication (discussed in Chapter 34).

For pet birds, the dose is higher than for mammals because the clearance is faster and metabolic rate is higher. A comparison of enrofloxacin, danofloxacin, and marbofloxacin in quails (Haritova et al., 2013) showed half-lives of 2–4 hours, and variable oral absorption among drugs (data not shown in Table 37.4). Enrofloxacin had much lower oral absorption in these birds than the other drugs studied. The Haritova paper also included tables of pharmacokinetic parameters for other birds. Pharmacokinetic studies of enrofloxacin in birds indicate a dose of 15 mg/kg IM or orally every 12 hours (Flammer, 1998; Flammer et al., 1991). Enrofloxacin has been administered to ducks at a dose of 10 mg/kg every 24 hours IM or orally. Enrofloxacin added to drinking water at a concentration of 0.3–0.5 mg/ml has been used to treat susceptible bacteria (Flammer et al., 1990). Enrofloxacin was well absorbed by this route, and as long as the bird is drinking, effective plasma concentrations can be attained. One concern with the IM injection is that it can produce irritation at the site of injection, which is problematic because birds have a limited muscle mass into which one can inject.

Fish

Fluoroquinolones have been considered for treatment of infections in ornamental fish and for use in aquaculture. These drugs are active against important gram-negative bacterial pathogens of fish, and they appear to be well tolerated. Enrofloxacin has been administered orally to rainbow trout kept in water maintained at 10° and 15°C.

Although oral absorption is less than in mammals, it was sufficient to produce effective plasma concentrations (Bowser et al., 1992). MIC values for pathogens infecting fish range from 0.0064 to 0.032 μg/ml for the most sensitive organisms to 0.25–0.45 μg/ml for *Streptococcus* spp. Thus the dose of 5 mg/kg should produce effective plasma concentrations for most susceptible pathogens (Bowser et al., 1992). In Atlantic salmon, enrofloxacin administered at 10 mg/kg intraperitoneally, intramuscularly, and orally was well absorbed from these routes, with no advantage of one route over another, but it produced a wide range of half-lives and Vd. Oral absorption in salmon was 46%, but the authors concluded that at 5 mg/kg this route would be suitable for therapeutic treatment (Stoffregen et al., 1997). Tissue concentrations were high, with concentrations detected at 120 hours after dosing.

Enrofloxacin has also been studied in red pacu as a model for other ornamental fish (Lewbart et al., 1997). For treatment of bacterial infections in ornamental fish, Lewbart (1998) recommends enrofloxacin at a dose of 5 mg/kg. This can be administered IM, IP, or orally with a recommended interval of every 48 hours, but the IM route produces the most predictable plasma concentrations. The oral dose can be prepared as a mixture of 0.1% in fish food (10 mg per 10 g of food). Enrofloxacin also has been added to water and used as a bath for fish in which the drug is absorbed across the surface area of the gill to produce systemic levels. In this treatment 2.5–5.0 mg enrofloxacin per liter is used as a 5-hour treatment bath repeated every 24 hours (Lewbart et al., 1997). The resulting peak plasma concentration after such a treatment was 0.17 μg/ml. Studies of the stability of enrofloxacin in water at various degrees of salinity and pH showed enrofloxacin to be stable when added to a water bath (unpublished results from the laboratory of the author, MGP). However, the effect of the drug on nitrifying bacteria in the water should be considered.

Invertebrate marine species also have been examined. Studies in cuttlefish showed that clearance was surprisingly rapid, and doses of 5 mg/kg every 12 hours would be necessary to maintain effective concentrations (Gore et al., 2005). However, the same study indicated that systemic absorption from immersion of cuttlefish in an enrofloxacin medicated bath was possible. In sea urchins and sea stars population pharmacokinetic methods were used to examine injections and water bath treatments (Phillips et al., 2016; Rosenberg et al., 2016). In sea urchins and sea stars the half-life was long (39 hours, 43 hours, respectively), and concentrations were maintained above a level needed to treat susceptible bacteria. The medicated bath treatment produced lower concentrations, but may still be adequate for treating some susceptible bacteria.

Horses

In horses, there is no fluoroquinolone approved for use in the USA, but these drugs, especially enrofloxacin, are often used in horses to treat infections that may be resistant to other common equine antimicrobials. Several pharmacokinetic studies have generated data for these drugs in horses to guide dosing protocols. These data, as well as clinical experience, have shown that this class of drugs can be valuable for treating infections in horses. Their valuable properties include the following: (i) ability to administer by oral, IV, and IM routes, although only enrofloxacin is available in an injectable formulation in the United States; (ii) spectrum of activity that includes staphylococci and gram-negative bacilli such as *Klebsiella pneumoniae, E. coli,* and *Proteus* spp.; (iii) spectrum of activity that does *not* include anaerobic bacteria, therefore posing less risk of disrupting bacteria in the intestine than other oral antimicrobials; and (iv) good safety profile in adult horses. Despite these advantages, the oral absorption is less than other animals, and more variable. Therefore, the analysis of susceptibility testing breakpoints resulted in lower values for equine isolates compared to other animals (Table 37.2). These breakpoints are based on an oral dose of enrofloxacin of 7.5 mg/kg once daily.

The oral absorption of enrofloxacin in horses has generally been in the range of 50–70% (Table 37.4). Most bacteria that infect horses are susceptible, but resistance is expected for streptococci and anaerobes. Strains of *Pseudomonas aeruginosa* may be resistant or only moderately susceptible. *Rhodococcus equi* can be resistant, and success in treating *Rhodococcus* infections in horses with enrofloxacin has not been encouraging (see Chapter 36 for recommendations to treat *Rhodococcus* in horses). Based on the studies cited in this section, as well as clinical experience to date, an injectable dose of enrofloxacin at 2.5 to 5 mg/kg once daily or an oral dose of 7.5 to 10 mg/kg once daily is recommended (Giguère et al., 1996). The higher oral dose is used to accommodate the decreased systemic availability from an oral dose. For orbifloxacin (Orbax), an oral dose of 5 mg/kg once daily is recommended (Davis et al., 2006). These doses meet PK-PD criteria for susceptible bacteria discussed earlier in this chapter. For marbofloxacin (Zeniquin), IV doses of 2 mg/kg q 24 h may be adequate for treatment of most gram-negative infections caused by Enterobacteriaceae (Bousquet-Mélou et al., 2002; Peyrou et al., 2004; Carretero et al., 2002). However, this dose would not be adequate for many gram-positive bacteria, such as *Staphylococcus* spp., with MIC values of 0.25 μg/ml or higher. The injectable formulation is not available in the United States; therefore, oral tablets of marbofloxacin would be required for administration. Marbofloxacin has systemic availability of approximately 62% in horses.

(Bousquet-Mélou et al., 2002). Oral dosing at 2 mg/kg may be adequate for susceptible Enterobacteriaceae with MIC values less than 0.2 μg/ml, but not for other bacteria. Doses higher than 2 mg/kg have not been studied in horses. Ciprofloxacin is not recommended because the oral absorption was poor in ponies and adult horses (Dowling et al., 1995; Yamarik et al., 2010). Enteritis may occur in horses from ciprofloxacin owing to the poor absorption and disruption of intestinal bacteria (Yamarik et al., 2010).

The method of administration for horses has been to (i) crush up tablets used in small animals; (ii) administer the injectable solution (2.27% or 10%) IM (neck muscle) or IV; or (iii) administer the concentrated 10% solution orally (Baytril-100 cattle formulation). All these methods appear to produce adequate plasma concentrations, except for the administration of the concentrated 10% solution (Baytril-100) orally. This solution has produced inconsistent and incomplete absorption in horses, possibly because of its insolubility in solutions of low pH (Haines et al., 2000). In other studies, absorption of this solution was better when horses were fed (Boeckh et al., 2001). This solution also has been associated with oral mucosal lesions in horses. Some clinicians have produced more consistent oral absorption and reduced mucosal irritation when the 100 mg/ml solution was compounded into a gel (Epstein et al., 2004).

Moxifloxacin (Avelox) is a human drug of this group and has been used on a limited basis for treatment of infections in dogs and cats caused by bacteria that have been refractory to other drugs. When administered to horses, moxifloxacin had favorable pharmacokinetics that could make it suitable for oral use in horses (Gardner et al., 2004). However, oral administration also produced diarrhea in the experimental horses studied, and one of these horses tested positive for *Clostridium difficile* toxins A and B. The spectrum of activity of this drug may be broad enough to disrupt the normal flora.

Ruminants

In cattle and sheep, the pharmacokinetics of enrofloxacin and danofloxacin has been reported (Table 37.4) and doses have been derived from these studies, clinical trials, or the approvals in various countries. Enrofloxacin and danofloxacin are approved for use in cattle in the United States and some European countries. These fluoroquinolones have been highly active against important pathogens causing bovine respiratory disease (BRD) in cattle. The MIC_{90} values listed for *Histophilus somni, Mannheimia haemolytica,* and *P. multocida* are 0.03, 0.06, and 0.03 μg/ml, respectively. Extralabel use is not allowed in food-producing animals (see Chapter 52 and 55). For enrofloxacin, the dose ranges from a single SC dose of 7.5–12.5 mg/kg, or treatment for 3 days at 2.5–5.0 mg/kg, once daily, SC. The withdrawal time is 28 days. Comparisons of enrofloxacin pharmacokinetics between dairy cows and beef steers did not show significant differences in parameters between the groups (Idowu et al., 2010). It is not approved for lactating cattle or dairy calves, but disposition into milk of lactating cows has been studied. Enrofloxacin is highly excreted in milk. (See Section Administration to Nursing, Pregnant, or Young Animals.)

Danofloxacin (Advocin®) is also approved for treatment of BRD in cattle. Danofloxacin contains either 25 mg/ml or 80 mg/ml of danofloxacin as the mesylate salt, depending on the country in which it is approved. It has a broad spectrum of activity, similar to enrofloxacin, including bovine isolates of *Pasteurella, Mannheimia haemolytica*, *Haemophilus somnus*, and *Mycobacterium bovis.* The breakpoints are listed in Table 37.2. The US approved label dose allows either a dose of 6 mg/kg twice, 48 hours apart, or a single dose of 8 mg/kg SC. The European formulation of 2.5% also allows for the intramuscular or intravenous routes at a dosage rate of 1.25 mg/kg. At this dose, three treatments should be given at 24-hour intervals. However, the higher dose is preferred because it is more likely to hit PK-PD targets. (PK-PD properties are discussed in Section Section Pharmacokinetics / Pharmacodynamics.)

The danofloxacin manufacturer reported a half-life of 4.35, and volume of distribution of 3.6 l/kg at the label dose of 6 mg/kg. Some earlier work at lower doses (Giles et al., 1991) reported similar results (half-life 4 hours and volume of distribution of 2.76 l/kg). Lung tissue concentrations were higher than the corresponding serum concentration and persist above the MIC for 12–24 hours after injection (Giles et al., 1991; Apley and Upson, 1993). In a comparison between enrofloxacin and danofloxacin (TerHune et al., 2005), danofloxacin produced plasma concentrations that were 56 times the MIC of 0.03 μg/ml, which is the MIC reported for North American BRD isolates. Although it is not legal to use fluroquinolones in the USA in an off-label manner, studies have also shown danofloxacin to be effective for bacterial enteritis in calves and is registered for this use in other countries.

Marbofloxacin (Forcyl®) 160 mg/ml solution is approved in some countries for use in cattle, and has also been tested in buffalo calves (Baroni et al., 2014). The dose studied in cattle has been 2 mg/kg per day for 3–5 days, or a single injection of 10 mg/kg, injected IM in the neck muscle. Vallé et al. (2012) concluded that a single high dose was as effective, and may reduce resistance, compared to lower doses.

Pigs

Enrofloxacin is approved in the United States for use in pigs for treatment and control of swine respiratory

disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis*, and *Streptococcus suis*. Efficacy and microbiological data (MIC distribution) have been reported for these pathogens in pigs (Grobbel et al., 2007). The pharmacokinetics of fluoroquinolones in pigs are shown in Table 37.4 and breakpoints for testing shown in Table 37.2. Most of the pharmacokinetic studies in pigs (Nielsen and Gyrd-Hansen, 1997; Anadón et al., 1999; Wiuff et al., 2002; Bimazumute et al., 2009) showed that the half-life of enrofloxacin ranged from 9 to12 hours. These studies were performed at a low dose. At the dose most often used clinically, 7.5 mg/kg once SC, the pharmacokinetics and tissue distribution were reported by Messenger et al. (2012). The Messenger study showed that at the dose of 7.5 mg/kg, the free drug concentrations in tissue exceed the predicted unbound concentration and attained target levels for swine respiratory pathogens. In all but one study, the ciprofloxacin concentrations were low and difficult to quantify, accounting for less than 10% of the corresponding enrofloxacin concentrations, or were below the assay limit of quantification (LOQ). One study (Anadón et al., 1999) that used older pigs, was an outlier and reported ciprofloxacin concentrations that were 51.5% of the corresponding enrofloxacin concentrations.

Marbofloxacin (Forcyl®, Marbocyl®) 16%, 10%, or 2% solution for IV or IM injection is approved in some countries (not the USA) for treatment of swine respiratory disease and metritis–mastitis–agalactia syndrome in sows. The dose in pigs is 2 mg/kg once daily for 3–5 days, or 8 mg/kg administered once, IM. The absorption from IM injection in pigs is not affected by the dose or concentration of the formulation (Schneider et al., 2014).

Administration to Nursing, Pregnant, or Young Animals

Nursing Animals

Distribution also has been measured for milk in rabbits and cattle. Enrofloxacin is excreted rapidly in the milk after administration. In cattle after administration of enrofloxacin at 5 mg/kg, enrofloxacin concentrations in milk parallel the concentrations in serum, with a C_{max} of 1.3–2.5 μg/ml, but concentrations of the active metabolite ciprofloxacin exceed those of enrofloxacin (Kaartinen et al., 1995; Tyczkowska et al., 1994; Rantala et al., 2002). In another study, the concentrations of ciprofloxacin in milk of dairy cows exceed the plasma concentration by 45 times (Chiesa et al., 2013). Danofloxacin distribution into milk of cows also exceeded serum concentrations (Shem-Tov et al., 1998). In rabbits the milk-to-plasma ratios were 3.6 and 2.6 for enrofloxacin and ciprofloxacin, respectively, after administration of 7.5 mg/kg IV (Aramayona et al., 1996).

The high distribution of fluoroquinolones into milk is apparently caused by active transport by proteins in the udder. One of the transporters responsible is the breast cancer resistance protein (BCRP), an ATP-binding cassette transporter (Real et al., 2011). Protein binding is high in milk, which may also trap enrofloxacin and ciprofloxacin (Aramayona et al., 1996). Despite these concentrations of fluoroquinolones in milk, the activity of enrofloxacin in mastitic milk is decreased, possibly owing to lower pH, chelation with cations, or other factors in milk that inhibit fluoroquinolone activity (Kaartinen et al., 1995). Although an experimental study showed promising results for treating mastitis caused by *E. coli* (Rantala et al., 2002), clinical results have been more disappointing (Suojala et al., 2010). In the United States, this would be considered extralabel use and is prohibited.

When administering fluoroquinolones to nursing animals, the amount in the milk should be considered because fluoroquinolones may cause arthropathy in some species of young animals (discussed further in Section Safety). Disposition into milk was studied in two mares after administration, and it was shown that although both ciprofloxacin and enrofloxacin were present in milk at levels that were as high or higher than the mares' plasma concentrations, the total doses administered to the foals via suckling were small, and the plasma concentrations in the foals were negligible (author's observations). Likewise, when ciprofloxacin was administered to cows, the concentration in milk was high, and delivered an oral dose of 0.5 mg/kg to suckling calves (Chiesa et al., 2013). However, the drug did not accumulate in calves and concentrations in the calves were one-tenth to one-fifth the concentration in the cow. Young nursing animals may have decreased oral absorption caused by interference from calcium in milk (see information on kittens in Section Young Animals).

Pregnant Animals

When administering fluoroquinolones to pregnant animals, there will be some drug transfer across the placenta because these drugs are lipophilic and have low protein binding, and drug transfer is not limited by tissue barriers. Placental transfer has been specifically examined in rabbits, in which it was shown that the more lipophilic drug, enrofloxacin, crossed the placenta to a greater degree (80%) than ciprofloxacin (5% placental transfer), which is less lipophilic (Aramayona et al., 1994). Despite the rather high transfer of enrofloxacin across the placenta, there have been no reports of adverse effects when fluoroquinolones were administered to pregnant animals. Manufacturer studies have not shown any adverse effects on pregnancy or reproduction.

Young Animals

There is a risk that fluoroquinolones may cause damage to the developing cartilage of young animals. This is discussed more thoroughly in the Section Safety. There have been few pharmacokinetic comparisons of

young animals versus older animals, but the studies available demonstrate that young animals were exposed to more drug than adults because of slower clearance. After administration of enrofloxacin, calves at 1 day of age had smaller volume of distirubion, longer half-life, and decreased clearance than at 1 week of age (Kaartinen et al., 1997). There also was a smaller amount of metabolism of enrofloxacin to ciprofloxacin in 1-week-old calves than in older ones. Rabbit pups exhibited lower clearance and longer half-life for enrofloxacin than adult rabbits (Aramayona et al., 1996). This pattern was also seen in horses: foals at 1–2 weeks of age showed little metabolism of enrofloxacin to ciprofloxacin after administration of IV and oral doses. Foals also exhibited slower clearance and longer half-life than adults (Bermingham et al., 2000).

In kittens, enrofloxacin was administered at 5 mg/kg via various routes (oral, IV, SC) (Seguin et al., 2004). There was no evidence of adverse effects or impaired excretion of enrofloxacin in kittens. The half-life in young cats (2, 6, and 8 weeks old) was shorter, and clearance more rapid, than in adult cats, Volume of distribution in 6 to 8-week-old cats was larger, and combined with the shorter half-life, produced lower plasma concentrations than in adults. Oral administration to kittens produced low plasma concentrations and it was hypothesized that interference with milk in nursing kittens may lower oral absorption (see Section Drug Interactions).

Safety

The fluoroquinolones have had a good safety record. For enrofloxacin, the LD_{50} in laboratory rats is 5000 mg/kg. When high doses were administered to animals during safety testing, one of the most common problems was gastrointestinal disturbances (nausea, vomiting, diarrhea), but these were usually produced at high doses and were not serious. Because most of these drugs used in animals do not alter the anaerobic flora of the gastrointestinal tract, there usually is minimal disruption of the intestinal bacterial population, even when these drugs are administered orally to rodents. There have been no reports of cutaneous drug reactions resulting from fluoroquinolone administration in the veterinary literature, but some of the freedom of information (FOI) summaries from manufacturers report an occasional reddening of the skin of dogs when high doses were administered.

There have been no reports of adverse effects on reproduction or pregnancy from administration of fluoroquinolones. Although the use in pregnant animals has been discouraged because of toxicity to developing cartilage, there have been no clinical reports where this effect has been described in offspring of treated animals.

After administration of high doses, adverse central nervous system (CNS) effects have been observed. The mechanism responsible for the CNS effects is probably through antagonism of the inhibitory neurotransmitter GABA. Fluoroquinolones injected rapidly IV or administered at high doses can induce CNS excitement. Fluoroquinolones can precipitate convulsions in some animals and these agents should be administgered cautiously to animals that are prone to seizures.

Safety issues have arisen in people that have been the subject of a warning by the FDA. On May 12, 2016, the FDA issued a warning to physicians advising that the serious side effects associated with fluoroquinolone antibacterial drugs generally outweigh the benefits for patients with sinusitis, bronchitis, and uncomplicated urinary tract infections who have other treatment options. For patients with these conditions, fluoroquinolones should be reserved for those who do not have alternative treatment options. During the FDA review, they revealed that fluoroquinolones when used systemically (i.e., tablets, capsules, and injectable) are associated with disabling and potentially permanent serious side effects. These side effects can involve the tendons, muscles, joints, nerves, and central nervous system. Some signs and symptoms of serious side effects include tendon, joint and muscle pain, a "pins and needles" tingling or pricking sensation, confusion, and hallucinations. Subsequently, the FDA is requiring sponsors to update their drug labels for all fluoroquinolone antibacterial drugs. The joint effects in young dogs and horses are discussed in Section Problems in Young Animals, but to our knowledge tendon injuries and the other concerns cited by the FDA for people have not been reported from the use of fluoroquinolones in animals.

Blindness in Cats

High doses of fluoroquinolones have caused ocular problems in cats from drug-induced changes in the retina (Corrado et al., 1987). This concern was precipitated by a report by Gelatt and colleagues (Gelatt et al., 2001) in which retinal degeneration was associated with enrofloxacin administration. This was followed by studies by the manufacturer in which toxicosis from enrofloxacin was described and new dose labeling was announced. The most common ophthalmological abnormalities were mydriasis, lack of menace reflex, and poor papillary light reflexes. Acute blindness may occur and retinal lesions were observed, which include increased tapetal reflectivity and attenuation of retinal blood vessels. In one review of safety studies (Corrado et al., 1987) nalidixic acid (one of the earlier quinolones) at 100 mg/kg/day, but not norfloxacin at 200 mg/kg/day produced both electrical and histopathological changes in feline retinas. In another review (Schluter, 1987), the author states that feline retinas are particularly sensitive to fluoroquinolones. When cats received 100 mg/kg

of nalidixic acid there was suppression of electroretinographic waves and histological changes in the cones and rods, but ciprofloxacin treatment at the same dose to cats had no effect on the electroretinographic findings or on the fundus.

In studies performed by the manufacturer, enrofloxacin was administered to cats at doses of 0, 5, 20, and 50 mg/kg for 21 days (eight cats per group). There were no adverse effects observed in cats treated with 5 mg/kg/day of enrofloxacin. However, the administration of enrofloxacin at 20 mg/kg or greater caused salivation, vomiting, and depression. At doses of 20 mg/kg or greater, there were mild to severe fundic lesions on ophthalmological examination, including changes in the fundus and retinal degeneration. There was also abnormal electroretinograms, including blindness. Ford et al. (2007) reported on a study in 24 cats that received 3, 5, or 7 days of enrofloxacin at a high dose of 50 mg/kg (10 times the label dose). Ocular changes were observed by day 3 of the study. At this dose, enrofloxacin caused both retinal degeneration and systemic toxic effects. Because the blindness has been associated with high doses, the manufacturer has limited the dose to 5 mg/kg/day in cats.

The other fluoroquinolones approve for use in cats are orbifloxacin (Orbax), pradofloxacin (Veraflox), and marbofloxacin (Zeniquin). The current approved dose of orbifloxacin for cats is 2.5–7.5 mg/kg/day. In a published abstract (Kay-Mugford et al., 2001), orbifloxacin oral liquid was administered to cats at 0, 15, 45, and 75 mg/kg for at least 30 days (eight cats/group). This represents 6, 18, and 30 times the lowest label dosage. No ocular lesions were observed in any cats treated with 15 mg/kg. At the higher doses (18 and 30 times dose) there was tapetal hyperreflectivity in the area centralis and minimal photoreceptor degeneration. When marbofloxacin was administered to cats at 5.55, 16.7, and 28 mg/kg, representing 2, 6, and 10 times the lowest label dose, for 6 weeks, there were no ocular lesions in cats (manufacturer's data). At 55.5 mg/kg (10 times the lowest label dose) for 14 days there were also no lesions from marbofloxacin. As discussed above in the Section Pradofloxacin Use in Dogs and Cats, it has been administered safely to cats without producing ocular problems.

Problems in Young Animals

Fluoroquinolones can produce an arthropathy in young rapidly-growing animals (Gough et al., 1992; Burkhardt et al., 1997). The species most susceptible to developmental arthropathy are rats and dogs. Dogs between the ages of 4 and 28 weeks are the most susceptible. Affected dogs may show signs of lameness and joint swelling, but when the drug was discontinued, the lesions were reversible. Kittens, calves, and pigs are more resistant to this effect. For example, feeder calves and 23-day-old calves were administered 25 mg/kg for 15 days without evidence of articular cartilage lesions. Young foals are susceptible to the joint arthropathy from enrofloxacin at 10 mg/kg orally (Bermingham et al., 2000). However, studies in adult horses treated with enrofloxacin have not demonstrated articular toxicity (Bertone et al., 2000; Giguère et al., 1999).

The risk increases with higher doses and in most instances has been more clinically obvious only when the highest maximum dose was exceeded (e.g., at 25 mg/kg of enrofloxacin); however, even enrofloxacin dosages of 10 mg/kg/day have induced cartilage damage in young dogs. Lesions may occur in as few as 2 days after initiation of treatment (Yabe et al., 2001). The use of fluoroquinolones has been discouraged in children, but thousands of children have been treated with these drugs under a compassionate protocol with no reports of joint arthropathy.

Joint arthropathy is caused by toxicity to the chondrocyte that causes vesicles to form on the articular surface. The mechanism for damage to cartilage is via chelation of magnesium by the drug (Egerbacher et al., 2001). Magnesium is necessary for proper development of the cartilage matrix, especially in young, growing animals. Chelation of the magnesium results in a local magnesium deficiency leading to loss of proteoglycan in the articular cartilage. Studies in which magnesium was supplemented to decrease cartilage damage had equivocal results. (Magnesium added to the diet while oral drugs are administered would cause a chelation and significantly decrease oral absorption.)

Diseases or Conditions

There has been limited study of the disposition of fluoroquinolones in animals that have other conditions. In most of these instances, there were no changes in the drug's pharmacokinetics that would necessitate a change in dosage. Since the fluoroquinolones rely on both the kidneys and liver for clearance, insufficiency in one organ may result in compensation by the other clearance route. For example, renal failure may result in more reliance on hepatic clearance. In dogs with renal impairment, clearance of marbofloxacin was only slightly decreased and there was no significant effect on volume of distribution or mean residence time (Lefebvre et al., 1998). In camels deprived of water for 14 days and lost 12.5% of their body weight, there was little effect on the distribution, clearance, or half-life of enrofloxacin. However, water deprivation resulted in a slower and less complete absorption from a SC injection compared to normal camels or camels injected IM (Gavrielli et al., 1995).

Drug Interactions

Combinations with other antibiotics neither antagonize nor enhance the microbiological effects of fluoroquinolones. Fluoroquinolones will kill bacteria whether or not they are dividing (Lode et al., 1998). Therefore, use of a bacteriostatic agent should not interfere with the action of a fluoroquinolone. Although there is no evidence that other antibiotics produce a synergistic effect when administered with fluoroquinolones, they may broaden the spectrum of activity.

Fluoroquinolones are involved in some drug interactions, but few of these are serious. Drugs containing di- and trivalent cations (e.g., Ca^{2+}, Mg^{2+}, Al^{+3}, Fe^{+3}), such as antacids, sucralfate, and nutritional supplements, can inhibit oral absorption (Simon et al., 2010; Nix et al., 1989). The effect on oral absorption is caused by chelation of the di- and trivalent cations to the quinolone, which inhibits permeability through the intestine, and can also affect drug solubility in the intestine that is needed for absorption (Simon et al., 2010). Although these effects are well documented in people, especially for ciprofloxacin, the results have been inconsistent in animals. Sucralfate (containing Al^{+3}) did not decrease oral absorption of enrofloxacin in dogs, and ciprofloxacin absorption was so inconsistent that it was not possible to detect a statistically significant interaction (KuKanich et al., 2016).

Cations mixed with fluoroquinolones in extemporaneously compounded preparations, can chelate the drug and decrease oral absorption. Chelation occurs on the carboxyl group at the 3-carbon position (Figure 37.1). Fluoroquinolones may inhibit metabolism of some drugs through an interaction with hepatic metabolism. One such example is the inhibition of theophylline metabolism by enrofloxacin (Intorre et al., 1995), in which enrofloxacin significantly increased the peak concentration and decreased systemic clearance of theophylline in dogs. This interaction occurs because enrofloxacin inhibits the cytochrome P540 enzyme CYP 1A2 enzyme (Martinez et al., 2013). There are other substrates for this enzyme, but there have not been reports of studies to examine if other interactions are possible in animals.

Fluoroquinolone interactions with pharmacokinetics of some nonsteroidal antiinflammatory drugs (NSAIDs) have been observed in animals (Ogino et al., 2005; Sidhu et al., 2005). However the interaction is inconsistent. Enrofloxacin inhibited the clearance of flunixin, and vice versa (Ogino et al., 2005), but marbofloxacin increased the clearance of tolfenamic acid and decreased the concentrations (Sidhu et al., 2005). If a reaction with an NSAID is possible, it is likely because of competition for tubular secretion.

Formulations Available

Fluoroquinolones approved for small-animal use are available as oral preparations as tablets or chewable tablets. There are no oral liquid preparations currently available in the United States, but oral solutions are available in other countries (5 and 25 mg/ml). When oral liquid formulations have not been available, veterinarians have used compounding pharmacists to create oral liquid formulations from tablets dissolved in an aqueous vehicle (Petritz et al., 2013; Carpenter et al., 2009). There are two formulations of enrofloxacin, an IM injectable preparation for dogs (2.27% solution, 22.7 mg/ml), and an injectable for cattle (100 mg/ml). The solutions are not approved for IV administration, but veterinarians have occasionally administered this preparation IV. The IV administration has not produced serious problems, but one should avoid rapid IV injection; otherwise, CNS reactions are possible. In addition, mixing a solution with some intravenous solutions may cause chelation and precipitation in the IV line. Fluids of particular concern are those that contain calcium or magnesium. The formulation approved for SC injection in cattle in the United States is 100 mg/ml in an L-arginine base. This preparation may cause tissue irritation if injected in small animals. It is very alkaline and has produced oral mucosal lesions when used for oral administration to horses (Boeckh et al., 2001).

There is an otic preparation available (Baytril otic) containing 5 mg/ml enrofloxacin and 10 mg/ml silver sulfadiazine. Some veterinarians have also used topical administration of enrofloxacin for otitis externa caused by pseudomonads (Griffin, 1993, 1999; Rosychuk, 1994). Other otic preparations have been mixed extemporaneously by veterinarians, even though these are not approved or evaluated for efficacy. For example, veterinarians have mixed the 2.27% injectable solution of enrofloxacin with saline, water, or other topical ear solutions in a 1 : 1 to 4 : 1 ratio (e.g., 4 parts saline, 1 part enrofloxacin). Stability studies by the author (MGP) with HPLC analysis confirmed these solutions to be stable for 2 weeks at room temperature.

Danofloxacin mesylate (Advocin), available as 180 mg/ml (and in some countries 25 mg/ml), is used for administration to cattle, SC in the neck area. It has a 2-pyrrolidone and polyvinyl alcohol vehicle. Marbofloxacin (Zeniquin) is available in tablets for dogs and cats. There is an injectable formulation available in some countries for small animals, cattle, pigs, and horses (Marbocyl, Forcyl), but not in the United States. Orbifloxacin is available in tablet form for dogs and cats. There are many more formulations available around the world not listed here. For example, the European Medicines Agency web site

(http://www.ema.europa.eu/ema/) lists 120 pages of fluoroquinolone formulations approved for animals in various countries. There is not enough space in this chapter to list all these formulations but a visit to the EMA web site, and the FDA approved drug web site (http://www.accessdata.fda.gov/scripts/animaldrugsatfda/).

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38

Antifungal and Antiviral Drugs

Jennifer L. Davis and Lara Maxwell

Antifungal Drugs

The need for safe and effective antifungal drugs has become important, particularly in small-animal medicine, with the recognition of serious systemic fungal diseases as well as the need for effective drugs to treat skin infections caused by dermatophytes and yeasts. Some animals are at a greater risk of fungal infections because they have other diseases or received medications that can produce immunosuppression, including hyperadrenocorticism, cancer chemotherapeutics, radiation therapy, or prolonged courses of corticosteroids. Fortunately, there have been many advances in the development of antifungal drugs in the last 20 years. Effective oral drugs are more widely used and there are newer, safer formulations of injectable agents. Figure 38.1 illustrates the sites of drug action for common antifungal drugs used in veterinary medicine. Unfortunately, there are only a few antifungal drugs that are approved for veterinary species (notable exceptions are topical products). Therefore, veterinarians often administer human-label drugs in an extralabel manner to animals.

Griseofulvin

Griseofulvin (Fulvicin U/F®, Fulvicin P/G®, Grifulvin V®, Grisactin®, Grisactin ultra®), is a fungistatic antibiotic produced by *Penicillium griseofulvin dierckx*. It is colorless, slightly bitter, and virtually insoluble in water. There are two types of preparations, the microsized and the ultramicrosized. Due to increased surface area, the ultramicrosized formulations have almost 100% bioavailability, whereas oral absorption of microsized formulations is lower and more variable (25–70%). The ultramicrosized preparations are not used often in veterinary medicine because of the higher cost. If the ultramicrosized form is used, the dose must be decreased to account for differences in absorption.

Mechanism of Action

Griseofulvin's selective toxicity is based on an energy-dependent uptake into susceptible fungi that occurs preferentially in fungal cells rather than mammalian cells. Once inside the cell, griseofulvin disrupts the mitotic spindle by interacting with polymerized microtubules, thus causing mitotic arrest in metaphase. Grossly this may appear as shortened fungal hyphae that have fewer branching points. This is known as the *curling* phenomenon. Griseofulvin may also interfere with cytoplasmic tubule formation, thereby inhibiting normal cellular trafficking.

Spectrum of Activity

Griseofulvin's activity is limited to organisms causing dermatophytosis, *Microsporum* spp., *Trichophyton* spp., and *Epidermophyton*. Fungal resistance to griseofulvin, caused by decreased energy-dependent uptake into the fungal cell, has not been reported to be a clinically important problem in veterinary medicine until recently. A 2013 study of feline and canine dermatophytosis showed therapy with griseofulvin failed to achieve both mycological and clinical cure in 16 dogs (39%) and four cats (40%), with some *M. gypseum* isolates from these animals reaching MIC values of >150 μg/ml (Nardoni et al., 2013).

Pharmacokinetics

Pharmacokinetic properties were reviewed by Hill et al. (1995). Griseofulvin distributes to the keratin of skin, hair, and nails and can be detected in the stratum corneum within hours of administration. Because of low water solubility, griseofulvin absorption is enhanced when given with a meal with high fat content. Only a small fraction of the dose is present in other body fluids or tissues. Absorption is nonlinear, and increases in doses may lead to a decreased fraction absorbed, as the rate-limiting step for absorption shifts from dissolution to solubility (Tanaka et al., 2013). The plasma half-life in the dog is 47 minutes (Harris and Riegelman, 1969);

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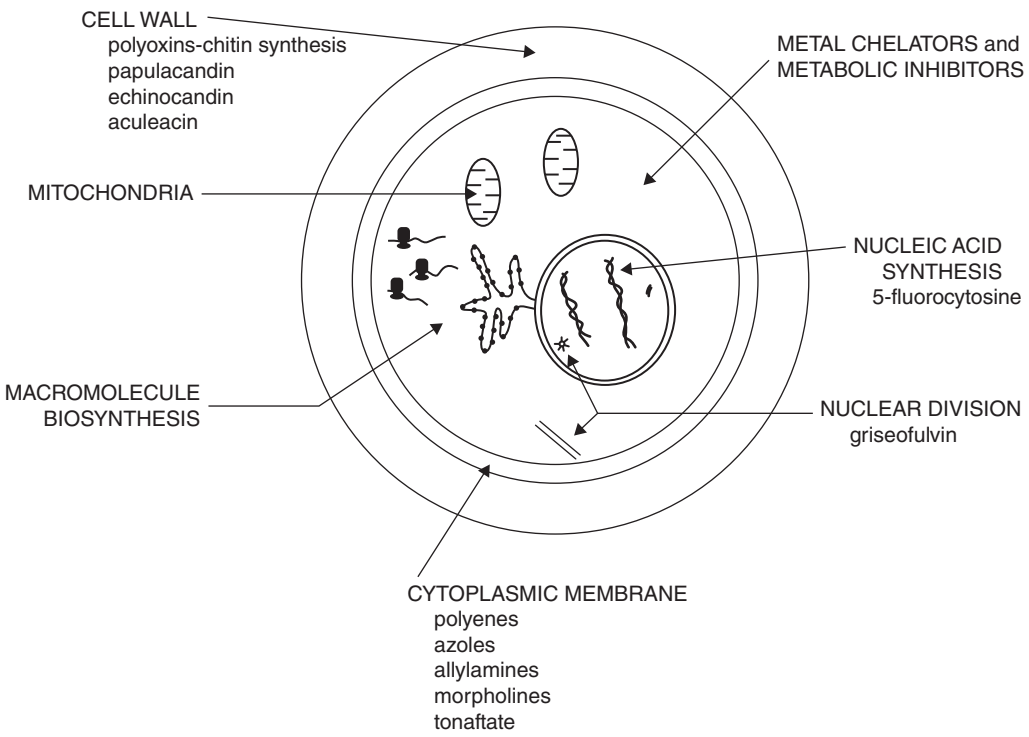

Figure 38.1 Schematic anatomy of a fungal cell and potential sites where antifungal drugs act.

however, the half-life at the site of action — the stratum corneum — is prolonged because the drug is bound tightly to keratinocytes and remains in the skin until these cells are shed. Thus, new hair or nail growth is first to become free of disease as keratin infected by fungus is replaced by new cells.

Griseofulvin is metabolized primarily by the liver to demethylgriseofulvin and the glucuronide. It is metabolized approximately six times faster in animals than in people, which is the reason animal doses are higher than human doses (half-life in dogs is less than 1 hour, compared to 20 hours in people) (Shah et al., 1972).

Clinical Use

Small animals: Griseofulvin is still used for treating dermatophytosis, but is being gradually replaced by azole drugs (discussed in Section Azole Antifungal Drugs). The recommended doses have varied, depending on the author. The label dose in dogs and cats for Fulvicin U/F® tablets is 11 to 22 mg/kg/day, but recommendations by specialists in dermatology have ranged from 44 mg/kg/day to 110–132 mg/kg/day in divided treatments (Scott, 1980). One review suggested a dose of 50 mg/kg once a day of the microsize formulation (Hill et al., 1995), and another review listed 25 mg/kg every 12 hours (deJaham et al., 2000), but the dose can be doubled for refractory cases. The most common dose is in the range of 50 mg/kg/day, which was confirmed in a report in which it was used in cats and was as effective as itraconazole for treatment of dermatophytosis (Moriello and DeBoer, 1995).

Griseofulvin is available in 125 and 250 mg capsules; 125, 250, and 500 mg tablets; and 125 mg/ml oral syrup. Often, at least 4 weeks are needed for successful therapy, and some patients require 3 months (or more) of continuous therapy. As long as 4 months may be necessary to treat infections of the nail bed (onychomycosis).

Large animals: Griseofulvin is approved at a dose of 2.5 g/day orally in adult horses for a minimum of 10 days. This translates to one packet of the powder formulation or one bolus per day, administered on feed. The dose for foals is half packet or half bolus per foal. Its use is limited to cases of dermatophytosis. There are currently no approved griseofulvin products for use in food animals in the United States. Nevertheless, when used off-label, griseofulvin has been effective in the prevention and treatment of dermatophytes in cattle (Reuss, 1978). Doses used are approximately 7.5–10 mg/kg for 7 to 35 days. At doses of 7.5 mg/kg orally once a day for 7 days, drug metabolites were still detectable in the liver of treated cattle at 10 days following the last administration (Tarbin and Fussell, 2013). A dose rate of 1 g/100 kg has been recommended for pigs for a duration of 30–40 days (Kielstein and Gottschalk, 1970). Because this drug is not approved, veterinarians must determine proper food

animal withdrawal times for this drug prior to administration.

Adverse Effects

The most serious adverse effects associated with griseofulvin occur in cats and include leukopenia, anemia, increased hepatic enzyme activity, and neurotoxicosis (Helton et al., 1986). Ataxia in a kitten (Levy, 1991) and bone marrow hypoplasia in an 8-year-old cat (Rottman et al., 1991) have been reported. Prolonged treatment of eight cats with griseofulvin at the high end of the dosage range resulted in no untoward clinical, hematological, or hepatic side effects, suggesting that griseofulvin toxicity may be idiosyncratic (Kunkle and Meyer, 1987). Cats with feline immunodeficiency virus (FIV) appear to be at increased risk for griseofulvin-associated neutropenia (Shelton et al., 1990); however, toxicity has also been reported in FIV-negative cats (Rottman et al., 1991). The mechanism of this increased risk is unknown but may involve griseofulvin-enhanced binding of immune complexes to granulocytic cells in infected cats (Shelton et al., 1991).Griseofulvin should never be administered to pregnant cats. Its teratogenicity has been well-documented (Scott et al., 1975; Gruffydd-Jones and Wright, 1977). The teratogenic effects include cranial and skeletal malformations as well as ocular, intestinal, and cardiac problems (Scott et al., 1975). It has been given to pregnant horses with no apparent ill effect (Hiddleston, 1970); however, this may be dependent on the stage of pregnancy in which the drug was given. One report documented bilateral microphthalmia, brachygnathia superior, and palatocheiloschisis of the drug when given to a mare in the second month of pregnancy (Schutte and Van den Ingh, 1997). The labeled products for horses state not to administer to animals with impaired hepatic function.

Amphotericin B

Amphotericin B (Fungizone®, Abelcet®, Amphotec®, AmBisome®) is a polyene antibiotic with a large macrolide ring with a hydrophobic conjugated double-bond chain and a hydrophilic hydroxylated carbon chain and attached sugar (Figure 38.2) (Mechlinsk et al., 1970).

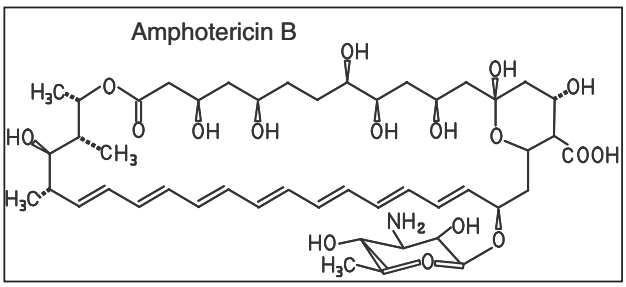

Figure 38.2 Amphotericin B.

It is a yellowish powder that is insoluble in water and somewhat unstable (Bennett, 1990). There are several formulations of amphotericin B available, including the conventional formulation, which is a micellar complex with the bile salt deoxycholate, and newer formulations that are lipid-based complexes. These are less toxic, but also more expensive (reviewed by Plotnick, 2000).

Amphotericin B lipid complex (Abelcet) is a suspension of amphotericin B complexed with two phospholipids. Amphotericin B cholesteryl sulfate complex (Amphotec, ABCD) is a colloidal dispersion of amphotericin B. The liposomal complex of amphotericin B (AmBisome) is a unilamellar liposomal formulation which, when reconstituted, produces small vesicles of encapsulated amphotericin B. Some investigators have attempted to achieve the benefits of lipid formulations without the added cost by mixing the deoxycholate salt in a 10 or 20% lipid solution (Intralipid). This formulation is stable for up to 3 weeks after mixing (Walker et al., 1998); however, the reports of the benefit of this emulsion versus the conventional formulation are inconsistent.

In comparison to the conventional formulation of amphotericin B, lipid formulations can be administered at higher doses to produce greater efficacy with less toxicity (Hiemenz and Walsh, 1996). Decreased toxicity is attributed to a selective transfer of the lipid complex amphotericin B, releasing the drug directly to the fungal cell membrane and sparing the mammalian cell membranes. Reduced drug concentrations in the kidneys and diminished release of inflammatory cytokines from amphotericin lipid complex compared to the conventional formulation may also prevent adverse reactions.

Mechanism of Action

The major action of amphotericin B is to bind ergosterol in the fungal plasma cell membrane, making the membrane more permeable and resulting in leakage of cell electrolytes and cell death (Brajtburg et al., 1990). At high concentrations, amphotericin B is thought to cause oxidative damage to the fungal cell (Warnock, 1991) or disruption of fungal cell enzymes. The selective toxicity of amphotericin B is based on its decreased binding to the major cell membrane sterol of mammalian cells (cholesterol) as compared to that of fungal cells (ergosterol).

Amphotericin B demonstrates concentration-dependent fungicidal activity. There is also a postfungal effect whereby an antifungal effect persists after drug concentrations have declined. This property allows for intermittent therapy (e.g., every other day in dogs).

Spectrum of Activity

The growth of strains of most veterinary fungal pathogens is inhibited in vitro at amphotericin B concentrations between 0.05 and 1.0 μg/ml and there is

good correlation between the MIC values and clinical response (O'Day et al., 1987). Because of concentration-dependent killing, peak (C_{max}) concentrations should be two to four times above the MIC (C_{max}/MIC ratio of 2–4 : 1) (Goodwin and Drew, 2008).

Susceptible fungi include *Histoplasma capsulatum, Cryptococcus neoformans, Coccidioides immitis, Blastomyces dermatitidis, Candida* spp., and various species of *Aspergillus*. Amphotericin B has been indicated for treatment of mucormycosis, sporotrichosis, and phycomycosis (Drouhet and Dupont, 1987). Most strains of *Pseudallescheria boydii,* as well as some agents causing chromoblastomycosis and phaeohyphomycosis, are resistant to amphotericin. Clinical fungal resistance to amphotericin B, either primary or acquired, does not appear to occur commonly, although resistant strains occur in vitro. In most cases, these resistant strains contain decreased levels of membrane ergosterol (Pierce et al., 1978) and increased catalase levels may allow these fungi to be resistant to oxidative damage (Sokol-Anderson et al., 1988). The MIC concentrations were increased in some human patient populations, such as neutropenic patients (Dick et al., 1980), transplant patients (Powderly et al., 1988), and patients undergoing cytotoxic therapy.

The spectrum of activity also includes the protozoa *Leishmania* and is often included in protocols to treat human and canine leishmaniasis. The treatment of protozoa is discussed in Chapter 42.

Pharmacokinetics

Despite its long history of use, much is still unknown concerning the pharmacokinetics of amphotericin B, especially in veterinary medicine. It is poorly absorbed from the gastrointestinal (GI) tract and therefore must be given intravenously, locally, or intrathecally. Amphotericin B binds extensively (~95%) to serum proteins, mainly β-lipoprotein (Bennett, 1977). Much of the drug is thought to leave the vascular space and bind to cholesterol-containing membranes. The highest concentrations are found in liver, spleen, kidney, and lungs, with little accumulation in either muscle or adipose tissue. Concentrations of amphotericin B in fluids from inflamed pleura, peritoneum, synovium, and aqueous humor are about two-thirds of those in serum. Amphotericin B readily crosses the human placenta. Penetration into normal or inflamed meninges, vitreous humor, and normal amniotic fluid is poor. This differential distribution may explain treatment failures for infections in some tissues. Although amphotericin B binds ergosterol with higher affinity than cholesterol, it was suggested that because there are more binding sites for cholesterol in the body than for ergosterol, amphotericin B may be sequestered from its site of action (Bennett, 1977).

Clinical Use

Amphotericin B is used to treat a variety of fungal diseases caused by susceptible fungi, as listed above. Numerous dosage protocols for amphotericin B have been described in the veterinary literature. These are summarized in Table 38.1. One such protocol for small animals was published by Rubin (1986) that is still used today. During infusion, it should be mixed with 5% dextrose solution because it will precipitate if added to an electrolyte containing solution (e.g., lactated Ringer's solution). A solution of amphotericin B with 0.45% saline and 2.5% dextrose has been used successfully subcutaneously without any visible precipitation (Malik et al., 1996).

Amphotericin B is used only sporadically as a systemic antifungal in equine medicine and there are no pharmacokinetic data available on amphotericin in the horse. It is more often used as a local treatment in the eye, limbs, and upper airway. In cases of ocular fungal disease that do not respond to typical therapy, amphotericin B (0.2 ml of a 5 mg/ml solution) injected subconjunctivally q 48 h for up to three treatments can be used. This should provide a higher level of drug to the eye; however, it may produce localized toxic effects, including conjunctivitis and conjunctival necrosis. Intravenous regional limb perfusion (IRLP) has also been reported to successfully treat pythiosis of the lower limbs at a dose of 50 mg for one to two treatments (Dória et al., 2012). Side effects include limb edema with pain on palpation, and inflammation of the injection site; however, these signs are considered manageable and resolve after 14 days. Topical and intralesional therapy with amphotericin B has also been reported as a successful treatment for nasal conidiobolomycosis in the horse (French et al., 1985; Zamos et al., 1996). There are no reports of use of this drug in food animals, and there are no approved formulations for use in these species.

Combination therapy using amphotericin B and flucytosine has been shown to be synergistic against cryptococcal infections (see Section Flucytosine). Combinations of amphotericin B and azole antifungals have been less successful. Azole-induced depletion of fungal cell membrane ergosterol results in fewer binding sites on which polyene antifungals can exert their effect. Antagonism and synergism between these two classes of antifungal agents have been reported experimentally (Polak et al., 1982; Dupont and Drouhet, 1979). Because of the slower onset of action of azole antifungals, many clinicians recommend initial therapy of serious systemic fungal infections with amphotericin B, followed by longer, follow-up treatment with an azole therapeutic protocol.

Adverse Effects

The most important clinical toxicosis associated with amphotericin B therapy is nephrotoxicity. It is this effect

Table 38.1 Selected dosing protocols for amphotericin B in companion animals

Species	Formulation	Disease treated	Dosing protocol	Reference
Canine	Fungizone	Unspecified	Pretreatment with 0.9% sodium chloride followed by infusion of 0.5 mg/kg in 5% dextrose (D5W) over 4–6 hours IV q48h; a test dose of 0.25 mg/kg is sometimes recommended.	Rubin, 1986
Canine	Abelcet	Blastomycosis	Pretreatment with LRS at 2.5 times maintenance for 30 minutes followed by flushing the line with D5W and infusing 1 mg/kg amphotericin in D5W over 2.5 hours IV followed by LRS at 2.5 times maintenance for an additional 2 hours after treatment. Repeat q48h to a total cumulative dose of 8–12 mg/kg.	Krawiec et al., 1996
Canine	Abelcet	Unspecified	2–3 mg/kg IV 3 times per week diluted in 5% dextrose to a concentration of 1 mg/ml for a total of 9–12 treatments (cumulative dose of 24–27 mg/kg).	Grooters and Taboada, 2003
Canine	AmBisome	Leishmaniasis	3–3.3 mg/kg IV.	Oliva et al., 1995
Canine	Fungizone 40 ml sterile water and 10 ml 10% Intralipid	Leishmaniasis	Pretreatment with 50 ml/kg of 0.9% sodium chloride followed by 10 ml/kg 20% mannitol. Drug mixture infused over 30–60 minutes at incrementally increasing dosing from 1–2.5 mg/kg IV twice a week for a minimum of 8 injections.	Lamothe, 2001
Canine/feline	Fungizone in 0.45% saline with 2.5% dextrose	Cryptococcosis	0.5–0.8 mg/kg SC in 400 ml for cats or 500 ml in dogs given twice a week for a cumulative dose of 8–26 mg/kg.	Malik et al., 1996
Feline	Abelcet	Unspecified	1 mg/kg IV 3 times per week diluted in 5% dextrose to a concentration of 1 mg/ml for a total of 12 treatments (cumulative dose of 12 mg/kg).	Grooters and Taboada, 2003
Equine	Fungizone	Phycomycosis	0.38 gradually increased up to 1.47 mg/kg IV in 1 l 5% dextrose once daily.	McMullan et al., 1977
Equine	Fungizone	Pulmonary histoplasmosis	0.3–0.6 mg/kg IV in 1 l 5% dextrose once a day or every other day.	Cornick, 1990
Equine	Fungizone	Systemic candidiasis	0.1–0.5 mg/kg IV in 1 l 5% dextrose infused over 4–6 hours once daily.	Reilly and Palmer, 1994
Equine	Fungizone	Candida arthritis	0.33–0.89 mg/kg IV in 1 l 5% dextrose once a day or every other day.	Madison et al., 1995
Equine	Fungizone	Cryptococcal pneumonia	0.5 mg/kg IV in 1 l 5% dextrose as a 1-hour infusion once a day.	Begg et al., 2004
Avian	Fungizone	Aspergillosis	1.5 mg/kg q8–12h reconstituted in sterile water and then diluted 1 : 50 with 5% dextrose for 3–7 days.	Tully, 2000

on kidneys that is the most common reason for discontinuing therapy with amphotericin B. It is a dose-related, predictable toxic effect that occurs in almost every animal treated with the conventional formulation. Direct tubular damage occurs because amphotericin B binds to cholesterol in the tubular cells, which results in electrolyte leakage from the cells (primarily K^+ loss) and renal tubular acidosis (Bennett, 1990). Induced renal vasoconstriction and impaired acid excretion may also contribute to amphotericin B's renal toxicity (Greene, 1990). Renal vasoconstriction may be caused by induced increases in the eicosanoid synthesis in renal blood vessels. The tubular damage, along with the renal vasoconstriction, leads to both an acute and a chronic cumulative renal toxicosis. Clinically, the signs of kidney injury are seen as increases in creatinine and blood urea nitrogen (BUN). Electrolyte loading, fluid diuresis, and slow infusion of amphotericin B have all been shown to decrease the severity and the rate of development of renal toxicity. Therefore, common protocols for administration of amphotericin B to animals include pretreatment with sodium chloride IV solution (Rubin, 1986) with or without mannitol (Legendre et al., 1984), and a slow infusion. Slower infusion times are associated with less kidney injury (Rubin, 1986). If the dose administered during a single infusion exceeds 1 mg/kg, acute renal injury is likely (Butler and Hill, 1964).

Careful clinical monitoring will help decrease the risk of permanent renal injury. Urine sediment evaluation has been suggested to detect kidney injury earlier than serum biochemical alterations (Greene, 1990); thus, urine should be evaluated for proteinuria, cylinduria, and hematuria, as well as specific gravity. In addition, BUN, creatinine, and electrolyte concentrations should be monitored. Therapy should be temporarily discontinued when active urine sediment is detected or the serum creatinine increases. After stopping therapy, patients may undergo a fluid diuresis to decrease the azotemia. If BUN and creatinine return to near-normal reference values, treatment may be resumed. If azotemia does not improve, one should consider an alternative treatment.

Other adverse effects from amphotericin that are frequently observed in animals include phlebitis, fever, nausea, and vomiting. Measures to prevent the nausea and vomiting have included administration of antiemetic drugs such as chlorpromazine, maropitant, or metoclopramide prior to infusion (see Chapter 46). Hypokalemia, bronchospasm, and anemia/hemolysis have frequently been reported in humans and therefore should be monitored for in veterinary patients.

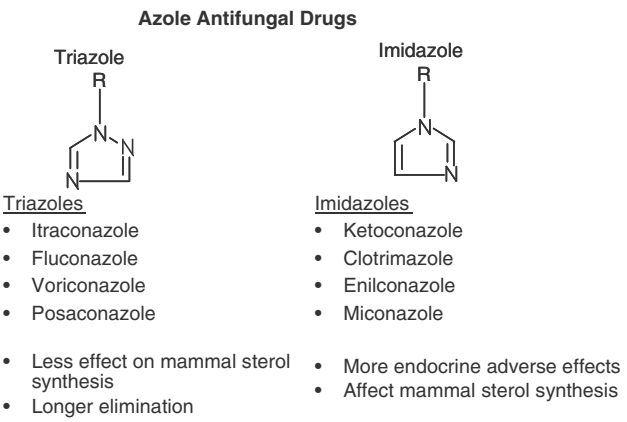

Figure 38.3 Azole antifungal drugs

Azole Antifungal Drugs

The azole antifungal drugs have a high safety profile, a broad spectrum of activity, and are available in topical, oral, and intravenous formulations. There are two main categories of azole antifungal drugs, the imidazoles (clotrimazole, miconazole, ketoconazole) and the triazoles (fluconazole, itraconazole, voriconazole) (Figure 38.3). Clotrimazole and miconazole are discussed in the section on topical therapy. The important physicochemical differences between azole antifungal drugs are summarized in Table 38.2.

Mechanism of Action

All azoles exert their antifungal effect on the cell membrane of fungi by inhibiting synthesis of the primary sterol of the fungal cell membrane, ergosterol. Inhibition of the P450–dependent lanosterol C_{14}-demethylase enzyme results in depletion of ergosterol and

Table 38.2 Comparison of the physicochemical properties and in vitro activity of commonly used azole antifungal drugs

					Activity		
Drug	**Solubility**	**pH Dependent**	**LogP**	**Protein binding**	**Yeasts**	**Aspergillus**	**Fusarium**
Ketoconazole	pi	Yes	3.78	>90%	+	±	–
Fluconazole	ss	No	0.54	10–12%	+	–	–
Itraconazole	pi	Yes	5.66	>98%	+	±	–
Voriconazole	vss	No	1.81	32–58%	+	+	±

pi, practically insoluble (<0.01 mg/ml); ss, slightly soluble (1–10 mg/ml); vss, very slightly soluble (0–1 mg/ml).

accumulation of C_{14}-methyl sterols in the cytoplasmic membrane of yeasts and filamentous fungi. This enzyme is also known as CYP51A or Erg11p and is encoded by the *ERG11* gene. Inhibition of this cytochrome P450 enzyme occurs via binding of the nitrogen (N_3 of imidazoles and N_4 of triazoles) to the heme iron atom of ferric cytochrome P450. This prevents the formation of the superoxide Fe^{+3} complex ($Fe^{+3}O^{-}$) needed for hydroxylation of methyl sterols. The result is an inability to demethylate C_{14}-methyl sterols and reduced synthesis of ergosterol. Sterols with less planar configurations are then incorporated into the fungal cell wall, which changes membrane fluidity and interferes with the barrier function of the membrane and with membrane-bound enzymes.

Azole drugs are generally fungistatic at concentrations achieved clinically, although there are exceptions for some fungal species, and for some drugs. The parameter that is best associated with clinical cure for azole drugs is the total exposure as measured by the area-under-the-curve in relation to the MIC (AUC/MIC ratio) (Goodwin and Drew, 2008).

The potency of each azole drug is related to its affinity for binding the P450 enzyme. The selective toxicity of each compound is directly dependent upon its specificity for binding fungal P450 more readily than mammalian P450. Imidazoles are less specific than triazoles and produce side effects in animals attributed to inhibition of P450 enzymes that are responsible for the synthesis of cortisol and reproductive steroid hormones. Azoles may decrease cholesterol, cortisol, androgen, and testosterone biosynthesis and may interfere with hepatic CYP450 enzymes that are important for drug metabolism and carcinogenic agents (Polak, 1990). These drugs also may inhibit the membrane transporter known as P-glycoprotein.

Interactions with Drug Metabolism

The inhibition of mammalian P450 enzymes is also responsible for drug–drug interactions that have been observed with the azole antifungals. When azoles are administered concurrently with other drugs that are metabolized by these enzymes, they can significantly increase the plasma concentrations of those drugs. Alternatively, when azoles are administered concurrently with drugs that induce the P450 enzymes, the concentrations of the azole drugs may be significantly decreased. The drug–drug interactions important to veterinary medicine are summarized in Table 38.3. The ability to inhibit mammalian P450 enzymes, and therefore the likelihood of drug–drug interactions, is greatest with ketoconazole (Aidasani et al., 2008) followed by itraconazole, voriconazole, and fluconazole.

Another method by which the azole antifungals can interfere with the absorption and pharmacokinetics of concurrently administered medications is through inhibition of the P-glycoprotein efflux pumps. These efflux pumps can be found in the intestine, where they limit the absorption of some substrates, as well as in the liver, kidney, eye, and CNS. At the intestinal level, there is a relationship between the P-glycoprotein efflux pump and the metabolism by intestinal CYP450 enzymes (Benet, 2009). Inhibition of both can have profound effects on systemic drug concentrations. Azole antifungals have the ability to inhibit P-glycoprotein pumps and therefore increase the oral absorption and tissue distribution of drugs within the body, particularly into protected sites, such as the blood–brain barrier and the blood–retinal barrier. The ability to inhibit P-glycoprotein is greatest with itraconazole, followed by ketoconazole and voriconazole (Wang et al., 2002). Fluconazole has little interaction with P-glycoprotein, which may explain why it has fewer significant drug interactions compared to the other azole antifungals (Yasuda et al., 2002; Wang et al., 2002).

Ketoconazole

Ketoconazole (Nizoral®), one of the imidazoles, became available in 1979. The results of successful use in veterinary medicine were published shortly thereafter (Legendre et al., 1982; Medleau et al., 1985). Ketoconazole is available in 200 mg tablets, and generic formulations are inexpensive and readily available.

Spectrum of Activity

Ketoconazole is most effective against yeast and dimorphic fungi such as *Candida*, *Malassezia pachydermatis*, *C. immitis*, *H. capsulatum*, and *B. dermatitidis*, as well as most dermatophytes with MIC values less than 0.5 µg/ml. It is less effective against *C. neoformans*, *S. schenckii*, and *Aspergillus* spp., with MIC values varying from 6 to >100 µg/ml (Hume and Kerkering, 1983).

Pharmacokinetics

Ketoconazole is relatively insoluble, except in an acid environment. It is not well absorbed orally unless there is acid secretion, such as after a meal. Ketoconazole is highly protein bound (>98%) and therefore does not penetrate into the cerebrospinal, seminal, or ocular fluid to a significant degree; although it does partition into milk. It distributes throughout the skin and subcutaneous tissue, making it effective for treatment of superficial and systemic fungal skin infections. The drug demonstrates nonlinear absorption and elimination kinetics, most probably due to saturation of solubility or metabolizing enzymes. It is biotransformed in the liver via O-dealkylation and aromatic hydroxylation and excreted mainly in the bile. Elimination half-life is approximately 2 hours in dogs.

Because ketoconazole is soluble only in acid aqueous environments (pH <3), gastric alkalizing agents (e.g.,

Table 38.3 Antifungal drug–drug interactions of significance in veterinary medicine[a]

	Drug/drug class	Result
Griseofulvin	Anticoagulants/coumarin or inandione derivatives	Griseofulvin is a hepatic enzyme inducer, which may increase the metabolism of these drugs, resulting in decreased anticoagulant effects.
	Barbiturates	Impaired absorption and therefore possibly impaired effectiveness of griseofulvin.
Amphotericin B	Bone marrow depressants	Increased risk of anemia or other blood dyscrasia.
	Corticosteroids	Exacerbation of hypokalemia, particularly with those drugs that have significant mineralocorticoid activity.
	Digoxin	Hypokalemia caused by AmpB increases the potential for digitalis toxicity.
	Neuromuscular blocking agents	Hypokalemia caused by AmpB enhances the blockade of nondepolarizing agents.
	Diuretics	Potassium depleting diuretics will exacerbate hypokalemia.
	Flucytosine	Synergism of AmpB with flucytosine may decrease the dose of AmpB necessary, therefore reducing the nephrotoxicity, however AmpB-induced renal dysfunction may increase 5-FC concentrations, thus increasing the potential for blood dyscrasias.
Azole Antifungals	Drugs that increase gastric pH	Decreases the absorption of those drugs with a pH-dependent solubility (ketoconazole and itraconazole only).
	Digoxin	Increased plasma concentrations of digoxin resulting from P450 inhibition may lead to increased digitalis toxicity.
	Benzodiazepines	Increased plasma concentrations of benzodiazepines, particularly midazolam, resulting from P450 inhibition may result in potentiation of the sedative effects of these drugs.
	Glipizide	Increased plasma concentrations of glipizide resulting from P450 inhibition may cause hypoglycemia.
	Second-generation antihistamines	Although identified in people, and not animals, there may be increased plasma concentrations of antihistamines resulting from P450 inhibition, which may result in cardiac arrhythmias, including ventricular tachycardia and torsades de pointes; not seen with fluconazole except at very high doses.
	Warfarin	Increased plasma concentrations of warfarin resulting from P450 inhibition may cause increased anticoagulant effects and bleeding.
	Cisapride	Although identified in people, and not animals, there may be increased plasma concentrations of cisapride resulting from P450 inhibition, which may result in ventricular arrhythmias, including torsades de pointes.
	Cyclosporine	Increased plasma concentrations of cyclosporine resulting from P450/P-gp inhibition may require adjustment of cyclosporine doses; has been used clinically to decrease the cost of cyclosporine treatment.
	Quinidine	Increased plasma concentrations of quinidine resulting from P450/P-gp inhibition may lead to increased quinidine toxicity.
	Nifedipine	Increased plasma concentrations of nifedipine resulting from P450/P-gp inhibition.
	Hydrochlorthiazide	Hydrochlorthiazide decreases the renal elimination of fluconazole, resulting in increases of fluconazole plasma concentrations.
	Carbemazapine	Induction of P450 enzymes by carbemazapine may decrease the plasma concentrations of antifungal drugs.
	Rifampin	Induction of P450 enzymes by rifampin may decrease the plasma concentrations of antifungal drugs.
	Phenytoin	Induction of P450 enzymes by phenytoin may decrease the plasma concentrations of antifungal drugs.
	Phenobarbital	Induction of P450 enzymes by phenobarbital may decrease the plasma concentrations of antifungal drugs.
	Prednisolone	Down-regulation of intestinal P-glycoprotein results in a subsequent increase in the AUC of orally administered prednisolone.
	Methadone	Inhibition of P450 enzymes results in significantly increased AUC and plasma concentrations of methadone after oral administration to healthy Greyhound dogs.
	Colchicine	Increased risk of colchicine toxicity when coadministered with azole antifungals.
	Other azole antifungals	Ketoconazole inhibits its own elimination, resulting in possible increased plasma concentrations over time.
Flucytosine	Bone marrow depressants	May exacerbate bone marrow toxicities.
	Nephrotoxic agents	May decrease flucytosine clearance, increasing the potential for bone marrow toxicity.
Terbinafine	*There are no reported drug–drug interactions with terbinafine.*	

[a]Not all of these interactions have been documented in veterinary medicine, but are present in human medicine and should be monitored for in veterinary patients.

antacids, H_2 blockers, and parietal cell proton pump inhibitors) or diseases resulting in achlorhydria will decrease its dissolution and oral absorption. Because of lack of consistent gastric acidity, ketoconazole is absorbed poorly in horses. When ketoconazole was administered at 30 mg/kg to horses in corn syrup, the drug was not detected in serum; however, when it was administered with 0.2 N hydrochloric acid intragastrically, oral absorption increased but systemic availability was only 23% with peak serum concentrations of 3.76 μg/ml (Prades et al., 1989).

Clinical Use

In people, ketoconazole has been replaced in therapy by safer triazole antifungal drugs and is no longer marketed in some countries. But in veterinary medicine, owing to ketoconazole's efficacy, safety, cost, and ease of administration, it is still a popular antifungal agent. For dermatophytosis in cats, 10 mg/kg/day has been used (Medleau and Chalmers, 1992). For candidiasis, 10 mg/kg/day for 6–8 weeks is recommended. For canine blastomycosis, histoplasmosis, cryptococcosis, and coccidioidomycosis, the dosage is 10–20 mg/kg every 12 hours. Ketoconazole may also be effective in treating nasal cryptococcosis in a dog at a dose of 10 mg/kg/day (Noxon et al., 1986). For *Malassezia* dermatitis in dogs, dosages of 5–10 mg/kg/day have been recommended (Hill et al., 1995). The duration of treatment is highly variable. Four to six weeks is a minimum for most diseases; many patients with blastomycosis are treated for a minimum of 2 months and as long as 6 months. If there is CNS involvement, particularly with cryptococcosis, higher doses (40 mg/kg) may be necessary to improve penetration into the CNS. Cats have been successfully treated for cryptococcosis with a dosage of 10–15 mg/kg/day (Pentlarge and Martin, 1986; Legendre et al., 1982; Medleau et al., 1985). As complete eradication of the fungal organism is difficult, relapse is common. For this reason, infections should be treated beyond the time clinical signs have resolved. Ketoconazole is not absorbed well orally in horses and it is not recommended. There are no approved formulations for use in food animals.

The use of ketoconazole is not limited to the treatment of fungal infections. Because of its inhibitory effect on P450 and P-glycoprotein, administration of ketoconazole concurrently with cyclosporine for the treatment of immune diseases has been used to reduce the dose of cyclosporine by up to 75% and reduce the cost of cyclosporine therapy by 58% (Dahlinger et al., 1998). A study examining blood and skin concentrations of cyclosporine with concurrent administration of ketoconazole at 2.5 mg/kg each showed this regimen to be potentially as effective as cyclosporine alone at 5.0 mg/kg for treatment of canine atopic dermatitis (Gray et al., 2013). Although this may be the most common interaction manipulated for clinical use, other drug–drug interactions have been reported. Ketoconazole (100 mg/day) administered to healthy beagle dogs resulted in down-regulation of intestinal P-glycoprotein and a subsequent increase in the AUC of orally administered prednisolone (Van der Heyden et al., 2012). Concurrent administration of ketoconazole with methadone significantly increased the AUC and plasma concentrations of methadone after oral administration to healthy Greyhound dogs (Kukanich et al., 2011). Other studies have shown that ketoconazole inhibits its own elimination, as well as that of midazolam in healthy Greyhounds, without any significant effect on fentanyl or morphine elimination (Kukanich and Hubin, 2010; Kukanich and Borum, 2008). Colchicine toxicity has also been reported to be precipitated by ketoconazole in a dog, and these drugs are not recommended for coadministration (McAlister et al., 2014).

Ketoconazole inhibits the synthesis of steroid hormones (via inhibition of the cytochrome P450 enzymes), most notably cortisol and testosterone. Although this may be a side effect of therapy, it has been exploited for the temporary management of hyperadrenocorticism in dogs (Bruyette and Feldman, 1988; Feldman et al., 1990) and as an antiandrogen treatment. Steroid synthesis inhibition is a temporary effect that persists only during dosing with ketoconazole (e.g., for up to 8 hours). Although the effects are temporary, they are effective. A recent retrospective study showed that ketoconazole administration improved clinical signs of hyperadrenocorticism in 90% of treated dogs, and 69% of dogs had cortisol concentrations following adrenocorticotropic hormone (ACTH) stimulation that were within the normal range (Lien and Huang, 2008). Dogs in that study were treated for the remainder of their life, with a median survival time after diagnosis of 25 months. Ketoconazole will not produce permanent hypoadrenocorticism.

Adverse Effects

Nausea, anorexia, and vomiting are the most common adverse effects, and may require cessation of therapy, particularly in cats (Medleau and Chalmers, 1992). They are usually dose related and may be diminished by decreasing the dose, dividing the total dose into smaller doses, and administering each dose with food. With chronic therapy pruritus, alopecia, lightening and drying of the hair coat, and weight loss may occur (Greene, 1990). Slight to moderate elevations of inducible hepatic enzymes are expected and may not be accompanied by hepatic injury. However, high elevations in hepatic enzymes, accompanied by other parameters (hyperbilirubinemia and clinical signs consistent with hepatic disease), may indicate hepatotoxicosis. Idiosyncratic hepatitis has been reported in animals and people (Janssen and Symoens, 1983).

Drug Interactions

Ketoconazole is a very potent inhibitor of fungal P450, but it also inhibits mammalian P450 at relatively low concentrations (Aidasani et al., 2008); therefore, side effects and drug interactions can occur. Inhibition of P450(17α) catalyzed conversion of progestins to androgens occurs during treatment. Dose-related inhibition of testosterone has resulted in gynecomastia, sexual impotence, and azoospermia. Cats appear to be more sensitive to ketoconazole liver toxicity than are dogs but they are less sensitive to the hormonal suppressive side effects (Willard et al., 1986a, 1986b).

Ketoconazole has been shown to be teratogenic in the rat and has resulted in mummified fetuses and stillbirths in dogs. It is therefore not recommended for use in pregnant or lactating animals. Cataracts have been reported after long-term ketoconazole therapy in dogs (de Costa et al., 1996). The average duration of therapy in affected dogs was 15 months, and dosages ranged from 6 to 31 mg/kg/day. These dogs were not diabetic. The mechanism of this reaction is not known.

Fluconazole

Fluconazole (Diflucan® and generic) has replaced ketoconazole in small animals and birds for many indications. The triazole groups result in increased resistance to metabolic attack, in vivo potencies 100 times that of ketoconazole, and significantly increased aqueous solubility (8 mg/ml) (Richardson et al., 1990). Because of these properties, this compound has good efficacy in animal models and pharmacokinetic properties that are improved over other azole antifungal drugs such as ketoconazole or itraconazole. It is available in 50–200 mg tablets, powder for oral suspension, and a 2 mg/ml parenteral formulation. Compounded formulations have good oral bioavailability and can be used with a reasonable expectation of performance.

Spectrum of Activity

Fluconazole has been shown to be effective for animal infections caused by *Blastomyces, Candida, Coccidioides, Cryptococcus*, and *Histoplasma*. It is not particularly active against *Aspergillus*. Resistant *Aspergillus* strains have been increasing in human medicine with MICs often >256 μg/ml. For this reason, fluconazole should not be used as a first choice for the treatment of aspergillosis unless susceptibility has been determined. Efficacy of fluconazole in people has been associated with AUC/MIC ratios as being the best predictor of cures. A ratio above 25 is considered desirable for the best outcome (Goodwin and Drew, 2008).

Pharmacokinetics

Fluconazole has different solubility characteristics than ketoconazole and itraconazole and is absorbed well regardless of the circumstances. Feeding or formulation (liquid versus tablet) does not affect absorption. Fluconazole demonstrates linear absorption kinetics, with bioavailability greater than 90% in most species (Brammer et al., 1990); thus, oral and IV dosages are identical. Maximum fluconazole concentrations are reached 1–4 hours after an oral dose. Unlike other azole antifungals, fluconazole is not highly protein bound. Humphrey et al. (1985) found plasma protein binding to be between 10 and 12% at concentrations of 0.1 and 1 μg/ml in mice, rats, dogs, and humans. Similar protein binding has also been documented in horses (12.3%) at a concentration of 5 μg/ml. Fluconazole's low molecular weight, water solubility, and high unbound fraction allow it to be readily distributed throughout the body, including privileged spaces that ordinarily exclude many drugs. Drug concentrations in saliva, sputum, skin, nails, blister fluid, and vaginal tissue and secretions were found to be similar to plasma concentrations. The advantages of fluconazole lie in its ability to produce higher CSF concentrations than ketoconazole or itraconazole; therefore, it may be useful for treating mycotic meningitis (Kowalsky and Dixon, 1991). Fluconazole CSF/plasma or CSF/serum concentration ratios range from 0.49 in horses (Latimer et al., 2001) to 0.88 in cats (Vaden et al., 1997). The drug also penetrates well into the aqueous humor with ratios of aqueous : plasma of 0.37 and 0.79 in the horse and cat, respectively.

Fluconazole is eliminated principally by the kidney. A unique feature of fluconazole is that this drug is the only one of the azoles that is water soluble and excreted in the urine in an active form; therefore, it may be one of the few drugs useful for treating fungal cystitis. As can be expected with a renally excreted drug, renal dysfunction affects fluconazole's elimination such that dose adjustments are necessary. When patients with normal kidney function were compared with those with severe renal insufficiency, fluconazole's elimination half-life nearly tripled (from 30.1 hours to 84.5 hours) (Dudley, 1990). Reduced dosages as well as extended dosing intervals have been recommended for patients with chronic kidney disease. The disparity between renal fluconazole clearance and creatinine clearance suggests that net tubular reabsorption is responsible for the extended half-life. Half-life was measured to be approximately 14 hours in dogs, 13–25 hours in cats (Vaden et al., 1997; Craig et al., 1994), and 38 hours in horses (Latimer et al., 2001). Steady-state concentrations are achieved in 5–7 days; thus, the manufacturer suggests a two-times loading dose during the first 12–24 hours (Dudley, 1990). The lack of significant hepatic metabolism allows for linear elimination kinetics; that is half-life is independent of dose.

Clinical Use

Small animals: Fluconazole is most often used for treatment of dermatophytes. Although not as active against

Aspergillus or *Penicillium* as other azoles, it has also been used to treat canine nasal aspergillosis and penicilliosis. Ten affected dogs were treated with 2.5–5 mg/kg/day fluconazole orally for 8 weeks. Six dogs became free of disease 2–4 weeks after cessation of therapy and remained free of disease for at least 6 months. Serum alkaline phosphatase and alanine transaminase activity remained within normal ranges throughout the treatment period, and adverse side effects were not noted (Sharp, 1991). Doses as high as 10–12 mg/kg/day have also been recommended in dogs. Fluconazole is also at least as effective as ketoconazole for the treatment of dogs with *Malassezia* dermatitis (Sickafoose et al., 2010). Fluconazole is associated with survival to clinical remission in 75% of dogs with blastomycosis, which was not statistically different than the 90% survival with itraconazole (Mazepa et al., 2011). The cost of fluconazole therapy in that study was approximately one-third that of itraconazole, and both drugs caused a similar incidence of hepatotoxicosis (elevated alanine aminotransferase, ALT). For cats with systemic cryptococcosis, clinical studies have shown a benefit from a dose of 100 mg/cat/day in one or two divided doses. A practical dose is one 50-mg tablet per cat, once a day, or twice daily for refractory cases. Other reported doses are in the range of 2.5–5 mg/kg once a day (Hill et al., 1995). Pharmacokinetic studies support a dose of 50 mg/cat per day for nasal or dermal cryptococcosis (Vaden et al., 1997).

Exotic animals: The doses for exotic animals are listed in Table 38.4. The half-life can be prolonged in reptiles because of the dependence on renal elimination. Thus, the half-life was 138 hours in turtles when injected SC, which allows for treatment once every 5 days (Mallo et al., 2002).

Large animals: Oral absorption in horses is reported to be greater than 100% (Latimer et al., 2001). From this cited study a dosing regimen of a loading dose of 14 mg/kg orally, followed by 5 mg/kg q 24 h was derived for horses to produce sufficient concentrations in plasma and tissues. This dose has been successful in treating cryptococcal meningitis and optic neuritis (Hart et al., 2008), and nasal conidiobolomycosis lesions in adult horses (Taintor et al., 2004) as well as disseminated candidiasis in foals (Reilly and Palmer, 1994).

Adverse Effects

Fluconazole has been generally well tolerated, with mild adverse effects being reported in 5–30% of cases. The GI tract was most frequently involved, followed by the CNS and skin. Elevations in hepatic enzymes have been observed, in small animals and horses, sometimes necessitating termination of treatment. Hematological abnormalities, including anemia, leukopenia, neutropenia, and thrombocytopenia, have been reported in people. In subacute toxicity studies in dogs, the highest dose tested (30 mg/kg) caused slight increases in liver weight, hepatic fat, and plasma transaminase activity. Although there is no evidence of mutagenicity or carcinogenicity, its use in pregnant patients is not recommended. However, it has been used successfully with no observed adverse effects on the fetus in horses in the seventh and tenth month of gestation (Taintor et al., 2004).

Drug Interactions

Drug–drug interactions are less frequently reported with fluconazole than other azole antifungals; however, they do occur. Fluconazole has been shown to significantly increase cyclosporine concentrations in both normal and renal transplanted dogs (Katayama et al., 2008, 2010a). Treatment with fluconazole has been shown to significantly prolong anesthesia times in horses following induction regimens that include midazolam (Krein et al., 2014). Compared to ketoconazole, there is little evidence of testosterone or other steroid biosynthesis inhibition (Shaw et al., 1987) in human or animal patients (VanCauteren et al., 1987b).

Itraconazole

Itraconazole (Sporanox®) was approved for use in the United States in 1992. Of several triazole compounds screened, itraconazole, first synthesized in 1980, was selected for further clinical development due to several criteria: (i) 5–100 times better in vitro and in vivo potency than ketoconazole, (ii) good activity against *Aspergillus* spp., (iii) activity against meningeal cryptococcosis in animal models, (iv) fewer adverse effects compared to ketoconazole, and (v) favorable pharmacokinetics (Cauwenbergh et al., 1987).

Itraconazole is a weak base (pK_a = 3.7), is highly lipophilic (logP = 5.66), and is practically insoluble in water. There are several different formulations available. The intravenous formulation is rarely used in veterinary medicine due to expense as well as instability once reconstituted. There are three oral formulations available. The oral capsules were the first formulation marketed and they are still commonly used. They are available in 100 mg dose strength, and consist of drug coated onto small sugar spheres. The capsules require an acid environment for dissolution and therefore absorption is often highly variable. There is also an oral solution approved for use in humans that contains 10 mg/ml of itraconazole complexed with hydroxypropyl-β-cyclodextrin, to increase the solubility. This product has been demonstrated to have higher, less variable absorption in humans, cats, and horses (Willems et al., 2001; Boothe et al., 1997; Davis et al., 2005; Mawby et al., 2016) but is bioequivalent to the capsules in dogs

Table 38.4 Selected systemic antifungal drugs used in exotic animal species

Species	Drug	Disease treated	Dosing protocol	Reference
Passerine and Softbill Birds	Fluconazole	Candidiasis	2–5 mg/kg PO q24h for 7–10 days	Dorrestein, 2000
	Griseofulvin	Dermatophytosis	20 mg/kg PO q24h for 4–6 weeks	
	Itraconazole	Aspergillosis	5–10 mg/kg PO q12–24h in orange juice or 0.1N HCl for 14 days	
	Ketoconazole	Dermatophytosis	20–30 mg/kg PO q12h for 14–30 days	
	Miconazole	Candidiasis or cryptococcosis	10–20 mg/kg IM or IV q8–24h	
	Nystatin	Intestinal candidiasis	100,000 IU/l of drinking water or 200,000 IU/kg soft food for 3–6 weeks	
Psittacine Birds	Fluconazole	Candidiasis	2–5 mg/kg PO q24 h for 7–10 days	Tully, 2000
	Flucytosine	Aspergillosis	60–150 mg/kg PO q12h in adults; 100–250 mg/kg PO q12h in neonates. Usually given in combination with amphotericin B	
	Itraconazole	Aspergillosis	5–10 mg/kg q12h for 4–5 weeks; 5 mg/kg q24h in African grays	
	Ketoconazole	Candidiasis	20–30 mg/kg q12h in orange or pineapple juice, lactulose, or methylcellulose for 14–30 days	
	Voriconazole	Aspergillosis	12–18 mg/kg oral q12h	Flammer et al., 2008
	Miconazole	Candidiasis or cryptococcosis	20 mg/kg IV q8h	
	Nystatin	Intestinal candidiasis	100,000–300,000 IU/kg PO q8–12h	
Raptors	Fluconazole	Mycelial candidiasis, systemic mycosis	5–15 mg/kg PO q12h for 14–60 days	Huckabee, 2000
		Gastrointestinal and systemic candidiasis	2–5 mg/kg PO q24h for 7–10 days	
	Flucytosine	Aspergillosis	120 mg/kg PO q6h; 20–30 mg/kg PO q6h for 60–90 days; 50–75 mg/kg PO q8h in combination with amphotericin B	
		Candidiasis	250 mg/kg PO q12h	
	Itraconazole	Aspergillosis	15 mg/kg PO q12h for 4–6 weeks	
	Ketoconazole	Candidiasis	15 mg/kg PO q12h	
		Aspergillosis	30–60 mg/kg PO q12h for 14–30 days	
	Nystatin	Intestinal candidiasis	100,000–300,000 IU/kg PO q8–12h	
Pet Fish	Itraconazole	Systemic mycoses	1–5 mg/kg q24h in feed for 1–7 days	Mashima and Lewbart, 2000
	Ketoconazole	Systemic mycoses	2.5–10 mg/kg PO, IM or ICe	
Ferrets	Amphotericin B	Systemic mycoses	0.4–0.8 mg/kg IV once a week to a total dose of 7–25 mg	Williams, 2000
	Griseofulvin	Dermatophytosis	25 mg/kg PO q24h for 3–4 weeks	
	Ketoconazole	Systemic mycoses	10–30 mg/kg PO q12h–24h	
Hedgehogs	Griseofulvin	Dermatophytosis	25–50 mg/kg PO q24h	Lightfoot, 2000
	Itraconazole	Systemic mycoses	5–10 mg/kg PO q12–24h	
	Ketoconazole	Systemic yeast/fungal infections	10 mg/kg PO q24h	
	Nystatin	Yeast infections	30,000 IU/kg PO q8–24h	
Marsupials	Griseofulvin	Dermatophytosis	20 mg/kg PO q24h for 30–60 days	Johnson-Delaney, 2000
	Nystatin	Candidiasis	5000 IU/kg q8h for 3 days	

(*continued*)

Table 38.4 (*Continued*)

Species	Drug	Disease treated	Dosing protocol	Reference
Rabbits	Amphotericin B	Systemic mycoses	1 mg/kg IV q24h	Ivey and Morrisey, 2000
	Fluconazole	Systemic mycoses	25–43 mg/kg slow IV q12h	
	Griseofulvin	Dermatophytosis	12.5–25 mg/kg PO q12–24h for 10–42 days	
	Ketoconazole	Dermatophytosis	10–40 mg/kg PO q24h for 14 days	
Rodents	Amphotericin B	Candidiasis	0.43 mg/kg PO or 0.11 mg/kg SC in mice	Adamcak and Otten, 2000
	Griseofulvin	Dermatophytosis	25 mg/kg PO q24h for 14–28 days in gerbils, guinea pigs, hamsters, and rats; 14 days in mice; 28–40 days in chinchillas	
	Itraconazole	Systemic mycoses	5 mg/kg PO q24h in guinea pigs; 50–150 mg/kg q24h in mice; 2.5–10 mg/kg PO q24 in rats	
	Ketoconazole	Systemic mycoses/candidiasis	10–40 mg/kg PO q24h for 14 days in all species	
Amphibians	Amphotericin B	Systemic mycoses	1 mg/kg ICe q24h for 14–28 treatments	Walker and Whitaker, 2000
	Fluconazole	Systemic mycoses	60 mg/kg PO q24h for 7 days	
	Itraconazole	Superficial and systemic mycoses	2–10 mg/kg PO q24h for 14–28 days	
	Ketoconazole	Systemic mycoses	10–20 mg/kg PO q24h for 14–28 days	
Chelonians	Amphotericin B	Aspergillosis	1 mg/kg ICe q24h for 2–4 weeks	Bonner, 2000
	Griseofulvin	Dermatophytosis	20–40 mg/kg PO q72h for 5 treatments	
	Itraconazole	*Sceloporus* sp.	20–30 mg/kg PO q6–8h in the spiny lizard	
	Ketoconazole	Systemic mycoses	25 mg/kg PO q24h for 2–4 weeks in turtles; 15 mg/kg PO q24h (27°C) in the gopher tortoise	
	Nystatin	Enteric fungal infections	100,000 IU/kg PO q24h for 10 days in turtles	
Reptiles	Amphotericin B	Systemic mycoses	1 mg/kg IT q24h for 14–28 days	Funk, 2000
	Fluconazole	Systemic mycoses	2–5 mg/kg PO q24h for 5–21 days in lizards and snakes; can also mix 100 mg with 20 ml of nystatin and give PO at 0.5–0.6 ml/kg	
	Fluconazole		For sea turtles, a loading dose of 21 mg/kg, followed by 10 mg/kg every 5 days, injected SC (Mallo et al., 2002)	
	Griseofulvin	Dermatophytosis	20–40 mg/kg PO q72h for 5 treatments in snakes	
	Ketoconazole	Superficial and systemic mycoses	25 mg/kg PO q24h for 3 weeks in snakes	
	Nystatin	Enteric fungal infections	100,000 IU/kg PO q24h	

(Hasbach et al., 2017). In cats, the oral solution was absorbed five times higher than the oral capsule (Mawby, Whittemore, and Papich unpublished data). A third oral formulation is licensed for use in cats in the United States (Itrafungol, Elanco). This formulation is also an oral solution (10 mg/ml itraconazole) with similar solubilizing agents and excipients as the human formulation.

Spectrum of Activity

Itraconazole has been tested both in vitro and in vivo against a wide variety of fungi (for review see Perfect et al., 1986; VanCutsem et al., 1987; VanCutsem, 1990; Cauwenbergh and DeDonker, 1987). It was found to be effective against virtually all medically important fungi, including *Microsporum, Trichophyton,*

Candida, Malassezia, Sporothrix, Pythium, Histoplasma, Aspergillus, Blastomyces, Coccidioides, and *Cryptococcus*. It has little activity against *Fusarium* sp.

Like other azole antifungal drugs, the AUC/MIC is the best surrogate marker to predict efficacy. However, the most often reported drug concentrations from studies in humans have been the plasma concentrations measured at the lowest point (trough, or C_{min}) during multiple dosing. In these studies (Goodwin and Drew, 2008) the trough concentrations greater than 0.5 to 1.0 μg/ml have been associated with clinical success.

Pharmacokinetics

Absorption is increased by an acid environment and when taken with meals and is less variable than ketoconazole absorption. Bioavailability increases from 40% after fasting to 99.8% when given with a meal (VanCauteren et al., 1987a), except in horses. Due to the low solubility of itraconazole, commercially available formulations include solubility enhancers. Without these specialized formulations, absorption is negligible. Comparison of the oral absorption of Sporanox® capsules, generic and compounded itraconazole capsules showed that, in dogs, the formulations are not bioequivalent (Mawby et al., 2014). Although therapeutic concentrations are reached with the generic formulations, relative bioavailability of the compounded capsules is only approximately 5%. The compounded suspension and capsule had negligible oral absorption in cats (Mawby et al., 2016). This feature of itraconazole also has been demonstrated in other species as well, including birds and horses. Thus, the use of compounded itraconazole formulations is not recommended.

Itraconazole is highly (99.8%) protein bound (95% to albumin and 5% to red blood cells) (Heykants et al., 1987); however, due to its lipophilicity and even higher affinity for tissue proteins, it is extensively distributed throughout the body. Tissue to plasma concentration ratios range from 1 : 1 in brain to 8 : 1 in keratin to 25 : 1 in fat stores. Highest tissue levels are seen in the liver and adrenal cortex (Heykants et al., 1987). High tissue binding also produces a very large volume of distribution (Troke et al., 1990; Heykants et al., 1990) and low plasma concentrations. Although it does not reach high concentrations in the CSF compared to fluconazole, itraconazole was found to be effective in treating meningeal cryptococcosis in both mouse and guinea pig models (Perfect et al., 1986).

Itraconazole is extensively metabolized, with less than 1% of the active drug and approximately 35% of inactive drug (as more than 10 metabolites) excreted in the urine. The major metabolite, hydroxyitraconazole, has similar antifungal activity to the parent drug, and is often found at concentrations two to three times higher than itraconazole in the plasma in humans (Willems et al., 2001). The metabolite to parent drug ratio is reported to be similar in dogs (Yoo et al., 2002); however, this metabolite has not been found in either cats (Itrafungol®, package insert) or horses (Davis et al., 2005). The predominant route of elimination for itraconazole is in the bile. Because of the increased metabolic stability of the triazole ring versus the imidazole ring (Richardson et al., 1990), itraconazole has a longer half-life (17–25 hours) than ketoconazole (8 hours) in humans. There is disagreement about the elimination rate since the terminal half-life in the dog has been reported to be 8–12 hours (VanCauteren et al., 1987a) and 44–58 hours (Heykants et al., 1987). Differences in study methods, assay sensitivity, and pharmacokinetic analysis may account for this discrepancy. More important than plasma half-life, therapeutically active concentrations are maintained much longer in tissues than in plasma. For example, itraconazole can be detected for 4 days in vaginal epithelium and for 4 weeks in skin and nails after cessation of therapy. These long-lasting tissue concentrations account for the ability to administer this drug intermittently for some fungal infections, as will be discussed below in Section Clinical Use. Itraconazole, like ketoconazole, exhibits nonlinear pharmacokinetics; steady-state concentrations were found to be three times higher after 14 days of therapy than those predicted by a single dose, and the half-life was seen to increase from 24 to 36 hours (Heykants et al., 1990).

Pertinent to the pharmacokinetics is the ability of itraconazole to inhibit drug metabolizing enzymes. Itraconazole and its metabolites are cytochrome P450 inhibitors (Templeton et al., 2008). Metabolizing enzymes may be saturated producing nonlinearity in elimination. In addition, repeated dosing may produce a time-dependent decrease in clearance and accumulation (Templeton et al., 2008). Therefore, with repeated dosing, the clearance may decrease and half-life increase. Coadministration of itraconazole and cyclosporine has been shown to result in increased cyclosporine concentrations in cats (Katayama et al., 2010b).

Clinical use

Small animals: Itraconazole is one of the most commonly administered oral antifungal agents for small animals. It has no endocrine effects compared to ketoconazole and is better tolerated. Itraconazole is highly bound in plasma and there is strong binding to keratin producing drug concentrations in skin that persist 2–4 weeks after cessation of drug therapy. It may be excreted into the sebum, increasing the concentrations in skin. This allows for pulse dosing for some diseases. *Histoplasma, Cryptococcus*, and *Blastomyces* are highly susceptible; *Candida, Aspergillus,* and *Penicillium* are less sensitive. Itraconazole also has been used to treat cutaneous leishmaniasis

because the *Leishmania* organism has ergosterol in high concentrations in its cell wall.

Cats: Itraconazole is probably better tolerated in cats than ketoconazole. Nevertheless, adverse effects are still possible. Since most adverse effects are dose related, one is advised to lower the dose in animals in which adverse effects are observed. One report indicated that there were dose-related GI effects of anorexia and vomiting in cats from administration of itraconazole (Mancianti et al., 1998).

The dosing regimens for cats were reviewed by Moriello (2004). Doses in cats vary from 5–10 mg/kg once a day, orally for at least 56 days, to 10 mg/kg once a day, for 28 days, followed by pulse therapy of 1 week on/1 week off. Lower doses of 1.5 to 3 mg/kg once daily for cycles of 15 days at a time are also used. The most recent regimen to be studied is 100 mg capsule per cat every other day for up to 8 weeks. This regimen yielded average therapeutic trough plasma concentrations (>0.5 μg/ml) within 3 weeks, however two of the ten cats in the study developed reversible adverse effects (Middleton et al., 2016).

The availability of a commercial form for cats has helped to define the use in this species. As mentioned previously, itraconazole (Itrafungol®) 10 mg/ml oral solution is registered for use in cats to treat dermatophytosis (not registered in the USA). The treatment schedule consists of once-daily doses of 5 mg/kg for three 1-week cycles. After each week of treatment, it should be followed by a week without treatment (week on/week off schedule). This schedule has been evaluated in cats and maintains drug concentrations in hair during the non-treatment phase (Vlaminck and Engelen, 2004).

Itraconazole has been compared to ketoconazole, with each drug administered at doses of 10 mg/kg/day for the treatment of experimentally induced feline disseminated cryptococcosis (Medleau et al., 1990). After 3 months of therapy, the infection had been cleared by both drugs as determined by cryptococcal antigen titers and CSF culture. Three months following therapy all animals remained clinically normal, and titers and CSF cultures remained negative. Although both antifungals brought about resolution of the disease, all cats receiving ketoconazole became anorectic and lost weight, requiring dosage adjustments. This was not seen with itraconazole, and in fact the animals receiving this drug gained weight during the study. Itraconazole has also been used in naturally occurring cryptococcal infections (Medleau, 1990), where an increase in treatment failure was noted in cats that were seropositive for FIV or feline leukemia virus (FeLV).

Dogs: In dogs, the most extensive study has been for treatment of blastomycosis (Legendre et al., 1996). In a study of 112 dogs, 5 mg/kg/day was as effective as 10 mg/kg/day. With a 60-day course of therapy, 54% of dogs were cured. Itraconazole has been used to treat ocular and systemic blastomycosis in dogs. When given 5 mg/kg itraconazole twice a day for 60 days, 76% of eyes with posterior segment disease other than optic neuritis and 18% and 13% of eyes with anterior uveitis or endophthalmitis, respectively, recovered (Brooks et al., 1992). Pulse dosing has also been evaluated in dogs. Itraconazole doses of 5 mg/kg PO q 24 h for 2 consecutive days per week for 3 weeks was found to be as effective as a dose of 5 mg/kg PO q 24 h for 21 consecutive days in the treatment of *Malassezia* dermatitis and otitis (Pinchbeck et al., 2002).

Itraconazole has been successfully used in both the prevention and the treatment of aspergillosis in caged birds. A dose of 20 mg/kg daily for at least 30 days was used to successfully treat five of 12 presumed cases of *Aspergillus* infections in penguins. This same author suggests its prophylactic use in penguin chicks (Shannon, 1992). A different treatment protocol was recommended for aspergillosis in raptors. Birds are treated with 10 mg/kg twice daily in combination with amphotericin B nebulization three times a day for 20 minutes. Treatment for some cases lasted as long as 6 weeks. These authors also recommend the prophylactic use of itraconazole whenever the clinician expects increased risk for the disease (Forbes et al., 1992). Other antifungal dosing regimens in birds and other exotic animal species are listed in Table 38.4.

Large animals: Itraconazole has been reported to be effective in horses for the treatment of mycotic rhinitis, osteomyelitis, and guttural pouch mycosis (Korenek et al., 1994; Foley and Legendre, 1992; Davis and Legendre, 1994). A pharmacokinetic study showed that the oral solution at a dose of 5 mg/kg q 24 h will produce adequate levels in blood and tissues for successful treatment (Davis et al., 2005). However, the use of the oral liquid will require large volumes — most likely requiring intragastric administration in horses — and the drug is very expensive. The oral capsules have a lower bioavailability and higher doses or more frequent dosing intervals are recommended. There are no reports of the use of this drug in food animals, and there are no approved formulations for these species.

Adverse Effects

Itraconazole is up to 125 times more selective for fungal P450 systems than mammalian liver enzymes in certain in vitro preparations (Vanden Bossche, 1987). It also does not inhibit P450 systems in the testis, adrenal, or liver in vivo (Vanden Bossche et al., 1990). In clinical studies, 100 mg of itraconazole given to humans each day for 30 days had no effect on serum testosterone or

cortisol levels (DeCoster et al., 1987). Similarly, there were no changes in testosterone and cortisol concentrations in rats and dogs receiving daily itraconazole for at least 1 month.

The biochemical basis for the specificity of itraconazole toward fungal P450 is thought to be dependent upon the hydrophobic nonligand portion of the molecule and its affinity for the apoprotein portion of the cytochrome molecule (Vanden Bossche et al., 1990). The resulting lack of significant inhibition of liver microsomal enzymes results in itraconazole's inability to affect other drugs' metabolism. Although the clinical significance is as yet unknown, drugs that can inhibit or stimulate liver degradative enzymes are able to alter the pharmacokinetics of itraconazole. Even though itraconazole is primarily cleared by hepatic metabolism, there appears to be no need for dosage adjustments in patients with liver disease (Heykants et al., 1987). As with ketoconazole, itraconazole's oral absorption is pH dependent; therefore, dosage adjustments may be necessary when gastric pH is increased.

Because itraconazole is better tolerated than ketoconazole, it is used as the drug of choice for long-term treatment. Dogs, cats, and exotic and zoo animals have received this drug for weeks without adverse effects. The capsules have been administered for up to 6 months in horses with no reported adverse effects. Nevertheless, adverse effects are still possible. Since most adverse effects are dose related, one is advised to lower the dose in animals in which adverse effects are observed. According to Legendre (1995) about 10% of dogs receiving recommended doses develop hepatic toxicosis. Liver enzyme elevations may occur in 10–15% of dogs. Itraconazole has been well tolerated by clinically ill cats, although one case of fatal drug-induced hepatitis has been reported (Medleau, 1990). Anorexia may occur as a complication of treatment, especially with high doses and high serum concentrations. It usually develops in the second month of therapy in dogs. In cats there seem to be dose-related GI effects of anorexia and vomiting (Mancianti et al., 1998). Drug-related cutaneous vasculitis has also been reported as a complication of itraconazole therapy in dogs (Nichols et al., 2001).

Dogs chronically administered itraconazole (2.5, 10, or 40 mg/kg daily for 3 months) had no significant alterations in mortality rate, behavior, appearance, food consumption, body weight, hematological values, serum and urine chemistry, or gross pathology (VanCauteren et al., 1987b). Subacute toxicity studies in rats revealed increased adrenal gland weight and the accumulation of proteinaceous material in the mononuclear phagocyte system at doses of 40 and 160 mg/kg. Since the mononuclear phagocyte system is responsible for clearing the host of a fungal infection, the clinical importance of this toxic effect is undetermined. Although not teratogenic at 10 mg/kg, maternal toxicity, embryo toxicity, and teratogenicity were observed at 40 and 160 mg/kg in rats (VanCauteren et al., 1987b); therefore, its use in pregnant animals is not recommended.

Postmarketing drug monitoring in humans has shown that itraconazole may cause or exacerbate underlying heart conditions. There is a dose-related negative inotropic effect seen in both healthy human volunteers and in anesthetized dogs. Owners should therefore be counseled to monitor the patients for signs of heart failure and to discontinue the drug if clinical signs are observed.

Mild to moderate kidney disease has not been reported to change the pharmacokinetic clearance of itraconazole in humans. However, if the intravenous formulation or the oral Sporanox® solution is used, renal failure may decrease the elimination of the carrier molecule hydroxypropyl-β-cyclodextrin. Therefore, the oral capsules are recommended for use in these patients.

Voriconazole

The newest triazole to be investigated in animals is voriconazole (Vfend®). Voriconazole is similar in structure to fluconazole (Figure 38.4); however, the substitution of a fluoropyrimidine ring for one of the triazole moieties and the additional of a methyl group to the propanol backbone increases the spectrum of activity and potency as well as the fungicidal activity against some species of molds, including *Aspergillus* and *Fusarium* spp. In a survey of fungal pathogens, voriconazole inhibited greater than 95% of *Aspergillus* with a concentration less than or equal to 1 μg/ml (Diekma et al., 2003). It is more lipophilic than fluconazole, more water soluble than itraconazole or ketoconazole, with intermediate protein binding. These properties allow for excellent oral bioavailability and tissue distribution. In people, the plasma concentrations were highly variable among individuals, which is caused by variations in hepatic metabolism, other medications coadministered, and nonlinear elimination. The pharmacodynamic parameters associated with clinical cure are an AUC/MIC plasma concentration of 20–25, or a plasma concentration above 2 μg/ml.

Pharmacokinetics

The pharmacokinetics of voriconazole have been investigated in dogs, horses and avian species, with preliminary data available for llamas and cats. Across species, the pharmacokinetics vary widely. Experimental studies in dogs have shown rapid and complete absorption of the drug following oral administration (Roffey et al., 2003). Half-life is short (approximately 3 hours), and at a dose of 6 mg/kg/day orally, plasma concentrations remained above the target MIC of 1 μg/ml for only 15 hours and the target AUC : MIC ratio of 20–25 for free drug concentrations were not reached (Lemetayer et al., 2015).

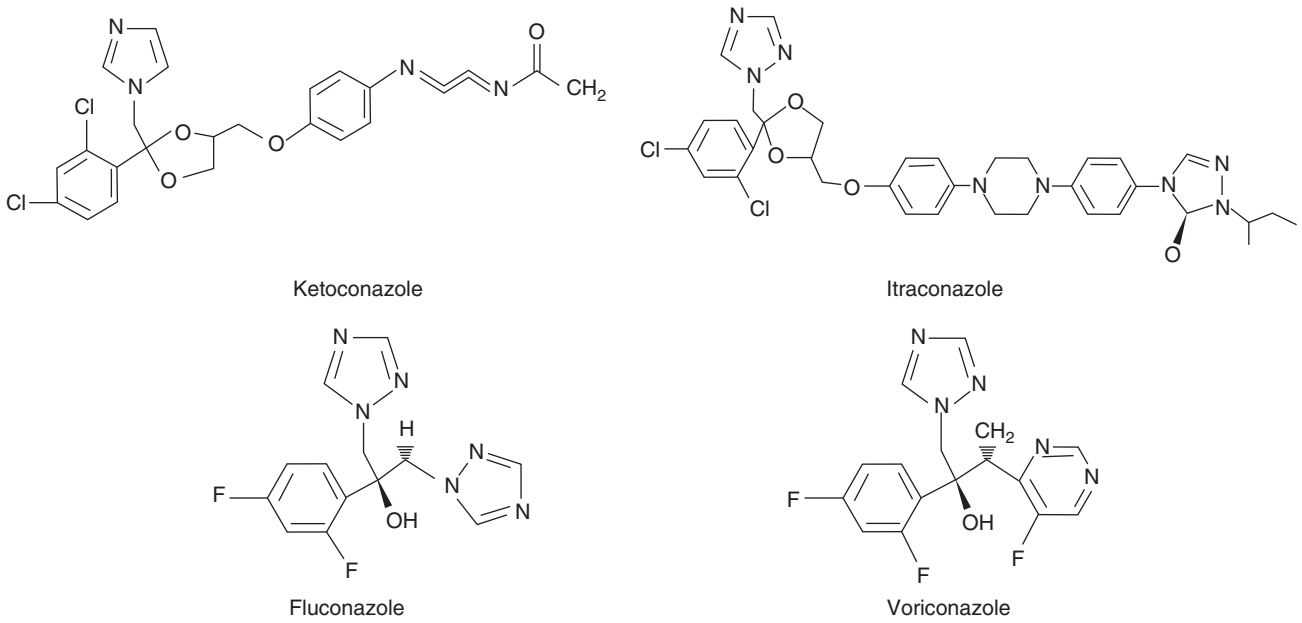

Figure 38.4 Chemical structures of commonly used systemic azole antifungal drugs.

Although the authors of that study state twice-daily dosing may be necessary, an effective dose cannot be extrapolated from current data as voriconazole exhibits nonlinear pharmacokinetics in this species, with a ninefold increase in plasma concentrations seen following a fourfold increase in dose (Lemetayer et al., 2015; Roffey et al., 2003). Interestingly, autoinduction of drug metabolism has been shown to occur in dogs, further complicating dosing recommendations as an increase in dose may be required with multiple administrations. Although drug was detected in body fluids, including CSF, aqueous humor and synovial fluid, it was found at a lower percentage than other reported species (Lemetayer et al., 2015).

Whereas the metabolism appears to increase with repeated doses in dogs causing lower concentrations after multiple doses, the opposite phenomenon occurs in cats. In the studies by Vishkautsan et al. (2016), the half-life was much longer in cats than dogs. The IV half-life was 12.4 hours, but after oral administration of 4–6 mg/kg, the half-life was 43 hours (± 9.02) producing an inflated oral absorption of 264%. Moreover, in the multiple dosing study of 25 mg per cat loading dose followed by 12.5 mg per cat every 48 hours, accumulation was observed steadily to 14 days and steady state was not achieved.

The pharmacokinetics of voriconazole have been studied following single and multiple dose administration in the horse (Davis et al., 2006; Colitz et al., 2007). In these cited equine studies, voriconazole had excellent oral absorption (95% and 100%) and a moderate half-life (8–13 hours) following oral administration. The oral dose used in the study by Davis et al. (2005) was 4 mg/kg and produced plasma concentrations higher than necessary for the treatment of most common veterinary pathogens, with the exception of *Fusarium* sp. The study by Colitz et al. (2007) used 3 mg/kg orally twice daily and produced concentrations above the minimum level necessary for successful treatment. When administered at 4 mg/kg orally once a day for 2 weeks in nonfasted horses, there was no statistically significant difference between voriconazole concentrations in plasma and body fluids when comparing days 7 and 14, suggesting enzyme induction may not be prominent in this species (Passler et al., 2010). In horses, voriconazole has good distribution into the aqueous humor, CSF, peritoneal fluid, pulmonary epithelial lining fluid, synovial fluid, urine, and periocular tear film. The concentrations in plasma, tissues, and other fluids exceed the minimum concentration recommended for successful therapy (Goodwin and Drew, 2008).

After oral administration of single doses ranging from 6 to 18 mg/kg in African grey parrots, a follow-up study examined multiple doses at the highest dose (18 mg/kg every 12 hours for 9 days). Compared to mammals, the elimination half-life was short at 1.1 to 1.6 hours (Flammer et al., 2008). A similar study looked at 18 mg/kg q 8 h for 11 days in Hispaniolan Amazon parrots, with similar results (Sanchez-Migallon Guzman et al., 2010). With multiple doses the kinetics changed, suggesting induction of the hepatic metabolism as mentioned for dogs above, and requiring dose adjustment for long-term treatment. Polyuria was observed in the treated birds, but no other adverse reactions were reported.

A single dose pharmacokinetic study is published on voriconazole in alpacas (Chan et al., 2009). Oral absorption is low in this species, and high daily doses would likely be needed for treatment success.

Clinical Use

Clinical experience with this drug is currently limited due to potential adverse effects, as well as cost. Successful reports of treatment of miscellaneous fungal infections are available, including intracranial phaeohyphomycotic granuloma and *Exophilia dermatitidis* in dogs, and pulmonary aspergillosis in a foal (Bentley et al., 2011; Murphy et al., 2011; Hilton et al., 2009). In these species, the most common clinical use of voriconazole is as a topical or local ocular therapy. Topical administration of the commercially available intravenous voriconazole solutions diluted to 1%, has good ocular penetration through an intact cornea with little to no local irritation (Clode et al., 2006). Intracorneal administration of 5% voriconazole solution has also been reported to result in resolution of clinical disease, specifically stromal fungal abscessation and secondary uveitis (Smith et al., 2014). At this time, the use of voriconazole in cats cannot be recommended.

Birds: The experience with voriconazole in birds has been reported by Flammer et al. (2008). On the basis of this study, the authors concluded that 12–18 mg/kg orally twice daily would be sufficient for treatment of some *Aspergillus* infections; higher doses may be needed for some infections and to maintain concentrations during long-term treatment.

Adverse Effects

Voriconazole appears to be safe for use in horses following multiple doses. The only adverse event reported in this species was in a single horse that developed pruritis after drug administration; however, these signs were controlled with administration of an antihistamine 2 hours prior to dosing (Passler et al., 2010). Except for the polyuria observed in birds, the drug also appears to be safe in the avian species studied. In dog studies using multiple doses, several dogs showed mild to moderate gastrointestinal disturbances (loss of appetite and diarrhea) and one dog had mild increases in liver enzymes (Lemetayer et al., 2015). Intravenous injection of 10 mg/kg in dogs results in severe, acute toxicity.

Cats appear to be the most susceptible to adverse drug events caused by voriconazole administration. Adverse effects in cats involving the gastrointestinal tract, eyes, and neurological function have been reported (Quimby et al., 2010; Smith and Hoffman, 2010; Vishkautsan et al., 2016). Inappetance, lethargy, and weight loss are common. Ataxia and hindlimb paresis that resolved following withdrawal of drug administration were noted in several cats. One proposed mechanism for the neurotoxicity associated with azole antifungals is through inhibition of voltage-gated calcium channels in neuronal cells (Heusinkveld et al., 2013). Visual abnormalities noted include mydriasis, decreased to absent pupillary light responses, and decreased menace response. On the other hand, in the study by Vishkautsan et al. (2016), miosis occurred from an unknown mechanism. Also noted in that study was excess salivation from administration of the oral suspension, but not from the oral tablet. Azotemia has also been noted, although it may be attributable to dehydration secondary to GI disturbances, or concurrent NSAIDs administration, rather than voriconazole having a direct nephrotoxic effect. One cat was also described to have a cutaneous drug reaction. In the case reports involving cats, they were administered doses that were similar to the canine dose, or a dose extrapolated from people (i.e., up to 13 mg/kg per day). As the study by Vishkautsan et al. (2016) showed, these doses are probably too high because of the slow clearance of voriconazole in cats compared to other animals. Prolonged QT intervals and arrhythmias have been reported in both dogs and cats. Because of adverse effects in people, it is recommended that in humans doses be adjusted to produce trough concentrations no higher than 4–6 μg/ml in order to avoid toxicity (Ashbee et al., 2014).

Posaconazole

Posaconazole (Noxafil®) is one of the newest azole antifungal drugs introduced. It is approved for use in people but its use in animals has been limited to just a few case reports and pharmacokinetic studies. Posaconazole resembles itraconazole in structure. It is used for invasive fungal infections, including those caused by *Aspergillus* and *Candida*. It is also active against dermatophytes, *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis*, and *Cryptococcus neoformans*. Its advantage over other azole drugs is the activity against *Fusarium* and *Mucorales* (formerly called Zygomycetes), such as *Mucor* and *Rhizopus*. It has some chemical properties that are similar to itraconazole and is a substrate for CYP450 enzymes and P-glycoprotein. As with other azoles, the pharmacokinetic/pharmacodynamic parameter that best correlates with clinical success is the plasma concentration AUC/MIC. For treating *Aspergillus* this ratio was above 200; for treating *Candida* the ratio was only 15. If AUC/MIC ratios cannot be monitored, it is suggested to maintain plasma concentrations above (C_{max}) 1.48 μg/ml or an average concentration at least 1.25 μg/ml (Goodwin and Drew, 2008). Additional information has indicated that prophylactic efficacy for invasive fungal infections is optimal when plasma concentrations of posaconazole exceed 0.7 μg/ml 3–5 hours after dosing in a multiple-dose regimen. It is available as an oral suspension containing 40 mg/ml posaconazole

Table 38.5 Topical antifungal products for dogs and cats

Antifungal agent	Product	Indication	Other ingredients
Clotrimazole	Otomax	*Malassezia pachydermatis*	Betamethasone, Gentamicin
Ketoconazole	Keto-Tris Flus	*Malassezia pachydermatis*	Tromethamine (Tris) EDTA
Miconazole	Surolan, and generic	*Otitis externa*	Polymyxin
Nystatin	Dermalone	*Malassezia pachydermatis* (weak activity)	Neomycin
Posaconazole	Posatex	*Malassezia pachydermatis* (with activity against other fungi)	Mometasone, orbifloxacin
Thiabendazole	Tresaderm	*Malassezia pachydermatis*	Dexamethasone, Gentamicin

(Noxafil) and 100 mg delayed-release tablets (Noxafil). The dose in people is 400 mg twice a day with a meal. If not given with a meal, dosage of 200 mg four times a day is recommended. Posaconazole is also one of the ingredients in an approved ear medication for dogs (Table 38.5). This product (Posatex®), contains orbifloxacin, mometasone (glucocorticoid), and 0.1% posaconazole in an otic suspension for the treatment of otitis externa in dogs associated with *Malassezia pachydermatis* and bacteria.

No dose ranges have been established for animals, but in two case reports on its use in cats with successful treatment, a dose of 5 mg/kg orally, every 24 hours was used without ill effects (Wray et al., 2008). Although it is eliminated via UDP-glucuronidation – which may be deficient in cats – the pharmacokinetics of posaconazole in cats resemble dogs, with oral absorption and half-life that are similar to dogs (Mawby et al., 2016b). Based on preliminary pharmacokinetics, the recommended dose for the oral administration of the suspension in cats is 12–15 mg/kg once daily. In dogs, the oral absorption of the suspension is approximately 26% and the half-life is 24 hours. In dogs, there is a food effect on absorption with oral systemic availability of 11% and 27% in fasted and fed dogs, respectively. The delayed-release tablets also offer a good treatment option for dogs. Bioavailability is increased and the half-life is prolonged (42 hours) (Kendall and Papich, 2015). The suggested dosing regimen for the delayed release tablets is 5 mg/kg orally every other day. It is highly protein bound with binding greater than 97% in dogs. In toxicity studies, dogs have tolerated 30 mg/kg/day for 1 year without any clinical signs. However, histologically, some neuronal vacuolation was observed at this dose. It should not be used during pregnancy because of inhibition of steroidogenesis.

Other Antifungal Agents

Terbinafine

Terbinafine (Lamisil®) is a highly fungicidal agent. It is a synthetic drug of the allylamine class. A closely related drug of the same class is naftifine (Naftin®), which is used as a topical cream for dermatophyte infections in people. Terbinafine inhibits squalene epoxidase to decrease synthesis of ergosterol. Fungal cell death results from disruption of the cell membrane (Balfour and Faulds, 1992).

Spectrum of activity: Terbinafine is active against yeasts and a wide range of dermatophytes. It is fungicidal against *Trichophyton* spp., *Microsporum* spp., and some *Aspergillus* spp. (often excluding *A. fumigatus*). It is also active against *Blastomyces dermatitidis, Cryptococcus neoformans, Sporothrix schenckii, Histoplasma capsulatum, Candida*, and *Malassezia* yeast. In people, it was more effective than griseofulvin for treating dermatophytes, with fewer relapses. There may also be some activity against protozoa (e.g., *Toxoplasma*).

Pharmacokinetics: Oral bioavailability in most species is moderate to high, ranging from 31% in cats (Wang et al., 2012) to >46% in the dog to >85% in mice (Jensen, 1989). Absolute bioavailability in horses is unknown; however, the relative bioavailability of terbinafine in horses was only 16% compared to Greyhounds (Williams et al., 2011). Reported half-lives after oral administration of 20–30 mg/kg to cats, dogs, and horses are approximately 8 hours. Maximum concentrations in cats and dogs (3–4 μg/ml) are much higher than those achieved in horses (0.31 μg/ml) (Wang et al., 2012; Williams et al., 2011). Oral absorption of terbinafine has also been studied in Hispaniolan Amazon parrots, with a dose of 60 mg/kg via intragastric gavage resulting in peak plasma concentrations of 0.11–0.67 μg/ml (Evans et al., 2013). In penguins, a dose of 15 mg/kg terbinafine q 24 h is suggested as a potential treatment option for aspergillosis (Bechert et al., 2010).

The lipophilic nature of terbinafine results in high concentrations in tissues such as stratum corneum, hair follicles, sebum-rich skin, and nails. In people, after 12 days of therapy, the concentrations in stratum corneum exceed those in plasma by a factor of 75. Concentrations in skin may be detected in people as early as 24 hours after oral administration, but maximum concentrations are reached at 7 days. Fungicidal concentrations in nails may require 3 weeks of treatment. Concentrations of terbinafine in the hair of cats being treated for dermatophytosis reach approximately 3.62 μg/g after 120 days of

treatment with a dose of 30–40 mg/kg. Like itraconazole, terbinafine is highly protein bound, with values reaching >99% in dogs and rabbits (Jensen, 1989).

Clinical use: Terbinafine has some efficacy for the treatment of dermatophytosis in dogs and cats, as well as *Malassezia* dermatitis in dogs. However, there has been conflicting clinical results. For dermatophytes the most common doses are 30–35 mg/kg once daily in dogs; and for cats, approximately 30 mg/kg per day, or one-quarter tablet for small cats (62.5 mg), half tablet for medium size cats (125 mg), and one tablet for large cats (250 mg), all administered once daily. It should be administered for at least 14 days, but may be extended to 60 days. (Moriello, 2004). Results suggest that it is effective for the treatment of *Malassezia* dermatitis in dogs when given at 30 mg/kg PO, or 30 mg/kg two times per week for at least 3 weeks (Berger et al., 2012). However, the results also showed that this treatment produced insufficient resolution and only partial remission even though there was clinical improvement in both groups. In other studies, terbinafine was as effective as itraconazole in treating shelter cats with *Microsporum canis* dermatophytosis when administered in doses of approximately 20–40 mg/kg/day (Moriello et al., 2013). For treatment of *Malassezia* dermatitis, terbinafine (30 mg/kg) was as effective as ketoconazole in reducing yeast counts on the skin (Rosales et al., 2005). Pharmacokinetic studies of 30–35 mg/kg per day in dogs (Williams et al., 2011; Sakai et al., 2011) showed that sufficient concentrations can be maintained for most of the dose interval for susceptible fungi, but clinical studies are needed to confirm efficacy. By comparison, doses in people are much lower at 125 mg twice daily (approximately 1.8 mg/kg q 12 h), with pediatric doses in the range of 4–8 mg/kg, once a day (Jones, 1995).

When combined with surgical excision, terbinafine has been reported to be successful as an adjunct treatment for intestinal pythiosis and *Cryptococcus neoformans* in dogs (Schmiedt et al., 2012; Olsen et al., 2012). It has been safely used in combination with other drugs, including itraconazole and mefenoxam (Hummel et al., 2011) and a synergistic effect against *Pythium* sp. has been shown when combined with itraconazole (Argenta et al., 2008). In both experimental and clinical trials, the average treatment length lasted approximately 60 days. Most treatments in cats are for at least 14 days, but may extend to 60 days. There are currently no published reports of the use of terbinafine in horses, although it has been used clinically at doses of 20–30 mg/kg orally once daily with some success against nasal and guttural pouch fungal masses.

Terbinafine is available as a 1% topical cream (Lamisil®, available over the counter) and 125 and 250 mg tablets. It has also been compounded as a 0.2% solution for ophthalmic use; however, administration did not result in detectable AH or plasma levels following administration to normal equine eyes, suggesting its use may be limited to superficial infections (Clode et al., 2011).

Adverse effects: In dogs treated with 30 mg/kg, serum ALT concentrations were mildly to moderately elevated in 4 of 10 dogs and ALP was increased in 2 of 10 dogs. Owner reported adverse effects include gastrointestinal disturbances and excessive panting (Berger et al., 2012). Gastrointestinal problems, including vomiting, can be common in cats, and facial dermatitis and pruritus has also been reported. This reaction is a problem because it may be confused with an ongoing dermatophyte infection. Pawing at the ground, curling lips, head shaking, anxiety, and circling was noted in one horse after oral administration, but these signs resolved spontaneously within 30 minutes of onset (Williams et al., 2011). In people, there is a rare incidence of severe hepatic failure and death. Terbinafine does not bind to P450 enzymes as do other antifungal drugs; therefore, it does not cause drug interactions or inhibition of steroid synthesis in animals. No teratogenic effects of the drug have been noted in people.

Lufenuron

Lufenuron (Program®), is an orally active inhibitor of chitin synthesis that is commonly used in dogs and cats for control of flea infestations (see Chapter 43 of this book for ectoparasite treatment). It has been evaluated as an antifungal agent, since fungi also have chitin in their outer cell wall. Although there are reports of successful treatment of dermatophytosis in dogs and cats, the success of this treatment has been controversial. If used for dermatophyte treatment, antifungal doses of lufenuron are higher than those recommended for flea control and have ranged from 50 mg/kg to greater than 250 mg/kg. The dose recommended by one group of investigators is 80–100 mg/kg orally, once every 2 weeks until mycological cure (Ben-Ziony and Arzi, 2000). However, endorsement of this use has diminished and dermatologists have disputed the efficacy because of a high incidence of recurrence. It does not have any in vitro effect on *Aspergillus fumigatus* or *Coccidioides immitis*.

It was hypothesized that lufenuron may have a positive effect against dermatological disease not caused by fungi, and an immunomodulatory effect (Zur and Elad, 2006). This is borne out in a study by Mancianti et al. (2009) in which pretreatment with lufenuron followed by treatment with either griseofulvin or topical enilconazole resulted in clinical and mycological cures at a higher rate than expected with any treatment alone.

Topical or local use of lufenuron may be more efficacious. It has been successfully used as a uterine lavage to treat fungal endometritis caused by *Candida* or

Aspergillus spp. in horses (Hess et al., 2002). However, this too is controversial because lufenuron had no in vitro antifungal activity against *Aspergillus* and *Fusarium* sp., pathogens important to horses (Scotty et al., 2005). If administered orally, absorption in horses is poor and an oral dose cannot be recommended. Lufenuron may also be used in water baths for the treatment of aquatic species and amphibians (Wolfe et al., 2001).

Flucytosine

Flucytosine (5-fluorocytosine, 5-FC, Ancobon) is a synthetic antifungal agent available as an oral preparation. Flucytosine must be taken into the fungal cell by cytosine permeate and then converted to the active form, 5-fluorouracil (5-FU), by a fungal cytosine deaminase enzyme. The 5-FU either is incorporated into RNA, disrupting protein synthesis, or is converted to a related compound that inhibits DNA synthesis. Mammalian cells do not have cytosine deaminase, which results in a selective toxicity of this compound; however, conversion to 5-FU may occur by microbes in the gastrointestinal (GI) tract resulting in 5-FU being taken up by mammalian cells, leading to anemia, leukopenia, and thrombocytopenia (Bennett, 1990).

Fungal mutations leading to alterations in the permease or deaminase enzyme activity has led to the development of resistance to flucytosine, both in vitro and during therapy. To decrease emergence of resistance, the use of flucytosine is limited to adjunct therapy with amphotericin B in systemic infections caused by *Candida* or *Cryptococcus neoformans*. Synergy between these two medications, with as much as a fourfold reduction in the MIC, has been demonstrated, and combination therapy has been successful, particularly in the treatment of cryptococcal meningitis (Medoff et al., 1971; Utz et al., 1975; Bennett et al., 1979). One explanation of this synergism involves the membrane-permeabilizing effects of amphotericin B facilitating flucytosine's entrance into the cell cytoplasm (Medoff et al., 1972). This combination of antifungal drugs is more effective than amphotericin B alone in the treatment of cryptococcal meningitis (Bennet et al., 1979; Utz et al., 1975). Advantages of this combination include a reduction in the amphotericin B dose, thereby limiting nephrotoxicity, as well as prevention of mutants to flucytosine (Drouhet and Dupont, 1987). The combination has been administered to treat cryptococcosis in cats at a dose of 250 mg per cat every 8 hours (25–50 mg/kg every 6–8 hours also has been used). This combination has also been suggested for therapy of acute hematogenously disseminated candidiasis (Horn et al., 1985). Although flucytosine is synergistic with amphotericin B, there is no evidence of synergism for azole antifungal drugs and this combination should not be used.

Adverse effects: In one report, the combination of flucytosine and ketoconazole was administered to two cats and liver injury occurred in one cat (Pukay and Dion, 1984). Myelosuppression is also a concern in cats. Cutaneous and mucocutaneous eruptions have been observed with use of flucytosine in dogs and it is not recommended to be used in dogs for this reason.

Sodium or Potassium Iodide

Iodide compounds were one of the first antifungal drugs used and they are still used today in veterinary medicine. They are inexpensive and can be administered orally, which makes them useful for long-term treatment. The mechanism of action of iodide compounds against fungal organisms is largely unknown, but it may involve stimulating the host's immune response or increasing the elimination of the fungi through the skin or hair. Iodide compounds have been used to treat sporotrichosis in dogs, cats, and donkeys, as well as nasal fungal granulomas caused by *Basidiobolus*, *Conidiobolus*, and *Pseudallescheria* spp. in horses (Koehne et al., 1971; Gonzalez Cabo et al., 1989; Irizarry-Rovira et al., 2000; Owens et al., 1985; Zamos et al., 1996; Davis et al., 2000). Ethylenediamine dihydroiodide (EDDI) is used as a nutritional source of iodine in cattle but also has been used to treat fungal granulomatous disease and infections associated with zygomycetes. The antifungal treatment has been questioned because the efficacy is not established.

The iodide compounds are seldom used as a sole therapy, but rather are used as an adjunct to surgical excision, intralesional injection of other antifungals, or systemic antifungal therapy. Toxic effects are possible (iodism) and may occur secondary to excessive iodide levels. Clinical signs attributable to excess iodine include lacrimation, salivation, coughing, anorexia, dry scaly skin, and tachycardia. Abortion and infertility may also be observed; therefore care should be taken when administering this drug to breeding animals. The recommended doses of 20% sodium iodide solution for dogs is 44 mg/kg PO q 24 h, for cats is 22 mg/kg PO q 24 h, and for horses is 125 ml IV q 24 h for 3 days followed by 30 grams PO q 24 h. Treatment is recommended to extend 30 days beyond the resolution of clinical signs. Iodoject IV is labeled for use in cattle for the treatment of actinomycosis and actinobacillosis at 66 mg/kg IV once a week. The use of this product for fungal infections is considered off label.

Topical Antifungal Agents

Enilconazole

Enilconazole (Imaverol®) is also called *imazalil* in some countries. It is an azole antifungal that has excellent activity against dermatophytes and filamentous fungi and it has a residual effect after application. It has been used in some countries for the topical treatment of dermatophyte infections in dogs and horses. For this treatment,

a 10% solution is diluted 50 : 1 to form an emulsion. It may be sponged on the animal every 3 or 4 days for four treatments. It may be applied to the premises as well to prevent recurring infections. In an evaluation of topical therapies for treatment of dermatophyte infections in dogs and cats (White-Weithers and Medleau, 1995), enilconazole was more effective than chlorhexidine, povidone iodine, ketoconazole, sodium hypochlorite, and Captan. The safety of enilconazole has been demonstrated in dogs, even at high doses. One study also showed that enilconazole is safe for treatment of dermatophytes in Persian cats (deJaham, 1998). Enilconazole also has been used to treat nasal aspergillosis in dogs. It has a unique vapor effect and, if instilled into the nasal cavity of dogs, will control fungal growth (Sharp et al., 1991; Sharp and Sullivan, 1992). A dose of 10 mg/kg in a volume of 5–10 ml is infused twice a day for 7–14 days. In one study using this protocol (Sharp et al., 1993) 26 of 29 affected dogs became asymptomatic. A similar protocol has been used for the successful medical treatment of guttural pouch mycosis caused by *Aspergillus* spp. in the horse (Davis and Legendre, 1994).

In the United States, enilconazole is available as Clinafarm®-SG or Clinafarm®-EC. Both are approved for use in poultry hatcheries to control *Aspergillus* organisms on facilities and equipment. Clinafarm®-SG comes in a canister used for smoke generation. Clinafarm-EC is available in a 750 ml bottle containing 13.8% (138 mg/ml) enilconazole. The other ingredients listed on the Material Safety Data Sheet are benzyl alcohol and dioctyl sodium sulfosuccinate. It also contains ethoxylated castor oil. The Canadian formulation of Imaverol® contains polysorbate 20 and sorbitan monolaurate as its inert ingredients with 10% enilconazole as the active drug. The Clinafarm-EC formulation is registered for controlling *Aspergillus* organisms in poultry facilities and equipment by making a 1 : 100 dilution and spraying or fogging the area to be treated. Although there are no published toxicology studies on this particular solution in animals, it has been used in a 50 : 1 dilution applied topically to dogs and cats without adverse effects. One study applied 100 ml of a 2 mg/ml formulation of Clinafarm-EC to cats and was judged to be safe (Hnilica et al., 2000). However, monitoring of liver enzymes was recommended by these authors.

Clotrimazole

Clotrimazole (Lotrimin®) is an imidazole antifungal. It is limited to topical use because after oral administration, the metabolism produces undetectable concentrations of the drug in plasma following repeated dosing. In veterinary medicine, it has been used for treatment of nasal aspergillosis in dogs following infusion of a 1% solution over 1 hour through a nasal catheter (Matthews et al., 1998). It has also been infused into the bladder of dogs and cats with fungal candiduria (Toll et al., 2003; Forward et al., 2002). Clotrimazole can be found in combination with gentamicin sulfate and betamethasone valerate as the product Otomax® and other generic forms that are labeled for the treatment of otitis externa caused by *Malassezia pachydermatitis* or susceptible bacteria in dogs (see Table 38.5).

Miconazole

Miconazole (Conofite® cream) is an imidazole antifungal effective against some fungi refractory to amphotericin B. Rapid clearance and poor oral absorption necessitated frequent IV infusions during hospitalization. In addition, the solubilizing agent in the parenteral form induces histamine-related toxic side effects, making its use hazardous. The intravenous formulation is no longer marketed in the United States. Miconazole has a broad spectrum of activity against yeasts and filamentous fungi, although recent evidence suggest that resistance in yeast isolates has been increasing over the last several years (Beltaire et al., 2012). In veterinary medicine, miconazole is used as a 2% cream or 1% spray or lotion for the treatment of dermatophytosis in dogs and cats. It is also commonly compounded as a 1% solution for topical treatment of keratomycosis. The commercially available cream and lotion formulations should not be used in the eye, as they can cause significant irritation. Miconazole can also be found combined with chlorhexidine as a shampoo for the adjunct treatment of dermatophytosis in animals. Antibacterial activity for miconazole has also been demonstrated, and it is a potential treatment for superficial pyoderma caused by methicillin resistant *S. aureus* (Clark et al., 2015), and otitis externa caused by *E. coli* and *P. aeruginosa* (Pietschmann et al., 2013). Synergism in these cases can be achieved by combining miconazole with chlorhexidine for MRSA and polymyxin B for otitis externa. Other products containing antifungal medications are listed in Table 38.5.

Mefenoxam

Mefenoxam (Subdue MAXX®) is an agricultural fungicide that works by blocking ribonucleic acid (RNA) synthesis via inhibition of ribosomal RNA polymerases. It is used to control plant-pathogenic oomycetes. In vitro, mefenoxam has an MIC_{90} of 1 μg/ml against *Pythium insidiosum* isolates (Brown et al., 2008). Results of acute and chronic dosing studies in dogs performed for the EPA certification revealed a no-effect level (NOEL) of 8 mg/kg/day when dosed for 6 months. Although pharmacokinetic data for dogs are not available, rodent studies showed that mefenoxam administered orally to rats at 2 mg/kg produced maximum blood concentrations of 0.48 μg/ml in males and 0.93 μg/ml in females. Extrapolation of these data has led to dosing dogs at 8 mg/kg/day divided into two treatments orally for the therapy of intestinal pythiosis, with some treatment success when

combined with itraconazole and terbinafine (Hummel et al., 2011). The drug has been well tolerated in dogs and no persistent clinical, hematological, or biochemical abnormalities have been detected during up to 18 months of administration. It has also been used both systemically and topically in combination therapy for the treatment of canine cutaneous pythiosis and lagenidiosis, as well as topically for the treatment of equine pythiosis.

Natamycin

Natamycin (Natacyn®) is a polyene antifungal with a mechanism of action similar to amphotericin B. It is approved for use in humans as a 5% ophthalmic suspension. Natamycin has excellent activity against yeasts and filamentous fungi, and it is considered the treatment of choice for *Fusarium* keratomycosis in the horse. It is most commonly used in veterinary medicine for treatment of keratomycosis in horses, although it has also been reported as a topical therapy for nasal aspergillosis as well as guttural pouch mycosis and dermatophytosis in this species (Brooks et al., 1998; Greet, 1981, 1987; Oldenkamp, 1979). Its systemic use is prohibited by expense, as well as toxicity.

Nystatin

Nystatin (Mycostatin®) is also a polyene antifungal that is limited to topical use due to systemic toxicity. Nystatin is not absorbed well from the gastrointestinal tract; therefore, it can be given orally as a "topical" treatment for oral and intestinal candidiasis, particularly in exotic animal species (see Table 38.4). In veterinary medicine, it is most commonly used in combination with antibiotics (neomycin, thiostrepton) and antiinflammatory (triamcinolone) drugs in ointments such as Panalog® and other generic formulations as well as otic preparations (Table 38.5).

Antiviral Therapy

Compared to other classes of antimicrobial drugs, the use of antiviral drugs in veterinary medicine has been limited, with all specific antiviral agents used in an extralabel manner. In human medicine, the use of these drugs is much more prominent due to the success of multiple agent protocols to control human immunodeficiency virus (HIV) and associated AIDS, as well as successful single agent therapies for the treatment of herpesvirus infections, influenza, hepatitis C, and other diseases. In veterinary medicine, the most successful use of antiviral agents has primarily been associated with the treatment of herpesvirus infections, with more limited success against retroviruses. Several antiviral drugs are administered systemically, whereas others are only used topically for the treatment of ocular lesions. Consequently, compared to our human medicine counterparts, the consideration of these agents in this book is limited. Readers are encouraged to consult a human pharmacology textbook for more in-depth discussion of mechanism of action and antiviral spectrum of these agents.

There are key differences between the principles of antiviral therapy as compared to antibacterial therapy. Because viruses use host cellular machinery for replication, it is difficult to achieve selective action against viral replication. In this way, many of the most successful of the antiviral drugs act more similarly to the cytotoxic anticancer drugs, by targeting DNA replication. As a consequence the therapeutic indices of antiviral and anticancer drugs may be similarly narrow. For the nucleoside analogs, which are primarily used in the therapy of DNA viruses, there tends to be an inverse relationship between selectivity for viral replication and toxicity to host cells. Another difference between antiviral and antibacterial drugs is that in vitro testing of the antiviral drugs must utilize a cell culture system to allow viral replication. The concentration of drug that inhibits viral growth by 50% is termed the IC_{50}, but this parameter depends upon numerous factors, and is not as robustly associated with efficacy as is the MIC of antibacterial drugs (Hussein et al., 2008a). Antiviral drugs tend to be narrow in spectrum with little cross-activity against multiple viruses. Another feature of antiviral agents that differs from that of antibacterial drugs is that only antiseptic and disinfectant agents can be classified as "virocidal" (antiseptics and disinfectants are discussed in Chapter 31 of this book), so antiviral drugs can only have virostatic actions, limiting replication until the host's immune system eliminates the viral infection. For this reason, antiviral drugs will be inactive against any nonreplicative or latent viral particles. This activity of antiviral drugs only against replicative virus also suggests why timing is critical to successful antiviral therapy, with early therapy resulting in optimal efficacy (Sawtell et al., 2001).

Antiherpesvirus Agents: Nucleoside Analogs

Acyclovir and Valacyclovir

Acyclovir (Zovirax®) is the prototypic nucleoside analog of the purine, deoxyguanosine. In veterinary medicine, the use of acyclovir is restricted to the therapy of herpesviruses. Acyclovir exemplifies selective activity against herpesviruses with little direct effect on host cells at therapeutic concentrations. This selectivity arises from the selective phosphorylation of acyclovir to its active form, acyclovir triphosphate, in infected cells. Acyclovir is monophosphorylated by viral thymidine kinase with 200 times greater efficiency than by the mammalian enzyme, which contributes to its favorable selectivity and high therapeutic index. Sequential phosphorylation

by cellular and viral enzymes then form the triphosphate form, which selectively inhibits viral DNA polymerase by competing with deoxyguanosine triphosphate. Acyclo-GTP that is incorporated into viral DNA strands causes termination of elongation. Virally infected cells are 40–100 times more efficient in converting acyclo-GMP to acyclo-GTP than are noninfected cells. Penciclovir and ganciclovir (discussed in Section Penciclovir and Famciclovir and Section Cidofovir, Ganciclovir, and Valganciclovir) have similar mechanisms of action against herpesvirus. Valacyclovir is a prodrug that is itself inactive but is rapidly metabolized to its active form, acyclovir, after oral absorption. The presence of valine allows active transport of valacyclovir into enterocytes, with metabolism by plasma and hepatic esterases to acyclovir.

Spectrum of activity: Whereas herpes simplex viruses (HSV) of people are exquisitely sensitive to acyclovir, few herpesviruses of veterinary importance share this sensitivity. Feline herpesvirus type-1 (FHV-1) is only weakly susceptible to acyclovir with an IC_{50} of 58 μg/ml, more than 100 times higher than that of HSV-1 (Maggs and Clarke, 2004; Gaskell et al., 2007). In contrast, equine herpesvirus type-1 (EHV-1) shares a sensitivity to acyclovir that is similar to HSV, with an IC_{50} of 0.3–3 μg/ml (Wilkins, 2004; Garré et al., 2007b).

Pharmacokinetics: Pharmacokinetic data for acyclovir are available for horses, dogs, and cats, as well as Quaker parakeets, pheasants, and other species (de Miranda et al., 1982, 1981). Oral bioavailability of the drug is high in dogs (>80%), moderate in people (10–30%), but low and variable in horses (<5%). In dogs, oral absorption of acyclovir decreases with escalating doses (Krasny et al., 1981). In horses, the oral bioavailability was undetectable in one study (Wilkins et al., 2005), and only 2.8–4% in other studies (Garré et al., 2007a; Bentz et al., 2006). However, the elimination half-life of acyclovir administered intravenously to horses was reported as 5–53 hours. The high variability is attributed to sampling time and the study conditions (Garré et al., 2007a; Maxwell et al., 2008a). This equine elimination half-life was much longer than in other species (~2 hours in dogs and cats) and suggested the presence of a "deep compartment" in horses. The elimination half-life of acyclovir administered orally to pheasants was similar to that in dogs (3 hours), but was prolonged (15 hours) in box turtles after oral administration of valacyclovir (Rush et al., 2005; Allender et al., 2013). In the species in which mechanisms of clearance have been assessed, acyclovir was primarily eliminated via glomerular secretion (de Miranda et al., 1981, 1982; Krasny et al., 1981). An IV infusion of 10 mg/kg for 1 hour produced effective concentrations in horses for 8 hours and could be administered twice daily for EHV-1 (Wilkins et al., 2005). However, the IV route of administration is seldom used in veterinary medicine (see Section Adverse effects). Because the oral bioavailability of acyclovir is poor in most species, a much better absorbed valine ester prodrug, valacyclovir (Valtrex®), is preferred. In cats and horses, orally administered valacyclovir has much higher bioavailability (more than two times greater in cats; 6–15 times greater in horses) than does acyclovir (Garré et al., 2007a; Owens et al., 1996; Maxwell et al., 2008a).

Clinical use in cats: Clinical signs of herpesvirus infections (feline herpesvirus type-1, FHV-1) in cats prominently consist of upper respiratory disease and ocular lesions (Thomasy et al., 2007; and in review by Gaskell et al., 2007). Since the ocular lesions can progress from conjunctivitis to keratitis, it is the ocular form of the disease that generally warrants antiherpetic therapy. Since acyclovir is poorly absorbed and FHV-1 is relatively insensitive to acyclovir, high doses of valacyclovir were tested in cats in an experimental model of FHV-1. At a dose of 240 mg/kg/day of valacyclovir, nearly six times the maximum labeled dose in people, administration of valacyclovir to cats did not suppress FHV-1 replication but did produce signs of toxicity (Nasisse et al., 1997).

Clinical use in horses: Herpesvirus infections are also important in horses, where EHV-1 is associated with neonatal infections, respiratory disease, abortion, and myeloencephalopathy, depending on the age class of the horse. Oral acyclovir has been used as a treatment for neonatal disease and for equine herpesvirus myeloencephalopathy (EHM) (Murray et al., 1998; Friday et al., 2000). Acyclovir has also been administered to horses with multinodular pulmonary fibrosis (EMPF) attributed to EHV-5, although the sensitivity of EHV-5 to nucleoside analogs has not been determined (Wong et al., 2008). Although it is possible that oral acyclovir may accumulate with multiple doses and result in therapeutic concentrations for highly sensitive isolates associated with a slowly progressive disease such as EMPF, oral acyclovir is too low in horses to justify its use in rapidly progressing herpesvirus infections, such as EHM (Wong et al., 2010). Acyclovir may be more useful as a treatment of neonatal herpesvirus infection. In one herd outbreak of EHV-1 in foals, two of three foals treated with acyclovir survived, whereas the two foals not treated died (Murray et al., 1998). Since this disease is almost universally fatal in foals, acyclovir shows promise as a therapeutict agent.

For the treatment of EHV-1, therapeutic studies have tested the safety and efficacy of valacyclovir, which has been become a more economically feasible option in horses with the availability of generic tablets. Results of experimental trials of the use of valacyclovir against EHV-1 infection have been conflicting. Weanling ponies inoculated with EHV-1 showed neither signs of EHM nor reduction in viremia when valacyclovir was

administered at 40 mg/kg every 8 hours for 5–7 days (Garré et al., 2009). However, aged mares appeared to have lower viral loads and be protected from EHM by valacyclovir administration early in the course of disease, at a loading dose of 36 mg/kg, oral, every 8 hours for 2 days, followed by a maintenance dose of 12 mg/kg every 12 hours for 7–14 days (Maxwell et al., 2008b, 2011).

Clinical use in psittacines: In birds, herpesvirus infections can be an important cause of morbidity. Acyclovir has been shown to decrease mortality in psittacine birds with herpesviral infections if the drug is administered prior to the onset of clinical signs (Smith, 1987). Orally administered acyclovir at 80 mg/kg IM every 24 hours after herpesvirus infection in Quaker parakeets was shown to be more effective in preventing death than either low- or high-dose (40 or 250 mg/kg) intramuscular injections. At the highest dose tested, acyclovir toxicity, as seen by local muscular necrosis, was thought to contribute to bird mortality (Norton et al., 1991). In pheasants, a dose of 120 mg/kg of acyclovir administered orally every 12 hours was necessary to maintain concentrations above 1.0 μg/ml, although the safety of this dose rate was not tested (Rush et al., 2005).

Adverse effects: The adverse effects of acyclovir and related drugs have not been well-documented in animals because of their infrequent use. Intravenous administration of acyclovir is seldom used in veterinary medicine due to cost and the increased risk of adverse effects that necessitate IV infusion over 1 hour to diminish the risk of presumed CNS effects (Bentz et al., 2006). Clinical signs of toxicity in cats that received high doses of valacyclovir included nephrotoxicity, as well as the bone marrow suppression that may have been an extension of pharmacological effects, since nucleoside analogs can inhibit cellular DNA replication at high enough concentrations (Nasisse et al., 1997). However, the reported renal tubular necrosis is a known adverse effect associated with high plasma concentrations of acyclovir and is attributed to crystalluria (Sawyer et al., 1988).

Penciclovir and Famciclovir

Penciclovir is administered topically as a 1% cream (Denavir®) for the therapy of herpes labialis in people. When considering the role of nucleoside analogs in veterinary herpesvirus infections, it is worth noting that efficacious topical penciclovir administration in people occurred with early therapy, beginning within 1 hour of lesion appearance, and with application every 2 waking hours. Even then, the main effect of penciclovir administration was to decrease the duration of clinical signs, rather than abolishing such signs. Penciclovir shares a similar mechanism of action as acyclovir, but the intracellular half-life in virus-infected cells is 10–20 times longer (Gill and Wood, 1996). This intracellular accumulation produces an indirect link between plasma penciclovir concentrations and its antiviral effect, and may also represent a therapeutic advantage of penciclovir as compared to acyclovir. Most HSV isolates are similarly sensitive to penciclovir and acyclovir, reflecting their similar mechanisms of action.

Spectrum of activity: As with acyclovir, HSV is quite sensitive to penciclovir, whereas FHV-1 is less so. However, FHV-1 is more sensitive to penciclovir than it is to acyclovir, with an IC_{50} of 3.2–10 μg/ml (Maggs and Clarke, 2004; Hussein et al., 2008b). With a reported IC_{50} of 1.6 μg/ml, EHV-1 may also be more sensitive to penciclovir than it is to acyclovir, although penciclovir sensitivity has been tested in only a single isolate (de la Fuente et al., 1992).

Pharmacokinetics: In rodents and people, the oral absorption of penciclovir is even lower than that of acyclovir. Therefore, an oral diacetate ester prodrug, famciclovir, was developed. It is more lipophilic than the active form, penciclovir, and has much greater oral bioavailability (Gudmundsson and Antman, 2007). After the oral administration of famciclovir to people and rodents, there is extensive metabolism by intestinal esterases and hepatic aldehyde oxidase to produce the active drug, penciclovir (Gudmundsson and Antman, 2007). When 62.5 mg famciclovir (~15 mg/kg) was orally administered to cats, plasma concentrations of penciclovir increased more slowly than in other species, were variable, and suggested saturable absorption (Thomasy et al., 2007). After oral absorption the elimination half-life of penciclovir in cats (3–4 hours) was similar to that of other species. Follow-up studies confirmed the variable and saturable absorption, as bioavailability fell from 12.5% at 40 mg/kg to 7% at 90 mg/kg (Thomasy et al., 2012b). Consequently, dose rates exceeding 40 mg/kg may not produce corresponding increases in plasma penciclovir concentrations. Nonetheless, maximal plasma penciclovir concentrations of 1.3 μg/ml in plasma and of 1.0 μg/ml in tear fluid demonstrated the feasibility of famciclovir administration to cats with ocular signs of FHV-1 infection (Thomasy et al., 2012a). The disposition of penciclovir following the oral administration of 20 mg/kg famciclovir to horses revealed maximal plasma penciclovir concentrations of 2.9 μg/ml, which was higher than in cats (Tsujimura et al., 2010). Interestingly, the elimination half-life of penciclovir after famciclovir administration to horses was prolonged (34 hours) as compared to other species, but similar to the prolonged elimination half-life of acyclovir after valacyclovir administration to horses (Maxwell et al., 2008a; Tsujimura et al., 2010). Despite the theoretical advantages of penciclovir as compared to acyclovir use in horses, studies of the efficacy of penciclovir or famciclovir in protecting horses from

signs of EHV-1 or EHV-5 infection are not currently available.

Clinical use: At present, famciclovir is the only systemically administered antiviral agent that is routinely used in cats with ocular or upper respiratory signs of FHV-1 infection. Its efficacy was studied using an oral dose of 90 mg/kg every 8 hours, in cats experimentally inoculated with FHV-1 (Thomasy et al., 2011). In this model, disease score, conjunctivitis, and shedding of FHV-1 were all lower in the cats treated with famciclovir, supporting its clinical utility. However, a lower dose is more commonly used in clinical practice (Malik et al., 2009). It is available as 125, 250, 500 mg tablets, with one-quarter to one 125-mg tablet administered every 8–12 hours most often selected by clinicians. Given that famciclovir absorption profiles are not proportional to dose, it is unknown whether these lower dose rates are as efficacious as was reported experimentally for the high dose rate.

Adverse effects: Whereas one initial case series on the use of famciclovir in cats reported anorexia and polydipsia (Thomasy and Maggs, 2008), a follow-up study in which 90 mg/kg was administered every 8 hours reported no adverse effects (Thomasy et al., 2011).

Cidofovir, Ganciclovir, and Valganciclovir

Cidofovir was the first nucleoside analog approved for clinical use and is still used today as an injectable or ophthalmic implant formulation to treat cytomegalovirus infections in immunocompromised people. Cidofovir is more broadly active against a variety of DNA viruses than are the other nucleoside analogs, as it can be phosphorylated by cellular rather than viral enzymes. Since cidofovir accumulates intracellularly, with a prolonged intracellular elimination half-life, cidofovir can be administered less frequently than the other nucleoside analogs. Cross-resistance between cidofovir and other nucleoside analogs is also unlikely to occur since activation by viral enzymes is unnecessary for the activity of cidofovir. Ganciclovir is also a nucleoside analog but is more potent than acyclovir against herpesviruses, including FHV-1, EHV-1, and human cytomegalovirus (Maggs and Clarke, 2004). Ganciclovir is administered systemically as an injectable formulation for intravenous administration or as an oral tablet. Similar to acyclovir and penciclovir, ganciclovir is poorly absorbed after oral administration, so it has also been formulated as a valine ester prodrug to enhance oral absorption. Although valganciclovir is moderately well absorbed after oral administration to horses, the high cost of this prodrug has precluded further testing in this species (Carmichael et al., 2013). As with the other nucleoside analogs that have been studied in horses, the ganciclovir elimination phase is prolonged as compared to that of other species.

Clinical use: A 0.5% compounded topical formulation of cidofovir is used in cats with herpetic keratitis (Davidson, 2006). Cidofovir ophthalmic solution administered every 12 hours reduced ocular FHV-1 viral load in experimentally inoculated cats, supporting this less frequent dosing interval (Fontenelle et al., 2008). Ganciclovir is a first-line therapeutic agent for cytomegalovirus infections in people, since acyclovir and penciclovir are less potent and less efficacious against these viruses. Ganciclovir is similarly potent when tested in vitro against several herpesviruses of veterinary importance, including the zoonotic, Monkey B virus, endemic to macaques (Brush et al., 2014). Ganciclovir has also been administered intravenously to horses at later stages of infection with EHV-1, when valacyclovir administration may be ineffective (Maxwell et al., 2011).

Adverse effects: Nephrotoxicity is the dose-limiting side effect associated with parenteral cidofovir administration, necessitating coadministration with probenecid to spare the kidneys. Therefore, parenteral cidofovir is not used clinically in veterinary species. However, ophthalmic administration of 0.5% cidofovir appears to be well-tolerated in cats (Fontenelle et al., 2008). Although ganciclovir is more potent than the other antiherpetic guanine analogs, it also has a narrower therapeutic index. Reversible bone marrow suppression, with neutropenia, thrombocytopenia, and anemia, can occur in people that require intravenous doses of ganciclovir, whereas temporary or permanent impairment of fertility as well as teratogenesis occurred in laboratory animals treated with ganciclovir for a prolonged period of time. When administered to mares at a maintenance dose of 2.5 mg/kg every 12 hours for 1 week, side effects were not noted (Maxwell et al., 2011).

Idoxuridine and Trifluridine

Idoxuridine (5-iodo-2′-deoxyuridine, formerly Herplex®, Stoxil®) and trifluridine (5-trifluoromethyl-2′-deoxyuridine, Viroptic®) are thymidine analogs that are only active against DNA viruses, primarily herpesvirus and poxvirus. Like other nucleoside analogs, the compounds are phosphorylated inside the host cell and then incorporated into growing mammalian and viral DNA strands. Only trifluridine is commercially available as a topical ophthalmic preparation for the treatment of herpetic keratitis. The commercial formulation for idoxuridine was withdrawn and is now only available as a compounded solution (0.1%) or ointment (0.5%). Trifluridine (also known as trifluorothymidine) is thought to have higher affinity for viral DNA than mammalian, and so is more potent; however, it also causes the most

conjunctival irritation in vivo (Nasisse et al., 1989). Since idoxuridine is better tolerated by cats with herpetic keratitis, it is the more popular choice (Stiles, 1995). As with most other antiherpetic drugs, antiviral effects do not persist once the drug is eliminated from the eye, so the ophthalmic preparations must be applied multiple times per day. Neither drug is administered systemically due to their propensity for toxic side effects.

Cytarabine and Vidarabine

Cytarabine (Ara-C®) also known as cytosine arabinoside) and vidarabine (Ara-A®), are nucleoside analogs of cytosine and adenine, respectively. They have in vitro activity against certain DNA viruses, including herpesviruses, poxviruses, vaccinia, rabies, cytomegalovirus, and probably hepatitis B virus. Cellular enzymes convert these compounds to the triphosphate form, which then act as competitive inhibitors of DNA polymerase. As with other antiherpetic drugs that are activated by cellular rather than viral enzymes, cytarbine and vidarabine are not very selective in their activity. As a result, side effects of these drugs have limited the clinical utility of parenterally administered formulations in both humans and veterinary species. Cytarabine is used as an antineoplastic agent in dogs and cats for treatment of leukemia and lymphoma. Its use as an anticancer drug is discussed in more detail in Chapter 44. Cytarabine also is used to treat meningoencephalomyelitis in dogs and is administered either subcutaneously or intravenously (Crook et al., 2013).

Vidarabine is no longer commercially manufactured in the USA, but is occasionally used topically as a compounded 3% ointment in the treatment of herpetic keratitis, although it is less potent against feline herpesviruses than is idoxuridine or trifluridine (Nasisse et al., 1989). The main advantage of vidarabine over the other antiherpetic agents discussed is that viral isolates may not be cross-resistant to both idoxuridine and vidarabine (Eriksson and Oberg, 1979).

Ribavirin

Ribavirin (Virazole®) is a guanosine analog that inhibits the replication of a wide range of RNA and DNA viruses in vitro (Te et al., 2007). In human medicine, ribavirin is primarily used in the therapy of selected RNA viruses, including hepatitis C, respiratory syncytial virus, Lassa fever virus, and influenza A and B. Ribavirin is thought to have multiple sites of action. After being monophosphorylated to ribavirin 5′-monophosphate by adenosine kinase, further phosphorylation to ribavirin 5′-triphosphate predominates intracellularly, and competitively inhibits RNA polymerase and viral replication. Most of the studies investigating the utility of ribavirin against veterinary diseases have been disappointing. Oral ribavirin worsened the condition of cats experimentally infected with calicivirus. Bone marrow suppression, weight loss, increased hepatic enzymes, and icterus were seen (Povey, 1978). These side effects were also seen in healthy cats given the drug (Weiss et al., 1993a); however, they were not seen in dogs when given 60 mg/kg for 2 weeks (Canonico, 1985). Ribavirin was shown to have in vitro antiviral activity against the feline infectious peritonitis (FIP) virus at concentrations of 150 μg/ml (Barlough and Scott, 1989). However, kittens experimentally infected with FIP virus had no significant difference in outcome when compared to those treated with placebo (Weiss et al., 1993b). In fact, similar to the cats infected with calicivirus, the ribavirin-treated FIP kittens had a worsening of clinical signs. In vitro activity against various viral pathogens, including bovine rhinotracheitis and bovine viral diarrhea has also been demonstrated, but there are no reports of the use of this compound in infected animals (Glotov et al., 2004). As is the case with the therapy of hepatitis C infections in people, the efficacy of ribavirin may be enhanced when interferons are coadministered (Carvalho et al., 2014).

Zidovudine, PMEA, and Lamivudine

Zidovudine (Azidothymidine®, AZT®, Retrovir®) is a thymidine analog that was key to the early antiviral success against human immunodeficiency virus (HIV). As such, AZT is a nucleotide analog that selectively inhibits viral reverse transcriptase (nucleoside reverse transcriptase inhibitor, NRTI), preventing viral RNA from making a DNA copy of itself. Like the nucleoside analogs discussed above, AZT is phosphorylated by cellular enzymes to the active triphosphate form. AZT inhibits the viral enzyme with greater affinity (~100 times) than mammalian DNA polymerases, resulting in selective activity and low mammalian toxicity. Adefovir (PMEA) is an acyclic purine nucleoside analog that is used in the therapy of hepatitis and herpesviruses in people, but has been investigated as an antiretroviral drug in cats. Lamivudine is also an older NTRI that has been similarly investigated in cats.

AZT can be given orally or IV in humans. Its oral bioavailability is 60–65%, and peak concentrations are achieved in about 1 hour. AZT is quickly metabolized to the 5′glucuronide, and both the metabolite and parent compound are eliminated in urine with a half-life of approximately 1 hour. The pharmacokinetics of AZT at a dose of 25 mg/kg have been studied in cats following IV, PO, or intragastric (IG) administration through a gastrostomy tube (Zhang et al., 2004a). Absorption following PO administration (95%) was higher than IG administration (70%), but effective plasma concentrations, as determined by the in vitro EC_{50}, were maintained for at least 12 hours following administration by all three routes. As in people, the elimination half-life was short (1.4 hours). No adverse effects, other than hemolysis after

IV injection, were observed in this study; however, only single doses were administered. These same investigators also studied the pharmacokinetics of lamivudine in cats (Zhang et al., 2004b). The pharmacokinetics were similar to those of AZT in cats, but with a high bioavailability, short half-life, and plasma concentrations maintained above the predicted EC_{50} for 12 hours following either IV, PO, or IG dosing of 25 mg/kg.

Clinical use: The use of AZT has been investigated in cats both as a clinical therapeutic and experimentally, using FIV as an animal model of HIV infection. Reverse transcriptase from FIV and HIV-1 viruses was shown to be nearly identical in sensitivity to several antiviral agents, including AZT. In addition, similar concentrations of AZT were required to inhibit replication of these viruses (North et al., 1989). Bovine leukemia virus has also been suggested as a possible animal model for investigation of retroviral infection. AZT inhibition of bovine leukemia virus reverse transcriptase was similar to that of FIV-RT (Reimer et al., 1989).

In experimental FeLV infections, when treated with high doses (20 mg/kg every 8 hours) of AZT within 1 week after virus challenge, kittens were protected from bone marrow infection and viremia. Administration of AZT did not eliminate viremia in kittens if treatment was delayed to 1 and 3 weeks after FeLV inoculation; however, antigen load in the blood was reduced (Tavares et al., 1987). AZT alone, or in combination with interferon or interleukin, was shown to prevent infection in cats challenged with virulent FeLV virus for a period of 6 weeks (Zeidner et al., 1989). However, in cats experimentally inoculated with FIV, even prophylactic use of high doses of AZT did not adequately protect cats from infection (Smyth et al., 1994). PMEA has been studied in the treatment of both FeLV and FIV infections in cats. PMEA was found to inhibit replication of FeLV in vitro and prevented the development of persistent antiginemia and the induction of the immunodeficiency disease in cats inoculated with the virus (Hoover et al., 1991).

AZT has been shown to reduce clinical signs when given to two FIV-positive cats at a dose of 10 mg/kg twice a day subcutaneously for a period of 3 weeks (Egberink et al., 1991). Although it did not eradicate the infection, the authors suggest it is of clinical benefit. Currently, AZT is primarily used to improve clinical signs, such as stomatitis, of cats with FIV and FeLV (Hartmann et al., 1992). In order to reduce dose-limiting side effects, a dose rate of 5 mg/kg PO every 12 hours has been used in symptomatic cats, although efficacy in cats naturally infected with FeLV was not documented (Stuetzer et al., 2013). Seropositive cats with symptoms of opportunistic infection showed improvement of clinical signs during PMEA therapy at 5 mg/kg/day (Egberink et al., 1990). A comparative study on the effects of PMEA and AZT on FIV- or FeLV-positive cats showed that PMEA was superior to AZT in diminishing the clinical signs of disease (Hartmann et al., 1992).

Overall, clinical utility of NRTIs in the therapy of retroviral infections in cats has been disappointing, with only moderate suppression of FeLV and FIV viral loads when therapy is initiated after the infection has become established. Although dose-limiting side effects may partially explain this suboptimal control of retroviral replication, there are also key differences between the much more successfully therapy of HIV in people and the therapy of retroviral diseases in cats. In people, AZT is always combined with other drugs, from a list of 25 antiretroviral drugs in six mechanistic classes, resulting in highly effective antiretroviral therapy (HAART) protocols that are tailored to the needs and responses of individual patients (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2015). Multiple drug protocols are always used to prevent the development of resistance to a single mechanistic class. Monotherapy in cats with AZT or AZT in combination with an immunomodulator, combined with dose-limiting side effects in cats, may limit the ability of antiretroviral therapy to successfully manage FeLV and FIV. Safe and effective combination antiretroviral therapy has not been established in cats. In vitro, lamivudine is synergistic in combination with AZT against FIV, but in chronically infected, experimental cats, the combination did not reduce FIV load and severe side effects were observed (Arai et al., 2002).

Adverse effects: Major toxicities of AZT include anemia and granulocytopenia, which occur in up to 45% of treated human patients (Richman, 1987). Dose escalation studies have demonstrated a dose-dependent and progressive anemia and neutropenia in cats chronically administered >30 mg/kg/day PO divided into three doses for 32–34 days. Marked bone marrow hypercellularity and extramedullary hematopoeisis were observed on postmortem examination (Haschek et al., 1990). In feline patients, Heinz body anemia is the major side effect observed with long-term AZT administration (Hart and Nolte, 1993). When AZT and lamivudine were coadministered to cats at high dose rates, some cats had severe hematological side effects and fevers (Arai et al., 2002). Although PMEA seemed to be more efficacious than AZT in feline patients, the adverse effects (mainly hematological) were more severe with PMEA, limiting its use (Hartmann et al., 1992).

Oseltamivir

Oseltamivir phosphate (Tamiflu®) is an ester prodrug that is converted by hepatic esterases to its active metabolite, oseltamivir carboxylate. In people, only a small percentage of oseltamivir phosphate reaches the

systemic circulation, whereas approximately 75% of the oral dose appears in systemic circulation as the active metabolite. Unlike the antiherpetic and antiretroviral drugs discussed above, oseltamivir does not act by directly inhibiting viral replication. Instead, oseltamivir is a competitive inhibitor of the enzyme neuraminidase, which influenza viruses use as part of the process of budding of the replicative viral particles from infected cells. The in vitro efficacy of oseltamivir has been tested against twelve strains of equine influenza A virus (EIV), and most isolates were quite sensitive at IC_{50} concentrations of 4.3–27.5 ng/ml (Yamanaka et al., 2006a). However, one isolate exhibited a much higher IC_{50} of 3785 ng/ml, demonstrating the variability in antiviral susceptibility among influenza strains. Although bioavailability was not determined, when 2 mg/kg of oseltamivir phosphate was administered orally to horses, the resulting plasma concentrations of oseltamivir carboxylate were similar to the plasma concentrations associated with a similar dose in people (Yamanaka et al., 2007). As in people, oseltamivir carboxylate is rapidly eliminated in horses, with a mean elimination phase half-life of 2.5 hours. Given that rapid rate of elimination, the dosing interval of oseltamivir administration in horses may need to be less than 10 hours.

Clinical Use

The efficacy of oseltamivir administered at a dose rate of 5 mg/kg twice daily for 5 days was investigated in a small group of horses experimentally inoculated with EIV (Yamanaka et al., 2006b). Although the small size of the study prevented definitive assessment of drug efficacy, oseltamivir administration did appear to decrease viral shedding, fever, and secondary bacterial pneumonia. Although there is interest in using oseltamivir in recent outbreaks of dogs infected with a canine influenza virus (CIV) that is apparently related to EIV, evidence for its efficacy against CIV is primarily anecdotal. As with most antiviral drugs, the timing of oseltamivir administration is critical to therapeutic outcome in people and must usually be initiated within 48 hours of illness (Rodriguez et al., 2011). Such early initiation of therapy is unlikely in a clinical veterinary setting. Since oseltamivir is an important component in the arsenal against influenza virus infections in people, and resistance to this and other drugs active against influenza can develop rapidly, reserving oseltamivir for human use may be the most prudent approach (Cheng et al., 2010). Indeed, due to concerns regarding pandemic avian influenza virus becoming resistant to oseltamivir (Lee et al., 2011), neuraminidase inhibitors are prohibited by the FDA for extralabel drug use in poultry. Although oseltamivir is not widely used in the therapy of CIV or EIV, it has been used with some regularity in the USA for the therapy of parvovirus infections in dogs. Since parvovirus does not use neuraminidase, the proposed rationale for therapy has instead centered on inhibition of secondary bacterial infections subsequent to parvovirus infections. A clinical trial was performed to test whether addition of oseltamivir at a dose rate of 2 mg/kg PO every 12 hours to a standard protocol improved outcomes in 19 dogs with parvovirus as compared to 16 dogs in the control group (Savigny and Macintire, 2010). Addition of oseltamivir to standard therapy did not affect disease scores, hospitalization time, or mortality, but treated dogs did lose less weight than control dogs and had a less profound decrease in circulating leukocytes. Given that the mechanism of action of oseltamivir does not support efficacy against parvovirus, only a small difference in outcome was associated with oseltamivir therapy, and public health concerns about spread of drug-resistant influenza from dogs to people, oseltamivir use in this setting is difficult to justify.

Adverse Effects

In people, gastrointestinal irritation is the most common side effect associated with oseltamivir administration. Nausea and vomiting have also been observed in dogs during administration of oseltamivir, although this effect may be minimized by dilution of the oseltamivir suspension (Savigny and Macintire, 2010). Side effects were not reported when a similar dose rate of oseltamivir was administered to horses (Yamanaka et al., 2006b).

Amantadine and Rimantadine

Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel®) and rimantadine (Flumadine®) are water-soluble cyclic amines with antiviral activity against a narrow range of RNA viruses, including myxoviruses, paramyxoviruses, togaviruses, and most strains of influenza A virus. Rimantadine has approximately three to four times greater in vitro activity against influenza A than amantadine (Betts, 1991). The mechanism of these two related compounds has been debated. They were thought at one time to prevent viral penetration and uncoating, but this was later refuted (Couch and Six, 1986). Their antiviral activity is now thought to involve inhibition of late-stage assembly of the virus.

Experimentally infected chicks that received amantadine via the drinking water were one-half as likely to die as untreated controls (Obrosova-Serova et al., 1976). However, the use of amantadine in poultry is thought to be responsible for the amantadine resistant influenza strains seen in the Chinese pandemic in 2005 (Ilyushina et al., 2005). Therefore, the FDA now prohibits the extralabel drug use of amantadine and rimantadine in poultry. These two compounds have been investigated for the treatment of influenza in the horse. In vitro

testing suggests that amantadine suppresses viral replication at concentrations of 300 ng/ml while rimantadine is more potent and has activity at concentrations as low as 30 ng/ml (Rees et al., 1997, 1999). Amantadine caused serious adverse effects, including fatal seizures, in horses at doses of 10–15 mg/kg IV. Absorption of the drug following oral administration was highly variable between individual horses; therefore, a dose could not be recommended (Rees et al., 1997). Rimantadine shows greater promise as an antiviral drug in horses. A multiple dose study examining the effects of oral rimantadine at a dose of 30 mg/kg q 12 h showed adequate absorption of the drug with plasma concentrations maintained above the estimated effective concentration (30 ng/ml) throughout the dosing interval. No side effects were reported. In challenge studies using influenza virus A2, prophylactic rimantadine administration caused a significant decrease in rectal temperature and lung sounds (Rees et al., 1999). However, both amantadine and rimantadine have fallen out of favor for the treatment of influenza in people. Although amantadine and rimantadine were originally introduced as antiviral agents, their poor efficacy in human influenza patients and the likelihood of adverse effects, such as gastrointestinal irritation and central nervous system effects, has resulted in recommendations against their use for influenza virus (Jefferson et al., 2006).

Treatment of Pain

An additional use of amantadine is for treatment of pain syndromes in animals. The proposed mechanism of action for amantadine is via inhibiting the neurotransmitter *N*-methyl-D-aspartate (NMDA) (Pozzi et al., 2006). NMDA produces central sensitization and pain in animals. Blocking of the NMDA central receptor by amantadine has been associated with relief of pain syndromes. Amantadine is well absorbed orally in practically all animals, but the precise duration of action and dosing regimens have not been fully investigated in animals. In one study, amantadine was administered for 21 days, in combination with meloxicam, for the alleviation of refractory osteoarthritis pain in dogs (Lascelles et al., 2008). At a dose of 3–5 mg/kg orally, once daily, with meloxicam, dogs responded better than if administered meloxicam alone. Other doses that have been cited for pain are 2–10 mg/kg orally every 8–12 hours in dogs and 2 mg/kg orally every 24 hours in cats (Pozzi et al., 2006).

Interferon

A complete discussion of the biochemistry, physiology, and immunological function of interferon-$\alpha_{2a,2b}$ (Roferon-A, Intron A) is beyond the scope of this chapter. Interferons are polypeptide molecules produced by certain mammalian cells in response to viral infections as well as other stimuli. They are potent cytokines that possess antiviral, immunomodulating, and anticancer properties (Pestka et al., 1987). Interferons are divided into three types: type I, which includes IFN-α, IFN-β, and IFN-ω, type II, composed of only IFN-γ, and type III, which are the IFN-λs (Hoffmann et al., 2015). The antiviral activities of interferons are indirect. They induce a variety of antiviral mechanisms via enhancement of IFN responsive gene promoters within the host cells. An interesting property of IFNs is that once they induce an antiviral state in one cell, that cell can then transfer this antiviral activity to other cells through cell-to-cell contact without requiring additional IFN. This has been demonstrated in vivo using IFN-α stimulated lymphocytes. This results in an amplification of the antiviral effect, which may explain why IFNs are efficacious despite low to undetectable plasma levels (Stanton et al., 1989). Some interferons are highly conserved between species and therefore their actions are not species specific. In people, multiple interferons have been used for treatment of HIV and cancer-associated diseases.

Interferons have been administered parenterally, intranasally, and orally. Parenteral and intranasal administration lead to an increased risk of adverse effects, including formation of neutralizing antibodies to IFN, as well as clinical signs of hyperthermia, anorexia, and malaise (Roney et al., 1985). Being peptides, interferons are inactivated by the digestive enzymes in the gastrointestinal tract, yet orally administered IFN has been shown to be an effective treatment for viral diseases in a number of species (Cummins et al., 1999). The probable mechanism for this is uptake of IFN by the oropharyngeal-associated lymphoid tissue, which sensitizes the lymphocytes in these organs. The lymphocytes are then released into the circulation where they can confer antiviral activity to cells at the site of infection (Bocci, 1991).

Alpha Interferon

In vitro studies have demonstrated that FHV-1 is susceptible to feline interferon (IFN-ω) or human IFN-α (Siebeck et al., 2006). Interferons and antiviral drugs, such as acyclovir, can act synergistically to inhibit viral replication (Weiss, 1989). The human formulation of IFN-α is available as an injectable formulation, but it has been administered in an extralabel manner by both oral and parenteral routes to veterinary species. Surprisingly, a dose as low as 0.5 U administered PO to cats appears to exert antiviral activity (Cummins et al., 1988). However, oral administration of IFN-α to horses at doses of 0.22 to 2.2 U/kg just before and after inoculation with EHV-1 failed to produce a protective response (Seahorn et al., 1990). Most of the use of IFN-α has been

Table 38.6 Clinical and experimental uses of interferon in veterinary species

Species	Disease treated	IFN Subtype	Dose and route of administration	Results	Reference
Feline	FeLV	Human IFN-α	1.6×10^4–1.6×10^6 U/kg with or without AZT at 10 mg/kg q 8 h	IFN administration decreased p27 antigen, but cats became refractory in 3–7 weeks, whereas AZT had no effect	Zeidner et al., 1990
	FIP (coronavirus)	Recombinant feline IFN-γ	1 million U/kg SC EOD until remission followed by weekly injections of the same dose	Produce complete remission (>2 years) in 4/12 cats, and partial remission (2–5 months) in 4/12 cats; only cats with the effusive form of FIP responded.	Ishida et al., 2004
	FeLV or FeLV/FIV coinfection	Recombinant feline IFN-ω	1 million U/kg SC q24h for 5 consecutive days in 3 series (day 0, 14, and 60)	Placebo controlled study; treated cats had significantly lower clinical scores in the first 4 months and significantly lower mortality rates at 9 and 12 months.	de Mari et al., 2004
	FeLV	Human IFN-α with or without *Staphylococcus* Protein A (SPA)	30 U/cat PO once daily on weeks 1,3,5,7, and 9	Placebo controlled study; no significant improvements seen in animals treated with IFN compared to controls; mild improvement noted based on owner perception in cats treated with SPA alone.	McCaw et al., 2001
	FIV	Natural human IFN-α	10 IU/kg PO q24h on alternating weeks for 6 months	Treated cats had a significant improvement in clinical signs and a longer survival than controls; no correlation with plasma viremia or viral load in leukocytes was noted.	Pedretti et al., 2005
	FeLV, FIV	Recombinant feline IFN-ω	1 million U/kg SC q24h for 5 consecutive days in 3 series (day 0, 14, and 60)	Cats treated with IFN had better improvement in clinical scores as compared with control cats, but proviral load and viremia were not affected.	Domenech et al., 2011
	FeLV	Human IFN-α	$1x10^5$ U/kg with or without AZT at 5 mg/kg q 12 h	No significant differences between placebo control and drug treatment groups in p27 antigen.	Stuetzer et al., 2013
Canine	Parvovirus (CPV-2)	Recombinant feline IFN-ω	1 million U/kg IV q24h for 3 consecutive days starting 4 days after viral inoculation	Placebo controlled study; treatment significantly reduced the severity of the enteritis within 12 hours of administration of the first dose; all dogs received standard supportive therapy.	Ishiwata et al., 1998
	Parvovirus	Recombinant feline IFN-ω	2.5 million U/kg IV q24h for 3 consecutive days	Multicentric, double-blind, placebo controlled field trial; treated dogs showed a significant improvement in clinical signs; mortality rate was 7% in treated dogs and 29% in controls; all dogs received standard supportive therapy.	de Mari et al., 2003

(*continued*)

Table 38.6 (*Continued*)

Species	Disease treated	IFN Subtype	Dose and route of administration	Results	Reference
Bovine	Vaccinia (Smallpox)	Natural human IFN-α2	10 million U/kg IM q24h starting 24 hours prior to viral inoculation	Placebo controlled study; complete protection was obtained at this dose.	Werenne et al., 1985
	IBR	Recombinant human IFN-α	0.05–5 U/kg PO q24h for 4 days starting 48 hours prior to intranasal viral inoculation	Placebo controlled study; the 0.05 and 0.5 U/kg groups showed significant improvement in mean rectal temperature and duration of antibiotic therapy; the 0.05 U/kg group also had significantly better weight gain.	Cummins et al., 1993
	IBR	Recombinant bovine IFN-α1	10 mg/animal intranasally 48 hours prior to viral inoculation	Significant reduction in morbidity and mortality in treated animals was noted, although treatment did not prevent clinical signs or affect viral shedding.	Babiuk et al., 1987
	BLV	Recombinant bovine IFN-τ	10^5–10^6 U/kg SC 3x/week for 3–4 weeks	Reduced titers of BLV in IFN treated cattle	Basu et al., 2006
	BVDV	Recombinant bovine IFN-τ	10^5–10^6 U/kg SC 5x/week for 2 weeks	Serum titers to BVDV decreased slightly in the high dose group, but only during the administration period	Kohara et al., 2012
Swine	FMDV	Adenovirus expressing porcine IFN-α or IFN-γ, alone and in combination	Low and high doses of each cytokine	Swine with the combination of the two IFNs were completely protected from challenge infection, in contrast to control animals	Moraes et al., 2007
Equine	EHV-1	Recombinant human IFN-α2a	0.22 or 2.2 U/kg PO 48 and 24 hours prior to viral inoculation and 24 hours after inoculation	No significant effects on clinical disease or duration of viral shedding were noted.	Seahorn et al., 1990
	Inflammatory airway disease	Recombinant and natural human IFN-α	90 U/horse (recombinant) or 50 U/horse (natural) PO q24h for 5 days	Placebo controlled study; all horses had a significant decrease in cough and nasal discharge; significantly fewer horses treated with either IFN product had a relapse after 4 weeks; viral etiology in this disease not proven.	Moore et al., 2004
	Shipping fever	Human IFN-α	1.25 g/head PO q24 h for 4 days	Placebo controlled study; elevations in white blood cells, fibrinogen, serum amyloid A were partially mitigated by IFN administration.	Akai et al., 2008

BLV, bovine leukemia virus; BVDV, bovine viral diarrhea virus; CPV, canine parvovirus; EHV, equine herpesvirus; FeLV, feline leukemia virus; FIP, feline infectious peritonitis; FMDV, foot-and-mouth disease virus; IFN, interferon; IBR, infectious bovine rhinotracheitis.

in experimentally infected animals or anecdotal, with few clinical trials to demonstrate efficacy for treating FHV-1 infections (Gaskell et al., 2007).

Omega Interferon

Omega interferons are licensed for use in the treatment of viral disease of dogs and cats in Europe, Japan, Australia, New Zealand, and Mexico. Omega interferon of feline origin, produced by genetic engineering, is a type 1 interferon closely related to α-interferon (Yang et al., 2007). This IFN-ω binds to the same receptors as IFN-α and IFN-β. Recombinant IFN-ω contained in Virbagen Omega is produced by silkworms previously inoculated with interferon-recombinant baculovirus, resulting in the synthesis of pure interferon.

After injection, IFN-ω has a half-life of 1.4 hours in dogs and 1.7 hours in cats. The IFN-ω is rapidly cleared by the kidneys and is not widely distributed. Instead, it is bound to receptors in cells infected by virus. Interferon has been used to stimulate immune cells in dogs with parvovirus and in cats with feline retrovirus (FeLV and FIV), which are the labeled indications for Virbagen Omega.

Doses and indications for animals have primarily been based on extrapolation of human recommendations, experimental studies in animals, or specific studies in cats and dogs with viral infections. The formulation used in veterinary medicine is available in 5 and 10 million units/vial, which is reconstituted before use. The suggested dose in dogs is 2.5 million units (MU)/kg IV once daily for 3 consecutive days. The dose in cats is 1.0 MU/kg SC for 5 consecutive days. Three separate 5-day treatments must be performed at day 0, day 14, and day 60. A summary of some of the reports of interferon use in natural and experimental viral disease of different species is presented in Table 38.6.

Adverse reactions: In people, injections of IFN-α have been associated with influenza-like symptoms. Other effects also have been reported in people, such as bone marrow suppression. In animals, interferon has been generally well tolerated, but may induce vomiting and nausea. In some animals it may induce hyperthermia 3–6 hours after injection. In cats, it may produce soft feces to mild diarrhea. A slight decrease in white blood cells, platelets, and red blood cells, and rise in the concentration of alanine aminotransferase may be observed. These parameters usually return to normal in the week following the last injection. In cats, it may induce transient fatigue during the treatment. Do not vaccinate dogs or cats receiving interferon.

L-Lysine

L-lysine is an essential amino acid that blocks the availability of arginine, which is necessary for the replication of herpesviruses. In vitro testing has demonstrated an inhibitory effect of L-lysine on FHV-1 replication in the presence of arginine (Maggs et al., 2000). Additionally, a dose of 400 mg per day, given in food, reduced viral shedding following the stress of changes in housing and husbandry (Maggs et al., 2003). This beneficial effect was blocked when the cats were given methylprednisolone to induce viral shedding. When given prophylactically at a dose of 500 mg 6 hours prior to viral challenge, and then continued at 500 mg PO q 12 h, it decreased the severity of the ocular lesions compared to controls; however, it had no effect on virus isolation (Stiles et al., 2002). Despite the promising results cited above, clinical results have been disappointing. A clinical study in shelter population cats did not show a benefit of treatment (Rees and Lubinski, 2008). When 144 treated shelter cats were compared to 147 controls, there was no difference in prevention of upper respiratory infection or conjunctivitis when cats were supplemented with 250 or 500 mg daily oral L-lysine. There was actually an increase in severity of some infections. For these reasons, the use of L-lysine to treat FHV-1 in shelter cats is not recommended until reevaluation is available.

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