Quinolones: a class of antimicrobial agents

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ABSTRACT: The fluoroquinolones are a series of synthetic antibacterial agents that are used in the treatment of a variety of bacterial infections. These agents inhibit the DNA gyrase, abolishing its activity by interfering with the DNA-rejoining reaction. The inhibition of the resealing leads to the liberation of fragments that are subsequently destroyed by the bacterial exonucleases. All fluoroquinolones accumulate within bacteria very rapidly, so that a steady-state intrabacterial concentration is obtained within a few minutes. Resistance develops slowly and is usually chromosomal and not plasmid mediated. However, development of resistance and transfer between animal and human pathogens has become a fervently argued issue among the microbiologists. Another concern regarding the use of new quinolones in the veterinary field is a possible detrimental effect on the environment. It still seems unlikely that the controlled use of veterinary quinolones will give rise to unfavorable effects on the environment.

Key words: fluoroquinolones; chemistry; pharmacokinetics; resistance; therapeutical use

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1. INTRODUCTION

Older members of the quinolone class of synthetic antimicrobial agents, particularly nalidixic acid, have been available for the treatment of urinary tract infections in humans for many years. These drugs are of relatively minor significance because of their limited therapeutic utility and the rapid development of resistance (Goodman and Gilman, 1992).

Over the last two decades, research on 4-quinolone-3-carboxylates has led to the discovery of a family of 6-fluoro-7-piperazinyl-4-quinolones active against gram-negative and gram-positive bacteria *in vitro* (Hooper and Wolfson, 1985) as well as intracellular pathogens (Fitzgeorge *et al*., 1988) and trimethoprim/sulfonamide resistant microbes (Preheim *et al*., 1987); in addition these antimicrobials are also active against mycoplasmas (Braunius, 1987). Collectively, these compounds are called fluoroquinolones. Although dozens of fluoroquinolones have been synthesized and reported, the most notable ones being developed, or used, in veterinary medicine worldwide include (in alphabetical order) amifloxacin, benofloxacin, ciprofloxacin, danofloxacin, difloxacin, enrofloxacin, marbofloxacin, norfloxacin and norfloxacin nicotinate, ofloxacin, orbifloxacin and sarafloxacin. Other major fluoroquinolones in human medicine include enoxacin, ofloxacin, sparfloxacin, temafloxacin, and tosufloxacin. Enrofloxacin was the first fluoroquinolone introduced into veterinary medicine. All fluoroquinolones are bactericidal and all act against the same bacterial target: the bacterial DNA gyrase (type II topoisomerase). No plasmidic resistance against them has been demonstrated (Hooper and Wolfson, 1985). However, after in vitro experimental selection (Desgrandchamps, 1989) or clinical administration (Kresken and Wiedemann, 1988), resistant mutants have been isolated. These isolated mutants show cross reactivity for the different quinolones and fluoroquinolones but no cross reactivity with other antimicrobial families.

These fluoroquinolones share a great oral bioavailability in all monogastric species, a large volume of distribution and a low binding to plasma proteins that allows them to cross membranes and reach the most remote parts of the body at concentrations above the minimum inhibitory concentrations (MIC's) of most pathogens. Tissues and sites demonstrating high concentrations following systemic administration include the kidney, liver and bile plus the prostate, female genital tract, bone and inflammatory fluids (Montay *et al*., 1984). They are eliminated for the most part in the urine and reach the levels 100 to 300 times higher in the urine than in the serum (Montay *et al*., 1984). All the fluoroquinolones exhibit distributional and antimicrobial properties that make them potentially useful in veterinary medicine.

2. CHEMISTRY

The 6-fluoroquinolones (also known as 4-quinolones or quinolones; Figure 1) are a series of synthetic antibacterial agents derived from, or related to, nalidixic acid and oxolinic acid.

Position 1 is nitrogen in the bicyclical aromatic ring structure, with an alkyl group (ethyl or perhaps cyclopropyl) often attached there. Carboxylic acid at position 3 is required for antimicrobial activity, similarly like a keto group at position 4. Many improvements on these early quinolone carboxylic acids have been made based in systematic structure-activity studies. A fluorine atom at position 6 on the quinolone carboxylic acid nucleus enhances the efficacy of these compounds against gram-negative pathogens and broadens the spectrum of activity against gram-positive pathogens: a basic nitrogen-containing moiety enhances tissue penetration and reduces the central nervous system toxicity. Modifications of the basic structure at positions 2, 5 and 7 alter the pharmacokinetics of the compound. A carbon, nitrogen or oxygen atom occupies position 8 on the heterocyclic aromatic ring, depending on the quinolone. Nitrogen atoms at positions 1 and 8 produce naphthyridine carboxylic acids (e.g. enoxacin or nalidixic acid), whereas nitrogen atoms at positions 1, 6 and 8 are called pyridopyrimidine carboxylic acids, which are not fluorinated at position 6 (e.g. pipemidic acid). Because of the presence of carboxylic acid and one or several basic amine functional groups, these antibacterial agents are amphoteric and considered zwitterionic: however, between the pK_a of the acidic and the basic functional groups (between pH 6 and 8), these compounds are sufficiently lipid-soluble to be able to penetrate tissues. In octanol/water partition experiments conducted at pHs ranging from 2.9–7.6, ciprofloxacin, norfloxacin and enoxacin did not pass significantly into octanol: though nalidixic acid showed an increasing passage into the lipid layer from pH 7.6 to 6.4 (Ashby *et al*., 1985). However, these classic study methods are unable to determine the true partition coefficient unless the relative abundance of the four potential ion combinations (i.e. [0.0]. [+.0]. [0.–]. [+.–]) is known (Takács-Novák *et al*., 1990). It appears that the uncharged species (i.e. [0.0]) is compared to a larger fraction of many quinolones in solution, this may account for diffusion across the membranes (Nikaido and Thanassi, 1993). What further complicates the issue is a possibility that initial interaction with membrane surfaces may be mediated by divalent cations (Nikaido and Thanassi, 1993), which may have effects that render classic octanol/water partitioning experiments not applicable. Water solubility at physiological pH varies across these compounds, depending on the substitutions on the quinolone carboxylic acid nucleus. Salt forms of the fluoroquinolones are freely soluble and are generally stable in an aqueous solution.

3. STRUCTURE-ACTIVITY RELATIONSHIP

As indicated above, the fluoroquinolones are based on the 4-quinolone ring (Figure 1). The structure of the ring has been largely modified to enhance the antimicrobial activity and to increase the volume of distribution of the molecule.

The substitution of a piperazinyl ring at position 7 has rendered the molecule active against *Pseudomonas* and the presence of a fluorine atom at position 6 extends the activity of the molecule to some but not all gram-positive bacteria (Neer, 1988). *Streptococcus* can be resistant (Berg, 1988). Additions of alkyl chains to the para position of the piperazinyl ring, and to the nitrogen at position 1, increase the lipid solubility and the volume of distribution of the compounds. The substitution of hydrogen atoms by fluorines at position 8 of the ring and on the methyl of the alkyl chain diminishes the rate of degradation and decreases the rate of elimination.

It was widely believed that 3-carboxylic acid and 4-carbonyl were necessary for the antimicrobial activity of the compounds. However, Chu *et al*. (1988) showed that the transformation of existing molecules in 2,3,4,9-tetrahydroisothiazolo [5,4-b] quinoline-3,4-diones produces a significant increase in their biological activity.

The quinolones bear both the acidic group (carboxylic acid) and the basic group (tertiary amine). This association gives them amphoteric properties. Their solubility is low, except between pH 6 and 8. Within this range, they have low water solubility and are prone to precipitate under more acidic conditions (Jenkins and Friedlander, 1988). It is apparently due to this property that crystalluria has been observed in man and animals (Ball, 1986).

4. ANTIMICROBIAL ACTIVITIES

Bacteria possess type II topoisomerase known as DNA gyrase: a tetrameric bacterial enzyme that folds and coils

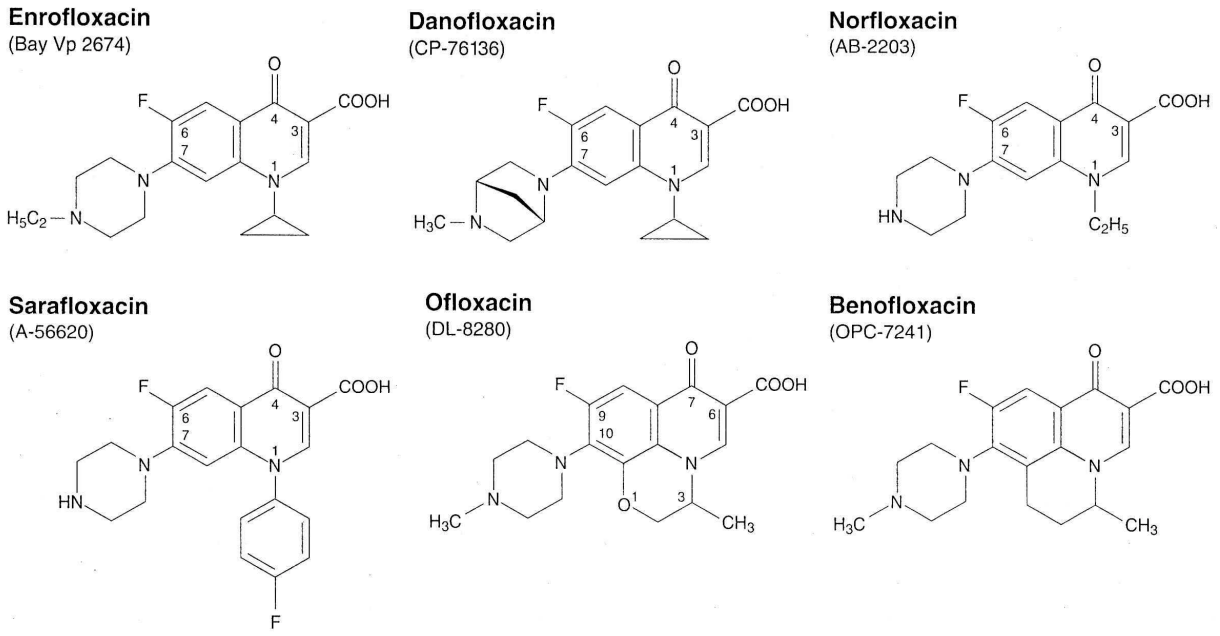

Figure 1. General chemical structure of some fluoroquinolones

1.0-0.3 m of circular bacterial DNA to such an extent that it can fit into the bacteria several thousand times shorter. Furthermore, the supercoiling of DNA that is catalyzed by DNA gyrase aligns DNA into a "relaxed" form that has decreased susceptibility to fragmentation and increased ease of separation during strand replication (Fernandes, 1988). This is accomplished by coiling DNA around an RNA core in a series of loops; each loop or domain is then negatively supercoiled by nicking both strands of DNA and passing that broken strand "behind" the accompanying double strand and then resealing the double nick. Quinolones inhibit the A sub-unit of DNA gyrase (produced by the *gyr* A gene) abolishing its activity, possibly by interfering with the DNA-rejoining reaction (Neu, 1988). The inhibition of resealing leads to the liberation of fragments that are subsequently destroyed by bacterial exonucleases (Smith, 1986).

DNA gyrase has also been described as working in a yin-yang mechanism with topoisomerase I where fluoroquinolones inhibit DNA replication by stimulating topoisomerase I resulting from the inhibition of DNA gyrase. Coumermycin and novobiocin act on the B sub-unit of DNA gyrase (Fernandes, 1988), and coumermycin has shown synergy with the fluoroquinolones (Fernandes, 1988). In fact, fluoroquinolones most likely bind in a co-operative manner to a pocket of single strand DNA created by DNA gyrase.

Interestingly, a *gyr* B mutation (*gyr* B is the gene that codes for the B sub-unit of DNA gyrase) that changes amino acid 447 into a negatively charged amino acid confers hyper-susceptibility to the of DNA gyrase. Coumermycin and novobiocin act on the B sub-unit of DNA gyrase (Fernandes, 1988), and coumermycin has shown synergy with fluoroquinolones with a positively charged piperazine substituent, suggesting that an electrostatic interaction between fluoroquinolones and the gyrase B sub-unit may result in increased stability of quinolone binding to the complex, thereby increasing susceptibility (Smith, 1984).

Sigmoidal fluoroquinolone binding kinetics suggests that four molecules (two pairs with opposing orientation and stacked above or below each other) can stereochemically fit into the pocket, acting co-operatively to inhibit DNA gyrase (Hooper and Wolfson, 1991) in a similar fashion to the co-operative binding of four oxygen molecules to hemoglobin. The result is rapid bactericidal activity at relatively low concentrations. The rate of bacterial cell may be accelerated if substituent 7 becomes a weaker base or if the carboxyl group becomes a stronger acid (Nikaido and Thanassi, 1993). One striking peculiarity of these antimicrobials is their biphasic concentration-response curve. Fluoroquinolones are considerably less effective against bacterial pathogens at concentrations much higher, as well as lower, than their minimum inhibitory concentrations (MICs). In the first phase, the percentage of killed bacteria increases with concentration; in the second phase, further increase in concentration causes a temporary decrease in the percentage of killed bacteria (Diver and Wise, 1986). This effect is seen during short-term exposures only. The percentage of bacteria killed after more than 1.5 hour exposure remains the same at any concentration above the MIC. Interestingly, the inhibition of pro-

tein synthesis caused by the concomitant administration of chloramphenicol (inhibitor of protein synthesis) and fluoroquinolones decreases the percentage of bacteria killed by fluoroquinolones. This is probably due to the inhibition of de novo synthesis of exonucleases. It is unlikely that the accidental overdosage of a treated animal would cause a decreased action; however, neither overdosage nor concomitant administration of a protein synthesis inhibitor is advisable. The specific and fundamental action on bacterial replication allows the fluoroquinolones to be active at very low concentrations and to show a post-administration activity. The concentration necessary to inhibit the mammalian replication enzymes is two orders of magnitude higher than the concentration inhibiting the corresponding enzymes in the bacteria (Oomori *et al*., 1988). This results in a favorable margin of safety for fluoroquinolones.

Mammals have an enzyme that makes couple-stranded cuts in DNA, similar to DNA gyrase, but it does not supercoil DNA and is not affected by fluoroquinolones (Fernandes, 1988). However, the increased activity of some fluoroquinolones at the mammalian topoisomerase II enzyme has been associated with genotoxicity (Hooper and Wolfson, 1991). Recent evidence suggests that there exists an asymmetric barrier between mammalian topoisomerase II and bacterial DNA gyrase, with those fluoroquinolones with cis-3,5-dimethylpiperazine configurations on the C7 carbon conferring much more selectivity for bacterial DNA gyrase than the *trans*-3,5-dimethyl analog (Gootz *et al*., 1994).

DNA gyrase is an intracellular enzyme, so the uptake of fluoroquinolones by the bacteria is critically important. The entry into cells is via porins (Chapman and Georgopapadakou, 1988), with subsequent entry across the cytoplasmic membrane occurring in dependence on the fluoroquinolone physicochemical properties. All fluoroquinolones accumulate within bacteria very rapidly, so that within a few minutes a steady-state intrabacterial concentration is obtained (Piddock, 1994). Accumulation is antagonized by cations such as magnesium and calcium, perhaps by binding to the cell surface resulting from chelation with divalent cations (Kotera *et al*., 1991). For gram-positive bacteria, an energy-dependent efflux transport system, similar to the tetracycline pump mediated by the TetA protein, pumps the fluoroquinolones out of the bacterial cell.

Post-antibiotic effects (decreased or abnormal growth of bacteria after an exposure to the antibacterial agent: PAE) lasting 4–8 hours were observed in a number of strains including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (Neu *et al*., 1987). The PAE is associated with decreased adherence to cells as a part of the phenomenon. Concentrations as low as 1 000 fold less than the MIC have been shown to decrease adherence of *Staphylococcus aureus* bacteria to buccal cells (Desnottes *et al*., 1987) even though the PAE is concentration dependent (Gould *et al*., 1990). The active efflux mechanism described above is depressed during the post-antibiotic effect, and can be inhibited by carbonyl cyanide m-chlorophenylhydrazone, which dissipates energy (Piddock, 1994). The inhibition of efflux mechanism results in an accumulation of fluoroquinolones inside the bacteria.

Fluoroquinolones are known to gain entry into phagocytic cells and remain microbiologically active inside the cells against bacterial pathogens such as *Legionella pneumophyla* (Carlier *et al*., 1990).

Microscopically, the morphologic alterations produced by fluoroquinolones include decreased cell division, filamentation, and cellular lysis (Foerster, 1987). Ultrastructurally, altered cell division is also evident, and bacterial cell "ghosts", i.e. remnants of the outer bacterial cell wall without internal cell components, are prominent after enrofloxacin treatment of bacterial cultures *in vitro* (Voight, 1987). These observations may be the result of the cascade of events resulting from the inhibition of DNA gyrase leading to general bacterial cellular dysfunction, disruption of normal cellular replication and repair processes, and cell death.

5. BACTERIAL RESISTANCE

Resistance occurs primarily by alterations in bacterial cell wall penetration, with mutant forms of DNA gyrase occurring only rarely (Chamberland *et al*., 1989). Permeability changes occur either via decreased permeability of the hydrophilic pores (OMP) or through alteration of the active transport (efflux) pump (Kaatz *et al*., 1991), thereby decreasing the intracellular content of fluoroquinolones. The enzymes that degrade quinolone antibacterial agents have not been observed.

One of the major reasons nalidixic acid fell out of favor with physicians and veterinarians was the high level of resistance that quickly developed in the early 1960's (Neu, 1988). Although low-frequency chromosomal mutations are the primary source of bacterial resistance to fluoroquinolones encountered to date, plasmid-mediated resistance to the older quinolones was encountered only in a single isolate of *Shigella dysenteriae* in Bangladesh (Neu, 1988). Plasmid-mediated resistance was not demonstrated in fluoroquinolones (Fernandes, 1988). The bacteria that contain R-plasmids carrying resistance to other antibacterial agents remain sensitive to many of the fluoroquinolones. The cross-resistance with β-lactam antibiotics, aminoglycosides, tetracyclines, macrolide and polypeptide antibiotics, sulfonamides, diaminopyrimidines, and nitrofurans does not generally occur. However, certain mutations conferring resistance to fluoroquinolones can also confer resistance to cephalosporins, tetracyclines, and chloramphenicol, although other mutations conferring fluoroquinolone resistance can cause

hypersusceptibility to β-lactams, aminoglycosides, and novobiocin (Piddock and Wise, 1989).

Single-step resistance to fluoroquinolones occurs in 10^{-7}–10^{-10} bacteria (Watanabe *et al*., 1990), with mutations in certain bacteria (e.g. *Enterobacter cloacae* and *Serratia marcescens*) developing at higher frequencies than in others (Watanabe *et al*., 1990). The frequencies of these mutations suggest a single mutation at a single locus (Piddock, 1994). When resistance does occur, cross-resistance between fluoroquinolones is generally observed to occur at higher frequencies for the older, and less potent, quinolones such as nalidixic acid and flumequine. Development of resistance generally means a 2-8-fold change in the minimum inhibitory concentration (MIC) (Fernandes, 1988). The decreased susceptibility caused by these mutations was not considered clinically significant in the mid-1990s as the MIC values are so low compared with clinical drug concentration ranges attainable in human beings (Smith, 1984). More recently, resistance has been reported most often for *Pseudomonas aeruginosa*, *Serratia marcescens*, and staphylococci in chronic infections or chronic bacterial exposure (e.g. indwelling venous catheter or urinary catheter). During oral administration to humans, aerobic fecal flora was almost entirely abolished while anaerobic bacteria remained little affected: after a week without selective pressure, fecal flora returned to normal (Brumfitt *et al*., 1984). The MIC values increased in the anaerobes although the anaerobic bacteria were not considered initially susceptible to fluoroquinolones. Furthermore, Brumfitt's study did not identify or type the anaerobes, and it was not proven without doubt that the same bacterial populations had developed resistance. Similar results were obtained with pefloxacin (Janin *et al*., 1987): the *Enterobacteriaceae* were eliminated, but streptococci and anaerobes were unaffected. In both studies, the yeast overgrowth did not occur. Resistance has developed to some of the fluoroquinolones during clinical use in humans, as evidenced by an increased MIC observed in *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* isolates from human patients with chronic respiratory infections treated with enoxacin, pefloxacin (Maesen *et al*., 1987) or norfloxacin (Rowan *et al*., 1988). When the fluoroquinolones are used, data support that intensive therapy, such as maximum concentrations achieved at the site of infection 4–8 times higher than the MIC or areas under the curve (AUC) to MIC doses of higher than 125, is associated with the minimization of resistance development (Felmingham *et al*., 1988; Forrest *at al*., 1993). Conversely, therapy over prolonged periods of time (4–10 days) in human beings is associated with the emergence of resistant strains of bacteria (Bayer *et al*., 1988). Clearly, intermittent dosing regardless of the route of administration is one of the methods of minimizing the development of bacterial resistance. Novel administration strategies, such as pulse dosing water medication, may provide a rational means of administering fluoroquinolones in herds or flocks.

Serial passage of *S. aureus* strains through a broth containing either enrofloxacin or flumequine at 0.5 times the MIC resulted in increases in the MIC for the organism although the cross drug resistance pressure (i.e. passage in media containing enrofloxacin on flumequine susceptibility, and vice versa) produced less development of resistance than direct drug resistance pressure (Semjén and Blaskó, 1994). This suggests that development of resistance to a human fluoroquinolone will be less if different fluoroquinolones are used in veterinary medicine. In addition, Dijkstra *et al*. (1994) indicated that gut models of resistance development were worse indicators of the emergence of bacterial resistance than broth cultures of sarafloxacin. In Dijkstra's *et al*. (1994) study, the susceptibility of isolates obtained from sarafloxacin-containing models was similar to those obtained from models without sarafloxacin, as well as being similar to the susceptibility of parent strains. There was no indication of emergence of resistance in their model. One of the major differences in the latter gut model compared with broth cultures is the presence of organic (fecal) material in the gut model, which is known and was shown to reduce the activity of fluoroquinolones (Dijkstra *et al*., 1994). Greenwood *et al*., (1984) showed that surviving bacteria were no less susceptible to ciprofloxacin after over 24 h than to therapeutic concentrations in conditions simulating bacterial cystitis.

Development of resistance is the greatest source of debate and political fallout for the use of fluoroquinolones in animals. Because fluoroquinolones are the drugs of choice for many refractory and/or nosocomial infections in human beings, there has been an attempt to minimize the development of resistance to them by the medical profession (Beam, 1994). Several multiyear surveys, particularly in Europe, have shown that resistance has developed slowly, coincidentally with the approval and increased use of fluoroquinolones in food-producing animals as well as in humans (Endtz *et al*., 1991; Pérez-Trallero *et al*., 1993). Increased prescription of fluoroquinolones by physicians also occurred during the same period of time, confounding any conclusions of a causal relationship between the veterinary use of fluoroquinolones and the development of resistance to human fluoroquinolones. In addition, these same discussions regarding the restriction of antimicrobial use in veterinary medicine to minimize development of resistance occurred several years ago, when firstly the aminoglycosides and then the cephalosporins were the classes of drugs reserved for refractory or hard to treat nosocomial infections in human beings. The nature of antimicrobial research is such that, as classes of antimicrobial agents become less effective, new classes are developed to address the problem infections.

It is clear that resistance to any class of antimicrobial agents increases as the level of use increases due to se-

lective pressure. Furthermore, the injudicious use of antimicrobial agents at insufficiently high doses, for inappropriate duration of therapy, or for use in clinically ill patients who do not warrant such treatment, exacerbates the development of resistance. Both the medical profession and the veterinary profession need to prescribe and/or administer agents like fluoroquinolones more conscientiously to minimize the development of resistance. The lack of solid data, and the potentially erroneous conclusions regarding the cause of the development of resistance to fluoroquinolones, speaks to the need for widespread and well-designed programs to monitor the development of bacterial resistance not only to fluoroquinolones but also to all antimicrobials in bacterial populations from normal animals and human beings. Such a monitoring program should investigate all sources of resistance pressure and the persistence of resistance in specific populations once it has been identified.

6. SPECTRUM OF ACTIVITY

In general, the fluoroquinolones have an excellent activity against *Enterobacteriaceae*, fastidious gram-negative bacteria and *Pseudomonas aeruginosa*, good to moderate activity against staphylococci, mycobacteria, chlamydia, mycoplasma and ureaplasma: and little or no activity against streptococci (particularly group D streptococci), enterococci, and anaerobic bacteria. The post-antibiotic effect of fluoroquinolones has been shown to be 4–8 h against *Escherichia coli*, *Klebsiella*, *Serratia*, and *Pseudomonas aeruginosa* (Neu *et al*., 1987). Comparison of ciprofloxacin, norfloxacin, pefloxacin, pipemidic acid and a variety of nonquinolone antibacterial agents (nitrofurantoin, sulfamethoxazole, trimethoprim, cephradine and amoxicillin) demonstrated that ciprofloxacin had the broadest spectrum of activity against all gram-negative bacteria and streptococci tested, with the exception of *Enterococcus faecalis* and *Streptococcus pneumoniae* (Hoogkamp-Korstanje, 1984). Compared with rosoxacin, norfloxacin, nalidixic acid and oxolinic acid the activity of ciprofloxacin against *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum* was found to be at least twice as high (Ridgway *et al*., 1984). Enrofloxacin has structural similarity to ciprofloxacin and has a similar antibacterial spectrum to ciprofloxacin against *Haemophilus* sp., *Pasteurella* sp. and *Actinomyces* sp. (Prescott and Yielding, 1990). Temafloxacin is more potent than either ciprofloxacin or ofloxacin against staphylococci and streptococci, but not against *Haemophylus influenzae*. Improved oral activity of temafloxacin is a function of both improved potency and better oral bioavailability (Swanson *et al*., 1991). The MIC of danofloxacin against 90% of the field isolates of *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophylus somnus* was found to be < 0.125 μ/ml (Jackson *et al*., 1990), and the range of MICs against Mycoplasma species was 0.008–0.5 μg/ml (Cooper *et al*., 1993). Many gram-negative bacteria that have become resistant to other classes of antibacterial agents, such as aminoglycosides, anti-pseudomonal penicillins, and third-generation cephalosporins, remain susceptible to fluoroquinolones.

Newer fluoroquinolones (either under development or already marketed) such as difloxacin, sparfloxacin, temafloxacin, tosufloxacin, and several other fluoroquinolones have increased activities against staphylococci, streptococci, enterococci, *Corynebacterium* sp., *Listeria monocytogenes* and *Bacillus* sp. (Furet and Pechére, 1991). These also show the activity against various anaerobic bacteria, including *Clostridium perfringens*, *Clostridium difficile*, and *Bacteroides fragilis*. Those containing a cyclopropyl group at position 1 show the activity against *Mycobacterium leprae* (Furet and Pechére, 1991). Recently, pefloxacin, ofloxacin, and ciprofloxacin were found to be active against *Plasmodium*, *Trypanosoma cruzi*, and *Leishmnia donovani* although *Toxoplasma gondii* was not susceptible (Furet and Pechére, 1991). Many of the newer fluoroquinolones with increased activity against gram-positive bacteria have the lower activity against *Pseudomonas aeruginosa* than older fluoroquinolones (Furet and Pechére, 1991).

Fluoroquinolones are more active in alkaline environments (pH > 7.4) for gram-negative bacteria (Blaser and Lüthy, 1988), but susceptibility of gram-positive bacteria to fluoroquinolones is not affected by pH (Fernandes, 1988). Susceptibility is not affected by the inoculum size (Fernandes, 1988), but activity is reduced by the presence of divalent cations (Blaser and Lüthy, 1988).

In general, aminoglycosides, β-lactams, imidazoles, macrolides, and lincosamides infrequently show synergy with fluoroquinolones against Enterobacteriaceae, gram-positive bacteria and anaerobes: but rarely do they show antagonism (Neu, 1991). Antipseudomonal penicillins and imipenem are synergistic with fluoroquinolones in 20–50% of the *in vitro* and *in vivo* models. Antagonism in streptococci and enterococci occurs between fluoroquinolones and either macrolides or tetracyclines (Neu, 1991) in general, fluoroquinolones are antagonistic with chloramphenicol.

7. PHARMACOKINETICS

Oral absorption of fluoroquinolones depends on the specific agent administered with ofloxacin adsorbed better than ciprofloxacin, pefloxacin or enoxacin; all of these were more readily adsorbed than norfloxacin (Lode *et al*., 1987; Neu, 1988), with the absolute oral bioavailability of norfloxacin in dogs of approximately 35% (Brown *et al*., 1990). Ciprofloxacin is absorbed primarily from the duodenum and jejunum when administered orally to monogastric animals (Wolfson and Hooper,

1991). Bioavailability is lower in ruminants although the mechanism for this anecdotal observation has not been determined. Bioavailability from parenteral injection sites is nearly 100% for all fluoroquinolones. Food generally inhibits the oral absorption of fluoroquinolones although there was no significant difference in enrofloxacin bioavailability in fed and fasted pigs (Gyrd-Hansen and Nielsen, 1994) or in ciprofloxacin bioavailability in humans on various high fat/high calcium diets (Frost *et al*., 1989a). Overall, oral bioavailability of fluoroquinolones ranges from 30–90% in chickens (Chen *et al*., 1994; Martinez-Larrañaga *et al*., 1994), turkeys (Gulkarov and Ziv, 1994) and pigs (Anadón *et al*., 1994; Gyrd-Hansen and Nielsen, 1994; Richez *et al*., 1994), although oral availability in donkeys was very low (Lavy *et al*., 1994).

The serum concentration peak is reached rapidly; the different fluoroquinolones display their maximum serum concentration peak within 1 and 2 hours after ingestion in man, and the times to the peak are similar in dogs, rodents and monkeys (Parpia *et al*., 1989). The time to serum peak concentrations after a single oral bolus administration of enrofloxacin is 2.5, 1.4, 0.9 and 0.5 hours, respectively, in the chicken, turkey, calf, dog and horse (VanCutsem *et al*., 1990). The concomitant administration of magnesium and aluminium containing anti-acids decreases the oral bioavailability of fluoroquinolones. This action is attributed to the chelation of carboxylate groups by the bivalent cations (Gasser *et al*., 1987). The low serum concentration when administered with milk replacer may be due to the presence of minerals that could chelate the antimicrobial.

Parenteral availability of most quinolones is approximately complete in pre-ruminant and ruminant cattle (Giles *et al*., 1991; Thomas *et al*., 1994a) although norfloxacin nicotinate availability from intramuscular injection sites was 70–90% (Soback *et al*., 1994a). Supra-availability from extravascular routes was observed in horses (Pyörälä *et al*., 1994) and may be a result of the enterohepatic recycling known to occur with some fluoroquinolones. The possibility of enterohepatic recycling of fluoroquinolones potentially confounds many of the pharmacokinetic calculations that assume the dose proportionally and no recycling (i.e. classical one- and two-compartment open pharmacokinetic models).

One of the most attractive pharmacokinetic characteristics of fluoroquinolones is their large volume of distribution. Distribution of fluoroquinolones to tissues is very good, owing to their physicochemical properties. Plasma protein binding of the quinolones varies, with the newer quinolones less bound to plasma proteins than nalidixic acid.

The steady-state volume of distribution of the fluoroquinolones is large, being 2–3 l/kg for danofloxacin in cattle (Grimshaw *et al*., 1990a; Giles *et al*., 1991), and 3.45 ± 0.72 l/kg in horses (Dowling *et al*., 1995), 1.47 l per kg for norfloxacin in dogs (Brown *et al*., 1990) and 0.75–0.96 l/kg for flumequine in calves (Mevius *et al*., 1990). In most species, this distribution volume is over 3 times greater than that for most β-lactam antibiotics and aminoglycosides, and probably represents intracellular sequestration of the drug in various tissues. Blister-fluid concentrations (indicative of interstitial fluid concentrations) equal serum concentrations within 2 h oral administration (Neu, 1988). Furthermore, tissue cage fluid concentrations of norfloxacin or ciprofloxacin were somewhat, but not substantially, higher than concurrent plasma concentrations after 6 h oral administration, and they were lower than concurrent plasma concentrations from 0–6 h dosing in normal dogs (Walker *et al*., 1989, 1990). The volume of distribution of enrofloxacin and ciprofloxacin increased in rabbits from 8 to 60 days of age, possibly due to changes in the body composition (Abadía *et al*., 1994a).

High concentrations of fluoroquinolones are achieved in saliva, nasal secretions and nasal mucosa, and bronchial epithelium (Neu, 1988), although these are not substantially higher than concurrent plasma concentrations. In fact, nasopharyngeal concentrations of ciprofloxacin were much higher than the MIC_{90} for meningococci and *H. influenzae*, but they were below the MIC for methicillin-resistant *Staphylococcus aureus* in human patients (Darouiche *et al*., 1990). Enrofloxacin concentrations that were up to 3 times higher than the serum concentrations were observed in tissue homogenates from calves taken 1 h after dosing, with 12 h concentrations in tissue homogenates exceeding the concurrent serum concentrations (Sheer, 1987). Similarly, danofloxacin lung homogenate concentrations over time were 3.5–4.5 times higher than the concurrent plasma concentrations (Giles *et al*., 1991). These danofloxacin concentrations in lung homogenate appeared somewhat related to the regional blood flow although danofloxacin concentrations in consolidated lung homogenates were proportionally higher than in the blood flow (Apley and Upson, 1993a). Furthermore, the concentrations of danofloxacin in bronchial secretions reproduced concurrent plasma concentrations in swine in spite of higher concentrations in bronchial mucosa and whole lung homogenates (Friis, 1994); similar relationships between bronchial secretion and lung tissue homogenate concentrations may apply to other species, including cattle. In the dog, enrofloxacin concentrations in bile and urine exceeded serum concentrations 10–20 times; tissue homogenate concentrations observed 1 h after drug administration in calves were in the following order: liver ≥ kidney > heart > lung ≥ spleen ≥ intestinal wall > serum = muscle = lymph nodes (Sheer, 1987). Concentrations of enoxacin in the skin were almost equal to concurrent plasma concentrations after multiple oral dosing (Malmborg and Rannikko, 1988).

Semen concentrations were half of those observed in the serum shortly after ciprofloxacin administration, but they were 10 times higher than serum concentrations in 12 h and 24 h after dosing (Dalhoff and Weidner, 1984).

Ciprofloxacin concentrations in expressed prostatic secretion after oral administration of 500 mg ciprofloxacin in human volunteers ranged from 0.9–15 µg/ml, indicating pronounced diffusion of ciprofloxacin into the prostatic fluid (Dalhoff and Weidner, 1984). Enrofloxacin showed similar penetration into the prostatic fluid and tissue in dogs, that means both were higher than concurrent serum concentrations (Dorfman *et al*., 1995) and no differences were noted in the presence of chronic *Escherichia coli* prostatitis. Good penetration of enoxacin into myometrium, cervix, and Fallopian tubes was demonstrated in human beings (Bates and Elder, 1988). In dogs, uterine and prostatic fluid concentrations were 2.2 and 1.4 µg/ml 1 h after an oral dose of 2.5 mg, whereas 1 h serum concentrations were 1.2 µg/ml after an oral dose of 5 mg enrofloxacin/kg (Sheer, 1987). In the cortical bone, enrofloxacin activity reached 29% of the concurrent serum activity (Duval and Budsberg, 1995) although it must be reminded that enrofloxacin and its dealkylated metabolite, ciprofloxacin, both contribute to *in vivo* activity. The ratio of concentrations of ciprofloxacin, pefloxacin, and ofloxacin in amniotic fluid compared to plasma ranged from 0.35 to 0.5 within 2–6 hours after dosing; comparable milk to plasma ratios were 0.75 to 1.84 (Giamarellou *et al*., 1989; Wolfson and Hooper, 1991). There is approximately 16 times higher placental transfer of enrofloxacin than of ciprofloxacin in rabbits (Aramayona *et al*., 1994), suggesting some very profound compound-specific transport processes through the placenta. In contrast, milk norfloxacin concentrations were up to 40 times higher than the corresponding serum concentrations after administration of norfloxacin nicotinate to ewes (Soback *et al*., 1994b). Enrofloxacin penetrates into milk to attain approximately twice the maximum concentration of ciprofloxacin at similar plasma concentrations, although the elimination of enrofloxacin from milk is approximately twice as fast as that for ciprofloxacin (Bregante *et al*., 1994). Penetration into the CNS is relatively good, and vitreous humor penetration is approximately 20% (Barza, 1991). Apart from nasal secretions (Dobbs *et al*., 1988) and ejaculate, body fluid concentrations of fluoroquinolones rarely reach plasma concentrations (Sorgel *et al*., 1989). Thus, the high tissue concentrations are a result of sequestration onto, or within, cells or cellular components of a tissue, although Carlier *et al*. (1990) found no specific subcellular structure affinity to pefloxacin. As an example, the intracellular concentrations of fluoroquinolones in polymorphonuclear leukocytes are 7–14 times higher than those found in the extracellular fluid (Zweerink and Edison, 1988).

The degree of the metabolism of fluoroquinolones varies widely. Biotransformation reactions involve predominantly the piperazinyl ring and its substituents. Most of the fluoroquinolone primary metabolites are active against bacteria: however, these metabolites have a shorter elimination half-life than their parent compound.

In general, phase I metabolism occurs primarily through hydroxylation and oxidation to oxoquinolones. Ofloxacin is not metabolized whereas pefloxacin is nearly completely metabolized. Nalidixic acid is hydroxylated and then glucuronidated. Enrofloxacin and pefloxacin are N-dealkylated to form ciprofloxacin and norfloxacin, respectively, similarly like fleroxacin (Lode *et al*., 1987; Küng *et al*., 1993). Other prominent metabolic pathways include oxidation to oxo-metabolites at the piperazine ring (Nix and Schentag, 1988), the major metabolites of ciprofloxacin, enoxacin, and norfloxacin (Lode *et al*., 1990; Anadón *et al*., 1995). Quite often, glucuronidation occurs, primarily on the carboxylic acid at position 3. The oxidized metabolites (like many of the N-desmethyl metabolites) have an antibacterial activity (Prescott and Yielding, 1990; Küng *et al*., 1993;) whereas the glucuronide conjugates are devoid of any activity (Nix and Schentag, 1988; Venezia *et al*., 1989). Other metabolic pathways include sulfoxidation and acetylation (Lode *et al*., 1990).

The excretion of fluoroquinolones is primarily via the kidney and secondarily via the liver. High urinary concentrations are achieved due to glomerular filtration and to probenecid-sensitive tubular secretion.

Excretion is decreased in individuals suffering from the renal failure and fluoroquinolones should be used in such patients with caution. The percentage of elimination through the bile varies among the species (Montay *et al*., 1984). For example, biliary excretion of the pefloxacin glucuronoconjugate is high in dogs and rats relative to all other species (Montay *et al*., 1984).

Nearly a half of the intravenous dose of ciprofloxacin is eliminated in the feces, with slightly more than a half of the dose being eliminated in the urine, after an oral dose more than 90% is excreted in the feces (Nix and Schentag, 1988). The glucuronide conjugates of the fluoroquinolones may be excreted in the urine or bile, depending on the fluoroquinolone and the species to which it was administered (Nix and Schentag, 1988). There are indications that the enterohepatic circulation of fluoroquinolones may occur, principally through the action of β-glucuronidases in the gastrointestinal tract that may liberate the parent agent or biologically active metabolites. Some studies also suggest that ciprofloxacin may be eliminated by active transepithelial elimination into the bowel lumen (Ramon *et al*., 1994; Wolfson and Hooper, 1991).

The renal excretion of fluoroquinolones is also variable although glomerular filtration occurs for the unbound fraction of all fluoroquinolones. Active tubular secretion by the organic anion transport system also occurs to a more variable extent (Drusano *et al*., 1986). Probenecid blocks the renal tubular secretion of norfloxacin and ciprofloxacin but because of the other routes of excretion, no large drug accumulation occurs (Wolfson and Hooper, 1991). Renal excretion accounts for 100% of cinoxacin (a non-fluoroquinolone) in 24 h (Drusano *et*

al., 1986), 60% of ciprofloxacin in 24 h in many species but only 30–40% in dogs (Abadía et al., 1994b) and 30–40% of norfloxacin and enrofloxacin in 24 h.

In normal animals, the biological half-life ($t_{1/2}$) of most fluoroquinolones ranges from 3 to 6 hours specifically, the $t_{1/2}$ of flumequine is 6–7 in calves (Mevius *et al*., 1990), 3.5–4.5 h for danofloxacin (IM.SC. or IV) in calves (Grimshaw *et al*., 1990b; Giles *et al*., 1991), 5.4 ± 0.9 h for enrofloxacin in calves (Sheer, 1987), 2–4 h for ciprofloxacin in dogs (Abadía *et al*., 1994b) and horses (Dowling *et al*., 1995), 3.6 h for norfloxacin (IV) in dogs (Brown *et al*., 1990), and 3 hours for enrofloxacin in laboratory Beagles compared to 5.0 ± 1.0 h in canine clinical patients. The interspecies differences are important: enrofloxacin has an elimination half-life of 7.3, 1.4, 1.2, 2.1 and 3.3 hours in the chicken, turkey, calf, dog and horse, respectively (VanCutsem *et al*., 1990). Fleroxacin has an elimination half-life of 1.6 hours in the rabbit, 9.4 hours in the dog (Sorgel *et al*., 1988) and 10.8 in man (Takayama *et al*., 1986).

Upon multiple dosing, ciprofloxacin, enoxacin and other fluoroquinolones have shown an increase in the $t_{1/2}$ and increased V_d from the first dose (Chang *et al*., 1988; Nix and Schentag, 1988); however, this phenomenon was not observed for norfloxacin in dogs using a dosage regimen of 5 mg/ kg every 12 h for 14 days (Brown *et al*., 1990) nor for ciprofloxacin in other studies (Höffler *et al*., 1984; Drusano *et al*., 1986) nor in dogs (Abadía *et al*., 1994b). The area under the concentration time curve normalized to a 1 mg/kg dose decreased as the dose of norfloxacin increased from 5 mg/kg to 20 mg/kg in healthy dogs (Brown *et al*., 1990). The multiple dose phenomenon described by Nix and Schentag (1988) and the non-linearity of the AUC with increasing doses in dogs observed by Brown *et al*. (1990) may reflect a decreased absorption of fluoroquinolones at higher doses, or may be the result of complicated enterohepatic recycling that may occur after repeated doses. The pharmacokinetics seems to be independent of the gender (Höffler *et al*., 1984) although individual fluoroquinolones may vary depending on the metabolic pathways and routes of excretion.

8. PHARMACOKINETICS IN DISEASE

The oral absorption is not altered in human patients with diarrhea or in those with cutaneous infections. In the cases of bacteriaemia, serum concentrations were still sufficient for effective treatment of gram-negative infections although differences and increased variability were observed (Wolfson and Hooper, 1991). Human beings with hepatic cirrhosis exhibited reduced metabolism of ciprofloxacin to oxociprofloxacin but not desethylene ciprofloxacin or sulfociprofloxacin, with no change in parent ciprofloxacin pharmacokinetics from that observed in healthy humans (Frost *et al*., 1989b). Danofloxacin pharmacokinetics and lung disposition were not altered dramatically in pneumonic calves compared with healthy ones although the volumes of distribution were somewhat larger in pneumonic calves (Apley and Upson, 1993b). Pneumonic and macroscopically normal lung homogenates had similarly high danofloxacin concentrations and similar depletion profiles. In pre-ruminant calves, the absorption from IM sites was not altered and elimination was not significantly slowed down by experimental pneumonic pasteurellosis (Thomas *et al*., 1994b).

9. PHARMACOKINETIC PREDICTORS OF EFFICACY

The fluoroquinolones are classified as bactericidal compounds, and in fact they have shown concentration-dependent bacterial killing within a couple of orders of magnitude of the MBC. Unlike β-lactam antibiotics, the efficacy of fluoroquinolones is related to both the maximum concentration and the time above the MIC (Blaser *et al*., 1987). *In vitro* pharmacokinetic systems have shown that peak concentrations exceeding 8 times the MIC were related with over 99% reduction in bacterial counts and prevention of bacterial regrowth for 24 h. The study did not separate peak concentrations from the time above the MIC by mimicking different pharmacokinetic profiles, precluding any definitive conclusions being made regarding the best pharmacokinetic predictor of efficacy. Similar results were observed in an *in vivo* model of *Streptococcus pneumoniae* in mice with ciprofloxacin (Sullivan *et al*., 1993), indicating that the peak concentration/MIC ratio had to reach a value of 10.6 for optimum protection in that model. Drusano *et al*. (1993) provided some additional insight by administering lomefloxacin to neutropenic rats with *Ps. aeruginosa* sepsis as a single daily dose which produced high peak concentration/MIC ratios (approximately 20/1) or as the same total daily dose fractionated into four daily injections, the latter producing a longer time above the MIC. The single daily dose produced significantly higher survival than the more fractionated regimen, indicating that peak concentration and/or intensity of exposure is linked more closely with efficacy than the time above the MIC intensity of exposure that has been quantified as the ratio of the area under the concentration-time curve to the MIC (AUC/MIC), otherwise known as the area under the inhibitory concentration curve (AUIC). Forrest *et al*. (1993) noted that, for ciprofloxacin, the probability of clinical and microbiological cures was above 80% when the AUIC was higher than 125; when the AUIC was lower than 125, the probabilities for clinical and microbiological cures were 42% and 26%, respectively. The time to eradication of the infection was similarly related to the AUIC, with 125 and 250 the cut off points for moderate and rapid eradication of the infection (Forrest *et al*.,

1993). The observation that the AUIC is closely related to efficacy may also be related to increased coverage of more resistant strains whereas current expectations are that C_{max} will be more closely related to reducing resistance. Optimizing one or both of these ratios may ultimately reduce the likelihood that the microbial flora will develop resistance. However, these are to date unproven hypotheses in the veterinary practice.

10. ADVERSE EFFECTS

With few exceptions, the adverse effects of fluoroquinolones are not of severe consequence when compared to the beneficial features they exhibit. The target tissues are the juvenile cartilage, central nervous system, urinary tract and digestive tract. Some skin eruptions were also observed in man (Ball, 1986). Embryonic losses in female monkeys exposed to very high doses were described (Neer, 1988).

Toxicity of the fluoroquinolones is mild at therapeutic doses, and generally consists of gastrointestinal disturbances such as nausea, vomiting and diarrhea (Norrby, 1991). At slightly higher doses, CNS signs of dizziness, restlessness, headache, depression, somnolence or insomnia may be seen (Neu, 1988). High serum concentrations may produce immediate toxic reactions, possibly due to overwhelming histamine release. These immediate reactions are believed to be principally CNS in nature, and consist of convulsions, defecation, urination, and emesis within 2–3 min of rapid IV injection of norfloxacin solution (Brown *et al*., 1990). These signs subsided within several minutes in the affected dogs, and slower infusion (for 2–3 minutes) did not produce such severe clinical signs. Others (Akahane *et al*., 1989) reported that the epileptogenic activity of fluoroquinolones possibly relates to the γ-aminobutyric acid (GABA)-like structures of the substituents at position 7 of some of the fluoroquinolones, which may allow them to act as GABA-receptor antagonists. Furthermore, enrofloxacin has increased the frequency and intensity of seizures in epileptic dogs (Van Cutsem *et al*., 1990). Other fluoroquinolones need not be likely to produce these CNS effects. Crystalluria can occur in dogs and humans at high doses of norfloxacin although the occurrence is rare in human beings treated with ciprofloxacin and has not been reported with either danofloxacin or enrofloxacin. Non-inflammatory, erosive arthropathies can be observed in growing animals treated with fluoroquinolones. Lesions of the weight-bearing cartilage of juvenile rats and beagle puppies were observed after an experimental exposure to nalidixic acid or fluoroquinolones (Kato and Onedara, 1988), causing lameness and pain severe enough to impose humanitarian euthanasia (McQueen and Williams, 1987; Kato and Onedara, 1988). Kato and Onedara (1988) observed the first histological changes as early as 5 hours after a very high dose of ofloxacin. It is apparently the reason why the manufacturer of enrofloxacin does not advocate the administration of this product to dogs younger than eight months of age. The articular cartilage forms vesicles after a single very high dose or after several moderately high doses, which can then progressively rupture and produce cartilaginous erosions. This observation is due to an early phase burst in oxidative metabolism in immature (but not mature) chondrocytes that may precipitate cell death (Hayem *et al*., 1994; Thuong-Guyot *et al*., 1994). These erosions are preferentially located at weight-bearing joints (Neu, 1988). For this reason, immature dogs, particularly those of large breeds, should not be treated with fluoroquinolones. In addition, most products labeled for human use state they should not be used in pregnancy although this warning may be precipitated by the lack of data. Furthermore, the use of fluoroquinolones in horses has not been recommended for similar reasons (Berg, 1988). Although the basis for that recommendation has been made with very little published supporting information.

Photosensitization occurs with all marketed fluoroquinolones, especially pefloxacin, although it is rare for norfloxacin and ciprofloxacin (Neu, 1988; Norrby, 1991). Topical administration to the eye shows less toxicity to the corneal epithelium than aminoglycosides (Cutarelli *et al*., 1991). However, ocular cataracts have been seen with prolonged use in humans (Neu, 1988).

Enrofloxacin has not been shown to be mutagenic by the Ames test or by the Chinese hamster ovary-HGPRT forward mutation assay and unscheduled DNA synthesis test (Altreuther, 1987). In the pregnant laboratory animals given very high doses of fluoroquinolones, maternotoxicity has occurred and some embryonic deaths have been reported in laboratory animals; no such observations have been made in the target species treated with fluoroquinolones at therapeutic doses.

Occasionally, laboratory tests may be altered in patients treated with fluoroquinolones, including increases in hepatocellular enzymes (alanine aminotransferase and aspartate aminotransferase), serum urea nitrogen and crystalluria, and decreases in haematocrit. These alterations may represent real perturbations of the organ systems of the animal or may be laboratory artifacts.

11. DRUG INTERACTIONS

The only possible drug interaction study that has been documented in animals is lack of effect of enrofloxacin on digoxin steady-state concentrations in dogs (Novotny and Shaw, 1991).

The following findings have been documented only in human studies. The oral absorption of fluoroquinolones is drastically decreased by antacids containing magnesium and aluminium (Nix *et al*., 1989), and other agents such as sucralfate also decrease the absorption of fluoroquino-

lones. Ranitidine did not alter the oral absorption of ciprofloxacin (Nix *et al.*, 1989) but it decreased the oral bioavailability of enoxacin (Grasela *et al*., 1989), suggesting that gastric pH affects the oral absorption of some fluoroquinolones, perhaps through alterations in dissolution.

After repeated administration the fluoroquinolones, including enrofloxacin, have been shown to decrease the hepatic clearance and to increase the elimination half-life of theophylline (Rybak *et al*., 1987; Bowles *et al*., 1988) and caffeine (Harder *et al*., 1988) reportedly by decreasing the demethylation of theophylline by the hepatic P450 enzymes, the 4-oxoquinolone metabolite. Ciprofloxacin administration over a period of 8–10 days prolonged the half-life of antipyrine from 9.45 to 14.9 h attributed to decreased clearance from 0.85 to 0.52 ml/min/kg in human patients (Ludwig *et al*., 1988). However, others have stated that oral doses of ofloxacin, enoxacin and norfloxacin showed no significant effect on the content of cytochrome P450, cytochrome b_5, NADPH- cytochrome P450 reductase, ethoxycoumarin O-deethylase, benzphetamine N-demethylase, or aniline hydroxylase in phenobarbital-responsive systems (Okazaki *et al*., 1988). Furthermore, the clinically important drug-drug interactions between theophylline and ofloxacin were not shown in several instances (Wolfson and Hooper, 1991). Enoxacin decreases the hepatic clearance of the R-enantiomer of warfarin but not the S-enantiomer, and the anticoagulant effects of warfarin are increased by the concurrent administration of ofloxacin (Wolfson and Hooper, 1991).

The concurrent administration of the non-steroidal anti-inflammatory agent fenbufen with enoxacin has been associated with seizures in human beings although patients given other fluoroquinolones concurrently with non-steroidal anti-inflammatory agents other than fenbufen did not develop seizures (Wolfson and Hooper, 1991). No drug-drug interaction studies have been published for danofloxacin.

12. THERAPEUTIC USES

The fluoroquinolones have shown efficacy against a variety of bacterial diseases and are indicated in the treatment of local and systemic diseases caused by a wide range of gram-positive and gram-negative bacteria, mycoplasma and chlamydia. Due to the wide array of spectrum the use of fluoroquinolones has been proposed in conditions such as deep-seated infections, prostatitis, CNS infections, bone and joint infections, and nosocomial infections resistant to other antibacterial agents.

In human beings, the fluoroquinolones are used for the treatment of a variety of severe infections that are either located in tissues inaccessible to other antibacterial agents or caused by bacterial pathogens resistant to other antimicrobial agents. These include (but are not limited to) purulent exacerbations of chronic respiratory infections (Maesen *et al*., 1987), complicated and uncomplicated urinary tract infections, *Salmonella* spp. infections, and other infections, such as otitis externa and ophthalmitis, which are resistant to agents (Barza, 1991). Norfloxacin and ciprofloxacin have received the most extensive clinical trials. Norfloxacin has mostly been used for the treatment of urinary tract infections. In one study (Friis, 1991), 408 out of 417 (98%) gram-negative isolates and 58 out of 62 (94%) gram-positive isolates were susceptible to norfloxacin. Norfloxacin is active against pathogens that often require parenteral therapy, and therefore, the entire spectrum of urinary pathogens can be treated with a single oral drug. Therefore many patients who once needed long-term hospitalization for parenteral therapy of difficult urinary tract infections can now be discharged earlier and treated with these oral fluoroquinolones.

In animals, enrofloxacin, marbofloxacin, norfloxacin, norfloxacin nicotinate, difloxacin and danofloxacin are approved for use in animals. Enrofloxacin is used in dogs for complicated and uncomplicated urinary tract infections (e.g. doses up to 11 mg/kg every 12 h) and for a variety of other infections, such as mycobacterial infections (Studdert and Hughes, 1992); prostatitis (Dorfman *et al*., 1995) and osteomyelitis (Duval and Budsberg, 1995) caused by susceptible bacteria. Higher recommended doses were calculated on the basis of an assumption that the concentrations of quinolones must exceed the MIC_{90} for the entire dosing interval (Walker *et al*., 1992), this was later shown to be an incorrect assumption (Drusano *et al*., 1993; Forrest *et al*., 1993). In dogs, a therapeutically equivalent dose of ciprofloxacin has been suggested to be 4–5 times the dose (on a mg/kg basis) of enrofloxacin which is 2.5 mg/kg twice a day; however, the scientific justification for this recommendation is questionable. Studies have been published indicating that enrofloxacin was effective in the treatment of acute salmonella infections in calves, and produced negative fecal cultures in salmonella carrier calves 5 and 12 days after treatment (Berg, 1988). In swine, enrofloxacin is reported to eliminate the carrier state for *Salmonella* with an oral dose of 200 ppm in the feed for 10 days (Berg, 1988). Clinical field studies were conducted with enrofloxacin and difloxacin in swine colibacillosis, poultry colibacillosis, and other poultry bacterial and mycobacterial diseases, with therapeutic success (Berg, 1988). Danofloxacin has undergone extensive field efficacy studies in bovine respiratory diseases, indicating that a dose of 1.25 mg/kg every day for 3–5 days is effective under a variety of management systems (Jackson *et al*., 1990). Other efficacy studies with danofloxacin brought about promising results for poultry mycoplasmosis (Kempf *et al*., 1992; Jordan *et al*., 1993). Parenteral enrofloxacin and oxytetracycline were both effective, and in terms of clinical efficacy, indistinguishable from each other, against *Actinobacillus pleuropneumoniae* in swine as determined by rectal temperature and lung weight (Pijpers *et al*., 1994).

Efficacy rates of enrofloxacin for treating pneumonia and diarrhea in cattle and swine are from 76% to 100% (Lekeux and Art, 1988; Yamamoto *et al*., 1992), those of danofloxacin for cattle and swine pneumonia from 83% to 86% (Grimshaw *et al*., 1990a; Giles *et al*., 1991). Enrofloxacin decreases mortality rates in poultry flocks with respiratory infections (Hinz and Rottmann, 1990), similarly like difloxacin, norfloxacin and danofloxacin. Danofloxacin may cause temporal sedentariness, and orbifloxacin may cause temporal walk failure.

The oral norfloxacin therapy of dogs suffering from acute enteritis removed the disease in 100% (Bhaumik, 1997), and in another study the urinary tract infection (Patil *et al*., 1995).

The pharmaceutical formulations of new veterinary quinolones are solutions and powders.

Enrofloxacin, danofloxacin, difloxacin and norfloxacin nicotinate are available as solutions for injection in cattle, and only enrofloxacin is available as a solution for oral use. For swine, all 4 drugs have been provided as solutions for injection. Danofloxacin, norfloxacin and norfloxacin nicotinate have been formulated as powder for feed and drinking water, and difloxacin, enrofloxacin, norfloxacin and danofloxacin as solutions for drinking water for swine. For poultry, danofloxacin, norfloxacin and norfloxacin nicotinate have been formulated as powder for adding to feed and drinking water, and difloxacin, enrofloxacin, norfloxacin and danofloxacin as solutions for adding to drinking water.

All drugs are administered for a maximum of 3 or 5 days. Injection sites should be changed when a large volume of drug is used, and the quinolones may cause indurations at the site of injection. Enrofloxacin should be used with caution because of its strong alkalinity.

13. INFLUENCE ON THE ENVIRONMENT

Further concern regarding the use of new quinolones in the veterinary field is a possible detrimental effect on the environment caused by the disposal of used drugs and from animal excreta. The first point to be considered is that new veterinary quinolones discarded into the environment are usually firmly adsorbed to soil and rarely pollute water. Furthermore, quinolones rapidly decompose when exposed to light. Finally, quinolones have virtually no effect on soil organisms such as protozoa and fungi or on insects and plants. It is therefore unlikely that the controlled use of veterinary quinolones will give rise to unfavorable effects on the environment.

Although there are no definite data implicating the veterinary use of anti-infectives in the development of drug resistance in human pathogens or in worsening environmental pollution, an urgent need exists for more appropriate selection and use of antimicrobial drugs. To this end, there are 3 important restrictions on the use of new veterinary quinolones. First, new veterinary quinolones are indicated only when the first-choice drugs are ineffective. Second, they are administered only by, or under the direction of, veterinarians. Third, professional and public education should be improved in the area of infectious diseases and antimicrobials to reduce inappropriate use of these compounds.

The curriculum of health professional (medical, dental, nursing, and veterinary) schools and postgraduate educational programs should be updated in the areas of sterilization, disinfection, hazards of inappropriate antimicrobial drug use, appropriate diagnosis and treatment of infectious diseases, and antimicrobial resistance. These efforts should result in a reduction of the spread of infectious agents and more prudent use of antimicrobials.

Better guidelines should be established and enforced to reduce the spread of infectious agents and antimicrobial resistance in the hospital environment, nursing homes, day care facilities, and food production industries.

Educational materials should be developed and widely distributed to patients and food producers. The need for partnerships in improving antimicrobial use for cost-effective treatment of infections and to preserve the effectiveness of antimicrobial drugs for the future should be emphasized.

Further basic research is needed to determine the mechanisms of the spread of pathogens, particularly in closed populations (i.e. hospitals, child care facilities and food production facilities).

The laws of evolution dictate that microbes will eventually develop resistance to nearly every antimicrobial. Thus, further basic research is needed to facilitate development of effective vaccines and other prevention measures. Vaccines are the most cost-effective method of disease control and prevention for many diseases (American Society for Microbiology, 1995).

14. CONCLUSION

Fluoroquinolones are one of the most useful classes of antimicrobial agents used in human and animal medicine today, both because of their spectrum and their physicochemical properties. As such, their popularity in clinical situations is increasing.

Recently, however, concerns have been aroused over the possible emergence of quinolone-resistant strains and the effects on the environment if such drugs are overused. At present it appears that physicians and veterinarians can prolong their usefulness for many years if they use appropriate clinical judgment and proper dosing principles when they prescribe and administer these drugs to patients.

If used in a well-controlled manner, quinolones will greatly contribute to stock farming management, without adversely influencing human chemotherapy.

15. REFERENCES

Abadía A.R., Aramayona J.J., Fraile L., Martinez C., Garcia M.A., Bregante M.A. (1994a): Pharmacokinetics of enrofloxacin and ciprofloxacin during ontogeny in the rabbit. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 231.

Abadía A.R., Aramayona J.J., Muñoz M.J., Pla Delfina J.M., Saez M.P., Bregante M.A. (1994b): Disposition of ciprofloxacin following intravenous administration in dogs. J. Vet. Pharmacol. Therap., *17*, 384–388.

Akahane K., Segiguchi M., Une T., Osoda Y. (1989): Structure-epileptogenicity relationship of quinolones with special reference their interaction with γ-amino-butiric acid receptor sites. Antimicrob. Agents Chemother., *33*, 1704–1708.

Althreuther P. (1987): Data on chemistry and toxicology of Baytril. Vet. Med. Rev., *2*, 87–89.

Anadón A., Martínez-Larrañaga M.R., Díaz M.J., Fernández R., Martínez M.A., Fernández-Cruz M.L., Fernández M.C. (1994): Pharmacokinetic properties of norfloxacin in pigs. In: Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 230–231.

Anadón A., Martínez-Larrañaga M.R., Díaz M.J., Bringas P., Martínez M.A., Fernández-Cruz M.L., Fernández M.C., Fernández R. (1995): Pharmacokinetics and residues of enrofloxacin in chickens. Am. J. Vet. Res., *56*, 501–506.

Apley M.D., Upson D.W. (1993a): Regional danafloxacin lung tissue concentrationsand their relationship to regional pulmonary blood flow in consolidated and nonconsolidated bovine lung. Am. J. Vet. Res., *54*, 944–951.

Apley M.D., Upson D.W. (1993b): Lung tissue concentrations and plasma pharmacokinetics of danofloxacin in calves with acute pneumonia. Am. J. Vet. Res., *54*, 937–943.

Aramayona J.J., Garcia M.A., Fraile L.J., Abadfa A.R., Bregante M.A. (1994): Placental transfer of enrofloxacin and ciprofloxacin in rabbits. Am. J. Vet. Res., *55*, 1313–1318.

Ashby J., Piddock L.J.V., Vise R. (1985): Correspondence: An investigation of the hydrophobicity of the quinolones. J. Antimicrob. Chemother., *16*, 805–808.

Ball P. (1986): Ciprofloxacin: an overview of adverse experiments. J. Antimicrob.Chemother., *18* (suppl. D), 187–193.

Barza M. (1991): Use of quinolones for treatment of ear and eye infections. Eur. J. Clin. Microbiol. Infect. Dis., *10,* 296–303.

Bates S.A., Elder M.G. (1988): An evaluation of pelvic tissue concentrations after oral administration of enoxacin. J. Antimicrob. Chemother., *21* (Suppl. B), 79–85.

Bayer A.S., Hirano L., Yih J. (1988): Development of β-lactam resistance and increased quinolone MICs during therapy of experimental *Pseudomonas aeruginosa* endocarditis. Antimicrob. Agents Chemother., *32*, 231–235.

Beam T.R. jr. (1994): Fluoroquinolones in Animal Feeds. Am. Soc. Microbiol. News, *60*, 348–349.

Berg J. (1988): Clinical indications for enrofloxacin in domestic animals and poultry. In: Quinolones: A new class of antimicrobial agents for use in veterinary medicine. In: Proc. West Vet. Conf, Las Vegas, Nevada: Mobay Corporation.

Bhaumik A. (1997): Effect of norfloxacin treatment in acute enteritis in dogs. Indian Vet. J., *74*, 246–247.

Blaser J., Stone B.B., Groner M.C., Zinner S.H. (1987): Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrob. Agents Chemother., *31*, 1054–1060.

Blaser J., Lüthy R. (1988): Comparative study on antagonistic effects of low pH and cation supplementation on in-vitro activity of quinolones and aminoglycosides against Pseudomonas aeruginosa. J. Antimicrob. Chemother., *22*, 15–22.

Bowles S.K., Popovski Z., Rybak M.J., Beckman H.B., Edwards D.J. (1988): Effects of norfloxacin on theophylline pharmacokinetics in steady-state. Antimicrob. Agents Chemother., *32*, 510–513.

Braunius W.W. (1987): Effect van Baytril™ (Bay Vp 2674) op jonge kalkoenen lijdende aan luchtweginfecties. Tijdschr. Diergeneeskd, *112*, 531–533.

Bregante M.A., Abadía A.R., Mora J., Aramayona J.J., Garcia M.A., Fraile L. (1994): Milk transfer of enrofloxacin and ciprofloxacin in the rabbit. In: Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 231–232.

Brown S.A., Cooper J., Gauze J.J., Greco D.S., Weise D.W., Buck J.M. (1990): Pharmacokinetics of norfloxacin in dogs after a single intravenous and single and multiple oral administrations of the drug. Am. J. Vet. Res., *51*, 1065–1070.

Brumfitt W., Franklin I., Grady D., Hamilton-Miller J.M.T., Iliffe A. (1984): Changes in the pharmacokinetics of ciprofloxacin and fecal flora during administration of a 7-day course to human volunteers. Antimicrob. Agents Chemother., *26*, 757–761.

Carlier M.B., Scorneaux B., Zenebergh A., Desnottes J.F., Tulkens P.M. (1990): Cellular uptake, localization and activity of fluoroquinolones in uninfected and infected macrophages. J. Antimicrob. Chemother., *26* (Suppl. B), 27–39.

Chamberland S., Bayer A.S., Schollaardt T., Wong S.A., Bryan L.E. (1989): Characterization of mechanism of quinolone resistance in *Pseudomonas aeruginosa* strains isolated in vitro and in vivo during experimental endocarditis. Antimicrob. Agents Chemother., *33*, 624–634.

Chang T., Black A., Dunkey A., Wolf R., Sedman A., Latts J., Welling P.G. (1988): Pharmacokinetics of intravenous and oral enoxacin in healthy volunteers. J. Antimicrob. Chemother., *21* (Suppl. B.), 49–56.

Chapman J.S., Georgopapadakou N.H. (1988): Routes of quinolone permeation in *E. coli*. Antimicrob. Agents Chemother., *32*, 438–442.

Chen Z., Fung K.F., Fang B., Song Y. (1994): Antimicrobial and pharmacokinetic studies of fluoroquinolones in chickens. In: Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 209–210.

Chu D., Fernandez P., Claiborne A., Shen L., Pernet A. (1988): Structure-activity relationships in quinolone antimicrobials: design, synthesis and biological activities of novel isothiazoquinolones. Drugs Exp. Clin. Res., *14*, 379–383.

Cooper A.D., Fuller J.R., Fuller M.K., Whitlestone P., Wise, D.R. (1993): *In vitro* activity of danofloxacin, tylosin and oxytetracycline against mycoplasmas of veterinary importance. Res. Vet. Sci., *54*, 329–334.

Cutarelli P.E., Laso J.H., Lazarus H.M., Putnam S.P., Jacobs M.R. (1991): Topical fluoroquinolones: antimicrobial activity and in vitro corneal epithelial toxicity. Curr. Eye Res., *10*, 557–563.

Dalhoff A., Weidner W. (1984): Diffusion of ciprofloxacin into prostatic fluid. Eur. J. Clin. Microbiol., *3*, 360–362.

Darouiche R., Perkins B., Musher D., Hamill R., Tsai S. (1990): Levels of rifampin and ciprofloxacin in nasal secretions: correlation with MIC_{90} and eradication of nasopharyngeal carriage of bacteria. J. Inf. Dis., *162*, 1124–1127.

Desgrandchamps D. (1989): Increasing rates of *in vitro* resistance to ciprofloxacin and norfloxacin in isolates from urine specimens. Antimicrob. Agents Chemother., *33*, 595–596.

Desnottes J.F., Diallo N., Moret G., Santonja R. (1987): Effects of subinhibitory concentrations of pefloxacin on the adherence of *Staphylococcus aureus* to human cells. Drugs Exp. Clin. Res., *13*, 69–73.

Dijkstra J.W., McConville M.L., Nouws J.F.M. (1994): Effects of srafloxacin hydrochloride on human enteric bacteria under simulated human gut conditions. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland. 75.

Diver J.M., Wise R. (1986): Morphological and biochemical changes in *Escherichia coli* after exposure to ciprofloxacin. J. Antimicrob. Chemother., *18* (suppl. D), 31–41.

Dobbs B.R., Gazeley L.R., Stewart I.A., Edwards I.R. (1988): Pharmacokinetics and sputum concentrations of enoxacin after twice daily oral dosing for seven days. J. Antimicrob. Chemother., *21* (Suppl. B), 61–66.

Dorfman M., Barsanti J., Budsberg S. (1995): Enrofloxacin concentrations in dogs with the normal prostate and dogs with chronic bacterial prostatitis. Am. J. Vet. Res., *56*, 386–390.

Dowling P.M., Wilson R.C., Tyler J.W., Duran S.H. (1995): Pharmacokinetics of ciprofloxacin in ponies. J. Vet. Pharmacol. Therap., *18*, 7–12.

Drusano G.L., Plaisance K.I., Forrest A., Standiford H.C. (1986): Dose ranging study and constant infusion evaluation of ciprofloxacin. Antimicrob. Agents Chemother., *30*, 440–443.

Drusano G.L., Johnson D.E., Rosen M., Standiford H. C. (1993): Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas spesis*. Antimicrob. Agents Chemother., *37*, 483–490.

Duval J.M., Budsberg S.C. (1995): Cortical bone concentrations of enrofloxacin in dogs. Am. J. Vet. Res., *56*, 188–192.

Endtz H.P., Rujis G.J., Van Klingeren B., Jansen W.H., Van der Reyden T., Mouton R. P. (1991): Quinolone resistance in campylobacter isolated from manand poultry following the introduction of fluoroquinolones in veterinary medicine. J. Antimicrob. Chemother., *27*, 199–208.

Felmingham D., Foxal P., O,Hare M.D., Webb G., Ghosh G., Grüneberg R.N. (1988): Resistance studies with ofloxacin. J. Antimicrob. Chemother., *22* (Suppl. C), 27–34.

Fernandes P.B. (1988): Mode of action, and in vitro and in vivo activities of the fluoroquinolones. J. Clin. Pharmacol., *28*, 156–168.

Fitzgeorge R., Featherstone A., Baskerville A. (1988): The effect of ofloxacin on the intracellular growth of *Legionella pneumophila* in guinea pig alveolar phagocytes. J. Antimicrob. Agents Chemother., *22* (Suppl. S), 53–57.

Foerster D. (1987): Visualization of the bactericidal action of Baytril by microphotography. Vet. Med. Rev., *2*, 100–103.

Forrest A., Nix D.E., Ballow C.H., Goss T.F., Birmingham M.C., Schentag J.J. (1993): Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. Antimicrob. Agents Chemother., *37*, 1073–1081.

Friis C. (1991): Danofloxacin in calves: pharmacokinetics and penetration into the respiratory tract tissue, Acta Vet. Scand., (Suppl.), *87*, 104–106.

Friis C. (1994): Penetration of danofloxacin into respiratory tract tissues and secretions in pigs. In: Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 208–209.

Frost R.W., Carlson J.D., Dietz A.J.,Jr., Heyd A., Lettieri J.T. (1989a): Ciprofloxacin pharmacokinetics after a standard or high-fat/high-calcium breakfast. J. Clin. Pharmacol., *29*, 953–955.

Frost R.W., Lettieri J.T., Krol G., Shamblen E.C., Lasseter K.C. (1989b): The effects of cirrhosis on the steady-state pharmacokinetics of oral ciprofloxacin. Clin. Pharmacol. Therap., *45*, 608–616.

Furet Y.X., Pechére J.C. (1991): Newly documented antimicrobial activity of quinolones. Eur. J. Clin. Microbiol. Infect. Dis., *10*, 249–254.

Gasser T., Graversen P., Madsen P. (1987): Treatment of complicated urinary tract infections with ciprofloxacin. Am. J. Med., *82* (Suppl. 4A), 278–281.

Giamarellou H., Kolokythas E., Petrikkos G., Gazis J., Aravantino D., Sfikakis P. (1989): Pharmacokinetics of three newer quinolones in pregnant and lactating women. Am. J. Med., *87* (Suppl. 5A), 49–51.

Giles C.J., Magonigle R.A., Grimshaw W.T.R., Tanner A.C., Risk J.E., Lynch M.J., Rice J.R. (1991): Clinical pharmacokinetics of parenterally administered danofloxacin in cattle. J. Vet. Pharmacol. Therap., *14*, 400–410.

Giles C.J., Grimshaw W.T.R., Shanks D.J. *et al*. (1991): The efficacy of danofloxacin in the therapy of acute bacterial pneumonia in housed beef cattle. Vet. Rec., *128*, 296–300.

Goodman L.S., Gilman A. (1992): The Pharmacological Basis of Therapeutics. 8th ed. New York, Macmillan. 1057.

Gootz T.D., McGuirk P.R., Moynihon M.S., Haskell S.L. (1994): Placement of alkyl substituents on the C7 piperazine ring of fluoroquinolones: Dramatic differential effects on mammalian topoisomerase II and DNA gyrase. Antimicrob. Agents Chemother., *38*, 130–133.

Gould I.M., Milne K., Jason C. (1990): Concentration-dependent bacterial killing, adaptive resistance and post-antibiotic effect of ciprofloxacin alone and in combination with gentamycin. Drugs Exp. Clin. Res., *26*, 621–628.

Grasela T.J.Jr., Schentag J.J., Sedman A.J., Wilton J.H., Thomas D.J., Schultz R.W., Lebsack M.E., Kinkel A.W. (1989): Inhibition of enoxacin absorption by antacids or ranitidine. Antimicrob. Agents Chemother., *33*, 615–617.

Greenwood D., Baxter S., Cowlishaw A., Eley A., Slater G.J. (1984): Antibacterial activity of ciprofloxacin in conventional tests and in a model of bacterial cystitis. Eur. J. Clin. Microbiol., *3*, 351–354.

Grimshaw W.T.R., Magonigle R.A., Giles C.J., Tanner A.C., Risk J.E., Lynch M.J., Rice J.R. (1990a): The pharmacokinetics of danofloxacin in cattle. In: XVI World Buiatrics Congress, Salvador, Bahia, Brazil.

Grimshaw W.T.R., Giles C.J., Cooper A.C. *et al*. (1990b): The efficacy of danofloxacin in the therapy of pneumonia associated with *Pasteurella* species in housed calves. Dtsch. Tierarztl. Wschr., *97*, 529–532.

Gulkarov A., Ziv G. (1994): Some pharmacokinetic features of norfloxacin nicotinate in turkeys. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 235.

Gyrd-Hansen N., Nielsen P. (1994): The influence of feed on the oral bioavailability of enrofloxacin, oxytetracycline, penicillin V and spiramycin in pigs. In: Proceedings of the Sixth Congress of the European Associassion for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 242–243.

Harder S., Staib A.H., Beer C., Papenburg A., Stille W., Shah P.M. (1988): 4-Quinolones inhibit biotransformation of caffeine. Eur. J. Clin. Pharmacol., *35*, 651–656.

Hayem G., Petit P.X., LeVacher M., Gaudin C., Kahr M.F., Pocidalo J.J. (1994): Cytofluorometric analysis of chondrotoxicity of fluoroquinolone antimicrobial agents. Antimicrob. Agents Chemother., *38*, 243–247.

Hinz K.H., Rottmann S. (1990): Studies *in vivo* on the efficacy of enrofloxacin against *Mycoplasma gallisepticum*. Avian Pathol., *19*, 511–522.

Höffler D., Dalhoff A., Gau W., Beerman D., Michael A. (1984): Dose- and sex independent disposition of ciprofloxacin. Eur. J. Clin. Microbiol., *3*, 363–366.

Hoogkamp-Korstanje J.A.A. (1984): Comparative *in vitro* activity of five quinolone derivates and five other antimicrobial agents used in oral therapy. Eur. J. Clin. Microbiol., *3*, 333–338.

Hooper D., Wolfson J. (1985): The fluoroquinolones: Structures, mechanisms of action and resistance and spectra of activity *in vitro*. Antimicrob. Agents Chemother., *28*, 581–586.

Hooper D.C., Wolfson J.S. (1991): Mode of action of the new quinolones: new data. Eur. J. Clin. Microbiol. Inf. Dis., *10*, 223–231.

Jackson J.A., Davidson J.N., Ter Hune T.N., Magonigle R.A. (1990): A dose response study of the fluoroquinolone, danofloxacin, against induced bovine pneumonic pasteurellosis. In: XVI World Buiatrics Congress, Salvador, Bahia, Brazil.

Janin N., Meugnier H., Desnottes J.F., Woehrle R., Fleurette J. (1987): Recovery of pefloxacin in saliva and feces and its action on oral and fecal floras of healthy volunteers. Antimicrob. Agents Chemother., *31*, 1665–1668.

Jenkins W., Friedlander L. (1988): The pharmacology of the quinolone antibacterial agents. In: Quinolones: A new class of antimicrobial agents for use in veterinary medicine. Proc. West Vet. Conf, Las Vegas, Nevada: Mobay Corporation Animals Health Division, Shawnee, Kansas. 5–16.

Jordan F.T.W., Horrocks B.K., Jones S.K., Cooper A.C., Giles C.J. (1993): A comparison of the efficacy of danofloxacin and tylosin in the control of *Mycoplasma gallisepticum* infection in broiler chicks. J. Vet. Pharmacol. Toxicol., *16*, 79–86.

Kaatz G.W., Seo S.M., Ruble C.A. (1991): Mechanisms of fluoroquinolone resistance of *Staphylococcus aureus*. J. Infect. Dis., *163*, 1080–1086.

Kato M., Onedara T. (1988): Effect of ofloxacin on the uptake of (3H) thymidine by articular cartilage cells in rat. Toxicol. Lett., *44*, 131–142.

Kempf I., Gesbert F., Guittet M., Bennejean G. (1992): Efficacy of danofloxacin in the therapy of experimental mycoplasmosis in chicks. Res.. Vet. Sci., *53*, 257–259.

Kotera Y., Watanabe M., Yoshida S., Inoue M., Mitsuhashi S. (1991): Factors influencing the uptake of norfloxacin by *Escherichia coli*. J. Antimicrob. Chemother., *27*, 733–739.

Kresken M., Wiedemann B. (1988): Development of resistance to nalidic acid and the fluoroquinolones after the introduction of norfloxacin and ofloxacin. Antimicrob. Agents Chemother., *32*, 1285–1288.

Küng K., Riond J.L., Wolffram S., Wanner M. (1993): Comparison of an HPLC and bioassay method to determine antimicrobial concentrations after intravenous and oral administration of enrofloxacin in four dogs. Res. Vet. Sci., *54*, 247–248.

Lavy E., Ziv G., Glickman A. (1994): Pharmacokinetics of norfloxacin in the donkey (*Equus asinus*). In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 52–53.

Lekeux P., Art T. (1988): Effect of enrofloxacin therapy on shipping fever pneumonia in feedlot cattle. Vet. Rec., *123*, 205–207.

Lode H., Höffken G., Prinzig C., Glatzel P., Wiley R., Olschewski P., Sievers B., Reimnitz D., Borner K., Koeppe P. (1987): Comparative pharmacokinetics of new quinolones. Drugs, *34* (Suppl. 1), 21–25.

Lode H., Höffken G., Boeckk M., Deppermann N., Borner K., Koeppe P. (1990): Quinolone pharmacokinetics and metabolism. J. Antimicrob. Chemother., *26* (Suppl. B4), 1–9.

Ludwig E., Székely É., Csiba A., Graber H. (1988): The effect of ciprofloxacin on antipyrine metabolism. J. Antimicrob. Chemother., *22*, 61–67.

Maesen F.P.V., Davies B.I., Geraeds W.H., Baur C. (1987): The use of quinolones in respiratory tract infections. Drugs, *34* (Suppl 1), 74–79.

Malmborg A.S., Rannikko S. (1988): Enoxacin distribution in human tissues after multiple oral administration. J. Antimicrob. Chemother., *21* (Suppl. B), 57–60.

Martínez-Larrañaga M.R., Díaz M.J., Bringas P., Fernández M.D., Fernández-Cruz M.L., Martínez M.A., Anadón A. (1994): Bioavalability and residues of enrofloxacin and its metabolite ciprofloxacin in broiler chickens. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 238–239.

McQueen C., Williams G. (1987): Effects of quinolone antibiotics in tests for genotoxicity. Am. J. Med., *82* (Suppl. 4A), 94–96.

Mevius D.J., Breukink H.J., Guelen P.J.M., Jansen T., De Gréve B. (1990): Pharmacokinetics, metabolism and renal clearance of flumequine in veal calves. J. Vet. Pharmacol. Therap., *13*, 159–169.

Montay G., Goueffon Y., Roquet F. (1984): Absorption, distribution, metabolic fate and elimination of pefloxacin mesylate in mice, rats, dogs, monkeys and humans. Antimicrob. Agents Chemother., *25*, 463–472.

Neer T.M. (1988): Clinical pharmacologic features of fluoroquinolone antimicrobial drugs. J. Am. Vet. Med. Assoc., *193*, 577–580.

Neu H.C. (1988): Quinolones: A new class of antimicrobial agents with wide potential uses. Med. Clin. North Am., *72*, 623–636.

Neu H.C. (1991): Synergy and antagonism of combinations with quinolones. Eur. J. Clin. Microbiol. Infect. Dis., *10*, 255–261.

Neu H.C., Kumada T., Chin N.X., Mandell W. (1987): The post-antimicrobial suppressive effect of quinolone agents. Drugs Exp. Clin. Res., *13*, 63–67.

Nikaido H., Thanassi D.G. (1993): Minireview: Penetration of lipophilic agents with multiple protonation sites into bacterial cells: Tetracyclines and fluoroquinolones as examples. Antimicrob. Agents Chemother., *37*, 1393–1399.

Nix D.E., Schentag J.J. (1988): The quinolones: an overview and comparative appraisal of their pharmacokinetics and pharmacodynamics. J. Clin. Pharmacol., *28*, 169–178.

Nix D.E., Watson W.A., Lener M.E., Frost R..W., Krol G., Goldstein H., Lettieri J., Schentag J.J. (1989): Effects of aluminium and magnesium antacids and ranitidine on the absorption of ciprofloxacin. Clin. Pharmacol. Therap., *46*, 700–705.

Norrby S.R. (1991): Side effects of quinolones: Comparisons between quinolones and other antibiotics. Eur. J. Clin. Microbiol. Infect. Dis., *10*, 378–383.

Novotny M.J., Shaw D.H. (1991): Effect of enrofloxacin on digoxin clearance and steady-state serum concentrations in dogs. Can. J. Vet. Res., *55*, 113–116.

Okazaki O., Kurata T., Tachizawa H. (1988): Effect of new quinolones on drugmetabolizing enzyme system of rat hepatic microsomes. Chemotherapy, *34*, 149–154.

Oomori Y., Yasue T., Aoyma H., Hirai K., Suzue S., Yokota T. (1988): Effects of fleroxacin on HeLa cell functions and topoisomerase II. J. Antimicrob. Chemother., *22* (Suppl. D), 91–97.

Parpia S., Nix D., Hejmanowski H., Wilton J., Schentag J. (1989): Sucralfate reduces the gastrointetinal absorption of norfloxacin. Antimicrob. Agents Chemother., *33*, 99–102.

Patil V.K., Keskar D.V., Jagadish S., Bhalerao D.P., Sharma L.K. (1995): Clinicopathology of urinary tract infections in dogs. Indian Vet. J., *72*, 374–377.

Pérez-Trallero E., Urbieta M., Jimenez D., Garcia-Arenzana J.M., Cilla G. (1993): Ten-year survey of quinolone resistance in *Escherichia coli* causing urinary tract infections. Eur. J. Clin. Microbiol. Infect. Dis., *12*, 349–351.

Piddock L.J. (1994): New quinolones and Gram-positive bacteria. Antimicrob. Agents Chemother., *38*, 163–169.

Piddock L.J.V., Wise R. (1989): Mechanism of resistance to quinolones and clinical perspectives. J. Antimicrob. Chemo-ther., *23*, 475–483.

Pijpers A., Vernooy J.C.M., Cruijsen A.L.M., Van Leengoed L.A.M.G., Koeman J., Hessels A.H., Vandenhoek J., Verheijden J.H.M. (1994): Efficacy of parenteral treatment with oxytetracycline and enrofloxacin against *Actinobacillus pleuropneumoniae* in swine. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 93.

Preheim L., Cuevas T., Roccaforte J., Mellencamp M., Bittner M. (1987): Oral ciprofloxacin in the treatment of elderly patients with complicated urinary tract infections due to trimethoprim/sulfamethoxazole-resistant bacteria. Am. J. Med., *82* (Suppl. 4A), 295–297.

Prescott J.F., Yielding K.M. (1990): *In vitro* susceptibility of selected veterinary bacterial pathogens to ciprofloxacin, enrofloxacin and norfloxacin. Can. J. Vet. Res., *54*, 195–197.

Pyörälä S., Panu S., Kaartinen L. (1994): Single-dose pharmacokinetics of enrofloxacin in horses. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland. 45–46.

Ramon J., Dautrey S., Farinoti R., Carbón C., Rubenstein E. (1994): Intestinal elimination of ciprofloxacin in rabbits. Antimicrob. Agents Chemother., *38*, 757–760.

Richez P., Dellac B., Froyman R. (1994): Pharmacokinetics of enrofloxacin in pigs after single and repeated in-feed medication with Baytril 2.5%. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology. Edinburgh, Scotland. 207–208.

Ridgway G.L., Mumtaz G., Gabriel F.G., Oriel J.D. (1984): The activity of ciprofloxacin and other 4-quinolones against *Chlamydia trachomatis* and Mycoplasmas *in vitro*. Eur. J. Clin. Microbiol., *3*, 344–346.

Rowan R.C., Mullenix T.A., Arroyo J.C., Voris J.C. (1988): Development of *Pseudomonas aeruginosa* resistance to norfloxacin during therapy. Drug Intellig. Clin. Pharm., *22*, 773–776.

Rybak M.J., Bowles S.K., Chandrasekar P.H., Edwards D.J. (1987): Increased theophylline concentration secondary to ciprofloxacin. Drug Intellig. Clin. Pharm., *21*, 879–881.

Semjén G., Blaskó É. (1994): *In vitro* emergence of resistance of flumequine and enrofloxacin in strains of *Staphylococcus aureus*. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland. 239–240.

Sheer M. (1987): Concentrations of active ingredient in the serum and in tissues after oral and parenteral administration of Baytril. Vet. Med. Rev., *2*, 104–118.

Smith J.T. (1984): Mutational resistance to 4-quinolone antibacterial agents. Eur. J. Clin. Microbiol., *3*, 347–350.

Smith J.T. (1986): The mode of action of 4-quinolones and possible mechanisms of resistance. J. Antimicrob. Chemo-ther., *18* (suppl. D), 21–29.

Soback S., Gips M., Bialer M. (1994a): Norfloxacin nicotinate pharmacokinetics in unweaned and weaned calves. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland. 63–64.

Soback S., Gips M., Bialer M., Bor A. (1994b): Effect of lactation on single-dose pharmacokinetics of norfloxacin nicotinate in ewes. Antimicrob. Agents Chemother., *38*, 2336–2339.

Sorgel F., Seelman R., Naber K., Metz R., Muth P. (1988): Metabolism of fleroxacin in man. J. Antimicrob. Agents Chemother., *22* (Suppl. D), 169–178.

Sorgel F., Jaehde U., Naber K., Stephan U. (1989): Pharmacokinetic disposition of fluoroquinolones in human body fluids and tissues. Clin. Pharmacokin., *16* (Suppl. 1), 5–24.

Studdert V.P., Hughes K.L. (1992): Treatment of opportunistic mycobacterial infections with enrofloxacin in cats. J. Am. Vet. Med. Assoc., *201*, 1388–1390.

Sullivan M.C., Cooper B.W., Nightingale C.H., Quintiliani R., Lawlor M.T. (1993): Evaluation of the efficacy of cipro-floxacin against *Streptococcus pneumoniae* by using a mouse protection model. Antimicrob. Agents Chemother., *37*, 234–239.

Swanson R.N., Hardy D.J., Chu D.T.W., Shipkowitz N.L., Clement J.J. (1991): Activity of temafloxacin against respiratory pathogens. Antimicrob. Agents Chemother., *35*, 423–429.

Takács-Novák K., Noszal B., Hermecz I., Keresztúri G., Podányi B., Szász G. (1990): Protonation equilibria of quinolone antibacterials. J. Pharm. Sci., *79*, 1023–1028.

Takayama S., Watanabe T., Akiyama Y. *et al*. (1986): Reproductive toxicity of ofloxacin. Arzneimittelforschung, *36*, 1244–1248.

Thomas V., Deleforge J., Bolsramé B. (1994a): Pharmacokinetics of marbofloxacin in preruminant and ruminant cattle. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland. 60–61.

Thomas V., Deleforge J., Bolsramé B., Espinasse J. (1994b): Pharmacokinetics of marboflaxicin in healthy and sick pre-ruminant calves. In: Proceedings of the Sixth Congress of the European Association for Veterinary Pharmacology and Toxicology, Edinburgh, Scotland. 61.

Thoung-Guyot M., Domarle O., Pacidalo J.J., Hayem G. (1994): Effects of fluoroquinolones on cultured articular chondrocytes flow cytometric analysis of free radical production. J. Pharmacol. Exp. Therap., *271*, 1544–1549.

VanCutsem P.M., Babish J.G., Schwark W.S. (1990): The fluoroquinolone antimicrobials: Structure, antimicrobial activity, pharmacokinetics, clinical use in domestic animals and toxicity. Cornell Vet., *80*, 173–186.

Venezia R.A., Prymas L.A., Shayegani A., Yocum D.M. (1989): *In vitro* activities of amifloxacin and two of its metabolites. Antimicrob. Agents. Chemother., *33*, 762–766.

Voight W.H. (1987): Electron microscopic studies on the effect of a quinolone carboxylic acid derivative on the ultrastructure of *Escherichia coli* and staphylococcal bacteria *in vitro*. Vet. Med. Rev., *2*, 119–121.

Walker R.D., Stein G.E., Budsberg S.C., Rosser E.J.Jr., MacDonald K.H. (1989): Serum and tissue fluid norfloxacin concentrations after oral administration of the drug to healthy dogs. Am. J. Vet. Res., *50*, 154–157.

Walker R.D., Stein G.E., Hauptman J.G., MacDonald K.H., Budsberg S.C., Rosser E.J.Jr. (1990): Serum and tissue cage fluid concentrations of ciprofloxacin after oral ad-

ministration of the drug to healthy dogs. Am. J. Vet. Res., *51*, 896–900.

Walker R.D., Stein G.E., Hauptman J.G., McDonald K.H. (1992): Pharmacokinetic evaluation of enrofloxacin administered orally to healthy dogs. Am. J. Vet. Res., *53*, 2315–2319.

Watanabe M., Kotera Y., Yosue K., Inoue M., Mitsuhashi S. (1990): *In vitro* emergence of quinolone-resistant mutants of *E. coli, Enterobacter cloaceae* and *Serratia marcescens*. Antimicrob. Agents. Chemother., *34*, 173–175.

Wolfson J.S., Hooper D.C. (1991): Pharmacokinetics of quinolones: newer aspects. Eur. J. Clin. Microbiol. Infect. Dis., *10*, 267–274.

Yamamoto T., Watanabe K., Horikita T. *et al*. (1992): Therapeutic efficacy of enrofloxacin to swine *Actinobacillus pleuropneumoniae* infection. Kachiku Shinryo, *352*, 19–27.

Zweerink M.M., Edison A.M. (1988): The uptake of 3H-norfloxacin by human polymorphonuclear leukocytes. J. Antimicrob. Chemother., *21*, 266–267.

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