A REVIEW OF THE THERAPEUTIC PROSPECTS OF FLUOROQUINOLONES

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Abstract
Fluoroquinolones are a class of antibiotics with potent bactericidal, broad spectrum activity against many clinically important pathogens which are responsible for variety of infections including urinary tract infections (UTI), gastrointestinal infections, respiratory tract infections (RTI), sexually transmitted diseases (STD) and skin infections. They are primarily used against urinary tract infections and are also clinically useful against prostatitis, infections of skin and bones and penicillin resistant sexually transmitted diseases. The growth in understanding of structure activity relationships with fluoroquinolones has enabled the development of even better compounds. The targets in fluoroquinolones research during the last few years include: improvements in pharmacokinetic properties, greater activity against gram-positive cocci and anaerobes, activity against fluoroquinolone-resistant strains, and improvements in activity against non-fermentative gram-negative species. The fluoroquinolones have a relatively simple molecular nucleus, which is can be subjected to many structural modifications. These agents have several favourable properties such as excellent bioavailability, good tissue penetrability and a relatively low incidence of adverse and toxic effects. This paper is an attempt to review the therapeutic prospects of fluoroquinolones antibacterials with an updated account on their development and usage.

Keywords: Fluoroquinolone, antibacterial, ciprofloxacin, pharmacokinetics, therapeutic use.

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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 [43]. 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 [48] as well as intracellular pathogens [39] and trimethoprim/sulfonamide resistant microbes [74]; in addition these antimicrobials are also active against mycoplasmas [20]. Collectively, these compounds are called fluoroquinolones. Although dozens of fluoroquinolones have been synthesized and reported, the most.

HISTORICAL BACKGROUND

The first clinically useful quinolone was nalidixic acid, discovered by Lesher and co-workers in 1962, which was generated from chloroquine, an antimalarial agent [9]. It was active against some Gram negative bacteria and had limited usefulness because of its high protein binding (approximately 90%) and little half life (about 1.5 h) [29]. Unfortunately, bacteria could develop a rapid resistance to this agent.

In 1968, Kaminsky and Melfezer discovered an oxolinic acid, which was lately approved by the United States Food and Drug Administration (USFDA). Since then, extensive efforts has been undertaken for the development and to derive an array of significantly active drugs of this class. Molecular modification for the lead optimization by bioisosteric replacements, homologation of side chain or branching of side chain, stereochemistry and other useful techniques of analogs design and development of fluoroquinolones have given rise to agents with broad spectrum activity and minimum toxic or side effects. Development of new antibiotics has been achieved from derivatives of known antimicrobial agents or by identification of novel agents active against previously unexploited targets.

The development has been focused on the following aspects [9]:

- Increasing activity against resistant strains of microbes, anaerobes and atypical organisms.
- Reducing rate of emergence of resistance and improving pharmacokinetics and pharmacodynamic profile.
- Targeted towards selectivity of drug.
Flumequine was the first fluoroquinolone which was patented in 1973, after that many fluoroquinolones have been patented and are still used today, including norfloxacin (1978), pefloxacin (1979), enoxacin (1980), fleroxacin (1981), ciprofloxacin (1981) and ofloxacin (1982) [9]. An advantage of these compounds over previous ones is their broad spectrum. A big revolution was made in 1980s when an analog of nalidixic acid, enoxacin was derived with significantly increased spectrum of activity against Gram negative or Gram positive bacteria [9]. The most successful and widely used fluoroquinolone, ciprofloxacin was marketed in 1986, and since then the value of fluoroquinolones for the treatment of a wide range of infections have become widely recognized [9]. This class of compounds has enhanced pharmacokinetic properties as well as extensive and potent activities against various parasites, bacteria and mycobacteria, including resistant strains as compared to previously existing bactericidal drugs.

**CLASSIFICATION**

Fluoroquinolones are classified (Table 1) on the basis of their spectrum of activity and their pharmacokinetic profile.

**Table 1: Classification of Fluoroquinolones**

<table>
<thead>
<tr>
<th>Generation</th>
<th>Drug</th>
<th>Characteristic Features</th>
</tr>
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<tbody>
<tr>
<td>First</td>
<td>Nalidixic Acid</td>
<td>Active against some Gram negative bacteria</td>
</tr>
<tr>
<td></td>
<td>Oxilinic Acid</td>
<td>Highly protein bound drug</td>
</tr>
<tr>
<td></td>
<td>Pipermidic Acid</td>
<td>Short life</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Second</td>
<td>Norfloxacin</td>
<td>Protein binding 50%</td>
</tr>
<tr>
<td></td>
<td>Enoxacin</td>
<td>Longer half life than the previous generation</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>Improved activity against Gram negative bacteria</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lomefloxacin</td>
<td></td>
</tr>
<tr>
<td>Third</td>
<td>Temafloxacin</td>
<td>Active against Gram negative bacteria</td>
</tr>
<tr>
<td></td>
<td>Sparafloxacin</td>
<td>Also active against Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td>Grepafloxacin</td>
<td></td>
</tr>
<tr>
<td>Fourth</td>
<td>Clinafloxacin</td>
<td>Show extended activity against both Gram positive and negative</td>
</tr>
<tr>
<td></td>
<td>Trevofloxacin</td>
<td>bacteria</td>
</tr>
<tr>
<td></td>
<td>Moxifloxacin</td>
<td>Active against anaerobes and typical bacteria.</td>
</tr>
<tr>
<td></td>
<td>Gatifloxacin</td>
<td></td>
</tr>
</tbody>
</table>
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 pKa 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 [11]. 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 [81]. 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 [65]. What further complicates the issue is a possibility that initial interaction with membrane surfaces may be mediated by divalent cations [65], 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.
STRUCTURE-ACTIVITY RELATIONSHIP (SAR)

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 [62]. *Streptococcus* can be resistant [15]. 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, [24] 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 [54]. It is apparently due to this property that crystalluria has been observed in man and animals [12].

ANTIMICROBIAL ACTIVITIES

Bacteria possess type II topoisomerase known as DNA gyrase: a tetrameric bacterial enzyme that folds and coils 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 [35]. 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 [64]. The inhibition of resealing leads to the liberation of fragments that are subsequently destroyed by bacterial exonucleases [78]. 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 subunit of DNA gyrase [35], and coumermycin has shown synergy with the fluoroquinolones [35]. 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 [35], 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 [78]. 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 [51] 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 [65]. 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 [28]. This effect is seen during short-term exposures only. The percentage of

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bacteria killed after more than 1.5 hour exposure remains the same at any concentration above the MIC. Interestingly, the inhibition of protein 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 [69]. 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 [35]. However, the increased activity of some fluoroquinolones at the mammalian topoisomerase II enzyme has been associated with genotoxicity [51]. 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 [42]. DNA gyrase is an intracellular enzyme, so the uptake of fluoroquinolones by the bacteria is critically important. The entry into cells is via porins [22], 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 [72]. Accumulation is antagonized by cations such as magnesium and calcium, perhaps by binding to the cell surface resulting from chelation with divalent cations [57]. 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 [63]. The PAE is associated with decreased adherence to cells as a part of the phenomenon. Concentrations as low as 1000 fold less than the MIC have been shown to decrease adherence of Staphylococcus aureus bacteria to buccal cells [27] even though the PAE is concentration dependent [89].
The efflux mechanism described above is depressed during the postantibiotic effect, and can be inhibited by carbonyl cyanide m-chlorophenylhydrazone, which dissipates energy [72]. 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 pneumophila* [21]. Microscopically, the morphologic alterations produced by fluoroquinolones include decreased cell division, filamentation, and cellular lysis [36]. Ultra structurally, 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* [85]. 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.

**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 postantibiotic effect of fluoroquinolones has been shown to be 4–8 h against *Escherichia coli, Klebsiella, Serratia*, and *Pseudomonas aeruginos* [62]. 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* [50]. 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 [90]. Enrofloxacin has structural similarity to ciprofloxacin and has a similar antibacterial spectrum to ciprofloxacin against *Haemophilus* sp., *Pasteurella* sp. And *Actinomyces* sp. [91]. Temafloxacin is more potent than either ciprofloxacin or ofloxacin against staphylococci and streptococci, but not against *Haemophilus influenzae*. Improved oral activity of temafloxacin is a function of both improved potency and better oral bioavailability [92]. The MIC of danofloxacin against 90% of the field isolates of *Pasteurella haemolytica, Pasteurella multocida,*
and *Haemophylus somnus* was found to be \(< 0.125 \mu\text{ml} [53]\), and the range of MICs against Mycoplasma species was 0.008–0.5 \(\mu\text{g/ml} [93]\). 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. [38]. 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* [38]. Recently, pefloxacin, ofloxacin, and ciprofloxacin were found to be active against *Plasmodium*, *Trypanosoma cruzi*, and *Leishmania donovani* although *Toxoplasma gondii* was not susceptible [38]. Many of the newer fluoroquinolones with increased activity against gram-positive bacteria have the lower activity against *Pseudomonas aeruginosa* than older fluoroquinolones [38]. Fluoroquinolones are more active in alkaline environments (pH > 7.4) for gram-negative bacteria [17], but susceptibility of gram-positive bacteria to fluoroquinolones is not affected by pH [35]. Susceptibility is not affected by the inoculum size [37], but activity is reduced by the presence of divalent cations [17]. In general, aminoglycosides, \(\beta\)-lactams, imidazoles, macrolides, and lincosamides infrequently show synergy with fluoroquinolones against Enterobacteriaceae, gram-positive bacteria and anaerobes; but rarely do they show antagonism [63]. 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 [63] in general, fluoroquinolones are antagonistic with chloramphenicol.

**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 [63], with the absolute oral bioavailability of norfloxacin in dogs of approximately 35% [19]. Ciprofloxacin is absorbed primarily from the duodenum and jejunum when administered orally to monogastric animals [87]. 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 [94] or in ciprofloxacin bioavailability in humans on various high fat/high calcium diets [37]. Overall, oral bioavailability of fluoroquinolones ranges from 30–90% in chickens [23]; [95], turkeys [46] and pigs [6]; [94]; [75], although oral availability in donkeys was very low [96]. 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 [70]. 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 [84]. 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 [97]. 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 [41]; although norfloxacin nicotinate availability from intramuscular injection sites was 70–90% [79]. Supra-availability from extravascular routes was observed in horses [73] 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 [45]; [40], and 3.45 ± 0.72 l/kg in horses [98], 1.47 l per kg for norfloxacin in dogs [19] and 0.75–0.96 l/kg for flumequine in calves [99]. 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 [63]. 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 [86]. 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 [1]. High concentrations of fluoroquinolones are achieved in saliva, nasal secretions and nasal mucosa, and bronchial epithelium [63], although these are not substantially higher than concurrent plasma concentrations. In fact, nasopharyngeal concentrations of ciprofloxacin were much higher than the MIC90 for meningococci and H. influenzae, but they were below the MIC for methicillin- resistant Staphylococcus aureus in human patients [100].

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 [77]. Similarly, danofloxacin lung homogenate concentrations over time were 3.5–4.5 times higher than the concurrent plasma concentrations [40]. 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 [8]. 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 [101]; 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 [77].

Concentrations of enoxacin in the skin were almost equal to concurrent plasma concentrations after multiple oral dosing [102]. 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 [26]. 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 [26].

Enrofloxacin showed similar penetration into the prostatic fluid and tissue in dogs, that means both were higher than concurrent serum concentrations [31] 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 [14]. 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 enorfloxacin/kg [77]. In the cortical bone, enorfloxacin activity reached 29% of the concurrent serum activity [34] although it must be reminded that enorfloxacin 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 [87]. There is approximately 16 times higher placental transfer of enorfloxacin than of ciprofloxacin in rabbits [10], 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 [79]. Enrofloxacin penetrates into milk to attain approximately twice the maximum concentration of ciprofloxacin at similar plasma concentrations, although the elimination of enorfloxacin from milk is approximately twice as fast as that for ciprofloxacin [103]. Penetration into the CNS is relatively good, and vitreous humor penetration is approximately 20% [13]. Apart from nasal secretions [29] and ejaculate, body fluid concentrations of fluoroquinolones rarely reach plasma concentrations [80]. Thus, the high tissue concentrations are a result of sequestration onto, or within, cells or cellular components of a tissue although [21] 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 [104].

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 [105]. Other prominent metabolic pathways include oxidation to oxo-metabolites at the piperazine ring [66], the major metabolites of ciprofloxacin, enoxacin, and norfloxacin [104]: [6]. 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 [106] whereas the glucuronide conjugates are devoid of any activity [66]. Other metabolic pathways
include sulfoxidation and acetylation [104]. 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 [61]. For example, biliary excretion of the pefloxacin glucurononoconjugate is high in dogs and rats relative to all other species [61]. 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 [66]. 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 [66]. 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 [87]. 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 [32]. Probenecid blocks the renal tubular secretion of norfloxacin and ciprofloxacin but because of the other routes of excretion, no large drug accumulation occurs [87]. Renal excretion accounts for 100% of cinoxacin (a non-fluoroquinolone) in 24 h [31], 60% of ciprofloxacin in 24 h in many species but only 30–40% in dogs [1] and 30–40% of norfloxacin and enrofloxacin in 24 h. In normal animals, the biological half-life (t1/2) of most fluoroquinolones ranges from 3 to 6 hours specifically; the t1/2 of flumequine is 6–7 in calves [99], 3.5–4.5 h for danofloxacin (IM.SC. or IV) in calves [45], 5.4 ± 0.9 h for enrofloxacin in calves [77], 2–4 h for ciprofloxacin in dogs [2] and horses [98], 3.6 h for norfloxacin (IV) in dogs [19], 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 [84]. Fleroxacin has an elimination half-life of 1.6 hours in the rabbit, 9.4 hours in the dog [80] and 10.8 in man [82]. Upon multiple dosing, ciprofloxacin, enoxacin and other fluoroquinolones have shown an increase in the t1/2 and increased Vd from the first dose [66]; however, this phenomenon was not observed for norfloxacin in dogs using a dosage regimen of 5 mg/ kg every 12 h for 14 days [19] nor for ciprofloxacin in other studies [49]; [32] nor in dogs.
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 [99]. The multiple dose phenomenon described by [66] and the non-linearity of the AUC with increasing doses in dogs observed, [19] 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 [49] although individual fluoroquinolones may vary depending on the metabolic pathways and routes of excretion.

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 [12]. Embryonic losses in female monkeys exposed to very high doses were described [62]. Toxicity of the fluoroquinolones is mild at therapeutic doses, and generally consists of gastrointestinal disturbances such as nausea, vomiting and diarrhea [67]. At slightly higher doses, CNS signs of dizziness, restlessness, headache, depression, somnolence or insomnia may be seen [63]. 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 [19]. 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 [3] reported that the epileptogenic activity of fluoroquinolones possibly relates to the γ-aminobutyric acid (GABA)-like structures of the substituent 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 [84]. 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 [56], causing lameness and pain severe enough to impose humanitarian euthanasia ([59]; [56]). Kato and

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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 ([48]; [83]). These erosions are preferentially located at weight bearing joints [63]. 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 [15]. Although the basis for that recommendation has been made with very little published supporting informations. Photosensitization occurs with all marketed fluoroquinolones, especially pefloxacin, although it is rare for norfloxacin and ciprofloxacin [63; 67]. Topical administration to the eye shows less toxicity to the corneal epithelium than aminoglycosides [25]. However, ocular cataracts have been seen with prolonged use in humans [63]. 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 [4]. 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.

**DRUG INTERACTIONS**

The only possible drug interaction study that has been documented in animals is lack of effect of enorfloxacin on digoxin steady-state concentrations in dogs [107]. The following findings have been documented only in human studies. The oral absorption of fluoroquinolones is drastically decreased by antacids containing magnesium and aluminium [66], and other agents such as sucralfate also decrease the absorption of fluoroquinolones. Ranitidine did not alter the oral
absorption of ciprofloxacin [66] but it decreased the oral bioavailability of enoxacin [44], suggesting that gastric pH affects the oral absorption of some fluoroquinolones, perhaps through alterations in dissolution. After repeated administration the fluoroquinolones, including enorfloxacin, have been shown to decrease the hepatic clearance and to increase the elimination half life of theophylline [76; 18] and caffeine [47] 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 [108]. However, others have stated that oral doses of ofloxacin, enoxacin and norfloxacin showed no significant effect on the content of cytochrome P450, cytochrome b5, NADPH- cytochrome P450 reductase, ethoxycoumarin O-deethylase, benzphetamine N-demethylase, or aniline hydroxylase in phenobarbital- responsive systems [68]. Furthermore, the clinically important drug-drug interactions between theophylline and ofloxacin were not shown in several instances [87]. 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 [87]. 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 [87]. No drug-drug interaction studies have been published for danofloxacin.

**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 [60], complicated and uncomplicated urinary tract infections, *Salmonella* spp. infections, and other infections, such as otitis externa and

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ophthalmitis, which are resistant to agents [13]. 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, enorfloxacin, marbofloxacin, norfloxacins, 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 [110]; prostatitis [31], and osteomyelitis [34] caused by susceptible bacteria. Higher recommended doses were calculated on the basis of an assumption that the concentrations of quinolones must exceed the MIC90 for the entire dosing interval [86], this was later shown to be an incorrect assumption [32; 111]. 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 [15]. 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 [15]. Clinical field studies were conducted with enrofloxacin and difloxacin in swine colibacillosis, poultry colibacillosis, and other poultry bacterial and mycobacterial diseases, with therapeutic success [15]. 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 [53]. Other efficacy studies with danofloxacin brought about promising results for poultry mycoplasmosis [55]. 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 [113]. Efficacy rates of enrofloxacin for treating pneumonia and diarrhea in cattle and swine are from 76% to 100% [58; 88], those of danofloxacin for cattle and swine pneumonia from 83% to 86% [45; 41]. Enrofloxacin decreases mortality rates in poultry flocks with respiratory
infections [114], 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% [16], and in another study the urinary tract infection [71]. 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.

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.

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