REVIEW ARTICLE

Development of Fluroquinolones as a potent antibacterial agents:A Review

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ABSTRACT

The fluoroquinolones are a series of synthetic antibacterial agents that are used in the treatment of 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 exo-nucleases. 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.

Keywords : Quinolones, Fluroquinolones, Antibacterial activity.

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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 [1].

The discovery of the fluoroquinolones (FQs) during the 1980s improved the treatment of infectious diseases, due to their fewer toxic side effects when compared with the existing drugs [2-4].Fluoroquinolones have gained stupendous importance during the last two decades because of their potent anti-bacterial activity against wide varieties of gram positive and gram-negative pathogenic bacteria with minimum toxic side-effects and somewhat different mechanism of action than other available antibacterial drugs. Over the last two decades, research on 4-quinolone-3-carboxylates has led to the discovery of a family of 6-fluoro-7-piperazinyl-4quinolones active against gram-negative and gram-positive bacteria *in vitro* as well as intracellular pathogens and tri methoprim/sulfonamide resistant microbes in addition these antimicrobials are also active against mycoplasma. 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 norfloxacinnicotinate, 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 fluoroquinolonesare bactericidal and all acts against the same bacterial target: the bacterial DNA gyrase (type II topoisomerase).No plasmidic resistance against them has been demonstrated. However, after invitro experimental selection or clinical administration, resistant mutants have been isolated. These isolated mutants show cross reactivity for the different quinolones and fluoroquinolonesbut no cross reactivity with other antimicrobial families.

Bacterial Resistance

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

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 *Shigelladysenteriae*in Bangladesh. Plasmid-mediated resistance was not demonstrated in fluoroquinolones. The bacteria that

contain R-plasmids carrying resistance to other antibacterial agents remain sensitive to many of the fluoroquinolones. 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. Single-step resistance to fluoroquinolones occurs in10^{-7}–10^{-10} bacteria, with mutations in certain bacteria (e.g. *Enterobacter cloacae* and *Serratiamarcescens*) developing at higher frequencies than in others. The frequencies of these mutations suggest a single mutation at a single locus .When resistance does occur, cross resistance between fluoroquinolones is generally observed to occur at higher frequencies for the older.

More recently, resistance has been reported most often for *Pseudomonas aeruginosa*, *Serratiamarcescens*, 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. The MIC values increased in the anaerobes although the anaerobic bacteria were not considered initially susceptible to fluoroquinolones. Resistance has developed to some of the fluoroquinolones during clinical use in humans, as evidenced by an increased MIC observed in *Streptococcus pneumonia* and *Pseudomonas aeruginosa*isolates from human patients with chronic respiratory infections treated with enoxacin, pefloxacin or norfloxacin. 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   It is clear that resistance to any class of antimicrobial agents increases as the level of use increases due to selective pressure. 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 [6].

Antimicrobial Activities

Bacteria possess type II topoisomerase known as DNA gyrase: a tetrameric bacterial enzyme that folds and coils1.0-0.3 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 [7]. 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 bothstrands 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 The inhibition of resealing leads tothe liberation of fragments that are subsequently destroyed by bacterial exonucleases [8]. DNA gyrase has also been described as working in ayinyang mechanism with topoisomerase I where fluoroquinolones inhibit DNA replication by stimulating topoisomeraseI resulting from the

inhibition of DNA gyrase. Coumermycin and novobiocin act on the B subunitof DNA gyrase and coumermycin has shown synergy with the fluoroquinolones. 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 confershyper-susceptibility to the of DNA gyrase. Coumermycin and novobiocin act on the B sub-unit of DNA gyrase 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 [9].

Sigmoidal fluoroquinolone binding kinetics suggests that four molecules (two pairs with opposing orientation and stacked above or below each other) can stereochemicallyfit into the pocket, acting co-operatively to inhibit DNA gyrase in a similar fashionto the co-operative binding of four oxygen moleculesto hemoglobin. The result is rapid bactericidal activity atrelatively low concentrations. The rate of bacterial cell maybe accelerated if substituent 7 becomes a weaker base orif the carboxyl group becomes a stronger acid. 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 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 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 theinhibition of de novo synthesis of exonucleases. It is unlikelythat the accidental overdosage of a treated animalwould 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 fluoroquinolonesto 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. This results in a favorable margin of safety for fluoroquinolones.Mammals have an enzyme that makes couple-strandedcuts in DNA, similar to DNA gyrase, but it does not supercoil DNA and is not affected by fluoroquinolones. However, the increased activity ofsome fluoroquinolones at the mammalian topoisomerase II enzyme has been associated with genotoxicity.

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. DNA gyrase is an intracellular enzyme, so the uptake of fluoroquinolones by the bacteria is critically important.The entry into

cells is via porins, with subsequent entry across thecytoplasmic 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 concentrationis obtained. Accumulation is antagonized by cations such as magnesium and calcium, perhaps by binding to the cell surface resulting from chelation with divalent cations. For gram-positive bacteria, an energy-dependent efflux transport system, similar to the tetracycline pump mediated by the Tet A 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*, *Klebsiellapneumoniae*,and *Pseudomonas aeruginosa*. The PAE is associated with decreased adherence to cells as apart of the phenomenon. Concentrations as low as 1 000 fold less than the MIC have been shown to decrease adherenceof *Staphylococcus aureus*bacteria to buccal cells even though thePAE is concentrationdependent. The active efflux mechanism described above is depressed during the postantibioticeffect, and can be inhibited by carbonyl cyanidem-chlorophenylhydrazone, which dissipates energy. The inhibition of efflux mechanism resulting 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* Microscopically, the morphologic alterations producedby fluoroquinolones include decreased cell division, filamentation,and cellular lysisUltrastructurally,altered cell division is also evident, and bacterialcell "ghosts", i.e. remnants of the outer bacterial cell wall without internal cell components, are prominent after enrofloxacintreatment of bacterial cultures *in vitro* These observations may be the result of the cascade of events resulting from the inhibition of DNAgyrase leading to general bacterial cellular dysfunction,disruption of normal cellular replication and repair processes.

Appropriate Fluoroquinolone Selection: Pharmacokinetic and PharmacodynamicConsiderations

Pharmacokinetic properties, including the concentration of drug in the serum over time (area under the curve [AUC]) and the peak serum concentration of the drug (Cmax), can be measured, and when considered in combination with in vitro activity, may be useful for predicting microbiologic success and clinical outcomes. In particular, the ratio of the Cmax to MICor AUC to MIC (AUIC) can be predictive of drug efficacy, although which parameter is most predictive of clinical outcome is the subject of some disagreement. Generally, the higher the ratio, the better the outcome.

Resistance Selection in vitro: Mechanisms and Implications

Pathogenic bacteria employ a variety of strategies to persist and replicate under adverse conditions such as exposure to an antimicrobial agent. The efflux pump system is a mechanism that allows immediate survival of bacteria in the presence of an antimicrobial agent by actively expelling that agent across the cell membrane, thereby reducing the intracellular

concentrations to sublethal levels. The pump's action is dependent on the antimicrobial's ability to bind to the bacteria.

While fluoroquinolones are generally concentrationdependent bactericidal agents, differences in antibacterialactivities exist among class members. Fluoroquinolones also differ in pharmacokinetic parameters, such as Cmax and AUC. These efficacy parameters, as they relate to *S.pneumoniae*and *P. aeruginosa,* for ciprofloxacin, levofloxacin, moxifloxacin, and gatifloxacin. Cmax/MIC and AUIC are highest for ciprofloxacin against *P.aeruginosa;* against *S. pneumoniae,* these values are highest with moxifloxacin.

Although AUC/MIC and Cmax/MIC ratios are useful for predicting antimicrobial efficacy, they may not be as useful for predicting the potential for drug resistance to develop. In this regard, Thomas et al. suggest that AUC/MIC should exceed 100 for gram-positive and gram-negative species to prevent resistance selection.

Alternatively, Zhao et al. have hypothesized that the rate at which resistance develops to a fluoroquinolone isrelated to its MICs and mutant prevention concentrations. Studies involving a range of bacterial species suggest that the concentration to prevent mutant emergence in the clinical setting can be derived in vitro and is 2 to 4 times higher than the MIC for most fluoroquinolones however, the clinical significance of these findings has not been clearly established. Derivation of the mutant prevention concentrations is a process involving spreading a high bacterial load onto a series of agar plates in which various concentrations of antimicrobial have been incorporated. The density of 1010 CFU/mL was selected to pinpoint frequency of mutation at levels of 10-7, 10-8, and 10-9, as well as to model the bacterial load at the site of infection. The inoculated plates are incubated overnight and the MIC of surviving colonies determined. This method has been applied to two species, *S. pneumonia* and *P. aeruginosa,* for several fluoroquinolones. Moxifloxacin exceeds the mutant prevention concentrations for *S. pneumoniae,* and ciprofloxacin exceeds the mutant prevention concentration for *P. aeruginosa*(both, 2 mg/L) by achieving maximum serum concentrations of 4.5 mg/L and 3.0 mg/L, respectively. These serum concentrations significantly exceed the mutant prevention concentrations; therefore, these agents are postulated to prevent mutant selection of *S. pneumoniae*and *P. aeruginosa,* respectively. Levofloxacin does not ume of usage exceeds that of 50 DDD/1,000 patients, a thresholdsuggested by Austin et al. as a predictive driver inselecting for antimicrobial resistance during a 2-year period.

Zambrano et al. at the same institution, recently reported a significant correlation between increased levofloxacin use and declining fluoroquinolone susceptibilities among ICU isolates of *K. pneumonia* (96% to 79% [p<0.008]) and *P. aeruginosa* (82% to 67% [p<0.01]). Similarly, another group reported that after levofloxacin was added to the formulary, levofloxacin use as a proportion of total fluoroquinolone use increased from <2% to >22% over a 6-month period (from 3rd quarter 1999 to 1st quarter 2000). During the period of 1st quarter 1998 to 2nd quarter 2000, the susceptibility of *P. aeruginosa*to ciprofloxacin decreased by 11% (82% to 71%). The use of parenteral antipseudomonal agents such as gentamicin, imipenem, ceftazidime, and piperacillin/tazobactam increased concurrently, suggesting that physicians

began using non-fluoroquinolone combination therapy when treating serious gram-negative infections. Furthermore, the antimicrobial cost reductions anticipated from switching to a less expensive fluoroquinolone on formulary were not realized. In 3rd quarter 2000, levofloxacin was replaced with ciprofloxacin as the main gram-negative fluoroquinolone, a substitution associated with a subsequent 6% increase in ciprofloxacin activity against *P. aeruginosa* during the next year. Because the ICU has been a focal point of antimicrobialresistance, the Centers for Disease Control and Prevention initiated.

Project ICARE in 1996. Specific data regarding fluoroquinolone use and fluoroquinolone susceptibility among *P.aeruginosa* isolates were presented for the period 1996–1999 by Hill et al. No correlation was found between prevalence of quinolone resistance and total use of ciprofloxacin/ ofloxacin. However, significant associations were found between fluoroquinolone resistance and combined use of ciprofloxacin, ofloxacin, and levofloxacin (p<0.019) and by use of levofloxacin alone (p<0.006). Likewise, recent studies suggest that using a less potent fluoroquinolone against *S. pneumoniae*for treating community and hospital respiratory tract infections may be affecting.

Clinical Consequences of in appropriate Fluoroquinolone Use

In appropriate use of antimicrobial agents has been associatedwith adverse consequences, including therapeutic failure, development of resistance, and increased health-care costs. One example of a mismatch between pharmacodynamics and clinical infection was in the use of ciprofloxacin for community- acquired pneumonia. The pharmacodynamics of the dose typically prescribed in these cases (ciprofloxacin 250 mg b.i.d.) are inappropriate for treating pneumococcal pneumonia, especially in seriously ill patients . By 1994, approximately 15 cases of *S. pneumoniae*infections that did not respond to ciprofloxacin had been reported, primarily in seriously ill patients and associated with contraindicated medications and other important medical issues. These events prompted the U.S. Food and Drug Administration to modify the package insert to warn against empiric use of ciprofloxacin for respiratory infections in which *S. pneumoniae*would be a primary pathogen. Consequently, ciprofloxacin has been used less frequently in these types of infections. By contrast, >50% of levofloxacin use has been for the treatment of respiratory infections. Since 1999, at least 20 case reports of pulmonary infections that did not respond to levofloxacin therapy have been published. Three of the patients died due to fulminant pneumococcal infections that were unresponsive to levofloxacin therapy at approved dosage. Very few of these cases were in immunosuppressed patients. Reports of pneumococcal failures on the standard dosage of levofloxacin, 500 mg every 24 h, have also been described in two clinical trials, one in a patient with acute exacerbation of chronic bronchitis and the other in a patient with community-acquired pneumonia. In some of the 21 case reports, the treatment failed, and the pathogen developed levofloxacin resistance during therapy.

Recently published details of four cases of pneumococcal pneumonia in which levofloxacin therapy failed. Two of the patients had no history of prior fluoroquinolone use and were levofloxacin susceptible beginning therapy, but their *S. pneumoniae*isolates were levofloxacin

in some patients with community-acquired pneumonia Though increased use of these agents would be expected to lead to increased resistance, a targeted approach to fluoroquinolone prescribing, emphasizing their appropriate use, may reduce development of antimicrobial resistance and maintain class efficacy.

CONCLUSION

The fluoroquinolone class of antimicrobial agents is beingincreasingly used empirically as resistance has developed to the more traditional antimicrobial agents. Guidelines now recommend fluoroquinolones as first-line empiric therapy for urinary tract infections in regions were trimethoprim sulfamethoxazole resistance is >10% to 20% (28), and fluoroquinolones are recommended as alternative empiric regimensresistant mutants; and 3) the inability to readily detect and respond to changes in antimicrobial susceptibilities. Traditionalreporting of susceptibility data may be misleading andmay not readily identify initial changes in resistance pattern or differences between agents of the same class.

To preserve fluoroquinolone activity, the activity of these agents must be continually assessed, and these agents must be used appropriately. The individual attributes of a given drugshould be matched with the likely pathogen at specific infectivesites. Expecting a single fluoroquinolone to be suitable for all infections is unreasonable, and excessive use of any single fluoroquinolone for all indications will lead to resistance thatwill adversely affect the entire class.

Given the defined strategy of selecting the agent with the best pharmacokinetic and pharmacodynamic profile against the known or suspected pathogen, an appropriate therapeutic choice for most serious infections, such as nosocomial pneumonia in which *P. aeruginosa*is a known or suspected pathogen, would currently include ciprofloxacin in combination with an antipseudomonalβ-lactam or an aminoglycoside antibiotic. This recommendation is based on the lower MIC90 andmutant prevention concentrations for this fluoroquinoloneagainst *P. aeruginosa*and higher Cmax/MIC and AUC/MIC ratios compared to other members of the class. Likewise, formost other gram-negative infections of the skin and urinary tract, including *P. aeruginosa*infections, ciprofloxacin monotherapyis appropriate. Ciprofloxacin, levofloxacin, and gatifloxain all achievehigh concentrations in urine; thus, they would all be appropriate choices for treating urinary tract infections in the community. Ciprofloxacin would be the most appropriate therapy incases where *P. aeruginosa*is a known or suspected pathogen. For other gram-negative infections, levofloxacin or gatifloxacin should be prescribed in appropriate doses to surpass the mutant prevention concentrations at the infection site.

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