Pharmacodynamics of Quinolone Antimicrobial Agents

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Pharmacodynamics describes the relationship between measures of drug levels in serum and tissue fluids and the antimicrobial and toxicologic effects of drugs (5, 10, 17, 49). While there have been numerous studies describing the pharmacokinetics of fluoroquinolones in serum and tissues, there has been much less emphasis on describing the time course of antimicrobial activity with these drugs. The MIC and minimal bactericidal concentration have been the major measures of the antimicrobial effect of the quinolones. Although these parameters are useful in defining the potency of the drug against a particular pathogen, they do not provide any information on the time course of antimicrobial activity. Other measures, such as the rate of bacterial killing with increasing concentrations of drug and persistent effects that last after antimicrobial exposure, provide a much better description of the time course of antimicrobial activity. This chapter focuses on the pharmacodynamic characteristics of the quinolone antimicrobials in in vitro models, in animal infection models, and in humans. We hope to demonstrate that there are many more similarities in results among the various models than there are differences.

TIME COURSE OF ANTIMICROBIAL ACTIVITY

Bacterial Killing

Numerous in vitro and in vivo studies have demonstrated that the quinolone antimicrobial agents exhibit concentration-dependent killing across a wide range of concentrations (2, 5, 8, 13, 18, 28, 31, 47). Thus, increases in the dose and concentration of the drug will result in faster and more extensive killing of bacterial organisms. Killing of some organisms such as Streptococcus pneumoniae is exceedingly rapid, and some investigators have suggested that the quinolones do not produce concentration-dependent killing with these organisms. However, in vivo studies, where the growth and killing are somewhat slower, have still demonstrated concentration-dependent killing for the quinolones with S. pneumoniae (2, 4, 7, 13).

A variety of different parameters have been used to characterize the killing characteristics of the quinolone antimicrobials. Three major types of analysis have been used (20, 33). Some measures reflect the time to a certain magnitude of organism reduction (e.g., 2 log kill and 3 log kill), while others measure the magnitude of log reduction at specific times (e.g., 12 or 24 h). Others depend on changes in bacterial numbers over time and are referred to as integrated measures. These would include the slope of the kill curve, area above or under the kill curves, and the "intensity of effect." With all of these measurements, increasing concentrations of the fluoroquinolones have resulted in a greater antimicrobial effect.

Persistent Effects

There are a variety of in vitro and in vivo persistent effects that have been characterized for the quinolone antimicrobial agents (11, 37, 38, 39, 48). The in vitro postantibiotic effect determines the delay in organism regrowth after short periods of drug exposure. Multiple studies with the fluoroquinolones with both gram-positive cocci and gram-negative bacilli have demonstrated in vitro postantibiotic effects (PAEs) of short to moderate duration (1 to 3 h) (11, 39). Much longer PAEs have been observed for fluoroquinolones with some slowly growing organisms such as mycoplasmas and Legionella pneumophila (11).

Further exposure of organisms during the PAE phase to sub-MIC concentrations can greatly
enhance the duration of the PAE (37, 38). For example, several gram-positive cocci and gram-negative bacilli in the PAE phase following exposure to trovafloxacin and grepafloxacin for 2 h at 10 times the MIC were placed in drug-free broth or exposed to drug concentrations at 3/10 of the MIC. The in vitro MIC was increased 2- to 19-fold by the sub-MIC concentrations.

In vivo PAE studies combine the effects of supra-MIC and sub-MIC concentrations (2, 4, 7). Studies in the neutropenic murine thigh infection model with various fluoroquinolones have exhibited in vivo PAEs of 1.5 to 5 h with both gram-positive cocci and gram-negative bacilli. The in vivo PAE can be significantly enhanced by the presence of neutrophils. Studies with ciprofloxacin and a strain of *Klebsiella pneumoniae* showed that an in vivo PAE of 2.4 h in neutropenic mice was increased to 7.5 h in normal mice (11). Recent studies with several fluoroquinolones have demonstrated that the duration of the in vivo PAE with a strain of *S. pneumoniae* is even more enhanced by the presence of neutrophils (more than 10-fold) than observed with *K. pneumoniae* (27).

**PK/PD PARAMETERS**

By using the MIC as a measure of potency, specific pharmacokinetic/pharmacodynamic (PK/PD) parameters have been identified to correlate measures of drug exposure with antimicrobial activity (10). The duration that concentrations exceed the MIC and peak and area under the curve (AUC) to MIC ratios have been the primary PK/PD parameters evaluated. However, identifying the major PK/PD parameter correlating with efficacy is complicated by the high degree of interdependence among these parameters. Comparing the effects of dosing regimens using different dosing intervals or half-lives of the drug have been able to reduce much of the interdependence among PK/PD parameters.

**In Vitro Models**

Studies using a combination of dose escalation, dosage regimen fractionation, and strains with different MICs have suggested that the AUC/MIC ratio is the major PK/PD parameter determining efficacy of fluoroquinolones against *Pseudomonas aeruginosa* and *S. pneumoniae* (28, 35). However, there are some differences, depending on which measure of antibacterial activity is used in the in vitro model. With fluoroquinolones initial killing as measured by the time-to-a-3-log kill was best related to the peak/MIC ratio, whereas regrowth and the intensity of effect were related more to the time above MIC (8, 20). When areas under and above the killing curve were used, the AUC/MIC ratio was the best parameter. Peak/MIC ratio has also been more important in dose-fractionation studies for organisms for which resistant subpopulations can readily emerge (8).

**Animal Models**

Dose-fractionation studies at several different total doses have been used to identify the primary PK/PD parameter determining in vivo activity (3). Studies in the neutropenic murine thigh infection and pneumonia models have consistently demonstrated that the 24-h AUC/MIC ratio best correlates with in vivo activity (2, 5, 10, 29, 30). The peak/MIC ratio also tends to show a reasonable correlation, while time of MIC does not. This is illustrated in Fig. 1 for trovafloxacin against *S. pneumoniae* ATCC 10813.

Drusano et al. used survival as an indicator of efficacy in rats infected with *P. aeruginosa* (18). The efficacy of lomefloxacin was related to the peak level when the peak/MIC ratio was greater than 10 and related to the AUC when the peak/MIC ratio was less than 10. This is similar to that reported in in vitro models that use strains from which resistant subpopulations can emerge. Thus, high peak concentrations appear to be important in preventing the emergence of resistant subpopulations of bacteria.

**Human Studies**

Although pharmacodynamic studies have been performed in humans, none of these studies have used dose fractionation to reduce the interdependence among the various parameters. For example, in the study with levofloxacin by Preston et al., all three PK/PD parameters were highly correlated with outcome, with P values from <0.001 to 0.006 (41).

**MAGNITUDE OF PK/PD PARAMETER REQUIRED FOR EFFICACY**

**In Vitro Studies**

The magnitude of the AUC/MIC ratio required in in vitro models depends markedly on which measure of antibacterial activity is used. AUC/MIC ratios greater than 40 generally result in 3 to 4 log reductions, while values greater than 250 are required for the lowest areas under the kill curve (20, 28, 34, 50, 51). However, it is not yet known which in vitro measure of antibacterial activity best correlates with drug efficacy in animals or humans.
Many of the animal infection models that use bacterial numbers as an indicator of efficacy have also not fully established a relationship between the reduction in organisms after short-treatment times with survival after more prolonged durations of therapy. Studies in our own laboratory using the neutropenic murine thigh infection and pneumonia models have demonstrated that the static dose (i.e., the dose producing no net change in bacterial numbers) after 24 h of therapy is very similar to the dose protecting 50% of the animals from death (i.e., PDso) following 4 to 5 days of therapy (5, 14). However, the degree of bacterial reduction at 24 h required to produce 90 to 100% survival has not yet been established and appears to vary depending on the type of organism. Still the static dose after 24 h of therapy has been useful to identify differences in the magnitude of the PK/PD parameter for various quinolones, dosing intervals, organisms, and sites of infection.

Fantin et al. used the neutropenic murine infection model to demonstrate with pefloxacin a linear relationship between the static dose and the drug's MIC (19). Because the slope of this relationship was approximately 1, the 24-h AUUMIC ratio for the static dose is a parameter that allows one to compare the activity of different quinolones against various bacterial pathogens. The 24-h AUUMIC values for most organisms have varied from 10 to 70 (5, 7, 10). Initial analysis suggested that the mean values were approximately 25 for gram-negative bacilli and 50 for gram-positive cocci. However, more recent studies with the newer fluoroquinolones and desfluoroquinolones have exhibited relatively similar values for Enterobacteriaceae, Staphylococcus aureus, and S. pneumoniae.

The impact of drug resistance on the magnitude of the 24-h AUUMIC ratio has been assessed primarily in S. pneumoniae. Studies with several of the newer fluoroquinolones have demonstrated that the static dose generally rises to the same degree as the MIC in ciprofloxacin-resistant strains (2, 4). Thus, the 24-h AUUMIC does not change for these more resistant organisms. However, studies with gemifloxacin against three strains with higher MICs due to efflux did not show much of an increase in the static dose (4). These initial data suggest that the efflux mechanism of resistance in pneumococci may not be as active in vivo as it is in broth. Similar differences between in vivo and in vitro resistance have been observed in strains of P. aeruginosa expressing the MexEF pump (25).

Differences in the 24-h AUUMIC values for the static doses among the various fluoroquinolones have been small unless there is significant protein binding. As shown in Fig. 2, the magnitude of the 24-h AUC for the static doses of the lowly bound sitafloxacin and gatifloxacin against six strains of S. pneumoniae were significantly lower than those for the highly bound gemifloxacin (2, 4, 7). However, similar values were observed when free drug levels of gemifloxacin were used to calculate the 24-h AUUMIC ratios for the static doses. Similar differences have been observed between garenoxacin with
Figure 2. Relationship between the magnitude of the 24-h AUC/MIC for the static doses of the lowly protein-bound sitafloxacin and gatifloxacin and the highly protein-bound gemifloxacin against six strains of *S. pneumoniae* in a murine infection model.

about 80% binding in mice and another desfluoroquinolone (PGE-9509924) with low protein binding (6, 15). These studies strongly suggest that free drug concentrations should be used for calculating the magnitude of PK/PD parameters.

Since the AUC is the primary determinant of in vivo activity, one would not expect that the dosing regimen would be an important determinant of the antibacterial activity of quinolones. The static dose for 24-h dosing regimens tends to be higher that those observed at 1- to 12-h dosing intervals (2, 4, 7). Because of the rapid half-life of these drugs in small animals, the duration of persistent effects is rarely long enough to span the time that serum drug concentrations are below the MIC when these drugs are administered once daily. Similar effects have been observed with fluoroquinolones and desfluoroquinolones in animal models of meningitis (32, 42). Thus, dosing regimens of 12 h or less are necessary to accurately establish the magnitude of the 24-h AUUMIC ratio in small animals.

We have also used the murine thigh infection and pneumonia models to compare the magnitude of the 24-h AUC/MIC for the static doses at different sites of infection and in the presence and absence of neutrophils (2, 4, 7). Very similar values have been observed in the thigh and lung models for multiple fluoroquinolones against a strain of *K. pneumoniae* that grows well in both tissues (2, 4, 7). The presence of neutrophils (i.e., values in normal mice compared with neutropenic mice) reduces the static dose about 25 to 50% when mice are infected with *K. pneumoniae*. However, marked reductions in the static dose (80 to 90%) are observed by the presence of neutrophils in mice infected with *S. pneumoniae*. These differences are largely due to differences in the enhancement of the in vivo PAE by the presence of neutrophils, as discussed previously (27). The presence of neutrophils also slightly increases the extent of killing of the pneumococcus but not of *K. pneumoniae*.

Many other animal studies of fluoroquinolones have used survival rather than bacterial numbers as a measure of outcome. For those studies that also give the pharmacokinetics of the drug, one can calculate the 24-h AUUMIC ratio for each dose studied. Fig. 3 examines the relationship between the 24-h AUUMIC ratio and mortality in various experimental infections (S, 13). The great majority of these studies were in neutropenic or other immunocompromised animals. Many of the studies that use survival as an outcome follow mortality for prolonged periods of time after the end of therapy. This practice may allow organisms that have not been eradicated to regrow and produce mortality.

Mortality at 7 to 12 days after the end of therapy has proven to correlate poorly with the 24-h AUUMIC values for the quinolone antimicrobial agents (S). Thus, only those studies that treated animals for at least 2 days, reported survival results within 24 h of the end of therapy, and observed at least 80% mortality by the end of therapy in untreated or saline-treated controls were used. This analysis included studies of pneumonia, peritonitis, sepsis, and soft tissue infections in mice, rats, and guinea pigs due to various organisms but primarily gram-negative bacilli. An excellent correlation between the 24-h AUUMIC ratio and mortality was observed. In general, AUUMIC ratios less than 30 were associated with greater than 50% mortality, whereas values of 100 or greater were associated with 90 to 100% survival.

Animal studies of endocarditis due to gram-negative bacilli or staphylococci have also been analyzed to determine the relationship between the 24-h
AUC/MIC ratio and bacterial numbers in the vegetations (3). Dosing regimens with 24-h AUC/MIC ratios of 100 or greater produced bacterial numbers in vegetations after 3 to 6 days of therapy that were at least 210gs lower than regimens with AUC/MIC ratios less than 100. Thus, the pharmacodynamic parameters predictive of efficacy of fluoroquinolones in the treatment of experimental endocarditis are similar to those for other infectious models.

Studies correlating mortality with the 24-h AUC/MIC ratios are also available for strains of S. pneumoniae in nonneutropenic animals (5, 12). Fig. 4 demonstrates the relationship between the 24-h AUC/MIC ratio and mortality for these infections. In some of these studies, specific genetic species of mice (e.g., CBA-J) were required to produce disease in the absence of neutropenia. In these studies the curve was shifted to the left and 90 to 100% survival could be observed as soon as the 24-h AUC/MIC value reached 25 to 35.

An earlier analysis, as shown in Fig. 5, examined the relationship between mortality and the 24-h AUC/MIC for intracellular and extracellular pathogens (13). The data for the extracellular pathogens are the same as many of the values shown in Fig. 3. The values for the intracellular pathogens were obtained from experimental studies in mice or guinea pigs infected with Chlamydia psittaci, L. pneumophila, Mycobacterium tuberculosis, and Salmonella enterica serovar Typhimurium. Although the intracellular concentrations of fluoroquinolones are higher than those in serum, this did not result in any difference in the relationship between the 24-h AUC/MIC and mortality for intracellular versus extracellular pathogens. Thus, concentrations of the quinolones in serum may also be good predictors of their in vivo activity against intracellular pathogens.

Human Studies

The first study correlating PK/PD parameters of the fluoroquinolones with clinical response in humans was published by Peloquin et al. (40). In this study, which evaluated intravenous ciprofloxacin in seriously ill patients with lower respiratory tract infections, time above MIC was reported to be the important parameter for eradication of the organism from respiratory secretions. However, patients infected with P. aeruginosa failed because of the emergence of resistance. These patients not only had a low time above MIC, they also had low peak/MIC and low AUC/MIC ratios. The results of a subsequent analysis of the data with additional patients by Forrest et al. demonstrated that the 24-h AUC/MIC value was the best predictor of the clinical and microbiologic efficacy of ciprofloxacin (24). As shown in Fig. 6, a 24-h AUC/MIC value of 125 or higher was associated with much higher rates of clinical and bacteriologic cure than values less than 125. These investigators also demonstrated that 24-h AUC/MIC values of 250 or higher resulted in faster eradication (mean, 1.9 days) of the organisms from respiratory secretions than patients that had values of 125 to 250 (mean, 7 days) (36). This is what one would expect for drugs that exhibit concentration-dependent killing.

Preston et al. correlated PK/PD parameters for levofloxacin with both clinical and microbiologic efficacy in patients with urinary tract, pulmonary, skin, and soft tissue infections (41). Although significant covariations existed among the PK/PD parameters, these investigators demonstrated that patients
who had a peak/MIC ratio of 12.2 or higher eradicated the infecting pathogen 100% of the time compared with 80.8% for those with peak/MIC ratios less than 12.2. The breakpoint for bacterial eradication using the peak/MIC ratio was similar to a 24-h AUC/MIC value of 100. Thus, the 24-h AUC/MIC ratios associated with microbiologic eradication in humans and high survival in animal infection models are very similar at values of 100 to 125.

One-third of the 21 patients infected with *S. pneumoniae* and treated with levofloxacin had 24-h AUC/MIC values from 30 to 100, and none of these patients failed therapy. This suggests that the 24-h AUC/MIC value for bacterial eradication of *S. pneumoniae* is lower than that for gram-negative bacilli. More recently, Ambrose and Grasela reported the relationship between the free drug 24-h AUC/MIC ratio and the eradication of *S. pneumoniae* from the blood or respiratory secretions of patients with community-acquired pneumonia and acute exacerbations of chronic bronchitis following treatment with levofloxacin or gatifloxacin (1). As shown in Fig. 7, bacterial eradication was less than 100% when the AUC/MIC ratio fell to the 30 to 40 range. More specifically, the rate of eradication was 100% when the AUC/MIC ratio was greater than 33.7 and only 64% when the ratio was less than 33.7. This breakpoint value is very similar to the 25 to 35 values observed for high survival in the animal models infected with *S. pneumoniae*.

There are also a few cases of clinical and bacteriologic failure with levofloxacin in patients infected with strains of *S. pneumoniae* that have MICs of 8 mg/liter (22, 23). The estimated 24-h AUC/MIC ratios in these patients would be less than 10. Failures in patients with pneumococcal pneumonia have also been reported for ciprofloxacin where the 24-h AUC/MIC ratio has been estimated to be about 10 to 20. These studies also support a 24-h AUC/MIC breakpoint of 25 to 35 for clinical and bacteriologic success with quinolones in patients infected with pneumococci.

### PK/PD PARAMETERS AND THE EMERGENCE OF RESISTANCE

A variety of in vitro and in vivo studies have examined the relationship between PK/PD parameters and the eradication or emergence of resistant organisms. Several animal and in vitro studies have suggested that peak concentrations of fluoroquinolones that are 8 to 10 times higher than the MIC of the starting inoculum can significantly enhance the probability of bacterial eradication or the emergence of resistant organisms.
reduce the emergence of resistant subpopulations (18, 26). More recent studies with in vitro models have determined the 24-h AUC/MIC ratios associated with enhancing and reducing the emergence of resistance (21, 33, 34, 44, 45). With P. aeruginosa, K. pneumoniae, and S. aureus the 24-h AUC/MIC values often range from 90 to 200 for preventing the selection of resistant organisms. On the other hand, the emergence of resistance in S. pneumoniae has been difficult to select in vitro models.

Thomas et al. studied the emergence of resistance in patients treated with ciprofloxacin as combination or monotherapy (46). As shown in Table 1, 86% of patients receiving ciprofloxacin monotherapy that had 24-h AUC/MIC ratios less than 100 developed resistance. This was 100% for strains of P. aeruginosa and 50% for other gram-negative bacilli. Ciprofloxacin monotherapy resulting in 24-h AUC/MIC ratios of 100 or higher developed resistance in only 10% of the patients. The value was 25% for P. aeruginosa and only 7% for other gram-negative bacilli. All the patients receiving combination therapy had 24-h AUC/MIC ratios greater than 100, and none of these patients developed resistance. Although the peak/MIC ratio was not evaluated in this report, the earlier publication by Peloquin et al. showed that a peak/MIC ratio of 8 or higher was similarly predictive as a 24-h AUC/MIC greater than 100 in reducing the selection of resistant organisms during therapy (40). It should also be pointed out that the 24-h AUC/MIC value of 100 is also incorrectly applied to infections caused by gram-positive organisms. The study by Thomas et al. had only a few of these organisms treated with ciprofloxacin, and none of the patients developed resistance.

The mutation prevention concentration (MPC) is another measurement that has been receiving increasing interest among many investigators (9, 16, 33, 43). It is defined as the lowest drug concentration in agar that prevents the growth of any colonies from very large inocula of susceptible organisms. Several studies have demonstrated that the MPC can vary for different organisms and for different fluoroquinolones. For S. pneumoniae, the MPC values have varied from four to eight times the MIC. The difference between the MIC and MPC has been called the selection window for resistance. Some investigators have suggested that concentrations of quinolones in serum should exceed the MPC for the entire dosing interval (9). A recent study with levofoxacin, gatifloxacin, and moxifloxacin in an in vitro model using S. aureus was designed to produce peak concentrations that were equal to the MIC, between the MIC and MPC, and above the MPC (21). Resistant subpopulations were primarily selected when peak concentrations were within the selection window. Much more data including animal and human studies are needed on the significance of the MPC and the relationship of PK/PD parameters to the emergence of resistance.

### APPLICATIONS OF QUINOLONE PHARMACODYNAMICS

Knowledge of the major PK/PD parameter determining efficacy and the magnitude of that target required for efficacy of specific pathogens has proven to be helpful for developing new quinolone agents, predicting the activity of new quinolone formulations, developing guideline recommendations, and establishing susceptibility and resistance breakpoints for susceptibility testing. Because the magnitudes of the PK/PD parameters appear to be relatively similar in animals and humans, animal studies can be useful for predicting clinical activity especially in situations where it is difficult to obtain sufficient clinical data (e.g., new emerging resistance). Initial studies suggest that pharmacodynamics can also be important in preventing the emergence of resistance. More in vitro, animal and human studies are necessary to fully document the usefulness of pharmacodynamic evaluation in optimizing therapy with the quinolone antimicrobial agents.

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<th>Therapy</th>
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1 GNB, gram-negative bacilli.
REFERENCES


