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Pharmacokinetic and Pharmacodynamic Activities of Ciprofloxacin against Strains of *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* for Which MICs Are Similar

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The serum bactericidal activity of ciprofloxacin against strains of *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* for which MICs are similar (0.4 μg/ml) was assessed with serum ultrafiltrates from five healthy volunteers receiving ciprofloxacin at 400 mg intravenously every 8 h. In addition, human serum was supplemented with ciprofloxacin to achieve a mean steady-state concentration (C_{ss}) that might be achieved in patients with renal failure, with total clearances of 3 to 4 liters/h (elimination rate constant, 0.08 h^{-1}). The area under the inhibitory titer curve from 0 to 24 h ($AUIC_{24}$) and the area under the bactericidal titer curve from 0 to 24 h ($AUBC_{24}$) were both measured and predicted as the area under the concentration-time curve from 0 to 24 h (AUC_{24})/MIC and AUC_{24}/MBC, respectively. We previously demonstrated that a breakpoint AUC_{24}/MIC of 125 for ciprofloxacin had a significantly higher probability of treatment success than lower values, with 250 to 500 being optimal. Volunteer sera (mean C_{ss}, 1.55 to 2.48 μg/ml) achieved AUC_{24}/MICs of 90 to 145. Supplemented serum (mean C_{ss}, 6.00 to 7.42 μg/ml) achieved AUC_{24}/MICs of 350 to 450. Correlation coefficients for measured and predicted values of AUC_{24}/MIC and AUC_{24}/MBC were 0.826 and 0.941, respectively. The mean percent errors were not significantly different from zero for either $AUIC_{24}$ or $AUBC_{24}$ values ($P > 0.1$, $P > 0.4$). Time-kill curve studies were performed with low (1.55 to 2.48 μg/ml), intermediate (6.00 to 7.42 μg/ml), and high (15 to 25 μg/ml) concentrations of ciprofloxacin for the three organisms. At low concentrations (3 to 6 times the MIC) AUC_{24}/MICs were <125 for two of five volunteers and the killing rates were considerably more rapid for *P. aeruginosa* than for *S. pneumoniae* or *S. aureus*. Intermediate concentrations (15 to 18 times the MIC) achieved optimal AUC_{24}/MICs, and the killing rates were similar for the three organisms. A paradoxical decrease in the killing rate was seen at high concentrations (35 to 60 times the MIC). At clinically achievable concentrations, ciprofloxacin killed *P. aeruginosa* more rapidly than it did either *S. pneumoniae* or *S. aureus*.

Many methods have been used to evaluate the pharmacodynamic properties of antimicrobial agents. Stationary measures such as MIC and MBC determinations provide ranges associated with inhibition or killing but only limited information on the rate of the inhibitory and bactericidal effects of an antibiotic acting on a specific microorganism. The serum inhibitory and bactericidal titer tests (SIT and SBT tests, respectively) were the first methods to incorporate patient pharmacokinetics into the testing of susceptibility. The SIT test is performed with a serum sample taken during antimicrobial therapy and measures the inhibitory activity of a patient's serum against the pathogen isolated from the patient. The SBT is obtained by subculturing samples showing inhibition of growth. Because the reciprocal of the bactericidal titer is roughly equivalent to the serum antibiotic concentration-to-MBC ratio, knowledge of the serum antibiotic concentration and the MBC for the bacteria can be used to predict the SBT (2, 15). Time-kill curve tests provide even more information, because these methods elucidate both the rate and the magnitude of bacterial killing that can be achieved with a fixed concentration of antibiotic.

More recently, the area under the inhibitory titer curve (AUIC) and the area under the bactericidal titer curve (AUBC) have been described as measures of patient-specific antibiotic pharmacokinetics integrated with bacteria- and antimicrobial agent-specific pharmacodynamics (1). In order to measure the AUBC, SBT tests are performed with patient serum at several time points following antibiotic dosing. The measured AUBC is calculated as the area under the reciprocal SBT versus time curve (1). The AUIC is a measure of inhibitory activity and is calculated as the AUC of the reciprocal SITs versus time. Ellner and Neu (5) coined the term inhibitory quotient to describe the peak concentration of an antimicrobial agent divided by the MIC. Since the reciprocal SIT may be predicted as the concentration of antibiotic in serum divided by the MIC, a partially predicted AUIC and AUBC can be calculated by dividing the measured AUC by the MIC and MBC, respectively, for a specific bacterium. These predicted values of AUIC and AUBC are actually AUC/MIC and AUC/MBC ratios, respectively. It is convenient to report steady-state AUIC (or AUC/MIC) and AUBC (or AUC/MBC) values per 24 h so that differences in dosing intervals and half-lives will not complicate the interpretation across different classes of antibiotics or between the various members of a class.

A study which assessed data that were obtained from three different clinical trials performed between 1985 and 1990 in patients with serious nosocomial infections, primarily pneumo-

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nia, revealed that the steady-state AUC from 0 to 24 h (AUC_{24})/MIC (referred to as $AUIC_{24}$) [6]) was the most important predictor of both clinical and microbiologic cure for ciprofloxacin. At an AUC_{24}/MIC of less than 125 $SIT^{-1} \cdot h$ (inverse SIT integrated over time [24 h]) the investigators reported an unacceptably low probability of treatment success. An AUC_{24}/MIC of 125 was considered to be the minimally effective value, with values of 250 to 500 exhibiting an increased in vivo bactericidal rate and a shorter time to bacterial eradication. Since the time above the MIC is the most important exposure parameter in predicting the response of gram-negative bacteria to β-lactam antibiotics (11), the duration of inhibitory activity must be considered along with target AUC_{24}/MICs. When these dosing interval considerations are respected, then, theoretically, AUC_{24}/MIC should also be predictive of the bacterial response to these agents, because of covariance between time greater than the MIC and the AUC/MIC ratio.

For the purposes of this report, the terms AUIC from 0 to 24 h ($AUIC_{24}$) and AUBC from 0 to 24 h ($AUBC_{24}$) are used in the traditional manner, describing the collection of serum following antimicrobial administration; this is followed by performance of the SIT or SBT test and then calculation of the 24-h area under the inverse SIT or inverse SBT curve by the trapezoidal rule. The terms AUC_{24}/MIC and AUC_{24}/MBC are used to describe the AUC divided by the MIC and MBC, respectively, by using antimicrobial concentrations measured in subjects following antibiotic administration.

In order to assess the effects of higher concentrations of ciprofloxacin than those achieved in the current study of healthy volunteers, we supplemented serum collected from healthy volunteers prior to antimicrobial dosing with ciprofloxacin and performed SIT and SBT tests. The purely in vitro measures of ciprofloxacin exposure are termed "serum-supplemented" $AUIC_{24}$, $AUBC_{24}$, AUC_{24}/MIC, and AUC_{24}/MBC.

It is common practice to examine the effects of different antibiotics on a single organism. The examination of the effects of various concentrations of ciprofloxacin on *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* has not been undertaken by time-kill curve methods and strains of the three organisms for which MICs and MBCs are similar. If a drug exhibits similar inhibitory and bactericidal activity against several organisms, as evidenced by similar MICs and MBCs, is the rate of killing similar, or are there other inherent differences in organisms that can alter the rate of bacterial killing over time?

A fundamental question is whether the MIC can describe differences between bacterial species, at least from the perspective of predicting the in vivo response, if the patient is treated to achieve concentrations in serum that are targeted multiples of the MIC for the isolated pathogen. We acknowledge that there are differences between bacterial isolates (i.e., capsule formation and ability to develop resistance), differences between patients (i.e., immune status, infection site, and bacterial inoculum), and differences in antimicrobial agents (i.e., ability to penetrate biofilm and the infection site, as well as postantibiotic effect) which can alter the response to treatment. However, our purpose is to search for treatment targets that will help to maximize treatment success.

In order to address the general objective of validating MIC as a measure of ciprofloxacin activity across species of bacteria, the study had the following specific objectives: (i) to measure SITs and SBTs for isolates of *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* for which MICs are similar at steady state in subjects receiving 400 mg of ciprofloxacin intravenously every 8 h and determine $AUIC_{24}$ and $AUBC_{24}$; (ii) to examine the killing rate for the three different bacterial species for which MICs and MBCs are similar; and (iii) to determine mathematical relationships between ciprofloxacin exposure and bacterial killing rate.

MATERIALS AND METHODS

Serum collection. On day 5 of a multiple-dose pharmacokinetic study of intravenously administered ciprofloxacin in five volunteers, serum was collected prior to initial dosing and at 0.25, 0.5, 1, 2, 4, and 8 h following the final dose, which was infused over 1 h. Volunteers were both male and female and greater than 18 years of age. Renal function was evaluated by 24-h urine collection for creatinine clearance, which was performed within 7 days of dosing. Creatinine clearance values for the five subjects ranged from 63 to 103 ml/min. Each volunteer received ciprofloxacin intravenously at 400 mg every 8 h.

In order to evaluate serum bactericidal activity and killing rates that might be observed at higher concentrations (i.e., patients with impaired renal function), additional human serum samples (obtained from the healthy volunteers described above prior to the first dose of ciprofloxacin) were supplemented with ciprofloxacin ex vivo to simulate the concentrations in patients with total ciprofloxacin clearances of 3 to 4 liters/h (normal CL is about 20 to 40 liters/h). These simulated cases were prepared (labelled "subjects" A, B, and C) with beginning concentrations of 8, 9, and 10 μg/ml, respectively, at time zero postinfusion. Monoexponential elimination was assumed, with an elimination rate constant of 0.08 h^{-1}. The concentrations were calculated at 0.25, 0.5, 1, 2, 4, and 8 h.

Antimicrobial solution preparation. Ciprofloxacin powder was obtained from the manufacturer (Miles Inc., West Haven, Conn.). Weighing was performed with a Mettler analytical balance (2% precision error at 2 mg). Ciprofloxacin solution was prepared by dissolving the powder in distilled water. The stock solution (1,000 μg/ml) was maintained at −20°C.

MIC determination. MICs were determined by broth microdilution techniques and with serial twofold dilutions of cation-adjusted Mueller Hinton broth (CAMHB) (8). Lysed horse blood (5%) was added to the broth for the testing of *S. pneumoniae* isolates. Stable cultures of *S. pneumoniae* (serial number JHSP001), *S. aureus* (serial number JHSA001), and *P. aeruginosa* (serial number JHPA001) were selected from our microorganism bank and were maintained on Trypticase soy agar with 5% sheep's blood. Colonies were touched with a sterile loop and were added to a tube containing 0.9% sodium chloride solution until the density was comparable to that of a 0.5 McFarland standard. Further dilution of the inoculum was achieved in CAMHB (MHB supplemented with Ca^{2+} [20 to 25 mg/liter] and Mg^{2+} [10 to 12.5 mg/liter]). The final inoculum was prepared from bacteria in the stationary growth phase and was approximately 5×10^5 CFU/ml. The final inoculum size was verified by counting the visible colonies by a spread plate technique. In order to determine more precise values, MICs were determined, in duplicate, with starting concentrations in the wells of column one of 5, 6, 7, and 8 μg/ml. The inoculum was added with a steel pin inoculator (MIC 2000; Dynatech, Alexandria, Va.). The microdilution trays were incubated at 35°C for approximately 18 h. Following incubation, the wells were examined by using a magnifying mirror with good lighting. The well containing the lowest concentration of antibiotic that prevented visible growth was defined as the MIC.

MBC determination. Ten-microliter samples from all microdilution wells lacking visible growth were subcultured onto blood agar and were incubated at 35°C for 18 h. Bactericidal

endpoints for MBC determinations were defined as a 99.9% reduction in the initial inoculum according to the tables of Pearson et al. (9).

Bactericidal activity in serum ultrafiltrate. SBTs were determined by microdilution techniques (7). An ultrafiltrate of volunteer serum was obtained by centrifugation at 1,000 × *g* at 25°C for 30 min through a Centrifree micropartition YMT ultrafiltration filter (Amicon Inc., Beverly, Mass.). Fifty microliters of CAMHB was added to the wells in columns 2 through 12. The ciprofloxacin-supplemented serum samples (subjects A, B, and C) were studied in the same manner as the volunteer samples. An autodilutor (AUTO III; Dynatech) was used to make twofold dilutions of ultrafiltrates in the 96-well plates. An additional 50 μl of CAMHB was added to all wells of the *S. aureus* and *P. aeruginosa* plates. Fifty microliters of CAMHB with 5% horse blood was added to all wells of the *S. pneumoniae* plates. Stable cultures of *S. pneumoniae* (serial number JHSP001), *S. aureus* (serial number JHSA001), and *P. aeruginosa* (serial number JHPA001) were maintained on Trypticase soy agar with 5% sheep's blood. Colonies were touched with a sterile loop and were added to a tube containing 0.9% sodium chloride solution until the density was comparable to that of a 0.5 McFarland standard. Further dilution of inoculum was achieved in CAMHB (MHB supplemented with Ca^{2+} [20 to 25 mg/liter] and Mg^{2+} [10 to 12.5 mg/liter]). The final inoculum was prepared from bacteria in the stationary growth phase and was approximately 5×10^5 CFU/ml. The final inoculum size was verified by counting the visible colonies by a spread plate technique. The microdilution plates were incubated at 35°C for approximately 24 h. The SIT was read as the well with the greatest dilution showing no visible growth. Each well showing no growth was subcultured onto blood agar with a calibrated 10-μl loop and was then incubated at 35°C for approximately 24 h. Bactericidal endpoints for SBT determinations were defined as a 99.9% reduction in the initial inoculum according to the tables of Pearson et al. (9).

$AUIC_{24}$ and $AUBC_{24}$ values (and serum-supplemented $AUIC_{24}$ and $AUBC_{24}$ values) were obtained from the reciprocal of the midpoint SITs and SBTs at different time points by the trapezoidal rule. The midpoint value is that which lies halfway between the measured titer and the next lowest titer. This point was chosen since it is known that the true SIT lies somewhere between the measured titer and the next lowest titer. Since MICs were determined by testing over a range of initial concentrations, it is felt that the MICs are relatively accurate and do not require midpoint correction. Without the midpoint correction for measured values, the measured SITs will almost always be systematically lower than the predicted values. The AUC_{24}/MICs and AUC_{24}/MBCs (and serum-supplemented AUC_{24}/MICs and AUC_{24}/MBCs) were obtained by dividing the value from the serum ciprofloxacin concentration versus time curve for each subject (calculated by the trapezoidal rule) by the MIC or MBC. Differences in measured ($AUIC_{24}$, $AUBC_{24}$) and predicted (AUC_{24}/MIC, AUC_{24}/MBC) values were assessed [(measured − predicted)/measured)]. The same method was used to assess differences in measured and predicted serum-supplemented values. Mean percent errors were reported as a measure of bias, and mean absolute errors were reported as a measure of precision. A paired samples *t* test was performed in order to test for statistical difference between the measured and the predicted values.

Antibiotic carryover. In order to prevent antibiotic carryover when performing time-kill curve studies, Amberlite XAD-4 polyadsorbant binding resin (Rohm and Haas, Philadelphia, Pa.) was used to bind ciprofloxacin. This method was chosen because it has previously been shown to be effective at removing >99% of ciprofloxacin from solution, at a concentration of 5 μg/ml, without affecting bacterial growth (16). Validation of the method was performed by using a spectrophotometer (Milton Roy Spectronic 1201) to read drug absorbances before and after vortexing with XAD-4 resin. Millex-GV 0.22-μm-pore-size filters (Millipore Products Division, Bedford, Mass.) were used to remove the resin prior to spectrophotometric analysis. A standard curve was made by preparing solutions of ciprofloxacin containing 10, 20, 50, and 100 μg/ml in distilled water. Two hundred microliters of these solutions was diluted 10-fold by adding 1.8 ml of distilled water. The A_{273} values of these solutions were plotted versus the concentration in order to establish a standard curve. Two milliliters of each solution was then added to glass tubes containing 1 g of XAD-4 resin, and the tubes were vortexed for <5 s. Samples were allowed to sit for 0, 5, 10, 15, and 20 min prior to absorbance determinations in order to assess whether an optimal binding time existed. Ciprofloxacin concentrations before and after 15 min of ciprofloxacin contact with XAD-4 resin were verified by a ciprofloxacin assay previously validated at The Clinical Pharmacokinetics Laboratory. The XAD-4 resin was autoclaved at 250°C (Sterilmatic; Market Forge, Everett, Mass.) prior to use, and through testing it was found not to affect bacterial growth.

Killing rate determination. For each subject and for the three simulated cases, the mean steady-state concentration was calculated as AUC/τ, where τ is the dosing interval (8 h). For each subject and case, the average concentrations at steady-state were prepared in CAMHB, which was used for killing rate studies. In addition, concentrations of 15, 20, and 25 μg/ml were prepared in CAMHB to establish the maximum killing rate for ciprofloxacin.

Initial killing curve studies were performed with each of the three organisms by using a rigorous sampling schedule (0, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, and 24 h) in order to characterize the early killing profiles and regrowth patterns (Fig. 1). These killing curve studies were performed with organisms in the exponential growth phase. Regrowth was noted with *S. pneumoniae* as well as with *P. aeruginosa* at low concentrations of ciprofloxacin at 8 h. Because of the nonlinear nature of the early killing profiles, subsequent killing curve studies were performed with samples obtained at 0, 4, and 6 h.

Two sets of experiments were performed. In experiment I, low (1.55 to 2.48 μg/ml), intermediate (6.00 to 7.42 μg/ml), and high (15.00 to 25.00 μg/ml) concentrations of ciprofloxacin were tested against each bacterial strain. In experiment II, the intermediate and high concentrations were tested against the same organisms in order to assess reproducibility. These killing curve studies were performed with bacteria in the exponential growth phase. For each of the three organisms, the greatest rate of killing was observed between 0 and 4 h. Therefore, these time points were chosen for determining growth and kill rates by using the following equations: kill rate = growth rate (control) − apparent kill rate (ciprofloxacin), Growth rate = $[\ln \text{CFU } (4\text{h})_{\text{control}} - \ln \text{CFU(initial)}]$/time interval (4 h), and apparent kill rate = $[\ln \text{CFU } (4\text{ h})_{\text{cipro}} - \ln \text{CFU(initial)}]$/time interval (4 h).

Linear regression of the growth rate versus the apparent killing rate was performed for all three organisms. The observed correlation coefficient (*r*) was tested for the difference from zero in order to determine whether there is a significant correlation between the growth rate and the rate of killing.

Inocula were prepared by inoculating brain heart infusion broth for *S. pneumoniae* or CAMHB for *S. aureus* and *P. aeruginosa* and incubating the organisms at 35°C to achieve

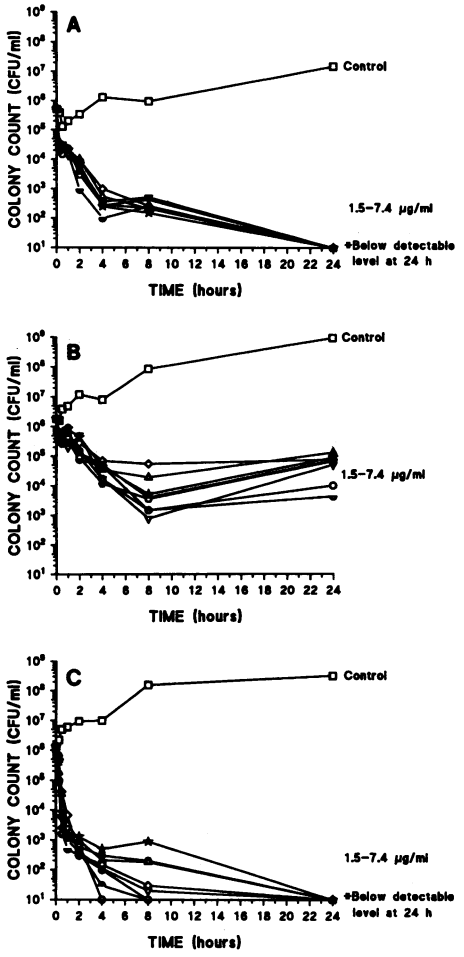

FIG. 1. Twenty four-hour killing curves for *S. pneumoniae* (A), *S. aureus* (B), and *P. aeruginosa* (C) for the control (□) and at the following ciprofloxacin concentrations: 1.5 μg/ml (△), 1.65 μg/ml (▽), 2.3 μg/ml (◇), 2.39 μg/ml (★), 2.48 μg/ml (●), 6.0 μg/ml ( ), 6.7 μg/ml (○), and 7.42 μg/ml ( ).

logarithmic growth. The final inoculum was prepared by taking 0.5 ml of the prepared inoculum at a 0.5 McFarland standard and adding 49.5 ml of the appropriate medium to achieve a final inoculum of approximately 10^6 CFU/ml. The final inoculum size was verified by counting the visible colonies by a spread plate technique. By using ciprofloxacin previously prepared at 100 times the average concentration for each subject and simulation, 50 μl was diluted with prepared inoculum to a final volume of 5 ml. Each of the prepared inoculum-drug combinations along with control cultures was incubated at 35°C. At the baseline and at 4 and 6 h after inoculation, samples were removed for determination of bacterial colony counts.

At each sampling time, 0.3 ml of the inoculum-drug solution was added to 2.7 ml of a 0.9% NaCl solution with 1 g of XAD-4 resin. Samples were vortexed for <5 s. The solutions were filtered with a SeraClear (Technicon, Plainfield, N.J.) filter to remove the resin. This filter was tested prior to use and was shown not to affect bacterial counts. One milliliter of solution was added to an empty petri plate for the 1:10 dilution. One hundred microliters of solution was added to an empty petri plate for the 1:100 dilution. One hundred microliters of the

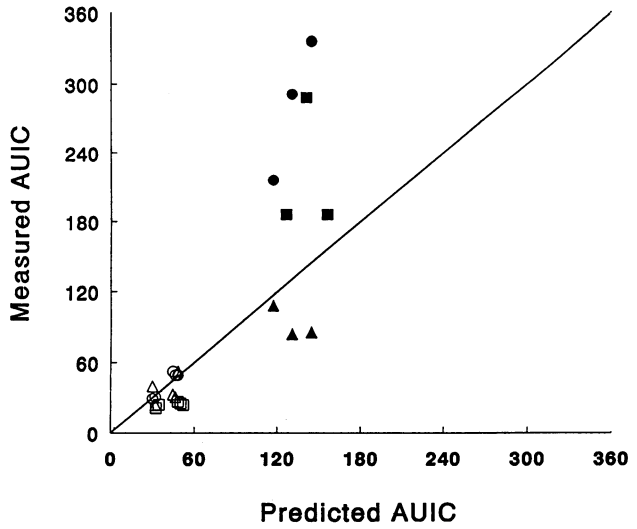

FIG. 2. Relationship between measured and predicted values of $AUIC_{24}$ (open symbols) and serum-supplemented $AUIC_{24}$ (closed symbols) for *S. pneumoniae* (△, ▲), *S. aureus* (□, ■), and *P. aeruginosa* (○, ●). The line represents a slope of unity.

solution was further diluted in 9.9 ml of 0.9% NaCl, and 1 ml of this solution was added to an empty petri plate for the 1:1,000 dilution. One hundred microliters of the diluted solution was added to an empty petri plate for the 1:10,000 dilution. Further dilutions were made in the same manner for control cultures. Ten milliliters of appropriate agar (brain heart infusion agar for *S. pneumoniae*, Mueller-Hinton agar for *S. aureus*, and Mueller-Hinton agar with additional 25% by volume of 0.9% NaCl solution for *P. aeruginosa*) maintained at 45°C was immediately added to each plate. Plates were incubated at 35°C for 24 to 48 h, until the colonies could be well visualized for counting. Plates containing between 20 and 200 colonies were counted. The bacterial count was reported as the

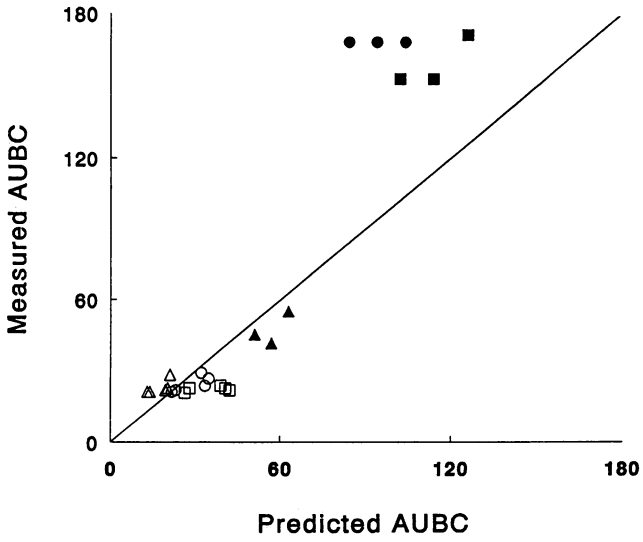

FIG. 3. Relationship between measured and predicted values of $AUBC_{24}$ (open symbols) and serum-supplemented $AUBC_{24}$ (closed symbols) for *S. pneumoniae* (△, ▲), *S. aureus* (□, ■), and *P. aeruginosa* (○, ●). The line represents a slope of unity.

TABLE 1. Predicted values (AUC_{24}/MIC) and measured values $AUIC_{24}$) at the midpoint[a]

Subject	*S. pneumoniae*		*S. aureus*		*P. aeruginosa*	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
1	30.2	39.8	32.6	21.4	30.2	29.3
2	48.4	52.5	52.2	24.0	48.4	49.5
3	32.2	24.0	34.7	24.4	32.2	30.8
4	44.8	33.0	48.3	27.0	44.8	52.5
5	46.6	30.75	50.2	25.5	46.6	49.5
A	117.2	108.0	126.4	186.0	117.2	216.0
B	130.8	84.0	141.1	288.0	130.8	291.0
C	144.7	85.5	156.1	186.0	144.7	336.0

[a] Mean percent error (standard error) was 14.1 (10.0); mean absolute percent error was 40.1. There was no significant difference between the predicted and the measured values ($P = 0.160$).

number of CFU per milliliter of culture medium after correcting for dilutions.

RESULTS

MIC and MBC data. MICs and MBCs were determined by eight replicates with starting ciprofloxacin concentrations of 5, 6, 7, and 8 μg/ml. The MICs and MBCs for *S. pneumoniae* were 0.41 μg/ml (range, 0.31 to 0.50 μg/ml) and 0.94 μg/ml (range, 0.63 to 1.50 μg/ml), respectively. The MICs and MBCs for *S. aureus* were 0.38 μg/ml (range, 0.25 to 0.44 μg/ml) and 0.47 μg/ml (range, 0.31 to 0.50 μg/ml), respectively. For *P. aeruginosa* the MICs and MBCs were 0.41 μg/ml (range, 0.25 to 0.44 μg/ml) and 0.57 μg/ml (range, 0.50 to 0.75 μg/ml), respectively. MICs for the three organisms were similar. The MBC for *S. pneumoniae* was higher than that for either *S. aureus* or *P. aeruginosa*, but it was still within 1 dilution of the values determined for the other two organisms.

AUIC and AUBC data. Figures 2 and 3 show the relationship between the predicted (AUC_{24}/MIC) and the measured ($AUIC_{24}$) values of AUIC (Fig. 2) and the predicted (AUC_{24}/MBC) and the measured ($AUBC_{24}$) values of AUBC (Fig. 3). For higher concentrations, bias was noted, with measured values being consistently higher. $AUIC_{24}$ and $AUBC_{24}$ values for individual volunteers or cases are shown in Tables 1 and 2 (A, B, and C are serum-supplemented cases). Mean percent errors for $AUIC_{24}$ and $AUBC_{24}$ were 14.1 and 3.9, respectively. The mean absolute percent errors for $AUIC_{24}$ and $AUBC_{24}$ were 40.1 and 32.1, respectively.

Antibiotic carryover data. The results obtained for the binding of ciprofloxacin to XAD-4 resin are shown in Table 3. Binding efficacy was shown to be time dependent, with 20 min of contact resulting in an average of 90% removal of drug. At 15 min, removal was about 85%. High-pressure liquid chromatographic measurement of these samples confirmed that an average of 85% of drug could be removed in 15 min, validating the spectrophotometric results. Resin contact time with samples during time-kill curve studies was 10 min or less. We found the XAD-4 resin to be much less efficient at removing ciprofloxacin than was earlier reported (16). Although we tested higher concentrations of ciprofloxacin than those tested in the previous study, it is unlikely that the discrepancy between the results of the two studies is due to this difference in concentration since we found no relationship between concentration and percent removal (Table 3). Since an insufficient amount of drug could be removed by this method, dilution of samples to dilute the drug concentration to at least 10-fold below the MIC was necessary, as described in Materials and Methods.

Time-kill curve data. The killing rate and ciprofloxacin concentration/MBC relationship for experiment I are shown in Fig. 4. The initial slopes for *S. pneumoniae* and *P. aeruginosa* were similar, although the killing rates were generally higher for *P. aeruginosa* at concentration/MBC ratios of less than 5. For *S. aureus*, the slope was less steep and the killing rates were generally lower at concentration/MBC ratios of less than 12. Maximal killing of *S. pneumoniae* was observed at about eight times the MBC. For *S. aureus*, maximal killing was seen at about 15 times the MBC. Maximal killing of *P. aeruginosa* was seen at about 27 times the MBC. It appears that the rate of killing was reduced for *S. aureus* at concentration/MBC ratios of greater than 15 and for *P. aeruginosa* at concentration/MBC ratios of greater than 27. *S. pneumoniae* was difficult to assess since the concentration/MBC ratio never exceeded 30; however, the killing rate appeared to reach an asymptote at about eight times the MBC.

Table 4 shows the average killing rates for experiments I and II as well as the percent difference in the rates between the two

TABLE 2. Predicted values (AUC_{24}/MBC) and measured values ($AUBC_{24}$) at the midpoint[a]

Subject	*S. pneumoniae*		*S. aureus*		*P. aeruginosa*	
	Predicted	Measured	Predicted	Measured	Predicted	Measured
1	13.2	21.4	26.3	21.0	21.7	21.4
2	21.1	28.5	42.2	22.1	34.8	27.0
3	14.0	21.4	28.1	22.9	23.1	22.1
4	19.5	22.1	39.1	24.0	32.2	29.3
5	20.3	22.9	10.6	22.9	33.5	24.0
A	51.1	45.0	102.2	153.0	84.3	168.0
B	57.1	41.3	114.1	153.0	94.1	168.0
C	63.1	54.8	126.2	171.0	104.1	168.0

[a] Mean percent error(S.E.) (standard error) was 3.9 (8.0); absolute percent error was 32.1. There was no significant difference between the predicted and the measured values ($P = 0.085$).

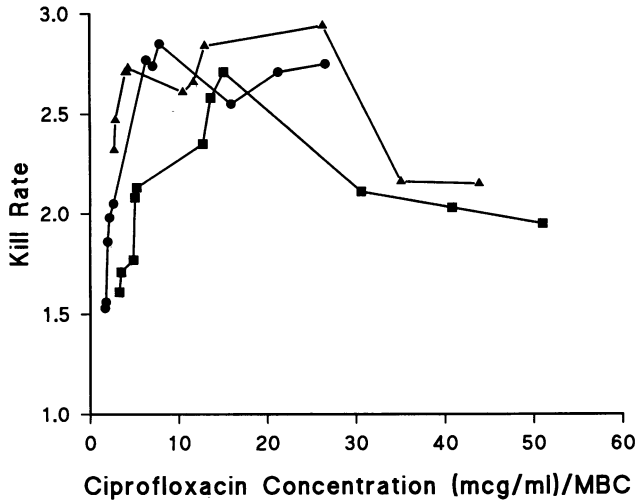

FIG. 4. Relationship between ciprofloxacin concentration/MBC ratio and killing rate for *S. pneumoniae* (●), *S. aureus* (■), and *P. aeruginosa* (▲).

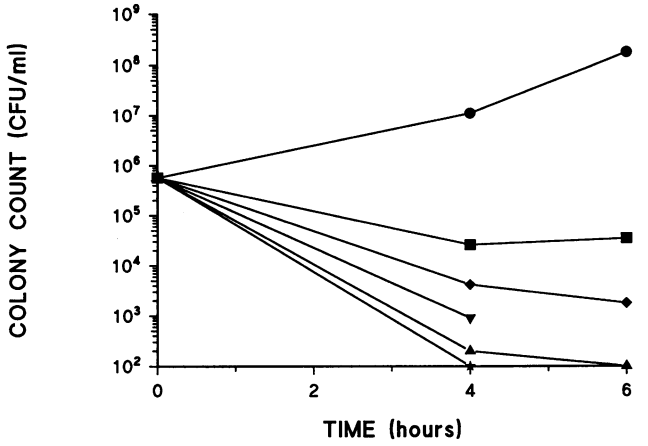

FIG. 5. Six-hour killing curves for *S. pneumoniae* for the control (●) and at the following ciprofloxacin concentrations: 1.55 μg/ml (■), 2.39 μg/ml (◆), 6.00 μg/ml (▲), 15.00 μg/ml (▼), and 25.00 μg/ml (★).

experiments. The results of experiment I were reproducible in experiment II, with the percent differences in killing rates between the two experiments ranging from 0 to 18.73%, with a mean difference of 5.72%. Figures 5 through 7 show the kill curves from experiment I for each organism. In Fig. 5, a concentration-effect relationship is apparent at low concentrations for *S. pneumoniae*, with some regrowth noted at 6 h. Killing rates were increased with concentrations increasing up to about 15 μg/ml, at which the rate of killing appeared to reach an asymptote. In Fig. 6, a concentration-effect relationship for the rate of killing is apparent at 4 h at all concentrations for *S. aureus*, except that a paradoxical decrease in the killing rate was noted at 15 to 25 μg/ml. Figure 7 demonstrates a concentration-effect relationship in the killing rates for *P. aeruginosa* at 4 h, except at 20 to 25 μg/ml, at which a decreased rate of killing was noted. Maximum killing rates for both *S. pneumoniae* and *S. aureus* were seen at 7.42 μg/ml, indicating that the maximum effect occurs at between 6.70 and 15 μg/ml (15 to 40 times the MIC). The maximum killing rate for *P. aeruginosa* was seen at 15 μg/ml, indicating that the maximum effect occurs at between 7.42 and 20 μg/ml (20 to 50 times the MIC).

The growth rates for *S. pneumoniae*, *S. aureus*, and *P. aeruginosa* were 0.74, 0.71, and 0.79, respectively. There was a trend toward an increasing killing rate with an increasing rate of bacterial growth. The correlation coefficient (r) was not different from zero, indicating that the growth rate and the apparent killing rate were not significantly correlated.

At low mean steady-state concentrations (1.55 to 2.48 μg/ml) the killing rates for *P. aeruginosa* were 1.3 to 1.5 times higher than those for *S. pneumoniae* or *S. aureus*. At intermediate mean steady-state concentrations (6.00 to 7.42 μg/ml) the killing rates were similar for all three organisms. It is possible that the rate of killing reaches an asymptote which is similar for all three organisms in this range of concentration/MIC ratios. At the highest mean steady-state concentrations (15.00 to 25.00 μg/ml) killing rates were similar for all three organisms and were generally lower than the killing rates at intermediate concentrations. The low concentrations tested represent the average steady-state concentrations achieved in healthy volunteers receiving ciprofloxacin at 400 mg intravenously every 8 h. Peak concentrations in these subjects averaged 4 to 8 μg/ml (10 to 20 times the MIC), but persisted only for a short time as the drug was distributed. The intermediate concentrations were the steady-state concentrations calculated for subjects A, B, and C. These represent peak ciprofloxacin concentrations of 8 to 10 μg/ml (20 to 25 times the MIC), which are not commonly seen in vivo. The highest concentrations (30 to 50 times the MIC) are generally not seen in vivo, although it is possible with a more susceptible organism to achieve a similar concentration/MIC ratio.

DISCUSSION

In our previous work, data pooled from several clinical trials of patients with nosocomially acquired pneumonia revealed that the AUC_{24}/MIC was the most important predictor of both clinical and microbiologic cure for ciprofloxacin (6). It was determined that a minimally effective AUC_{24}/MIC of 125 was

TABLE 3. Time-dependent removal of ciprofloxacin by adsorption to XAD-4 resin

Ciprofloxacin concn (μg/ml)	% (avg) Removal at:				
	0 min	5 min	10 min	15 min	20 min
10	15–46 (31)	48–53 (51)	67–88 (78)	69–94 (82)	74–93 (84)
20	41–46 (44)	49–72 (61)	67–86 (77)	79–91 (85)	83–96 (90)
50	29–47 (38)	64–65 (65)	76–87 (82)	85–92 (89)	90–95 (93)
100	39 (39)	53–69 (61)	69–88 (79)	79–91 (85)	86–94 (90)
Mean	38	60	79	85	90

TABLE 4. Average ciprofloxacin killing rates and percent difference at increasing concentrations for experiments I and II

Ciprofloxacin concn (μg/ml)	Killing rate by[a]:								
	S. pneumoniae			*S. aureus*			*P. aeruginosa*		
	Expt I	Expt II	% D	Expt I	Expt II	% D	Expt I	Expt II	% D
1.5	1.53			1.61			2.32		
1.65	1.56			1.71			2.47		
2.30	1.86			1.77			2.71		
2.39	1.98			2.08			2.71		
2.48	2.05			2.13			2.73		
6.00	2.75	2.78	1.10	2.35	2.35	0.00	2.80	2.41	13.9
6.70	2.64	2.83	6.71	2.38	2.78	14.4	2.83	2.49	12.0
7.42	2.72	2.98	8.72	2.43	2.99	18.7	2.83	2.84	0.35
15.00	2.37	2.72	12.9	2.05	2.16	5.09	2.94	2.94	0.00
20.00	2.76	2.66	3.62	1.92	2.14	10.3	2.18	2.14	1.83
25.00	2.92	2.57	12.0	1.80	2.10	14.3	2.13	2.16	1.39

[a] Killing rate was calculated as (change in CFU control/change in time) − (change in CFU with ciprofloxacin/(change in time). % D, percent difference.

necessary in order to achieve clinical cure. At AUC_{24}/MICs of 250 to 500, the in vivo bacterial killing rate was increased, and bacterial eradication in these patients occurred more rapidly. By targeting an AUC_{24}/MIC of 350 by a population pharmacokinetic model, the values for most patients would be predicted to be in the optimal range, with no patients having values predicted to be in the unacceptable category (AUC_{24}/MIC, <125) (6). The implication of this clinical work is that bacterial killing rates are determined by their MICs, at least at equal levels of exposure to antimicrobial agents.

At the lowest mean steady-state concentrations tested (three to six times the MIC), the killing rate of ciprofloxacin for *P. aeruginosa* was superior to those for both *S. pneumoniae* and *S. aureus*. This is in spite of the similar MICs for the organisms, and suggests that there are some interorganism susceptibility differences that cannot be discerned solely by measurement of the MIC. It should be noted, however, that regrowth was noted for *S. pneumoniae* at 6 h which may have been due to the higher MBC for this organism. Whether the early difference in the killing rate for *P. aeruginosa* has in vivo significance is uncertain. It has been demonstrated clinically that *P. aeruginosa* organisms for which MICs are similar to those tested in the present study may persist in the respiratory secretions of patients treated with ciprofloxacin for pneumonia (10, 12). Serum from healthy volunteers achieved AUC_{24} values of 90 to 145 for the three organisms tested. Marginal activity is the rule for these three organisms because of the high MICs for these organisms. Thus, the inability to achieve optimal AUC_{24}/MICs with *P. aeruginosa* may explain the persistence of this organism in the respiratory secretions of patients with pneumonia. Two of the five volunteers studied had average steady-state concentrations of <2.14 μg/ml (less than five times the MIC) and AUC_{24}/MICs of <125. All five volunteers received 400 mg of ciprofloxacin every 8 h intravenously for 5 days to achieve these steady-state concentrations. With normal renal function, 400 mg of ciprofloxacin administered intravenously every 8 h may not be sufficient to achieve a breakpoint AUC_{24}/MIC of 125 in infections caused by bacteria for which MICs are 0.5 μg/ml or greater. Further work will be necessary in order to determine whether higher doses of ciprofloxacin or the addition of a second active agent would be the recommended strategy in these patients.

At intermediate mean steady-state concentrations (15 to 18 times the MIC), a difference in the killing rates between the different bacteria was no longer apparent. Ciprofloxacin was as effective at eradicating *S. pneumoniae* as it was at eradicating *P. aeruginosa*. Since the MIC is the concentration of antimicrobial agent that inhibits growth of most, but not all, of the organisms

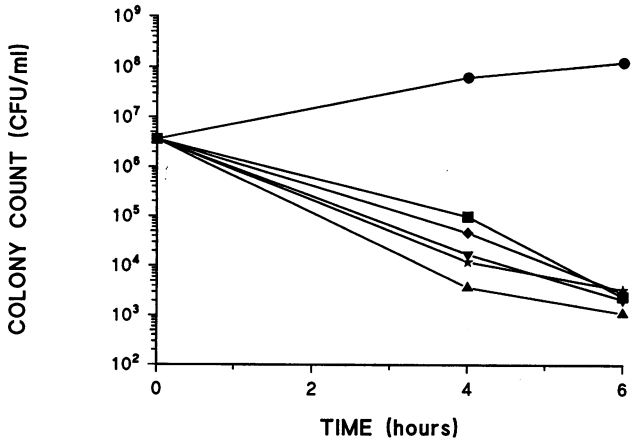

FIG. 6. Six-hour killing curves for *S. aureus* for the control (●) and at the following ciprofloxacin concentrations: 1.55 μg/ml (■), 2.48 μg/ml (★), 7.42 μg/ml (▲), 15.00 μg/ml (▼), and 25.00 μg/ml (◆).

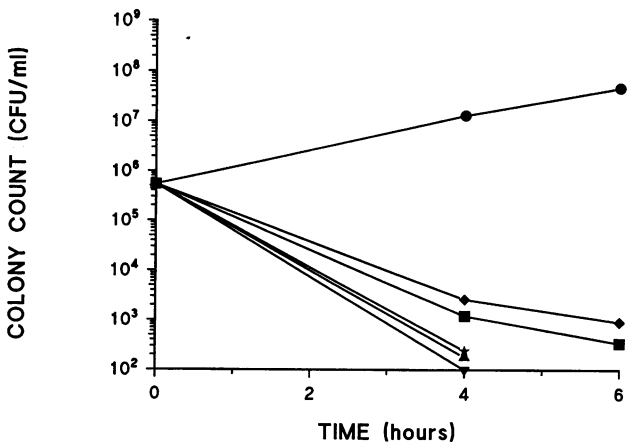

FIG. 7. Six-hour killing curves for *P. aeruginosa* for the control (●) and at the following ciprofloxacin concentrations: 1.55 μg/ml (■), 2.48 μg/ml (★), 6.00 μg/ml (▲), 15.00 μg/ml (▼), and 25.00 μg/ml (◆).

in a given inoculum, it is conceivable that at lower concentrations a greater proportion of a more resistant subpopulation of organisms may survive. If this hypothesis is correct, most of the more resistant organisms would be eradicated at the higher concentrations, and differences between bacterial strains would not be as apparent.

If the rate of killing is important to the clinical outcome, it is clear that for gram-positive and gram-negative organisms for which MICs are higher, higher concentrations of ciprofloxacin than those generally achieved in vivo or combination therapy may be necessary to achieve clinical cure in bacterial infections caused by organisms with intermediate susceptibilities (MICs, 0.5 to 1.0 μg/ml). Serum-supplemented AUC_{24}/MICs of 350 to 450 were achieved for subjects A, B, and C, in which serum was supplemented with ciprofloxacin at 15 to 18 times the MIC. All three organisms were killed at a similar, maximal rate at this concentration range. These results support previous evidence that demonstrated that an AUC_{24}/MIC of 350 to 500 is important for predicting bacterial killing or microbiologic cure. Perhaps the increased killing rate seen at concentrations that produce an AUC_{24}/MIC in the optimal range further explains why this range is associated with increased clinical efficacy (6), since most patients with multiples of the MIC in excess of 35 have clinical cures (10).

The fluoroquinolone antimicrobial agents exhibit rapid bactericidal activity. This bactericidal activity increases progressively as the concentration increases. Ciprofloxacin has been shown to exert a maximum killing effect at 40 to 50 times the MIC for *Escherichia coli*. Even higher concentration to MIC multiples resulted in a decrease in the bactericidal effect (13). It has been proposed that this paradoxical or Eagle effect (4) may be due to partial inhibition of RNA synthesis at higher ciprofloxacin concentrations (14).

At the highest concentrations tested in our study, an increase in bacterial survival was seen in comparison with that seen at slightly lower concentrations. This effect was seen at >20 to 40 times the MICs for *S. pneumoniae* and *S. aureus*. Whether this represents a paradoxical effect or an asymptote in the concentration-effect relationship is unclear. Decreased killing was also noted with *P. aeruginosa* at high concentrations of ciprofloxacin (>35 to 50 times the MIC). Although this paradoxical effect has been well documented in in vitro studies, an in vivo correlate has not been described. Peak concentration/MIC ratios greater than 50 have been associated with favorable outcomes in both animal models and human clinical studies (3, 6).

In conclusion, the predicted values of AUC_{24}/MIC correlated well with the measured values. Serum-supplemented AUC_{24}/MICs in the range of 350 to 435 were associated with similar and maximal killing rates in three different organisms selected because the MICs for the organisms were similar. A maximal effect for ciprofloxacin was seen at 15 to 40 times the MIC for the gram-positive organisms tested and at 20 to 50 times the MIC for *P. aeruginosa*. These data support the premise that MIC is a useful indicator of relative susceptibility across bacterial species, although there may be some subtle differences in killing rates. With attention to the MIC, ciprofloxacin dosage regimens can be designed to be effective against all three of these difficult-to-treat bacteria. However, because the MIC is usually 10- to as much as 100-fold higher for these bacteria than for members of the family *Enterobacteriaceae*, either higher doses of ciprofloxacin or combination therapy may be necessary in order to achieve optimal AUC_{24}/MICs. Further research will be necessary in order to determine the role of combination therapy.

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