ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1996, p. 627–632 Vol. 40, No. 3
0066-4804/96/$04.00+0

Twenty-Four-Hour Area under the Concentration-Time Curve/MIC Ratio as a Generic Predictor of Fluoroquinolone Antimicrobial Effect by Using Three Strains of *Pseudomonas aeruginosa* and an In Vitro Pharmacodynamic Model

KARL J. MADARAS-KELLY,[1]† BETH E. OSTERGAARD,[1,2] LAURIE BAEKER HOVDE,[2] AND JOHN C. ROTSCHAFER[1,2]*

College of Pharmacy, University of Minnesota, Minneapolis, Minnesota,[1] and St. Paul-Ramsey Medical Center, St. Paul, Minnesota[2]

Received 11 November 1994/Returned for modification 18 May 1995/Accepted 10 December 1995

Several investigators have suggested that the 24-h area under the concentration-time curve (AUC)/MIC ratio (AUC/MIC_{24} or $AUIC_{24}$) can be used to make comparisons of antimicrobial activity between fluoroquinolone antibiotics. Limited data exist regarding the generic predictive ability of AUC/MIC_{24} for the antimicrobial effects of fluoroquinolones. The purposes of the present investigation were to determine if the AUC/MIC_{24} can be used as a generic outcome predictor of fluoroquinolone antibacterial activity and to determine if a similar AUC/MIC_{24} breakpoint can be established for different fluoroquinolones. Using an in vitro pharmacodynamic model, 29 duplicate concentration time-kill curve experiments simulated AUC/MIC_{24}s ranging from 52 to 508 $SIT^{-1} \cdot h$ (inverse serum inhibitory titer integrated over time) with ciprofloxacin or ofloxacin against three strains of *Pseudomonas aeruginosa*. Each 24-h experiment was performed in cation-supplemented Mueller-Hinton broth with a starting inoculum of 10^6 CFU/ml. At timed intervals cation-supplemented Mueller-Hinton broth samples were collected for CFU and fluoroquinolone concentration determinations. Transformation of bacterial counts into the cumulative bacterial effect parameter of the 24-h area under the effect curve ($AUEC_{24}$) was performed for each concentration time-kill curve. Multivariate regression analysis was used to compare pharmacodynamic predictors (AUC/MIC_{24}, 24-h AUC, peak concentration [C_{max}] to MIC ratios [C_{max}:MIC], etc.) with ln $AUEC_{24}$. To identify threshold breakpoint AUC/MIC_{24}s, $AUEC_{24}$s were stratified by the magnitude of AUC/MIC_{24} into subgroups, which were analyzed for differences in antibacterial effect. The Kruskal-Wallis test and subsequent Tukey's multiple comparison test were used to determine which AUC/MIC subgroups were significantly different. Multiple regression analysis revealed that only AUC/MIC_{24} ($r^2 = 0.65$) and MIC ($r^2 = 0.03$) were significantly correlated with antibacterial effect. At similar AUC/MIC_{24}s, yet different MICs, C_{max}s, or elimination half-lives, the $AUEC_{24}$s were similar for both fluoroquinolones. The relationship between AUC/MIC_{24} and ln $AUEC_{24}$ was best described by a sigmoidal maximal antimicrobial effect (E_{max}) model ($r^2 = 0.72$; $E_{max} = 9.1$; $AUC/MIC_{50} = 119\ SIT^{-1} \cdot h$; $S = 2.01$ [S is an exponent that reflects the degree of sigmoidicity]). Ciprofloxacin-bacteria AUC/MIC_{24} values of $<100\ SIT^{-1} \cdot h$ were significantly different ($P < 0.05$) from the AUC/MIC_{24} values of $>100\ SIT^{-1} \cdot h$. An ofloxacin AUC/MIC_{24} of $>100\ SIT^{-1} \cdot h$ and an AUC/MIC_{24} of $<100\ SIT^{-1} \cdot h$ exhibited a trend toward a significant difference ($P > 0.05$ but < 0.1). The inverse relationship between drug exposure and MIC increase postexposure was described by a sigmoidal fixed E_{max} model (AUC/MIC_{24}, $r^2 = 0.40$; $AUC/MIC_{50} = 95\ SIT^{-1} \cdot h$; $S = 1.97$; C_{max}:MIC, $r^2 = 0.41$; C_{max}:$MIC_{50} = 7.3$; $S = 2.01$). These data suggest that AUC/MIC_{24} may be the most descriptive measurement of fluoroquinolone antimicrobial activity against *P. aeruginosa*, that ofloxacin and ciprofloxacin have similar AUC/MIC_{24} threshold breakpoints at approximately 100 $SIT^{-1} \cdot h$, that the concentration-dependent selection of resistant organisms may parallel the threshold breakpoint of the antimicrobial effect, and that AUC/MIC_{24} generically describes the antibacterial effects of different fluoroquinolones.

The 24-h area under the concentration-time curve (AUC)/MIC ratio (AUC/MIC_{24}) has been suggested as a generic pharmacodynamic predictor that can be used to make comparisons of fluoroquinolone activity irrespective of bacterial species (12, 15, 16). Other investigators have suggested that high peak concentration (C_{max}) to MIC ratios (C_{max}:MIC), the AUC above the MIC (AUC > MIC), and the time of dosage interval above the MIC ($T >$ MIC) are all associated with favorable outcomes in in vitro, animal, and human studies conducted with fluoroquinolones (2, 3, 9, 13).

In two clinical trials, the ability of AUC/MIC_{24} to predict a positive clinical response appeared to be promising (4, 5). For ciprofloxacin, a AUC/MIC_{24} of 125 $SIT^{-1} \cdot h$ (inverse serum inhibitory titer integrated over time) has been shown to be a significant breakpoint for predicting the probabilities of clinical and microbiologic cure in critically ill patients with nosocomial pneumonia (5). For OPC 17116, an experimental fluoroquinolone, a AUC/MIC_{24} of $>75\ SIT^{-1} \cdot h$ has been shown to be predictive of clinical and microbiologic cure for patients with acute exacerbation of chronic bronchitis (4).

Limited data exist regarding the generic predictive ability of AUC/MIC_{24} for fluoroquinolones other than ciprofloxacin or OPC 17116. To date, no comparative trials between fluoro-

* Corresponding author. Mailing address: St. Paul-Ramsey Medical Center, 640 Jackson St., St. Paul, MN 55101. Phone: (612) 221-3896. Fax: (612) 292-4031.

† Present address: College of Pharmacy, Idaho State University, Pocatello, Id. and Veterans Affairs Medical Center, Boise, ID 83702.

quinolone antibiotics have been conducted to determine if AUC/MIC_{24} is the optimal generic predictor of fluoroquinolone activity or if a generic breakpoint of antimicrobial effect exists for fluoroquinolone antibiotics (16).

The purposes of the investigation described here were to (i) determine the predictive ability of AUC/MIC_{24} with respect to other pharmacodynamic parameters as measurements of fluoroquinolone activity, (ii) investigate if AUC/MIC_{24} is a generic term that predicts interclass fluoroquinolone activity against different isolates of *Pseudomonas aeruginosa*, and (iii) to determine if an AUC/MIC_{24} breakpoint of antimicrobial activity could be identified for ofloxacin and could be confirmed for ciprofloxacin.

(This work was presented in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy [10a].)

MATERIALS AND METHODS

Twenty-nine concentration time-kill curve experiments simulating AUC/MIC_{24}s ranging from 52 to 508 $SIT^{-1} \cdot h$ were performed with ciprofloxacin or ofloxacin against three different strains of *P. aeruginosa.*

In vitro model. Concentration time-kill curves were conducted by using a previously described in vitro model (19). At the start of each experiment, an inoculum of *P. aeruginosa* was instilled into a chemostat along with a bolus injection of either ciprofloxacin or ofloxacin appropriate to produce the desired initial antibiotic concentrations for each experiment. Each experiment was run in duplicate for 24 h. Antibiotic-free cation-supplemented Mueller-Hinton broth (CAMHB; Ca^{2+}, 50 mg/liter; Mg^{2+}, 25 mg/liter) was pumped via a peristaltic pump into a chemostat at a predetermined fixed rate. Simultaneously, an equal volume of CAMHB was displaced from the chemostat into a waste reservoir, simulating a monoexponential pharmacokinetic process and the desired half-life ($t_{1/2}$) of the antibiotic.

Bacteria. Three strains of *P. aeruginosa*, ATCC 27853 and two clinical isolates (PSA 5279 and PSA 9284), were studied. Prior to concentration time-kill curve experiments, several colonies of the isolate were grown overnight in 50 ml of CAMHB and were then diluted 1:5 in fresh CAMHB approximately 1 h prior to the experiment to allow the organisms to attain exponential growth. Bacterial isolates were grown to a 0.5 McFarland standard in CAMHB. A volume equal to 1:100 of the flask volume was added to each chemostat. The resultant starting bacterial inoculum was approximately 10^6 CFU/ml.

Susceptibility testing. Susceptibility testing was performed in triplicate for each isolate prior to concentration time-kill experiments and on all isolates at 24 h postexposure. Susceptibility testing was performed by a commercial serial twofold microdilution colorimetric method (Alamar Biosciences, Sacramento, Calif.) in CAMHB with an inoculum of approximately 5×10^5 CFU/ml. All isolates were subcultured onto fresh blood agar plates for at least 3 consecutive days prior to susceptibility testing.

Antibiotics. Stock solutions (5,000 μg/ml) of ofloxacin (R. W. Johnson, Raritan, N.J.) and ciprofloxacin (Miles, New Haven, Conn.) were prepared by using the appropriate amounts of sterile distilled water and were frozen in aliquots at −70°C until they were needed for individual experiments. Antibiotics were administered as bolus injections at either 8-, 12-, or 24-h intervals.

Pharmacokinetics. Four groups of experiments were performed. In group 1, a range of AUC/MIC_{24}s between 52 and 508 $SIT^{-1} \cdot h$ was simulated by allowing the C_{max} to vary (for ofloxacin, 3.5 to 62.0 μg/ml; for ciprofloxacin, 1.1 to 20.0 μg/ml) while maintaining a constant $t_{1/2}$ of 4.5 h for ciprofloxacin and 6.0 h for ofloxacin. All experiments in group 1 were conducted with strain PSA 5729, of which MICs were typical for MICs of ciprofloxacin and ofloxacin at which 90% of isolates are inhibited. Dosage intervals of 8, 12, and 24 h were used in group 1 experiments. For group 2, AUC/MIC_{24}s were held constant at approximately 250 $SIT^{-1} \cdot h$ for the same clinical isolate (PSA 5729), but the $t_{1/2}$ and C_{max} were altered to reflect approximately one-half to two times the original pharmacokinetic parameters used in group 1. Dosage intervals in group 2 experiments were all 12 h. In groups 3 and 4, AUC/MIC_{24}s ranging from approximately 88 to 250 $SIT^{-1} \cdot h$ were simulated by varying the C_{max} while maintaining the $t_{1/2}$ similar to that for group 1 experiments. However, these experiments were performed with strains ATCC 27853 and PSA 9284, respectively. The dosage intervals in group 3 and 4 experiments were all 12 h. The target and observed AUC/MIC_{24}, C_{max}, and $t_{1/2}$ values, as well as microbiologic data, are outlined in Table 1.

The concentrations of ciprofloxacin and ofloxacin were determined by a previously reported high-pressure liquid chromatography (HPLC) technique (7). The lower limit of fluoroquinolone detection was 0.1 μg/ml, and the coefficient of variation was <5% over a range of 0.5 to 10.0 μg/ml.

C_{max}, minimum concentrations (C_{min}), and $t_{1/2}$ were calculated by standard monoexponential pharmacokinetics equations (14). The AUC_{24} was determined by Equation 1, where n is equivalent to the number of dosing intervals in the 24-h period and K_e is equivalent to the monoexponential elimination rate constant. The AUC/MIC_{24} was determined by dividing the cumulative 24-h AUC by the preexposure MIC (equation 2). In all phases of the comparative analysis, actual as opposed to target pharmacokinetic data were used. Antibiotic concentrations were maintained above the preexperiment MIC for the entire 24-h duration.

$$AUC_{24} = \{\int_0^n [(C_{max}/K_e) - (C_{min}/K_e)]\} \quad (1)$$

$$AUC/MIC_{24} = \{\int_0^n [(C_{max}/K_e) - (C_{min}/K_e)]\}/MIC \quad (2)$$

Pharmacodynamics. At predetermined timed intervals, samples of CAMHB were removed from the model for quantitation of the bacteria by a saline dilution technique. The number of predetermined timed intervals was dependent on the frequency of antibiotic administration; however, a minimum of 16 samples were removed for each 24-h experiment. Bacterial counts were determined by 1:10 serial dilution of 100 μl of medium into cold saline which was plated onto Trypticase soy agar supplemented with 5% sheep blood (Dimed, New Brighton, Minn.).

Antibiotic carryover was prevented by the combination of saline dilution and an adaptation of the previously published method of Zabinski et al. (18). For all samples in which the fluoroquinolone concentrations were >5 μg/ml, 2-ml CAMHB samples removed from the models were first exposed to 1 g of XAD-4 antimicrobial polymeric binding resin (Rohn and Haas, Philadelphia, Pa.). One milliliter of the previously exposed solution was then exposed a second time to 1 g of antimicrobial polymeric binding resin (Rohn and Haas) preceding serial plate dilution. For samples in which the fluoroquinolone concentrations were <5 μg/ml, 1-ml CAMHB samples were exposed to 1 g of XAD-4 antimicrobial polymeric binding resin prior to saline dilution.

After incubation for 18 to 24 h at 37°C, the numbers of CFU on each plate were counted. The theoretical lower limit of bacterial counting accuracy was 3.0×10^2 CFU/ml. Concentration time-kill curves were constructed by plotting the $\log_{10}$ CFU per milliliter versus time. Analysis of the antimicrobial effect was performed by using the 24-h area under the bacterial effect curve ($AUEC_{24}$) (equation 3) (11, 17).

$$AUEC_{24} = \int_0^{24} (C_2 - C_1) \cdot (T_2 - T_1)/(\ln C_2 - \ln C_1) \quad (3)$$

In equation 3, C_2 and C_1 represent bacterial concentrations (CFU per milliliter) of adjacent sampling times, whereas T_2 and T_1 represent respective adjacent sampling times from the time of initiation of the experiment for C_2 and C_1, respectively. The parameter $AUEC_{24}$ was inversely related to the antimicrobial effect. $AUEC_{24}$ incorporated regrowth portions of experiments and allowed for analysis of multiple antibiotic exposures. $AUEC_{24}$ only incorporated time-concentration datum points that were above 3.0×10^2 CFU/ml. To compensate for differences in the initial starting inoculum, $AUEC_{24}$ was standardized by dividing $AUEC_{24}$ by the numbers of CFU per milliliter at time zero.

$AUEC_{24}$ and MIC increase post concentration time-kill curve exposure versus AUC/MIC_{24} and C_{max}:MIC were modeled to an E_{max} sigmoidal nonlinear effect model (equation 4) by using Adapt II (University of Southern California, Los Angeles) (equation 4).

$$AUEC_{24} = \frac{E_{max} \times AUC/MIC_{24}^{(S)}}{AUC/MIC_{50}^{(S)} + AUC/MIC_{24}^{(S)}} \quad (4)$$

In this model, $AUEC_{24}$ was related to AUC/MIC_{24} by the two parameters E_{max} and the AUC/MIC_{50}, where E_{max} is equivalent to the maximal antimicrobial effect, AUC/MIC_{50} is the AUC/MIC associated with $E_{max}/2$, and S is an exponent that reflects the degree of sigmoidicity. Because ln $AUEC_{24}$ was inversely related to AUC/MIC_{24}, the reciprocal of $AUEC_{24}$ plus a constant were used to fit the data. Bacterial resistance was also inversely related to antibiotic exposure, so a transformation of the fold increase in MIC post antibiotic exposure was used to fit the data to an E_{max} sigmoidal model as well: reciprocal MIC fold increase post antibiotic exposure $= 1/(F + 1)$ (equation 5), where F is the magnitude of fold increase in MIC post antibiotic exposure.

Statistical analysis. Both univariate and stepwise multiple regression (Numbers Crunchers Statistical Software, Kayesville, Utah) analyses were used to compare the following pharmacodynamic predictors and study variables with ln $AUEC_{24}$: AUC/MIC_{24}, C_{max}:MIC, C_{max}, AUC_{24}, C_{min}:MIC, C_{min}, $t_{1/2}$, drug, dosing interval, bacterial isolate, and MIC. Statistical significance was defined as a P value of ≤0.05.

To determine if a significant breakpoint of antimicrobial effect existed for ciprofloxacin, ofloxacin, or both fluoroquinolones, $AUEC_{24}$ data were stratified by corresponding AUC/MIC_{24} magnitude into the following five subgroups:

TABLE 1. Target and observed pharmacokinetic and pharmacodynamic parameters and microbiologic data[a]

Experimental group	Drug	Target AUC/MIC ($SIT^{-1} \cdot h$)	Observed AUC/MIC (mean % difference [± SD])	Target C_{max} (μg/ml)	Observed C_{max} (mean % difference [± SD])	Target $t_{1/2}$ (h)	Observed C_{max} (mean % difference [± SD])	Time interval (h)	Isolate	MIC (μg/ml)
1	Ciprofloxacin	52–507	12.6 (8.9)	1.1–20.0	7.3 (4.4)	4.5	7.0 (4.2)	8, 12, 24	PSA 5729	0.25
	Ofloxacin	52–508	18.6 (19.6)	3.5–62.0	6.0 (7.2)	6.0	6.6 (0.8)	8, 12, 24	PSA 5729	1.0
2	Ciprofloxacin	250	7.4 (4.1)	12.8–44.0	6.7 (8.0)	2.0/8.0[b]	6.9 (7.2)	12	PSA 5729	0.25
	Ofloxacin	250	11.2 (8.0)	12.5–35.0	12.2 (3.3)	2.5/10.0[b]	7.9 (6.1)	12	PSA 5729	1.0
3	Ciprofloxacin	88–250	8.8 (5.3)	3.7–7.5	6.2 (5.3)	4.5	6.3 (5.8)	12	PSA 9284	0.5
	Ofloxacin	88–250	13.0 (11.0)	22.0–35.0	2.0 (1.5)	6.0	10.4 (5.9)	12	PSA 9284	4.0
4	Ciprofloxacin	88–250	7.9 (5.2)	1.9–5.5	8.8 (3.9)	4.5	10.1 (6.7)	12	ATCC 27853	0.25
	Ofloxacin	88–250	3.0 (2.6)	12.0–35.0	5.8 (5.1)	6.0	3.7 (3.6)	12	ATCC 27853	2.0

[a] $n = 58$ (29 duplicate experiments).
[b] Two different $t_{1/2}$ values were simulated.

>400, 301 to 400, 201 to 300, 101 to 200, or <101 $SIT^{-1} \cdot h$. The Kruskal-Wallis A test was used to detect a significant difference between all AUC/MIC_{24} groups. Tukey's multiple comparisons test with correction for unequal sample sizes was used to determine which groups were significantly different ($P \leq 0.05$) (20).

RESULTS

Pharmacokinetic parameters calculated from drug concentration-time data determined by HPLC indicated that in >90% of the experiments, actual values for $t_{1/2}$, C_{max}, and AUC/MIC_{24} were within ±15% of the target values. Measured versus observed AUC/MIC_{24}s in ofloxacin experiments generally had larger variability than those in ciprofloxacin experiments because of the greater dependence on the MIC (denominator) in calculating the ofloxacin AUC/MIC_{24}. A summary of the differences between predicted and observed AUC/MIC, C_{max}, and $t_{1/2}$ parameters are presented in Table 1.

At like AUC/MIC_{24}s but different $t_{1/2}$s, C_{max}s, and MICs, the cumulative ln $AUEC_{24}$ was similar for both ciprofloxacin and ofloxacin (Fig. 1). Similarities in cumulative AUC/MIC_{24} against PSA 5729 were observed over AUC/MIC_{24} from approximately 50 to 500 $SIT^{-1} \cdot h$, despite differences in $t_{1/2}$, C_{max}, and MIC.

In experiments in which the AUC/MIC_{24}s were held constant at approximately 250 $SIT^{-1} \cdot h$ against PSA 5729 but $t_{1/2}$ and C_{max} were altered to reflect approximately one-half and two times the original value, cumulative $AUEC_{24}$s were very similar for both ciprofloxacin and ofloxacin. When high C_{max} values were combined with a short $t_{1/2}$, a pronounced initial bacterial kill was followed by rapid regrowth. Conversely, experiments performed with a lower C_{max} and prolonged $t_{1/2}$ demonstrate an attenuated initial bacterial kill followed by a longer suppression of regrowth. Subsequent redosing of the fluoroquinolone resulted in a limited initial effect but a longer suppression of bacterial regrowth. For six sets of duplicate experiments conducted at an AUC/MIC_{24} of ~250 $SIT^{-1} \cdot h$ by using different $t_{1/2}$ and C_{max} values against PSA 5729 (ciprofloxacin MIC, 0.25 μg/ml; ofloxacin MIC, 1.0 μg/ml) no significant differences in total $AUEC_{24}$s were evident, despite large fluctuations in pharmacokinetic variables. The results for three separate pharmacokinetic regimens with similar cumulative $AUEC_{24}$s are presented in Fig. 2.

For AUC/MIC_{24} of <100 $SIT^{-1} \cdot h$, a decrease in the initial antimicrobial effect and elimination of the antimicrobial effect upon repeat dosing of both agents were evident. The magnitude of the antibacterial effect increased with increasing AUC/MIC_{24}; however, subsequent redosing of fluoroquinolone equivalent to an AUC/MIC_{24} of 90 and 55 $SIT^{-1} \cdot h$ did not result in an antibacterial effect. Five different bacterial concentration time-kill curves for ofloxacin (12-h dosing interval) with PSA 5729 at AUC/MIC_{24}s ranging from 55 to 350 $SIT^{-1} \cdot h$ are represented in Fig. 3.

Univariate regression analysis of the cumulative ln $AUEC_{24}$s generated from the 29 duplicate sets of concentration time-kill curves versus the pharmacodynamic predictors and pharmacokinetic parameters revealed that AUC/MIC_{24} ($r^2 = 0.58$),

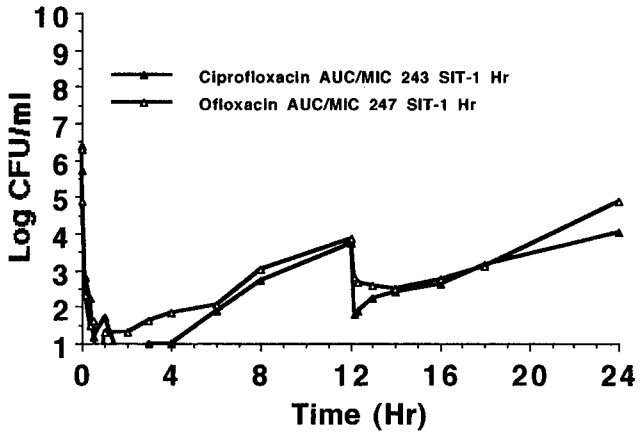

FIG. 1. Ciprofloxacin and ofloxacin against PSA 5729 at an AUC/MIC of ~250 $SIT^{-1} \cdot h$. Results are means of duplicate experiments.

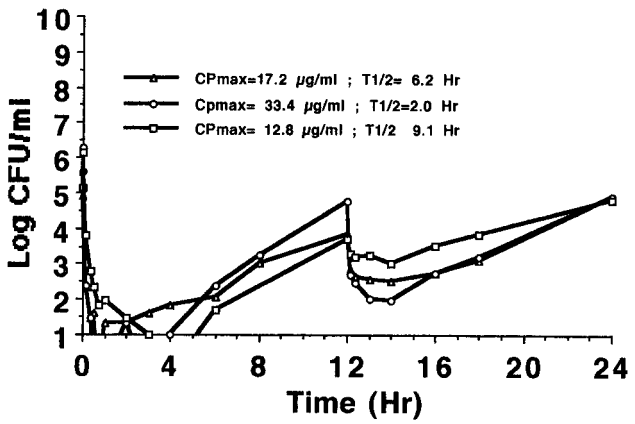

FIG. 2. Varied ofloxacin pharmacokinetics against PSA 5729 at an AUC/MIC of ~250 $SIT^{-1} \cdot h$. Results are means of duplicate experiments.

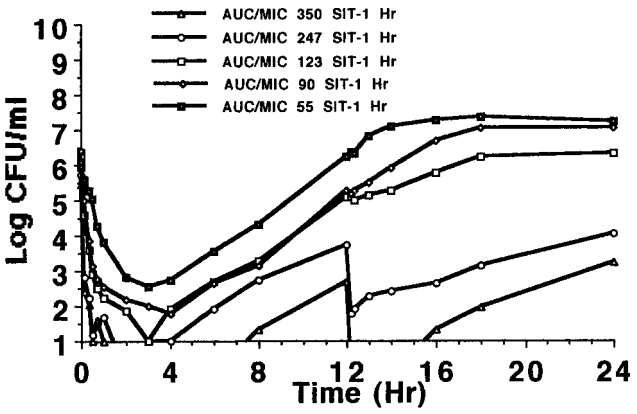

FIG. 3. Antimicrobial effect of ofloxacin against PSA 5729. AUC/MIC range, 55 to 350 $SIT^{-1} \cdot h$. Results are means of duplicate experiments.

C_{max}:MIC ($r^2 = 0.33$), C_{max} ($r^2 = 0.24$), AUC_{24} ($r^2 = 0.19$), C_{min}:MIC ($r^2 = 0.28$), and dosage interval ($r^2 = 0.12$) were all significantly correlated with antibacterial effect.

In the multiple regression analysis, only AUC/MIC_{24} ($r^2 = 0.65$) and MIC ($r^2 = 0.03$) were significantly correlated with antibacterial effect. The addition of other parameters into the stepwise model did not make a significant contribution to total variance. Analysis of variance also revealed that there were no significant differences between antimicrobial agent (independent of dose) or *P. aeruginosa* isolate with regard to antibacterial effect.

Pharmacodynamic modeling of ln reciprocal $AUEC_{24}$ transformations versus AUC/MIC_{24} demonstrated that the data best fit an E_{max} sigmoidal model with $r^2 = 0.72$ ($P < 0.005$). Parameters fitted by this model were $E_{max} = 9.1$, $AUC/MIC_{50} = 119\ SIT^{-1} \cdot h$, and $S = 2.01$. ln reciprocal $AUEC_{24}$ versus AUC/MIC_{24} for 29 duplicate sets of ciprofloxacin and ofloxacin bacterial concentration time-kill curves against three strains of *P. aeruginosa* are represented in Fig. 4.

Analysis of the relationship between $AUEC_{24}$ and AUC/MIC_{24} subgroup magnitude by the Kruskal-Wallis test indicated that significant differences in $AUEC_{24}$ existed between AUC/MIC_{24} subgroups. Tukey's multiple comparisons test indicated that the antibacterial effect associated with AUC/MIC_{24}s of ≤100 were significantly different from AUC/MIC_{24}s of >400, 301 to 400, 201 to 300, and 101 to 200 $SIT^{-1} \cdot h$ for ciprofloxacin or when combined fluoroquinolone data were analyzed. The antibacterial effect associated with ofloxacin AUC/MIC_{24}s of ≤100 $SIT^{-1} \cdot h$ exhibited a trend ($P > 0.05$ but < 0.1) toward a significant difference when compared with AUC/MIC_{24}s of 101 to 200 $SIT^{-1} \cdot h$. No other comparisons of antibacterial effect associated with AUC/MIC_{24} subgroups demonstrated a significant difference.

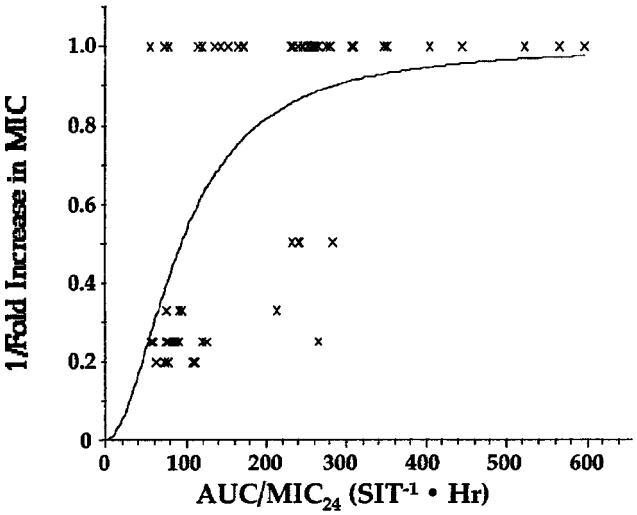

FIG. 5. Post experiment MIC changes and association with AUC/MIC.

Analysis of isolates recovered from the chemostats post antibiotic exposure revealed that an increase in the postexposure MIC magnitude was inversely related to AUC/MIC_{24} and C_{max}:MIC. Pharmacodynamic modeling of postexposure MIC magnitude increase versus AUC/MIC_{24} and C_{max}:MIC demonstrated that the data best fit an E_{max} sigmoidal model (AUC/MIC_{24}, $r^2 = 0.40$ [$P < 0.05$]; C_{max}:MIC, $r^2 = 0.41$ [$P < 0.05$]). Parameters fitted by this model were $E_{max} = 1$ (fixed), $AUC/MIC_{50} = 95\ SIT^{-1} \cdot h$, and $S = 1.97$ for AUC/MIC_{24}. Param-

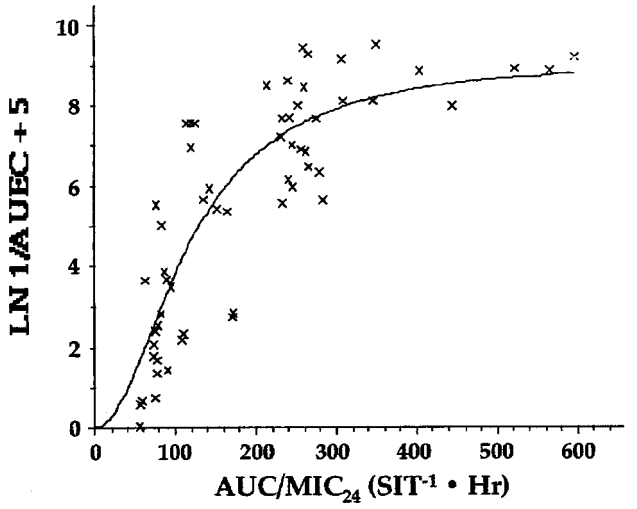

FIG. 4. Ciprofloxacin and ofloxacin against three strains of *P. aeruginosa*: relationship between AUEC and AUC/MIC.

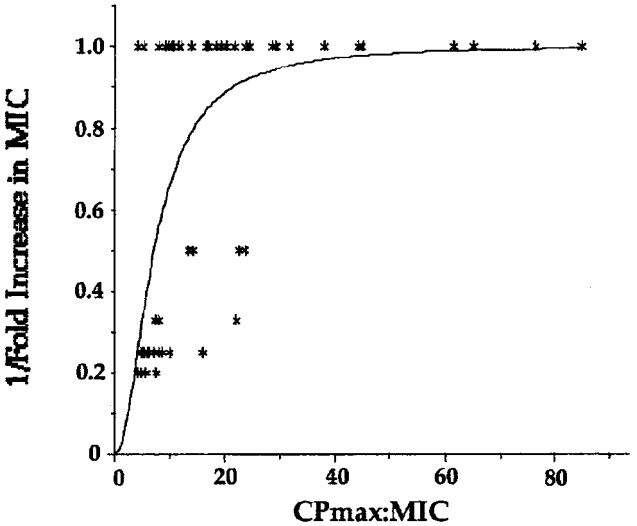

FIG. 6. Post experiment MIC changes and association with C_{max}:MIC.

eters fitted for C_{max}:MIC were $E_{max} = 1$ (fixed), C_{max}:MIC_{50} = 7.3, and $S = 2.01$. The association of postexposure MIC magnitude increase and AUC/MIC_{24} and C_{max}:MIC are presented in Fig. 5 and 6, respectively.

DISCUSSION

A number of in vitro, animal, and human trials have studied the pharmacodynamic activities of fluoroquinolone antibiotics (1–5, 8–10, 13, 16). Generally, fluoroquinolones are considered to act in a concentration-dependent manner (16). However, most of the pharmacokinetic and pharmacodynamic relationships such as AUC_{24}/MIC, C_{max}/MIC, time > MIC, and AUC/MIC_{24} that have been used to describe fluoroquinolone pharmacodynamics are highly covariant, and a definitive outcome parameter of fluoroquinolone activity is lacking.

These data suggest that AUC/MIC_{24} is the most descriptive pharmacodynamic predictor of the antibacterial activities of both ciprofloxacin and ofloxacin against *P. aeruginosa* over a broad range of drug exposure concentrations. In addition, these data imply that both the magnitude and the duration of fluoroquinolone exposure may be important for a maximal antibacterial effect. Both univariate and multivariate regression analyses indicated that the relationship between $AUEC_{24}$ and AUC/MIC_{24} is more predictive than the relationship between $AUEC_{24}$ and C_{max}:MIC for both ciprofloxacin and ofloxacin. However, AUC/MIC_{24} was highly interdependent with C_{max}:MIC because 75% of the variance could be attributed to C_{max}:MIC. These results are similar to those previously described by Forrest et al. (5).

In the present investigation, experiments which were conducted with different combinations of $t_{1/2}$, C_{max}, and MIC to produce similar AUC/MIC_{24}s resulted in a similar degree of antibacterial effect. While a range of $t_{1/2}$ (2.0 to 10.2 h) and C_{max}:MIC (12.8 to 33) were simulated at similar AUC/MIC_{24}s, the degree of variability associated with fluctuations in C_{max} or $t_{1/2}$ and their contributing effects to an optimal AUC/MIC_{24} could not be assessed in these limited experiments. While the majority of total $AUEC_{24}$ variance could be explained by AUC/MIC_{24}, the MIC was independently a statistically significant variable in the multivariate analysis. However, the *P. aeruginosa* isolate independent of MIC was not a significant variable. The MICs in the present experiments were calculated by serial twofold dilutions over a limited range of values, and extrapolation of the MIC as an independent variable to a broad range of MICs or other bacterial species merits caution.

Data from the present investigation support the generic application of AUC/MIC_{24} as a pharmacodynamic predictor for both of the fluoroquinolones ciprofloxacin and ofloxacin against *P. aeruginosa*. However, the relationship between an AUC/MIC_{24} of <100 $SIT^{-1} \cdot h$ and the loss of antimicrobial effect was more pronounced for ciprofloxacin than for ofloxacin. The variability in ofloxacin response may be explained by the greater dependence of AUC/MIC_{24} upon the larger ofloxacin MIC. The breakpoint of approximately 100 $SIT^{-1} \cdot h$ is slightly different from those reported previously for ciprofloxacin and OPC 17716 (4, 5). Differences in study design and the limited number of experiments in our study do not allow for a more precise breakpoint assessment. In addition, the present experiments were not designed to assess the effects of protein binding, tissue penetration, or immune function on the antimicrobial effects of fluoroquinolones.

Corresponding to the lack of an antimicrobial effect at lower AUC/MIC_{24}s, the magnitude and frequency of fluoroquinolone-resistant isolates of *P. aeruginosa* increased. Pharmacodynamic modeling of postexposure magnitude increase in the MIC versus AUC/MIC_{24} and C_{max}:MIC demonstrated that one-half of the maximal postexposure increase in the MIC was attributable to an AUC/MIC_{24} of 95 $SIT^{-1} \cdot h$ or a C_{max}:MIC of 7.3. While resistance did develop at higher levels of drug exposure, resistance did not develop at AUC/MIC_{24}s of >285 $SIT^{-1} \cdot h$ or a C_{max}:MIC of >24. High peak fluoroquinolone concentrations have previously been shown to prolong the duration of time necessary to develop resistance in other in vitro models, and resistance development associated with similar C_{max}:MIC values have been described (1, 2, 10). Only one clinical study has related the incidence of resistance with microbiologic or clinical failures. Peloquin et al. (13) reported that 70% of therapeutic failures in patients treated with ciprofloxacin and infected with *P. aeruginosa* developed resistance, with a C_{max}:MIC of ≤ 5, whereas approximately 62% of isolates from our study for which C_{max}:MIC ratios were <10 developed resistance. Perhaps longer fluoroquinolone exposure periods would still result in the development of resistance at AUC/MIC_{24}s of >285 $SIT^{-1} \cdot h$ in this in vitro system.

Selective pressure cannot completely explain the decrease in antibacterial effect apparent upon readministration of antibiotic, because organisms in experiments in which there were not postexposure increases in the MIC also exhibited regrowth. In addition, even at supraphysiologic fluoroquinolone concentrations, no experiments resulted in complete sterilization of the chemostat. Haag et al. (6) suggested that in vitro model bacterial regrowth is due to an adherent bacterial population that seeds the chemostat as antimicrobial concentrations drop below the MICs for the bacteria. In our experiments, fluoroquinolone concentrations remained above the preexposure MIC for the duration of all experiments, including experiments in which no increase in postexposure MIC was exhibited. Quantitative measurements of antibacterial effect (and selective pressure) in in vitro and other neutropenic models may be influenced by the inability to remove the remaining bacterial subpopulations. In addition, the influence of a starting inoculum of $>10^6$ CFU/ml on the selection of resistant organisms and $AUEC_{24}$ is unknown. The relationships between quantitative measurements of antibacterial effect obtained in this in vitro system and the variables that we studied should be viewed as a relative rather than an absolute measurement of antibiotic effectiveness.

In conclusion, these data support the generic application of AUC/MIC_{24} as the most descriptive measurement of fluoroquinolone antimicrobial activity against *P. aeruginosa*. A breakpoint of antibacterial effect was demonstrated at fluoroquinolone-bacterial AUC/MIC_{24} of 100 $SIT^{-1} \cdot h$, which is consistent with data generated in animal models and data reported from human trials. Furthermore, the development of subvariant bacterial populations over the course of our in vitro experiments closely correlated with the breakpoint of antimicrobial effect. Trials with additional fluoroquinolones and pathogens should be undertaken to further explore the value of AUC/MIC_{24} as a generic predictor of pharmacodynamic outcome with fluoroquinolone treatment.

ACKNOWLEDGMENTS

We thank Frank N. Konstantinides, David Wright, Robert Juola, and Edward Acosta for technical assistance.

This project was supported in part from an American College of Clinical Pharmacy/R. W. Johnson Research Institute Fellowship Award.

REFERENCES

1. **Bauernfeind, A., C. Petermuller, and B. Heinrich.** 1986. Dependence of bacterial activity and mutant selection of 4-quinolones on their serum concentration levels. Infection **14**(Suppl. 1)**:**S26–S30.

2. **Blaser, J., B. B. Stone, and M. C. Groner.** 1987. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrob. Agents Chemother. **31:**1054–1060.
3. **Drusano, G. L., D. E. Johnson, M. Rosen, and H. C. Standiford.** 1993. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas* sepsis. Antimicrob. Agents Chemother. **37:**483–490.
4. **Forrest, A., M. Amantea, D. A. Collins, S. Chodosh, and J. J. Schentag.** 1993. Pharmacodynamics (PDs) of oral OPC-17116 in patients (pts) with acute bacterial exacerbations of chronic bronchitis, abstr. 81, p. 134. *In* Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
5. **Forrest, A., D. E. Nix, C. H. Ballow, T. F. Goss, M. C. Birmingham, and J. J. Schentag.** 1993. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. Antimicrob. Agents Chemother. **37:**1073–1081.
6. **Haag, R., P. Lexa, and I. Werkhauser.** 1986. Artifacts in dilution pharmacokinetic models caused by adherent bacteria. Antimicrob. Agents Chemother. **29:**765–768.
7. **Herman, V. K., F. N. Konstantinides, R. A. Zabinski, A. Krinke, K. J. Walker, and J. Rotschafer.** 1991. Abstr. 336. *In* Program and abstracts of the 17th Meeting of the Federation of American Societies for Experimental Microbiology. Federation of American Societies for Experimental Microbiology.
8. **Hyatt, J. M., D. E. Nix, and J. J. Schentag.** 1994. Pharmacokinetic and pharmacodynamic activities of ciprofloxacin against strains of *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* for which MICs are similar. Antimicrob. Agents Chemother. **38:**2730–2737.
9. **Leggett, J. E., S. Ebert, B. Fantin, and W. A. Craig.** 1991. Comparative dose effect relations at several dosing intervals for beta-lactam, aminoglycoside, and quinolone antibiotics against gram negative bacilli in a murine thigh-infection and pneumonitis models. Scand. J. Infect. Dis. **74**(Supp.l)**:**179–184.
10. **Lerner, S. A., C. A. Lavieri, and E. M. Quimosng.** 1988. Selection of ciprofloxacin mutants *in-vitro* from clinical isolates of Pseudomonas aeruginosa and other bacteria in an *in-vitro* two compartment capillary model. Rev. Infect. Dis. **10**(Suppl. 1)**:**34–35.

10a. **Madaras-Kelly, K. J., B. E. Ostergaard, L. Baeker Hovde, and J. C. Rotschafer.** 1994. Identification of an area under the inhibitory curve (AUIC) threshold breakpoint for two fluoroquinolones utilizing an *in vitro* pharmacodynamic model, abstr. A85, p. 146. *In* Program and abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

11. **McFarland, M. M., E. M. Scott, and A. L. W. Po.** 1994. Time-survival studies for quantifying effects of azlocillin and tobramycin on *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **38:**1271–1276.
12. **Nightingale, C. H.** 1993. Pharmacokinetic considerations in quinolone therapy. Pharmacotherapy **13**(2 pt 2)**:**34S–38S.
13. **Peloquin, C. A., T. J. Cumbo, and D. E. Nix.** 1989. Evaluation of intravenous ciprofloxacin in patients with nosocomial lower respiratory tract infections: impact of plasma concentrations, organism, minimum inhibitory concentration and clinical condition of bacterial eradication. Arch. Intern. Med. **149:** 2269–2273.
14. **Sawchuk, R. J., D. E. Zaske, R. J. Cipolle, W. A. Wargin, and R. G. Strate.** 1977. Kinetic model for gentamicin dosing with the use of individual patient parameters. Clin. Pharmacol. Ther. **21:**362–369.
15. **Schentag, J. J., D. E. Nix, and M. H. Adelman.** 1991. Mathematical examination of dual individualization principles. Relationships between AUC above MIC and area under the inhibitory curve (AUC/MIC) for cefmenoxime, ciprofloxacin, and tobramycin. DICP-Ann. Pharmacother. **25:**1050–1057.
16. **Schentag, J. J., D. E. Nix, and A. Forrest.** 1993. Pharmacodynamics of the fluoroquinolones, p. 259–271. *In* D. C. Hooper and J. S. Wolfson (ed.), Quinolone antimicrobial agents, 2nd ed. American Society for Microbiology, Washington, D.C.
17. **Yeh, K. C., and K. C. Kwan.** 1978. A comparison of numerical integrating algorithms by trapezoidal, Lagrange, and spline approximations. J. Pharmacokinet. Biopharm. **6:**79.
18. **Zabinski, R. A., A. J. Krinke, K. J. Walker, S. S. Gilliland, and J. C. Rotschafer.** 1993. Elimination of quinolone carryover through the use of antibiotic removal binding resins. Antimicrob. Agents Chemother. **37:**1377–1379.
19. **Zabinski, R. A., K. Vance-Bryan, K. J. Walker, A. Krinke, J. A. Moody, and J. C. Rotschafer.** 1993. Evaluation of the activity of temafloxacin versus *Bacteroides fragilis* using an in vitro pharmacodynamic system. Antimicrob. Agents Chemother. **37:**2454–2458.
20. **Zar, J. H.** 1984. Multiple comparisons, p. 185–206. *In* Biostatistical analysis, 2nd ed. Prentice-Hall, Inc., Englewood Cliffs, N.J.