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Pharmacodynamics of a Fluoroquinolone Antimicrobial Agent in a Neutropenic Rat Model of *Pseudomonas* Sepsis

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We examined the impact of dose fractionation and altered MICs on survivorship in a neutropenic rat model of *Pseudomonas aeruginosa* sepsis employing the new fluoroquinolone antibiotic lomefloxacin. Once-daily administration of a drug dose which produced a high peak concentration/MIC (peak/MIC) ratio (ca. 20/1) produced significantly better survivorship compared with regimens employing the same daily dose but on a more fractionated schedule. The use of a smaller dose, producing lower (<10/1) peak/MIC ratios, did not show this effect, as once-daily and twice-daily regimens produced equivalent results (the area under the concentration-time curve/MIC ratio was linked to survivorship). Challenge with resistant mutants selected for altered MICs of fluoroquinolones (two and four times the MIC for the parent strain, respectively) resulted in markedly diminished survivorship. Challenge with the parent strain and use of a drug dose which produced a peak/MIC ratio identical to that for animals challenged with the mutant for which the MIC was four times that for the parent strain and treated with the larger drug dose produced survivorship curves which were not different. For this animal model, peak/MIC ratio was linked to survivorship, particularly when high ratios (10/1 to 20/1) were obtained. At lower doses, producing peak/MIC ratios <10/1, the area under the concentration-time curve relative to the MIC appeared to be most closely linked to outcome. The time that levels in plasma exceeded the MIC did not influence survivorship. The hypothesis most likely to explain these findings is that higher peak/MIC ratios can suppress the parent strain and mutant organisms (*gyrA* and transport mutants) for which the MIC is higher but limited (no more than eight times that for the parent strain).

Schedules of antibiotic dosing can be altered to improve the outcome for severely infected patients. Many years passed before convincing data indicating that different classes of antimicrobial agents (e.g., β-lactams and aminoglycosides) require different schedules for optimal outcome for severely infected patients were generated (3, 12, 13, 17). In general, in vitro and animal model systems have provided necessary insights which have guided the design of clinical trials to provide the data which have proven these hypotheses in the clinical arena (1, 6, 8).

Fluoroquinolone antimicrobial agents have been recently introduced into the physicians' therapeutic armamentarium. These agents are highly active against aerobic or facultative gram-negative bacilli. They have been shown to be very concentration dependent in their rate of kill. This property makes them resemble aminoglycosides more than β-lactam antibiotics in their microbiological properties. As these drugs have become more widely used clinically, we felt it important to investigate the relationship between the concentration in plasma-time profile and survivorship in a model of *Pseudomonas aeruginosa* sepsis in neutropenic rats.

MATERIALS AND METHODS

Bacteria. Several blood culture isolates of *P. aeruginosa* from patients at the University of Maryland Cancer Center were screened in vitro and in rats. *P. aeruginosa* strain 25 was selected for study because of the following characteristics: antibiotic susceptibility, as determined by a lomefloxacin MIC 1 μg/ml, and a 50% lethal dose (LD_{50}) of 2.6×10^6 organisms for intraperitoneally challenged neutropenic rats.

Organisms more resistant to lomefloxacin (MICs four to eight times that for the parent strain) were derived by serial passage of the *P. aeruginosa* 25 parent strain on lomefloxacin-containing agar. The MICs for these isolates were stable after six weekly passages of the isolates on antibiotic-free media.

Animals. Female Sprague-Dawley rats weighing 180 to 200 g (Harlan Sprague-Dawley, Inc., Walkersville, Md.) were conditioned in our laboratory for 1 week after being received from the breeder. During the conditioning period, stool cultures were examined to ensure that the animals were not colonized with *P. aeruginosa*. During conditioning and throughout the experiments, rats had free access to rat chow and fresh drinking water which was acidified to prevent contamination with *P. aeruginosa*. Noninfected neutropenic control rats remained free of *P. aeruginosa* throughout the experiment.

Induction of neutropenia. Cyclophosphamide (Mead Johnson, Evansville, Ind.) was administered intraperitoneally in doses of 100 mg/kg of body weight on day 0 and 75 mg/kg of body weight on day 4. Leukocyte counts were taken daily for noninfected rats to assess the effects of cyclophosphamide. Differential counts were taken on Wright-stained smears. As demonstrated previously (10), cyclophosphamide therapy effected a reduction in the mean leukocyte count from 12,700 leukocytes per μl on day 0 to 470 leukocytes per μl on day 5 and maintained the count at the latter level throughout the therapeutic period (days 5 to 8). A reduction in total leukocyte count was accompanied by a reduction in granulocytes. On day 0, the mean granulocyte

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count was 1,905 granulocytes per μl, and, on days 5 through 8, it was less than 50 granulocytes per μl.

LD_{50} determinations. Neutropenic rats were inoculated intraperitoneally on day 5 with 1 ml of a serial 10-fold dilution (10 rats per dilution) of an overnight culture of *P. aeruginosa* strain 25 grown in Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.). The LD_{50} of 2.6×10^6 organisms was calculated by the method of Reed and Meunch (16) from results 72 h after bacterial challenge (day 8). With a challenge inoculum level of 10^9 organisms, 10 of 10 rats died; at 10^8 organisms, 8 of 10 died; at 10^7 organisms, 7 of 10 died; at 10^6 organisms, 5 of 10 died; and at 10^5 organisms, only 1 of 10 died. The other more resistant mutant organisms had calculated LD_{50}s less than 1 log unit different from that determined with the parent strain. Challenges with the mutant organisms were corrected for these slight differences in LD_{50}.

Antimicrobial agents. The lomefloxacin used in the rats was from vials prepared for clinical use. Reference solutions of lomefloxacin were prepared from standardized antibiotic powders supplied by the manufacturer (Searle, Skokie, Ill.).

In vitro susceptibility studies. MIC and MBC were determined by microtiter broth dilution techniques (14). Serial twofold dilutions of lomefloxacin were prepared with Mueller-Hinton broth (BBL) containing 50 μg of calcium per ml and 25 μg of magnesium per ml (0.05 ml per well). For *P. aeruginosa* strain 25, a dilution of an overnight culture was added to each well (0.0015 ml per well) to yield a final concentration of approximately 5×10^5 CFU per ml. The contents of each microtiter tray were mixed, and the trays were covered and incubated at 37°C overnight. The MIC was defined as the lowest antimicrobial concentration that prevented visible growth. A 0.01-ml sample from each MIC microtiter well was inoculated onto a Trypticase soy agar plate, which was incubated at 37°C overnight. The MBC was defined as the lowest antibiotic concentration producing a ≥99.9% reduction in viable bacteria.

Concentrations of antimicrobial agents in rat serum. Studies to determine antimicrobial concentrations in rat serum were performed with infected animals. Lomefloxacin was administered to the animals subcutaneously. Animals (three rats per group) were injected with various doses of lomefloxacin (80, 40, and 20 mg/kg) and then were bled retro-orbitally at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, and 12 h after drug administration. Concentrations in serum were determined by modified cylinder plate procedures supplied by the manufacturer. The assay organism was *Klebsiella pneumoniae* ATCC 10031. The assay organism was added to molten agar (antibiotic medium 11 [Difco]) before distribution of the latter into assay plates. Agar wells (5 mm in diameter) were filled with reference antibiotic solutions or rat serum and were diluted with pooled normal rat serum (PEL-Freeze Biological, Rogers, Ark.). Zones of inhibition of bacterial growth were measured after incubation of the plates at 30°C overnight, and antibiotic concentrations in serum were calculated from the curves of the inhibition zone sizes from reference antibiotic concentrations. The assay was linear over a range of 0.5 to 40 μg/ml. The sensitivity was 0.5 μg/ml. Within-day and between-day coefficients of variation were less than 10% over the concentration ranges tested.

Therapeutic trials. Neutropenic rats (20 to 50 rats per group) were challenged intraperitoneally on day 5 (counting from the initial cyclophosphamide injection) with 1 ml of an overnight culture of *P. aeruginosa* diluted to yield multiples of the LD_{50}. Antibiotics were administered 2 h after the bacterial challenge. They were then administered in a multiple-dose fashion on a 24, 12, or 6-h schedule for 72 h after the initiation of antimicrobial therapy. The following controls (20 to 50 rats per group) were included in each experiment: rats receiving cyclophosphamide only; rats receiving cyclophosphamide, *P. aeruginosa*, and saline; and rats receiving cyclophosphamide and antibiotics. Mortality was recorded immediately before each antibiotic dose, and the final mortality was recorded 72 h after bacterial challenge (day 8).

TABLE 1. Pharmacodynamic variables for three lomefloxacin regimens[a]

Regimen	Peak/MIC ratio	AUC/MIC ratio/24 h	Time > MIC[b]/24 h
20 mg/kg every 6 h	4.7 ±0.5	57.2 ±7.0	16.8 ±0.6
40 mg/kg every 12 h	6.9 ±0.6	63.6 ±32.8	14.0 ±0.9
80 mg/kg every 24 h	20.8 ±6.3	64.3 ±9.1	9.6 ±0.1

[a] Three infected animals per regimen were evaluated. Data are means ± standard deviations.
[b] Time > MIC, time that the concentration in plasma was above the MIC.

Statistical analysis. Deaths recorded throughout the experiment were compared by Kaplan-Meier analysis. Differences were compared for statistical significance by the log rank and Wilcoxon tests. Data for concentration in serum and time were modeled through the use of weighted, nonlinear least-squares regression analysis. All data were weighted by the inverse of the assay variance in an attempt to counteract any heteroscedasticity. Multiple models were evaluated, and model discrimination was accomplished by employing the Akaike information criterion (18). A two-compartment open model with first-order input and first-order elimination fit the data best. The relationship between peak concentration/MIC (peak/MIC) ratio and survivorship was ascertained by employing a modified Hill's model (Sigmoid-Emax model) (4). The ADAPT II package of programs of D'Argenio was employed for all nonlinear regression analyses (2).

RESULTS

Concentrations of lomefloxacin in rat serum. Data for individual animals were modeled, and the identified parameter estimates were used to calculate the peak concentration achieved, the area under the concentration in plasma-time curve (AUC) from 0 to τ (at steady state), and the time that concentrations in plasma remained above the MIC for the challenge organism. The average data for the three dosing regimens are displayed in Table 1. The daily dose used for the fractionation experiments was chosen from a once-per-day dose escalation study. Doses of 10, 20, 40, and 80 mg/kg, all given once daily, were evaluated. We were seeking a daily dose for fractionation which would provide a 50 to 70% salvage rate for infected animals. Obviously, such a salvage rate would be on the steep part of the dose-response curve, and, consequently, an improvement or worsening in survivorship due to dose fractionation could be readily observed. This led to the choice of an 80-mg/kg/day dosage for further study.

The first set of challenge experiments was performed with four groups of animals with 50 animals per group. The groups received saline (control) or lomefloxacin at 80 mg/kg once daily, 40 mg/kg every 12 h, or 20 mg/kg every 6 h as

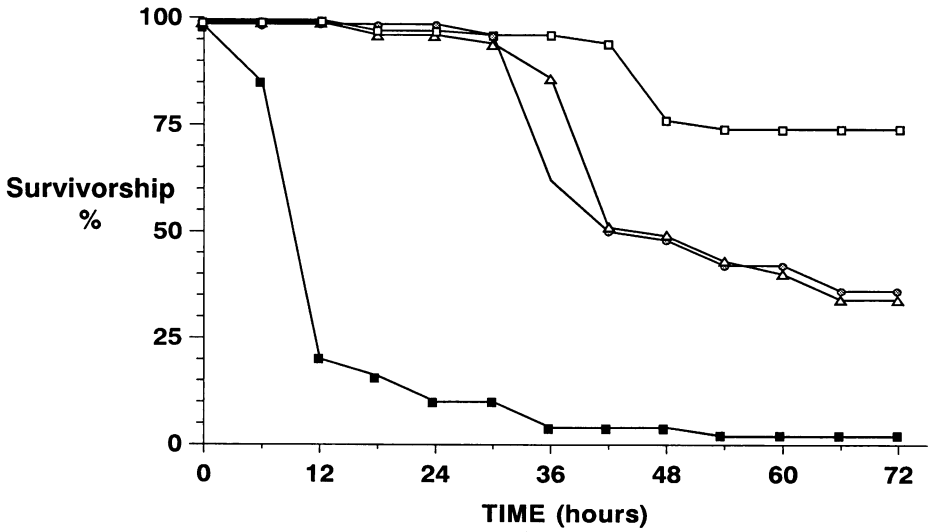

FIG. 1. Dose fractionation experiment 1. The MIC of lomefloxacin for the challenge organism was 1 μg/ml. Regimens of 80 mg/kg every 24 h (□), 40 mg/kg every 12 h (△), and 20 mg/kg every 6 h (⊗) were evaluated. Control animals received a saline placebo injection (■). There were 50 animals evaluated per group.

therapy for the neutropenic rats. The outcomes are displayed in Fig. 1. The once-daily administration of lomefloxacin resulted in 74% survivorship. Twice- and four-times-daily administrations of the same total daily dose of the drug resulted in 32 and 36% survivorships, respectively. The difference between the once-daily dosing group and the other two groups is statistically significant ($P < 0.001$, Kaplan-Meier analysis). Table 1 indicates that if the time that the concentration in plasma was above the MIC were the clinically important variable linked to survivorship, then the regimen of 20 mg/kg every 6 h should have performed best. Likewise, if the AUC was linked to outcome, then all three regimens should be equivalent or nearly so. Finally, if the peak/MIC ratio is important for the outcome, once-daily administration of lomefloxacin should have produced the best clinical outcomes. The results displayed in Fig. 1 indicate that the peak/MIC ratio was the pharmacodynamic variable most closely linked to survivorship in this model.

However, when one examines the twice-daily and four-times-daily regimen outcomes in Fig. 1, it is clear that there is little difference between them. This led us to the second set of experiments, in which a lower dose of lomefloxacin of 40 mg/kg was employed once daily and contrasted with 20 mg/kg administered every 12 h along with the control (saline, administered to a third group of animals). Twenty animals in each group were evaluated. The results are displayed in Fig. 2. There was no difference between the outcomes engendered by 40 mg of lomefloxacin per kg once daily versus 20 mg/kg administered on a 12-h schedule. Both were significantly different from the control ($P < 0.001$). Consequently, in this experiment it appears that the AUC relative to the MIC is the pharmacodynamic variable most closely linked to outcome.

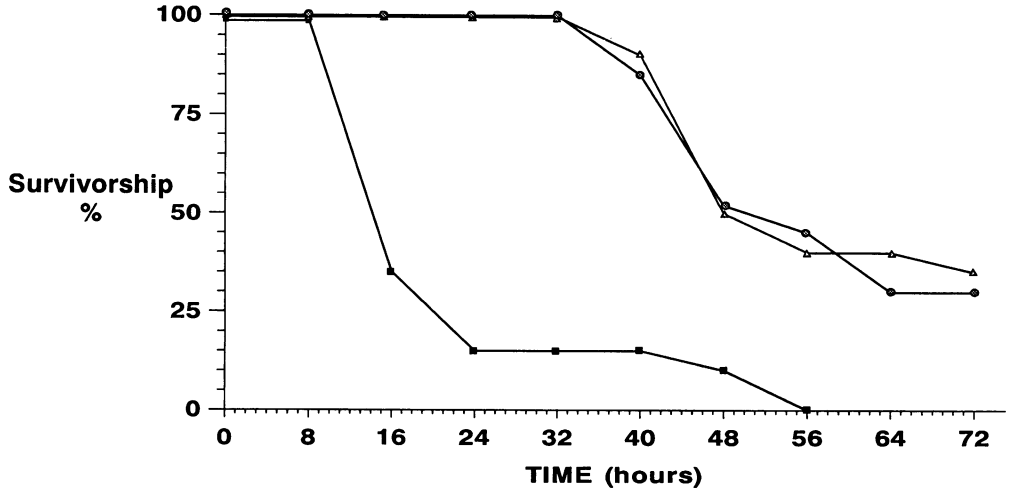

FIG. 2. Dose fractionation experiment 2. The MIC of lomefloxacin for the challenge organism was 1 μg/ml. Regimens of 40 mg/kg every 24 h (△) and 20 mg/kg every 12 h (⊗) were evaluated. Control animals received a saline placebo injection (■). There were 20 animals evaluated per group.

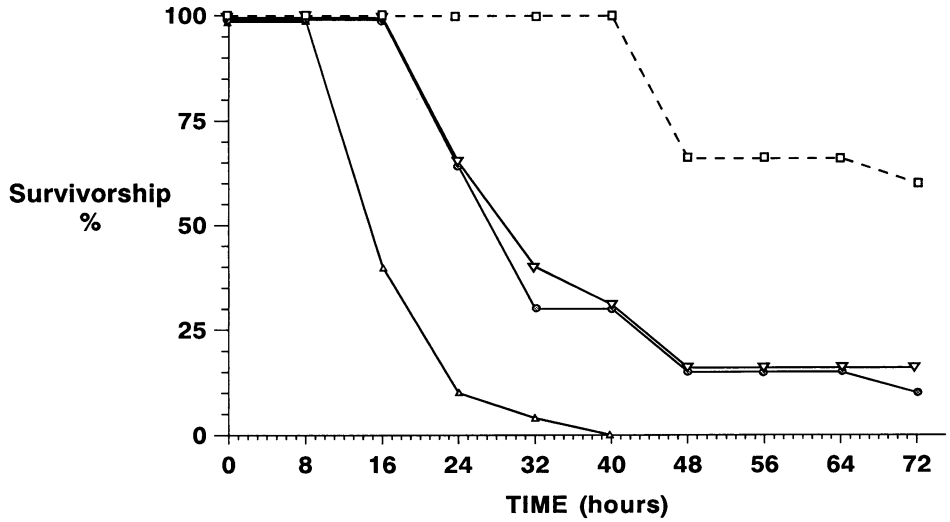

FIG. 3. Effect of altered MIC upon survivorship. Three isogenic organisms for which the lomefloxacin MICs were different served as the bacterial challenge. MICs were 1 (□), 4 (▽), and 8 (△) μg/ml. The three groups received 80 mg/kg every 24 h. A fourth group had the strain for which the MIC was 1 μg/ml (⊖) used as the challenge organism and a dosing regimen of 20 mg/kg every 24 h. This provided the same peak/MIC ratio as the challenge organism for which the MIC was 4 μg/ml used with animals treated with 80 mg/kg every 24 h. There were saline-treated controls for each challenge organism (all died). There were 20 animals evaluated per group.

In either event, whether outcome is linked to the peak/MIC ratio or AUC/MIC ratio, a once-daily administration schedule is clearly preferable for clinical utility. Consequently, in the next series of experiments, we employed only once-daily administration schedules for lomefloxacin. We had examined the impact of pharmacological variables on outcome by changing doses and administration schedules. We now wished to examine the impact of changing MICs on outcome in this animal system. Using strains of *Pseudomonas* for which the MICs were different would be unacceptable because of changes in virulence factors that may also change along with MICs. Consequently, we selected a series of mutant organisms derived from the parent strain (*P. aeruginosa* 25). For the first mutant, the MIC was 4 μg/ml (four times the MIC for the parent strain). For the second mutant, the MIC was 8 μg/ml (eight times that for the parent strain). LD_{50} determinations with each of the mutant strains were performed as described previously.

In the next experiment, we examined seven groups of 20 animals each. The first three groups were saline controls for each of the strains (*P. aeruginosa* 25 and mutants for which the MICs were four and eight times that for the parent strain). For group 4, the parent strain (for which the MIC was 1 μg/ml) was used and animals were treated with 80 mg of lomefloxacin per kg once daily. For group 5, the mutant for which the MIC was 4 μg/ml was used and animals were treated with 80 mg of lomefloxacin per kg once daily. For group 6, the mutant for which the MIC was 8 μg/ml was used and animals were treated with 80 mg of lomefloxacin per kg once daily. For group 7, the parent strain, *P. aeruginosa* 25 (for which the MIC was 1 μg/ml), was used and animals were treated with 20 mg of lomefloxacin per kg once daily. Two groups would have the same peak/MIC ratios: the one with the mutant for which the MIC was four times that for the parent strain and which was treated with 80 mg/kg/day and the group with the parent strain and which was treated with 20 mg/kg/day. All other groups would have differing peak/MIC ratios ranging from approximately 20:1 to 2.5:1. The outcomes of this experiment are displayed in Fig. 3. For clarity, the outcomes for the saline control groups are not displayed. All saline control groups had 100% mortality.

Group 4 (MIC for challenge strain, 1 μg/ml; treatment, 80 mg/kg/day) had a survivorship curve which was quite similar to and not significantly different from the survivorship curve of the once-daily therapy group in Fig. 1, with an ultimate survivorship of 65%. Group 5 (MIC for challenge strain, 4 μg/ml; treatment, 80 mg/kg/day) had an ultimate outcome of 15% survivorship. Group 6 (MIC for challenge organism, 8 μg/ml; treatment, 80 mg/kg/day) had an ultimate survivorship of 0%. Finally, group 7 (MIC for challenge strain, 1 μg/ml; treatment, 20 mg/kg/day), which had a peak/MIC ratio identical to that of group 5, had a survivorship curve which is virtually superimposable over that of group 5 and had an ultimate outcome of 10% survivorship. Each group, with the exceptions of groups 5 and 7, had significantly different survivorship curves.

Clearly, a changing peak/MIC ratio affects survivorship, and challenge groups with peak/MIC ratios which were the same have quite similar outcomes.

As it had been reported that very high concentrations of quinolones could paradoxically reduce the kill rate of facultative or aerobic gram-negative bacilli (5), we wished to examine whether higher doses of drug resulting in higher peak/MIC ratios would obtain better or worse outcomes in this neutropenic animal model of *Pseudomonas* sepsis. The final experiment examined the parent strain, *P. aeruginosa* 25, with saline or lomefloxacin at 80 or 160 mg/kg once daily as the treatment regimen. The results are displayed in Fig. 4. Twenty animals per group were examined. The dosage of 80 mg/kg once daily has outcomes which display excellent reproducibility, with a survivorship of 75% in this experiment. The dosage of 160 mg/kg once daily resulted in a survivorship of 95%. All saline-treated controls died, as in previous experiments.

The experimental results displayed in Fig. 1 and 2 demonstrate that either the peak concentration or the total AUC, depending on the concentrations achieved, influenced the outcome. In both instances, the time that concentrations in

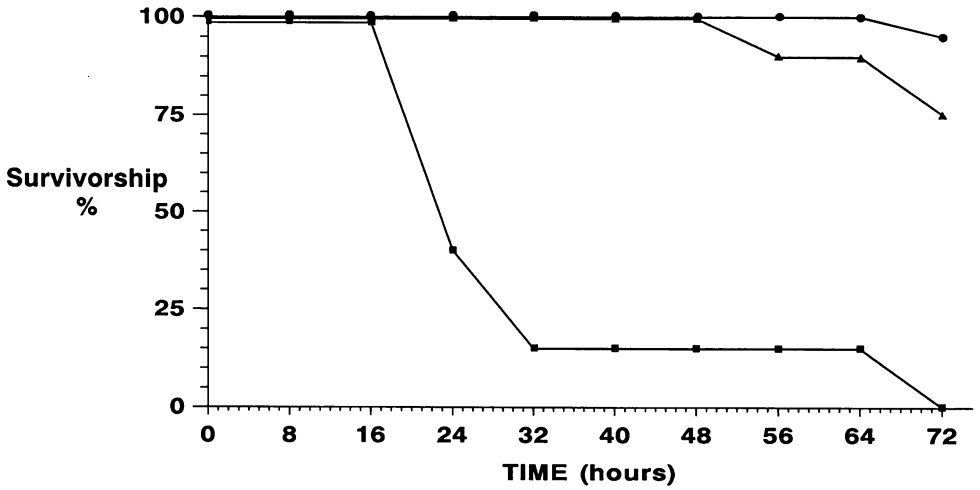

FIG. 4. Effect of a larger lomefloxacin dose on survivorship. The MIC of lomefloxacin for the challenge organism was 1 μg/ml. Regimens of 160 (●) and 80 (▲) mg/kg every 24 h were evaluated. Control animals received a saline placebo injection (■). There were 20 animals evaluated per group.

plasma remained above the MIC did not seem to be linked to the outcome for lomefloxacin in this animal model of *Pseudomonas* sepsis. We then wished to examine the relationship between peak/MIC ratio and percent survivorship as well as the relationship between AUC/MIC ratios and survivorship. In Fig. 5a, we show the relationship between the peak/MIC ratio and percent survivorship by employing a modified Hill's model (Sigmoid-Emax model). There is an excellent fit of the model to the data, with an r^2 of 0.99 ($P < 0.001$). However, in Fig. 5b, we demonstrate a similar relationship for the AUC/MIC ratio. This relationship is equally statistically significant compared with the relationship developed for the peak/MIC ratio. Figure 5c displays this relationship for the time that the concentration in plasma remained above the MIC. This relationship is not quite as good as those developed with either the peak/MIC ratio or AUC/MIC ratio but still remains statistically significant, demonstrating the high degree of covariance among peak/MIC ratio, AUC/MIC ratio, and time that the concentration in plasma remains above the MIC.

DISCUSSION

Fluoroquinolones are relatively recent additions to our therapeutic armamentarium. As with many other agents, there is considerable confusion over the most appropriate way to administer these drugs in order to obtain an optimal outcome for seriously infected patients. Work with other classes of anti-infective agents has demonstrated that the time that concentrations in plasma remain above the MIC for the infecting pathogen is important in the outcome of infections when β-lactam agents are employed and that either the peak/MIC ratio or AUC/MIC ratio is important when aminoglycosides are employed (1, 3, 6, 8, 12, 13, 17). These conclusions have been demonstrated in in vitro studies and animal models and by clinical data. We wished to develop insights into the pharmacodynamics of outcome for a new class of antimicrobial agents, the fluoroquinolones. On the basis of their rapid, concentration-dependent bactericidal activity, fluoroquinolones would, on first principles, be thought most likely to have outcome linked to either peak concentration or total AUC. Indeed, there has been some controversy regarding this. Previous in vitro studies of fluoroquinolones which have integrated the pharmacokinetic behavior of these compounds into the evaluation identified the peak/MIC ratio as the pharmacodynamic variable most closely linked to outcome (1). However, animal model data developed by the group at the University of Wisconsin have demonstrated total exposure or AUC as the variable most closely linked to outcome (11). Ironically, in clinical studies, outcome was originally linked to the time that concentrations in plasma remained above the MIC (15). However, this group also demonstrated that the emergence of resistance was most frequently linked with low peak/MIC ratios. This was seen particularly with *P. aeruginosa*.

Our experiments were designed to differentiate among these variables. Clearly, the dose fractionation experiment (experiment 1) demonstrates that when relatively high peak/MIC ratios are achieved (10/1 to 20/1), the peak/MIC ratio is the important pharmacodynamically linked variable. However, the examination of the data from this experiment also revealed that smaller doses on 12- and 6-h schedules gave indistinguishable survivorship curves. This led to the evaluation of a smaller dose given on a once-daily schedule. The lower peak/MIC ratio attained with the lower dose given once daily resulted in a survivorship which was indistinguishable from that of the fractionated dose (half the dose given twice a day).

We were unable to examine the organisms causing the septic deaths of these animals. However, it is clear that there was a change in survivorship by schedule as indicated by achieving peak/MIC ratios of 7 to 1 versus 20 to 1. This is consistent with the larger peaks being able to suppress not only the parent strain (for which the MIC was 1 μg/ml) but also the mutant organisms in the population. Spontaneous mutation to fluoroquinolone resistance via *gyrA* mutation occurs with a frequency of approximately $1/10^7$ to $1/10^9$ (9). As we were using relatively dense inocula, it is likely that these mutants were already present in the population of organisms with which we challenged these animals. Much the same argument can be made for transport mutants. For transport mutants and *gyrA* mutants, MICs are usually four to eight times that for the parent strain from which they were derived. Consequently, it is a reasonable hypothesis that

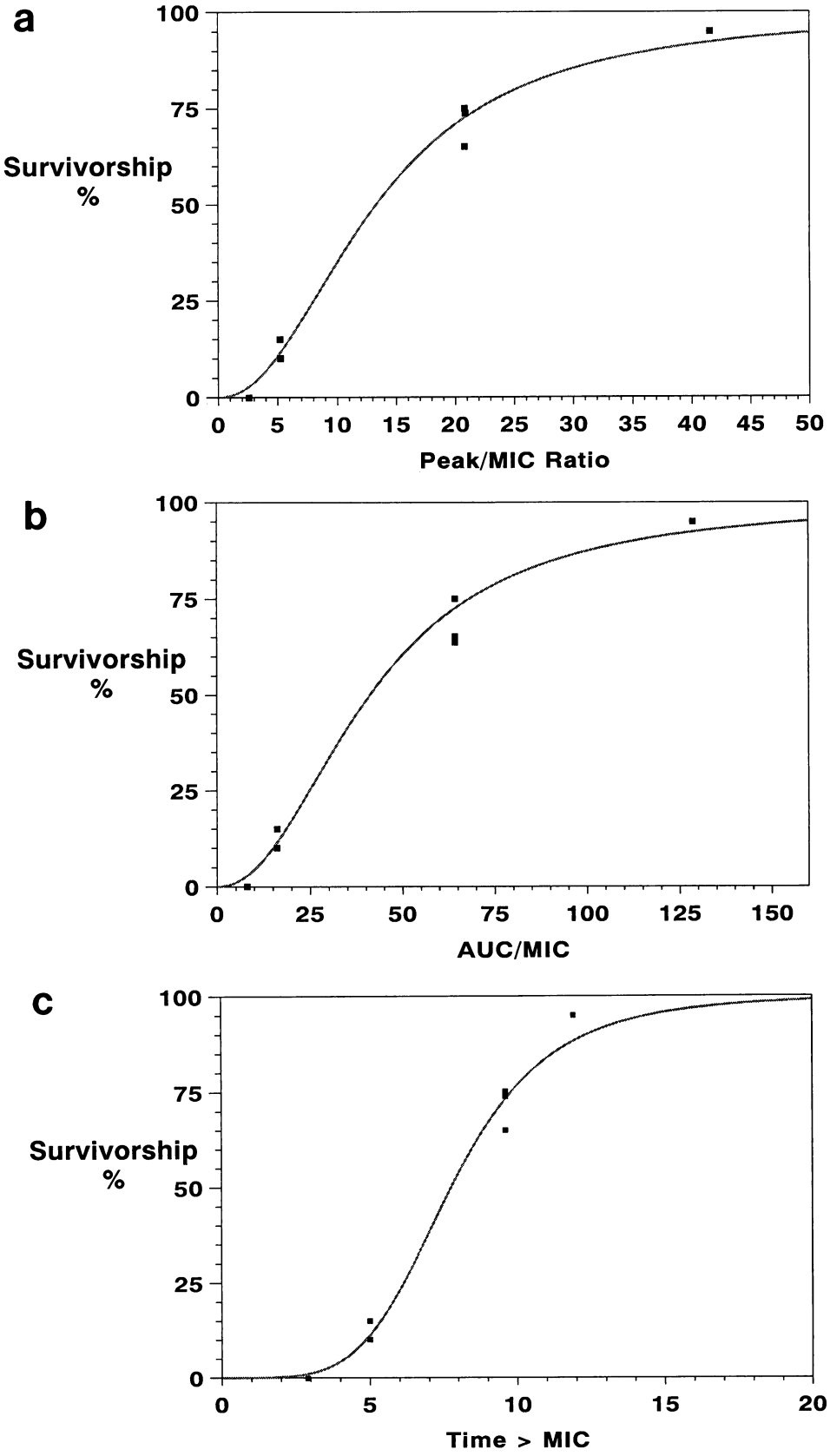

FIG. 5. Modified Hill's (Sigmoid-Emax) model evaluating survivorship as a function of three different independent variables, the peak/MIC ratio (a), the AUC/MIC ratio (b), and the time that the concentration in plasma was above the MIC (c). (a) % Survivorship = $100 \times (\text{peak/MIC ratio})^{2.18}/[(13.3)^{2.18} + (\text{peak/MIC ratio})^{2.18}]$. $r^2 = 0.989$; weighted sum of squares = 6.1314; $P < 0.001$. (b) % Survivorship = $100 \times (\text{AUC/MIC ratio})^{2.18}/[(41.1)^{2.18} + (\text{AUC/MIC ratio})^{2.18}]$. $r^2 = 0.989$; weighted sum of squares = 6.1315; $P < 0.001$. (c) % Survivorship = $100 \times (t > \text{MIC})^{4.71}/[(7.75)^{4.71} + (t > \text{MIC})^{4.71}]$, where $t >$ MIC is the time that the concentration in plasma was above the MIC. $r^2 = 0.985$; weighted sum of squares = 8.3915; $P < 0.001$.

quite high peak/MIC ratios (higher than 10:1) would suppress not only the parent strain but also the mutant organisms as well. Indeed, this 10:1 peak to MIC breakpoint had been predicted in the in vitro system of Blaser et al. (1). When once-daily dosing is insufficient to obtain peak/MIC ratios higher than 10:1, survivorship appears to be linked to total exposure or AUC. With an animal model, Leggett and colleagues (11) demonstrated that the AUC/MIC ratio was linked to outcome even though adequate (peak/MIC ratio > 10/1) peaks were achieved with some regimens. However, an important limitation of these mouse thigh data is that the usual bacterial challenge is approximately 10^6 organisms. As the mutational frequency to resistance is of a lower order, it is unlikely that resistant mutants would be in the population. Consequently, there would be a low likelihood of identifying the peak/MIC ratio as the important pharmacodynamically linked variable in such a system. In neither instance (in vitro or animal model data) did it appear that the time that the concentration was above the MIC was linked to outcome. This is important, as we were performing these experiments with a small animal system. The drug half-life for fluoroquinolone antimicrobial agents is much shorter in the small animal system than is observed clinically. Indeed, the half-life of lomefloxacin in rats in these experiments was on the order of 2 h. Consequently, dose fractionation, which would result in longer times that the concentration was above the MIC, would tend to exaggerate the importance of this parameter if, indeed, it were linked to outcome. The fact that, even in this animal model, in which the half-life of lomefloxacin was short, it was either the peak/MIC ratio or AUC/MIC ratio which was linked to survivorship emphasizes that the time that the concentration is above the MIC plays little role in determining the outcome of *Pseudomonas* sepsis in this model. Indeed, there are now clinical data to speak to this point. Forrest and colleagues have reanalyzed the data of Peloquin et al. by using a Cox proportional hazards model (7). These data indicate that the AUC/MIC ratio was the most important indicator of clinical success with the fluoroquinolone antimicrobial agent ciprofloxacin. This is not a surprising finding given the relatively large numbers of patients in this data set with pseudomonads and other relatively resistant pathogens as the causes of their nosocomial infections. The relatively resistant pathogens treated would be expected to result in quite low peak/MIC ratios for ciprofloxacin (i.e., akin to the experiment whose results are displayed in Fig. 2).

While we had examined alteration of the pharmacologic profile of lomefloxacin by dose fractionation and the resultant change in survivorship, we also wished to examine the influence of changing the MIC with an unchanging pharmacologic profile of a drug. Consequently, we derived two mutant organisms for which the MICs differed from that for the parent strain. This was done to prevent any changes in virulence properties from obscuring an underlying relationship between the changing MIC and the outcome. The results of the experiment were clearcut. Changing peak/MIC ratios as a consequence of altering the MIC had a major impact on survivorship in this animal model of *Pseudomonas* sepsis (please note that this experiment employed a dose of 80 mg/kg/day, with which the peak/MIC ratio had been shown to be the dynamically linked variable). We also examined a smaller dose of drug for use in a group of animals challenged with the parent strain, which produced the same peak/MIC ratio (and AUC/MIC ratio) obtained with one of the mutant organisms (for which the MIC was 4 μg/ml) with the larger 80-mg/kg/day single daily dose. The survivorship curves for the two groups of animals were virtually identical. This indicates that the ratio (either peak/MIC or AUC/MIC) controls the outcome, and it is irrelevant whether that ratio is obtained by altering the dose of the drug or by treating a more susceptible (or resistant) organism.

The paradoxical phenomenon of very high concentrations of drug actually reducing or shutting off the killing of gram-negative bacilli has been described to occur with fluoroquinolones. This is thought to be secondary to ongoing protein synthesis being necessary for the lethal effect of fluoroquinolones (5). We examined a very large dose of lomefloxacin given once daily (160 mg/kg). The hypothesis to be tested was that the very high concentrations of drug engendered by this dose could paradoxically make survivorship decrease in treated animals. We did not observe this, however, as the survivorship in the group treated with 160 mg/kg once daily was 95%, compared with 75% for the group treated with 80 mg/kg once daily.

The large number of experiments and experimental dosing groups allowed us to examine the dose-response curve for lomefloxacin for the challenge strain of *Pseudomonas* used in these experiments. We pooled data only for experiments in which a lomefloxacin dosage of 80 mg/kg once daily was included as a control and in which the survivorship for this dosing schedule was between 65 and 75% (this was the case in every experiment in which this dosage was employed). This ensured direct comparability across experiments. We modeled the dose-response relationship according to a modified Hill's model (Sigmoid-Emax model), as this has been shown to have utility for delineation of such relationships. In Fig. 5a we show the relationship between the peak/MIC ratio obtained and the percent survivorship in our animal model system. As can be seen, the fit of the model to the data is quite excellent, with an r^2 of 0.99. In Fig. 5b we show the same data but with a change in the independent variable, this time examining the AUC/MIC ratio of these dosing regimens. As can be seen, we have also derived an excellent relationship with AUC/MIC ratio and one that is statistically equivalent to that developed for the peak/MIC ratio. In Fig. 5c we display these data, this time looking at the time that the concentration in plasma was above the MIC as the independent variable. Once again, it is obvious that this is quite a good relationship but one that is slightly inferior to that developed by having either the peak/MIC ratio or AUC/MIC ratio serve as the independent variable.

These analyses point out that there is a tremendous degree of covariance among these parameters. That is, with a higher dose, the peak concentration, as well as the total exposure or AUC, must go up, and, as a consequence, the time that the concentration is above the MIC also increases. It is, therefore, not surprising that different experimental designs in different systems, such as have been seen with the in vitro hollow fiber system (1), the animal model system of Leggett and colleagues (11), and the clinical trials of Peloquin et al. (15), should identify different pharmacodynamically linked variables. A dose fractionation design, such as that employed in the experiments whose data are displayed in Fig. 1 and 2, was capable of easily differentiating among the variables examined and was able to identify the peak/MIC ratio as being most important at values higher than 10:1. It also demonstrates that at values less than 10:1, the AUC/MIC ratio does an equivalent job of describing the results.

In conclusion, these experiments demonstrate that the peak/MIC ratio or AUC/MIC ratio is most important in determining the outcome of serious infection with fluoroquinolone antimicrobial agents. In general, these pharmacody-

namic variables are equivalent in their ability to describe the outcome seen in the animal model system. However, when relatively high peak/MIC ratios are achieved (≥20:1), outcomes become significantly better, probably as a result of the suppression of the more resistant mutants in the population. These results explain much of what has been seen in the previous literature with in vitro and animal model systems (1, 11) and are bolstered by the results of clinical trials which have been reported recently (7). Whether dosing regimens which attain high peak/MIC ratios will result in less emergence of resistance clinically is unknown and requires study, but it is a worthy hypothesis for testing.

The implication for the physician in clinical practice is that the best results for seriously infected patients can be obtained with relatively infrequent dosing of fluoroquinolones, with the consequent attainment of high peak/MIC ratios. It is likely that once-daily dosing will be superior to other modes of administration, assuming a constant daily dose. However, one must keep in mind that very large doses of some fluoroquinolones, as would be given on a once-daily schedule, might prove to be toxic. This needs careful clinical evaluation. Nonetheless, in our animal model system, once-daily administration was as good as or superior to more fractionated dosing schedules, even when the half-life of the drug in question in the animal system was only 2 h. These results also suggest that it might be possible for clinicians to make judgments about the relative utility of different fluoroquinolones for clinically important pathogens by comparing peak/MIC ratios achieved with typical doses and schedules. Such predictions need to be tested against the response rates seen in clinical studies. The clinical relevance of peak/MIC ratios or AUC/MIC ratios needs to be further examined in clinical trials of fluoroquinolone antimicrobial agents.

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