ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2003, p. 1604–1613 Vol. 47, No. 5
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.5.1604–1613.2003

In Vitro Pharmacodynamic Evaluation of the Mutant Selection Window Hypothesis Using Four Fluoroquinolones against *Staphylococcus aureus*

Alexander A. Firsov,[1]* Sergey N. Vostrov,[1] Irene Y. Lubenko,[1] Karl Drlica,[2] Yury A. Portnoy,[1] and Stephen H. Zinner[3]

Department of Pharmacokinetics and Pharmacodynamics, Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, Moscow, Russia[1]; Public Health Research Institute, New York, New York[2]; and Mount Auburn Hospital, Harvard Medical School, Cambridge, Massachusetts[3]

Received 24 June 2002/Returned for modification 9 November 2002/Accepted 30 January 2003

To study the hypothesis of the mutant selection window (MSW) in a pharmacodynamic context, the susceptibility of a clinical isolate of methicillin-resistant *Staphylococcus aureus* exposed to moxifloxacin (MOX), gatifloxacin (GAT), levofloxacin (LEV), and ciprofloxacin (CIP) was tested daily by using an in vitro dynamic model that simulates human pharmacokinetics. A series of monoexponential pharmacokinetic profiles that mimic once-daily administration of MOX (half-life, 12 h), GAT (half-life, 7 h), and LEV (half-life, 6.8 h) and twice-daily administration of CIP (half-life, 4 h) provided peak concentrations (C_{max}) that either equaled the MIC, fell between the MIC and the mutant prevention concentration (MPC) (i.e., within or "inside" the MSW), or exceeded the MPC. The respective ratios of the area under the curve (AUC) over a 24-h dosing interval (AUC_{24}) to the MIC varied from 13 to 244 h, and the starting inoculum was 10^8 CFU/ml (6×10^9 CFU per 60-ml central compartment). With all four quinolones, the greatest increases in MIC were observed at those AUC_{24}/MIC values (from 24 to 62 h) that corresponded to quinolone concentrations within the MSW over most of the dosing interval (>20%). Less-pronounced increases in MIC were associated with the smallest simulated AUC_{24}/MIC values (15 to 16 h) of GAT and CIP, whose C_{max} exceeded the MICs. No such increases were observed with the smallest AUC_{24}/MIC values (13 to 17 h) of MOX and LEV, whose C_{max} were close to the MICs. Also, less pronounced but significant increases in MIC occurred at AUC_{24}/MIC values (107 to 123 h) that correspond to quinolone concentrations partly overlapping the MIC-to-MPC range. With all four drugs, no change in MIC was seen at the highest AUC_{24}/MIC values (201 to 244 h), where quinolone concentrations exceeded the MPC over most of the dosing interval. These "protective" AUC_{24}/MIC ratios correspond to 66% of the usual clinical dose of MOX (400 mg), 190% of a 400-mg dose of GAT, 220% of a 500-mg dose of LEV, and 420% of two 500-mg doses of CIP. Thus, MOX may protect against resistance development at subtherapeutic doses, whereas GAT, LEV, and CIP provide similar effects only at doses that exceed their usual clinical doses. These data support the concept that resistant mutants are selectively enriched when antibiotic concentrations fall inside the MSW and suggest that in vitro dynamic models can be used to predict the relative abilities of quinolones to prevent mutant selection.

Examination of time-kill curves of antibiotic-exposed bacteria using in vitro dynamic models allows pharmacokinetically related comparisons of antimicrobial effects but may or may not directly reflect the selective enrichment of resistant mutants. Bacterial resistance has been studied infrequently using these models. Limited observations reported from earlier time-kill studies (3, 8, 21–23) precluded delineation of relationships of the area under the concentration-time curve (AUC)/MIC ratio with resistance because the ranges of the simulated AUC-to-MIC ratios were too narrow. In fact, the first attempts to relate resistance to the AUC/MIC or peak concentration (C_{max})/MIC ratio were reported quite recently from studies that declared resistance analysis as a primary goal (1, 7, 17, 18, 20, 25–27, 30, 33, 34; A. MacGowan and K. Bowker, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., poster A-440, 2001). Despite wide ranges of AUC/MIC ratios simulated in some recent studies (17–20, 27, 33; MacGowan and Bowker, 41st ICAAC), reasonable relationships with resistance were not established. The relatively few studies of these relationships can be classified as those that directly attempt to relate resistance to the simulated pharmacokinetics but do not (17, 20) and those that imply the existence of relationships with the AUC/MIC ratio measured within a 24-h dosing interval (AUC_{24}/MIC) or with the C_{max}/MIC ratio but do not actually report them (26, 27, 30). One study did report a complex effect of AUC_{24}/MIC and duration of moxifloxacin treatment on bacterial resistance (MacGowan and Bowker, 41st ICAAC), but the three-dimensional plots masked rather than highlighted these links. For example, according to an analysis of these data (A. Firsov, S. Vostrov, I. Lubenko, S. Zinner, and Y. Portnoy, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-1210, p. 10, 2002), the reported 72-h area under the population analysis profile-time curve as an index of pneumococcal resistance did not correlate with simulated AUC_{24}/MIC ratios (r^2, 0.04).

Without AUC/MIC and C_{max}/MIC relationships to resis-

* Corresponding author. Mailing address: Department of Pharmacokinetics and Pharmacodynamics, Gause Institute of New Antibiotics, Russian Academy of Medical Sciences, 11 Bolshaya Pirogovskaya St., Moscow, 119021 Russia. Phone: 7 (095) 245-0154. Fax: 7 (095) 245-0295. E-mail: firsov@dol.ru.

tance, reports of AUC/MIC and C_{max}/MIC values that protect against the selection of resistant mutants appear to be contradictory. For example, with *Streptococcus pneumoniae*, "protective" AUC/MIC values for grepafloxacin varied from 32 h (17) to 80 h (7, 34) and those for levofloxacin varied from 9 h (17) to 26 h (20) and 50 h (34). Furthermore, although moxifloxacin-resistant *S. pneumoniae* was not found at the AUC/MIC values of 107 h (7) and 250 h (34), significant losses in susceptibility were seen at AUC/MIC values as high as 43,500 h (17).

There are many possible reasons for these contradictions. One is that simulated concentrations might or might not fall into the mutant selection window (MSW), i.e., the concentration range between the MIC and the mutant prevention concentration (MPC), within which it is proposed that resistant mutants are selected (35). To test the MSW hypothesis and to highlight the reasons for these contradictions, the abilities of moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin to selectively enrich resistant mutants of *Staphylococcus aureus* and the dynamics of antistaphylococcal effects were studied using in vitro simulations of the four fluoroquinolones at concentrations equal to the MIC, between the MIC and the MPC, and above the MPC.

(This study was presented in part at the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Diego, Calif., 27 to 30 September 2002.)

MATERIALS AND METHODS

Antimicrobial agents, bacterial strain, and susceptibility testing. Moxifloxacin and ciprofloxacin powders were kindly provided by Bayer Corporation (West Haven, Conn.), gatifloxacin was provided by Bristol-Myers Squibb (New Brunswick, N.J.), and levofloxacin was provided by Ortho-McNeill Pharmaceuticals (Raritan, N.J.). A clinical isolate of methicillin-resistant *S. aureus* 201 was selected for the study. MICs were determined prior to, during, and after a 3-day course of treatment with the quinolones. Susceptibility testing was performed in triplicate by broth microdilution techniques at 24 h postexposure with the organism grown in Ca^{2+}- and Mg^{2+}-supplemented Mueller-Hinton broth (MHB) at an inoculum size of 10^6 CFU/ml. In order to obtain more-precise values, MICs were determined by using doubling dilutions with starting concentrations of 3, 4, and 5 mg/liter as described previously (16). MICs for *S. aureus* 201 were 0.09 μg of moxifloxacin/ml, 0.3 μg of gatifloxacin/ml, 0.6 μg of levofloxacin/ml, and 0.8 μg of ciprofloxacin/ml.

MPCs were determined as described elsewhere (35). Briefly, the tested microorganisms were cultured in MHB and incubated for 24 h. Then the suspension was centrifuged (at 4,000 × *g* for 10 min) and resuspended in MHB to yield a concentration of 10^{10} CFU/ml. A series of agar plates containing known fluoroquinolone concentrations was then inoculated with ~10^{10} CFU of *S. aureus*. The inoculated plates were incubated for 48 h at 37°C and screened visually for growth. To estimate the MPC, logarithms of bacterial numbers were plotted against fluoroquinolone concentrations (Fig. 1). The MPC was taken as the point where the plot intersected the *x* axis, i.e., the lowest fluoroquinolone concentration that completely inhibited growth. The MPCs of moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin were estimated as 0.34, 1.17, 1.75, and 2.83 μg/ml, respectively.

Simulated pharmacokinetic profiles. A series of monoexponential profiles that mimic once-daily administration of moxifloxacin, gatifloxacin, and levofloxacin and twice-daily dosing of ciprofloxacin were simulated with half-lives ($t_{1/2}$) of 12 h for moxifloxacin, 7 h for gatifloxacin, 6.8 h for levofloxacin, and 4 h for ciprofloxacin. The simulated $t_{1/2}$ represented weighted means of the values reported for humans: 9.1 to 13.4 h (28; J. Sullivan, M. Woodruff, J. Lettieri, V. Agarwal, G. Krol, and A. Heller, 8th Eur. Congr. Clin. Microbiol. Infect. Dis., poster P-389, 1997), 6.0 to 8.4 h (24), 6.0 to 7.4 h (4–6, 19), and 3.2 to 5.0 h (2, 15, 32), respectively.

In vitro dynamic model. A previously described dynamic model (13) was used in the study. Briefly, the model consisted of two connected flasks, one containing fresh MHB and the other with a magnetic stirrer, the central unit, containing the same broth with either a bacterial culture alone (control growth experiments) or a bacterial culture plus an antimicrobial agent (killing-regrowth experiments). Peristaltic pumps circulated fresh nutrient medium to the flasks and from the central 60-ml unit at a flow rate of 3.5 ml/h for moxifloxacin, 6 ml/h for gatifloxacin, 6.1 ml/h for levofloxacin, and 10.4 ml/h for ciprofloxacin. The clearance provided by these flow rates plus the volume of the central unit ensured monoexponential elimination of the fluoroquinolones and bacteria from the system at an elimination rate constant of 0.06 h^{-1} for moxifloxacin, 0.1 h^{-1} for gatifloxacin and levofloxacin, and 0.17 h^{-1} for ciprofloxacin.

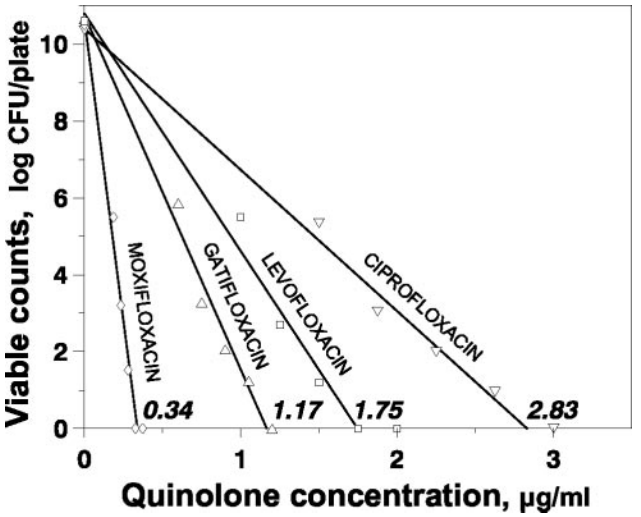

FIG. 1. Determination of MPC. Estimated values are given along the *x* axis.

The system was filled with sterile MHB and placed in an incubator at 37°C. The central unit was inoculated with an 18-h culture of *S. aureus.* After a 2-h incubation of the bacteria, the resulting exponentially growing cultures reached approximately 10^8 CFU/ml (6×10^9 CFU per 60-ml central compartment), and moxifloxacin, gatifloxacin, levofloxacin, or ciprofloxacin was injected into the central unit. All experiments were performed in duplicate within a 2-week interval. The reliability of fluoroquinolone pharmacokinetic simulations and the high reproducibility of the time-kill curves provided by the model have been reported elsewhere (11).

Study design. To establish the optimal duration of treatment, i.e., the minimal course of fluoroquinolone administration that provides stable increases in the MIC, a pilot study was performed with two fluoroquinolones. Daily dosing of moxifloxacin and levofloxacin was simulated for 5 consecutive days by using 50 h as a target AUC_{24}/MIC value. This value corresponds to fluoroquinolone concentrations falling into the MSW (the peak level is close to the MPC, and the trough level is close to the MIC), where resistance is expected to develop most readily (35).

In the main study, 3-day courses of fluoroquinolone administration were simulated over a 16-fold range of the AUC_{24}/MIC ratio. Daily doses of each fluoroquinolone were designed to correspond to comparable mean AUC_{24}/MIC values (averages of the values reached during the 1st, 2nd, and 3rd days) ranging from 13 to 17 h to 201 to 244 h, and the times when fluoroquinolone concentrations were inside the MSW (T_{MSW}) ranged from <20% of the dosing interval to 40 to 90% and then back to <20% (Fig. 2). In turn, the simulated AUC_{24}/MIC values corresponded to fluoroquinolone peak concentrations close to or 2 to 3, 4 to 6, 8 to 12, or 16 to 24 times greater than the respective MICs and trough concentrations close to or 1.5, 3, 6, 12.5, or 25 times less than the MPCs.

Quantitation of the antimicrobial effect and susceptibility changes. In each experiment, multiple sampling of bacterium-containing medium from the central compartment was performed throughout the observation period. One hundred-microliter samples were serially diluted as appropriate, and 100 μl was plated onto agar plates. The duration of the experiments was defined in each case as the time until antibiotic-exposed bacteria after the last dose reached the maximum numbers observed in the absence of antibiotic (≥10^9 CFU/ml). The lower limit of accurate detection was 2×10^2 CFU/ml.

Based on time-kill data obtained in the main study, the intensity of the antimicrobial effect (I_E, defined as the area between the control growth and time-kill curves [9, 13]) was determined from time zero to the time when the effect could no longer be detected, i.e., the time after the last fluoroquinolone

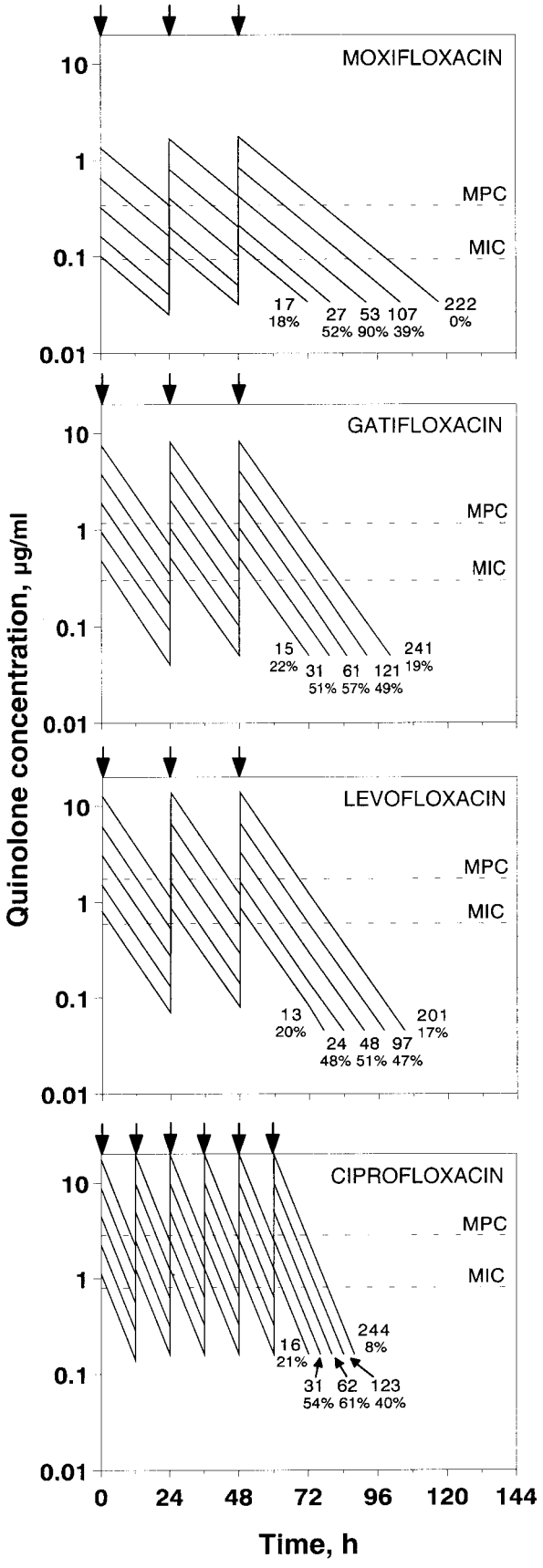

FIG. 2. In vitro-simulated pharmacokinetic profiles of the fluoroquinolones moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin. The numbers at the end of each profile are the AUC_{24}/MIC value and the percentage of the dosing interval that falls within the MSW. Arrows reflect quinolone dosing.

dose at which the number of antibiotic-exposed bacteria reached 10^9 CFU/ml. The upper limit of bacterial numbers, i.e., the cutoff level on the regrowth and control growth curves used to determine I_E, was 10^9 CFU/ml. The computation of I_E at comparable AUC_{24}/MIC values simulated with each drug is depicted graphically in Fig. 3.

To reveal possible changes in susceptibility during treatment, precise fluoroquinolone MICs of bacterial cultures sampled from the model were determined daily for 6 days in the pilot study and for 4 days in the main study. The stability of resistance observed in the pilot study was determined by consecutive passaging of *S. aureus* that was exposed to three, four, and five doses of moxifloxacin and levofloxacin onto antibiotic-free agar plates for 10 consecutive days. MICs were determined on days 1, 3, 7, and 10 as described above.

Relationships of the emergence of resistance to the AUC_{24}/MIC ratio and T_{MSW}. To combine the data obtained with all four fluoroquinolones, increases in the MIC observed at 72 h (MIC_{72}) were related to the respective initial MIC (MIC_0). The ratios of MIC_{72} to MIC_0 were fitted to the log AUC_{24}/MIC by using a Gaussian type function where Y is the MIC_{72}/MIC_0 ratio, x is log AUC_{24}/MIC, x_c is the log AUC_{24}/MIC that corresponds to the maximal value of MIC_{72}/MIC_0, and a and b are parameters:

$$Y = 1 + a\ exp\ [-(x - x_c)^2/b] \quad (1)$$

Equation 1 also was used to fit the MIC_{24}/MIC_0 ratios of levofloxacin and trovafloxacin reported in a study with *Bacteroides fragilis* (25) against simulated AUC_{24}/MIC ratios.

To visualize the sigmoid shape of the T_{MSW} relationship to resistance, the MIC_{72}/MIC_0 ratios were fitted to the T_{MSW} by using the Boltzmann function

$$Y = (1 - Y_{max})/\{1 + exp\ [(x - x_0)/dx]\} + Y_{max} \quad (2)$$

where Y is the MIC_{72}/MIC_0 ratio and Y_{max} is its maximal value, x is T_{MSW}, x_0 is the T_{MSW} that corresponds to $Y_{max}/2$, and dx is the width parameter.

Fluoroquinolone doses that prevent the selection of resistant mutants were calculated from AUC_{24}/MIC ratios at which no increases in MIC occurred by using dose-AUC relationships reported earlier (10, 31).

Relationships of the antimicrobial effect to the AUC_{24}/MIC ratio. The I_E was related to log AUC_{24}/MIC. With each fluoroquinolone, the I_E versus log AUC_{24}/MIC data were fitted by the logistic function

$$Y = Y_{max}/[1 + exp\ (b - ax)]. \quad (3)$$

where x is log AUC_{24}/MIC, Y is I_E, Y_{max} is the maximal value of I_E, and a and b are parameters reflecting the slope and amplitude of the curve whose ratio, b/a, corresponds to x_{50}, i.e., to the log AUC_{24}/MIC ratio that provides the antimicrobial effect equal to $Y_{max}/2$.

RESULTS

Validation of the optimal study design. To establish the minimal duration of fluoroquinolone treatment that allows detection of *S. aureus* resistance, daily measurement of the MICs of moxifloxacin and levofloxacin was performed during the 5-day courses at AUC_{24}/MIC values of approximately 60 h, which correspond to fluoroquinolone concentrations almost entirely within the MSW (Fig. 4). As seen in Fig. 4, significant increases in the MIC were found with both drugs beginning from the third dose. These increases were even more pronounced after the fourth dose and, especially, after the fifth dose. Serial passages of resistant isolates sampled 72, 96, and 120 to 125 h after fluoroquinolone exposure and placed on antibiotic-free plates revealed minimal or no changes in the elevated MICs, showing stable resistance after the 3rd, 7th, and

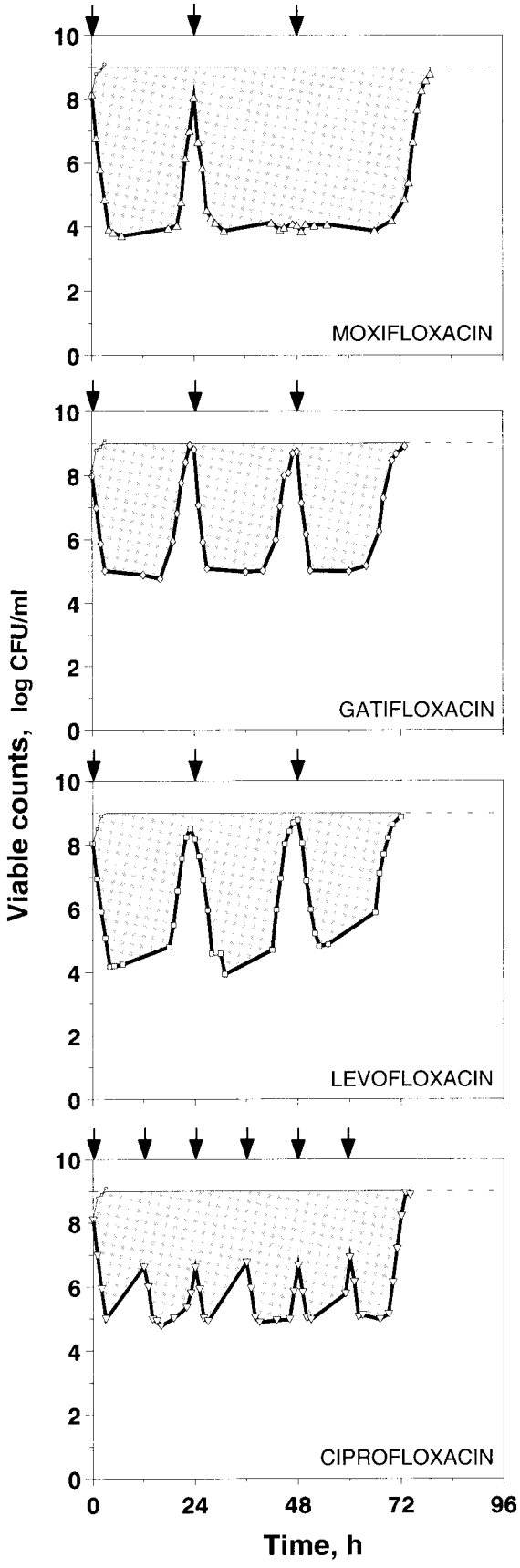

FIG. 3. Determination of I_E (shaded areas) at comparable AUC_{24}/MIC values for moxifloxacin (53 h), gatifloxacin (61 h), levofloxacin (48 h), and ciprofloxacin (62 h). Bold lines delineate the time-kill and regrowth curves, and thin lines delineate control growth curves. Arrows indicate quinolone dosing; the dotted line marks the cutoff level (13).

10th passages (Table 1). For example, after the 7th to 10th passage, the elevated MIC observed in the 3-day treatment with moxifloxacin was still twofold greater than the initial value. Even more stable resistance was documented in the 4- and 5-day treatments with both fluoroquinolones. The reduced susceptibility of *S. aureus* resulted in a gradual increase in the minimal numbers of surviving organisms (for both fluoroquinolones) that was concomitant with a slight increase in maximal bacterial counts (for levofloxacin only) (Fig. 4).

This pilot study shows that the relatively small but stable increases in MIC observed after the third doses of moxifloxacin and levofloxacin are predictive of more-pronounced changes in the susceptibility of *S. aureus* after 4- and 5-day fluoroquinolone exposures. Therefore, the shorter 3-day treatments were simulated in the main study.

Emergence of resistance. Results of repeated susceptibility testing in 3-day exposures with the four fluoroquinolones are summarized in Fig. 5. Most of the largest increases in MIC were observed after the third dose at those AUC_{24}/MIC values (from 24 to 31 h to 48 to 62 h) that correspond to fluoroquinolone concentrations falling into the MSW over most of the dosing interval (T_{MSW}, 50 to 90% of the dosing interval). Less-pronounced but significant increases in MIC occurred at AUC_{24}/MIC values (97 to 123 h) corresponding to fluoroquinolone concentrations that partly overlap the MIC-MPC range (T_{MSW}, 40 to 50% of the dosing interval). Less noticeable increases in MIC were associated with the lowest simulated AUC_{24}/MIC values (15 to 16 h), with C_{max}s exceeding the MICs of gatifloxacin and ciprofloxacin (T_{MSW}, $\leq$20% of the dosing interval (see Fig. 2). No such increases were observed with the lowest AUC_{24}/MIC values (13 to 17 h,) with C_{max}s close to the MICs of moxifloxacin and levofloxacin (T_{MSW}, $<$20% of the dosing interval). Also, no changes in MICs were seen at the highest AUC_{24}/MIC values (201 to 244 h), with fluoroquinolone concentrations exceeding the MPC over most of the dosing interval (i.e., with C_{max}s above the MPCs and trough concentrations comparable to [moxifloxacin and ciprofloxacin] or slightly less than [gatifloxacin and levofloxacin] the MPCs [T_{MSW}, $<$20% of the dosing interval]) (Fig. 2). Overall, no changes in susceptibility were seen when concentrations were so small or so large as to provide T_{MSW} equivalent to $\leq$20% of the dosing interval, whereas significant increases in MIC were associated with T_{MSW} of $>$20%.

These MIC changes with all four fluoroquinolones were observed at similar AUC_{24}/MIC or AUC_{24}/MPC values. This also applies to the minimal values of AUC_{24}/MIC (201 to 244 h) and AUC_{24}/MPC (60 to 69 h) that prevent the selection of resistant *S. aureus* mutants. However, these "protective" AUC_{24}/MIC and AUC_{24}/MPC values correspond to quite different daily quinolone doses (Fig. 5). With moxifloxacin, the respective protective dose is 33% lower than the clinical dose

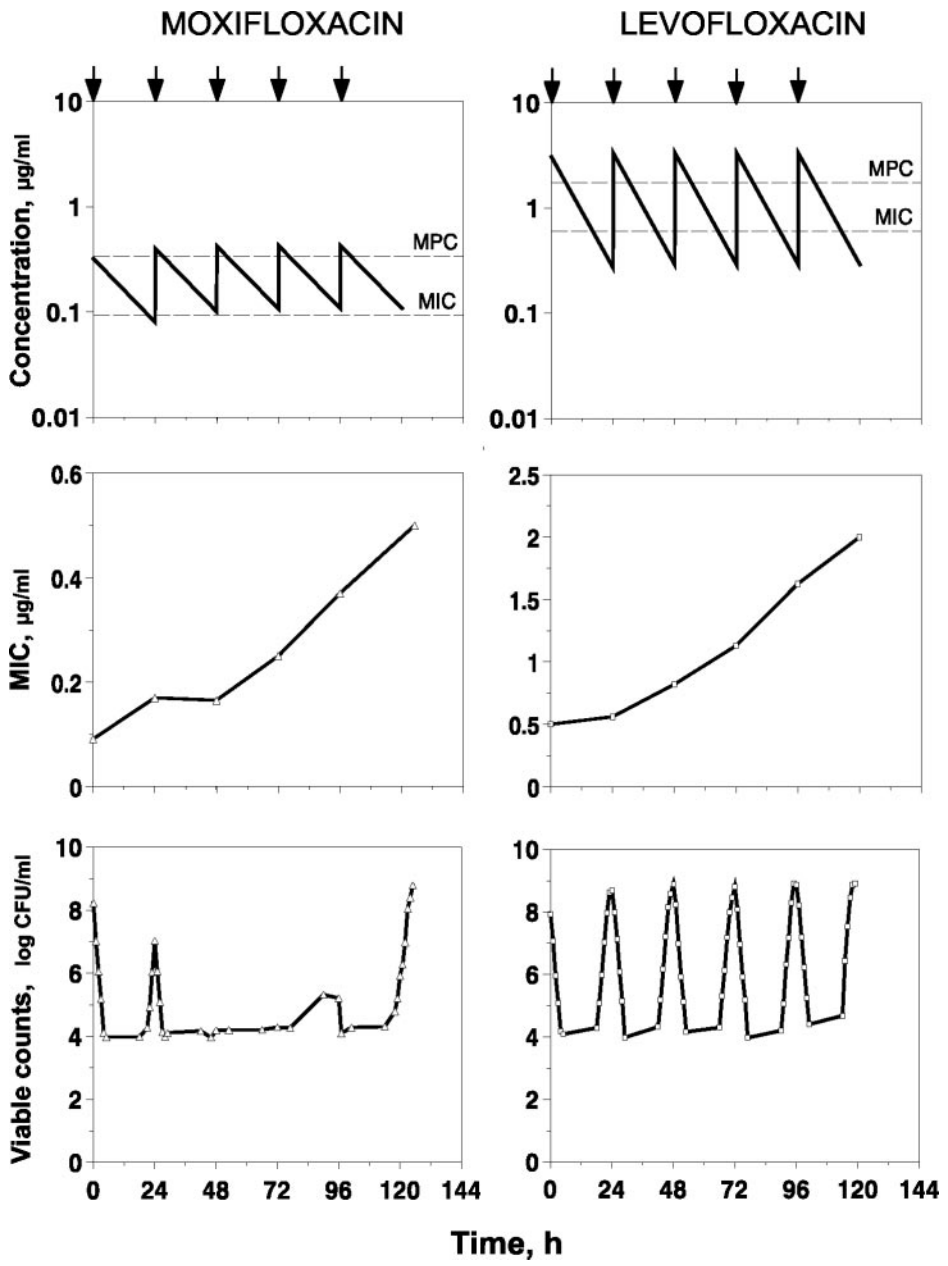

FIG. 4. Simulated pharmacokinetics, showing changes in the susceptibility and time-kill curves of *S. aureus* 201 during and after 5-day treatments with moxifloxacin (triangles) (AUC_{24}/MIC, 53 h) or levofloxacin (squares) (AUC_{24}/MIC, 48 h).

(400 mg), whereas the protective doses of gatifloxacin, levofloxacin, and ciprofloxacin are 90, 120, and 540% greater than their clinical doses (400 mg, 500 mg, and twice 500 mg, respectively). Therefore, AUC_{24}/MIC and AUC_{24}/MPC values achieved at the usual clinical dose of moxifloxacin but not at the usual clinical dose of the other three fluoroquinolones prevent the selection of resistant *S. aureus*.

Similar patterns of the AUC_{24}/MIC-dependent changes in the susceptibility of *S. aureus* to moxifloxacin, levofloxacin, gatifloxacin, and ciprofloxacin allow establishment of a single relationship between increases in MIC and log AUC_{24}/MIC. To normalize the increases in MIC observed at 72 h with the four fluoroquinolones, they were related to the respective initial MICs. As seen in Fig. 6, the MIC_{72}/MIC_0-versus-log AUC_{24}/MIC relationship was fitted by equation 1, with the central point at an AUC_{24}/MIC of 43 h, where the loss in staphylococcal susceptibility was maximal, whereas no resistance was associated with AUC_{24}/MIC values of ≥200 h. Unlike the log AUC_{24}/MIC plot, the T_{MSW} plot of the MIC_{72}/MIC_0 ratio was sigmoid in shape, and it was fitted by equation 2. Moreover, regardless of the simulated AUC_{24}/MIC ratio, the susceptibility of *S. aureus* declined when T_{MSW} exceeded 20% of the dosing interval, whereas it did not change when T_{MSW} was less than 20% of the dosing interval.

Pharmacodynamics. The time courses of killing and regrowth of *S. aureus* 201 exposed to moxifloxacin, gatifloxacin,

TABLE 1. MICs determined before and after exposure of *S. aureus* to two fluoroquinolones

Fluoroquinolone	Duration of treatment (days)	MIC (μg/ml)				
		Before treatment	Just after sampling	After 3rd passage	After 7th passage	After 10th passage
Moxifloxacin	3	0.09	0.25	0.25	0.19	0.19
	4		0.37	0.31	0.31	0.37
	5		0.5	0.5	0.5	0.5
Levofloxacin	3	0.6	1	1	0.75	0.75
	4		2	2	1.5	1.5
	5		2	2	1.5	1.5

levofloxacin, and ciprofloxacin are shown in Fig. 7. The lowest simulated AUC_{24}/MIC values (13 to 17 h), with fluoroquinolone peak concentrations close to the MICs (moxifloxacin and levofloxacin) or slightly exceeding the MICs (gatifloxacin and ciprofloxacin), resulted in only slight and transient reductions in bacterial numbers, with bacterial regrowth occurring at the beginning of each dosing interval. The twofold-increased AUC_{24}/MIC values (24 to 31 h), with fluoroquinolone concentrations exceeding the MICs over a considerable part of the dosing interval, produced more-pronounced reductions, al-

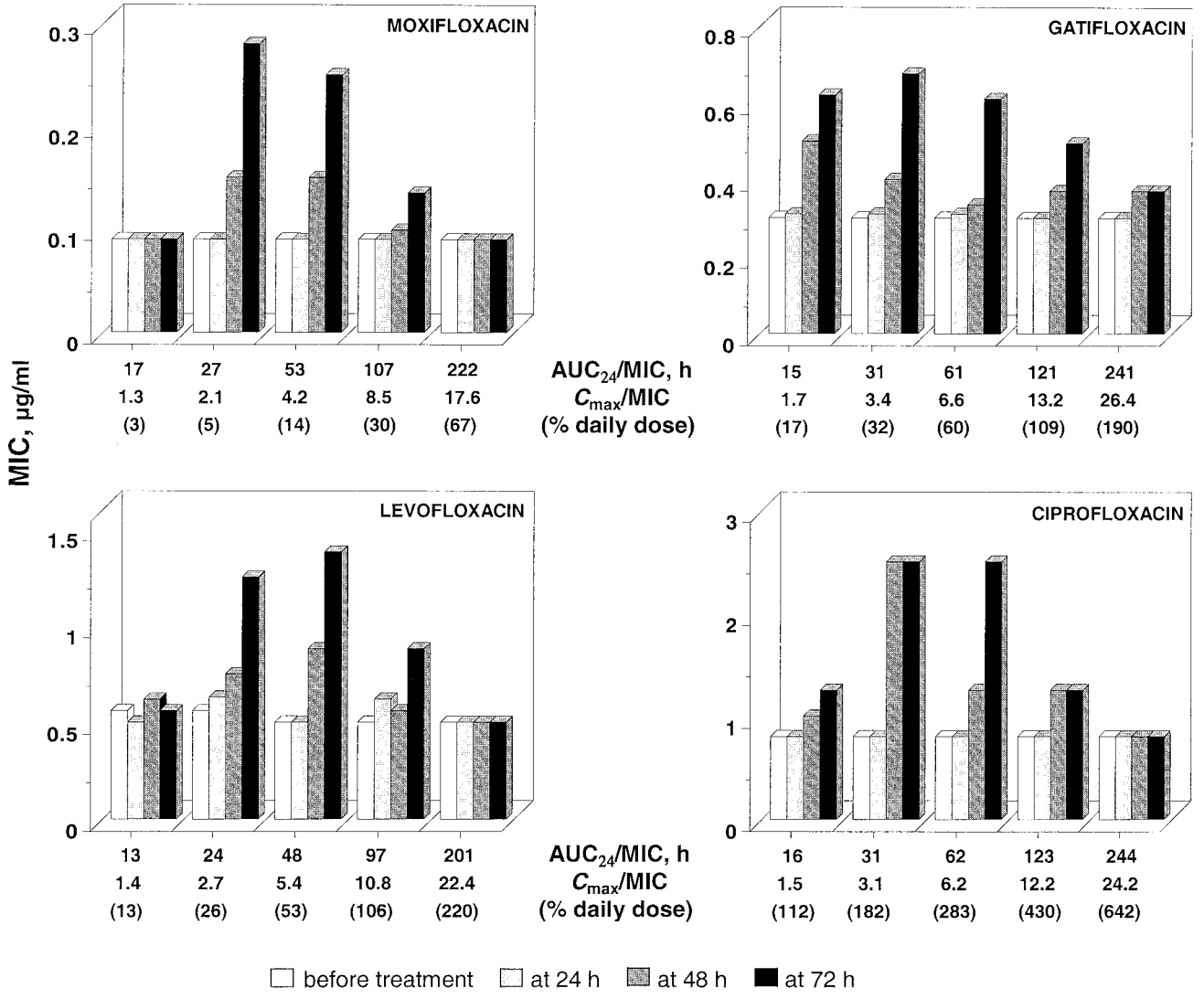

FIG. 5. Changes in the susceptibility of *S. aureus* 201 during and after 3-day treatments with four fluoroquinolones at different AUC_{24}/MIC ratios.

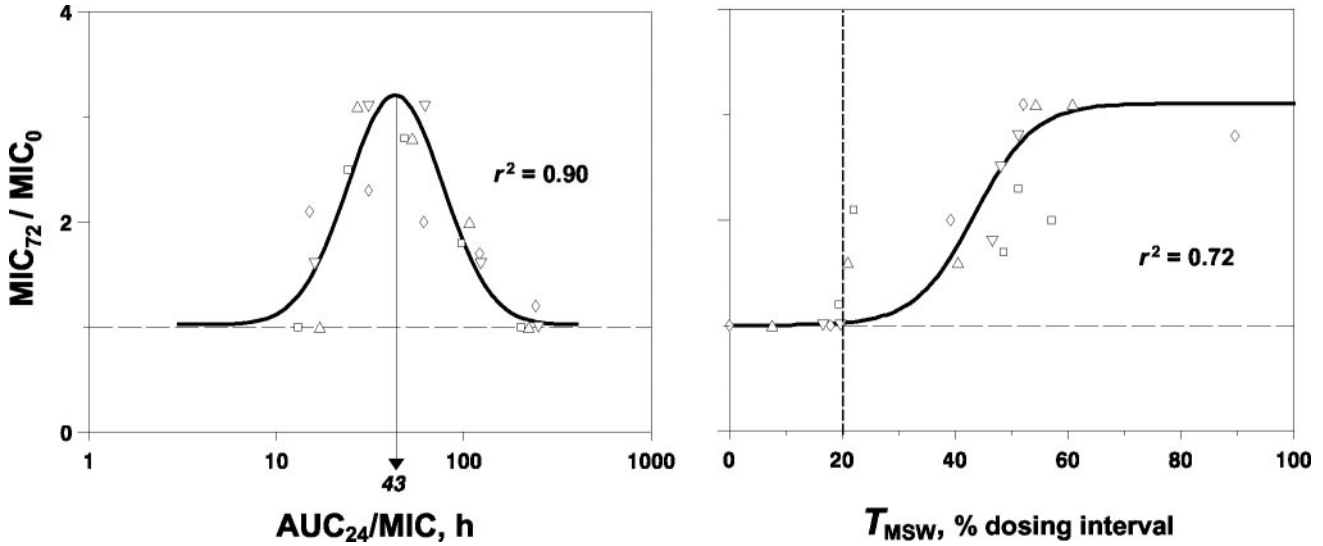

FIG. 6. Resistance of *S. aureus* 201 related to the simulated AUC_{24}/MIC value (left) and T_{MSW} (right) for four fluoroquinolones (combined data). For equation 1, $a = 1.7$, $b = 0.18$, and $x_c = 1.63$. For equation 2, $Y_{max} = 3.1$, $x_0 = 43$, and $dx = 5.1$.

though regrowth still occurred within each dosing interval. Increasing the AUC_{24}/MIC values to 48 to 62 h, where fluoroquinolone concentrations exceed the MICs over the entire dosing interval (moxifloxacin and ciprofloxacin) or most of it (gatifloxacin and levofloxacin), was accompanied by a further decrease in the minimal numbers of surviving organisms. Regrowth occurred by the end of each dosing interval with gatifloxacin, levofloxacin, and ciprofloxacin and only after the first and third doses of moxifloxacin. Further reductions in bacterial counts were observed at higher AUC_{24}/MIC values, where fluoroquinolone concentrations exceeded the MPC either over 50% of the dosing interval (AUC_{24}/MIC, 97 to 123 h) or over the entire interval with moxifloxacin (AUC_{24}/MIC, 222 h) and ciprofloxacin (AUC_{24}/MIC, 244 h) or most of the dosing interval with gatifloxacin and levofloxacin (AUC_{24}/MIC, 241 and 201 h, respectively). Regrowth occurred only after the third dose of moxifloxacin, gatifloxacin, or levofloxacin and after the sixth dose of ciprofloxacin, and it occurred later with moxifloxacin than with gatifloxacin, levofloxacin, and ciprofloxacin.

These differences resulted in different shapes of the AUC_{24}/MIC relationships with I_E (Fig. 8). Beginning from an AUC_{24}/MIC value of >60 h (moxifloxacin versus all other fluoroquinolones) and >100 h (gatifloxacin and levofloxacin versus ciprofloxacin), the I_E-log AUC_{24}/MIC curves differ both in terms of the slope (a) and the maximal I_E (Y_{max}). For example, at an AUC_{24}/MIC value of 125 h, the effect of moxifloxacin was 35% greater than those of gatifloxacin and levofloxacin and 47% greater than that of ciprofloxacin. As seen in Fig. 8, the described differences were inherent in the relatively high simulated AUC_{24}/MIC ratios, whereas at the lower AUC_{24}/MIC ratios no differences among the curves were detected.

DISCUSSION

Emergence of resistance. This study suggests that losses in the susceptibility of *S. aureus* exposed to four different quinolones occur at concentrations that fall into the MSW. The most pronounced losses occurred at AUC_{24}/MIC values of 25 to 60 h, when T_{MSW} was >20% of the dosing interval. No changes in susceptibility were associated with AUC_{24}/MIC values below 15 h (minimal bacterial killing) or above 200 h (maximal killing). Although similar AUC_{24}/MIC values might be considered to protect against staphylococcal resistance (201 h for levofloxacin, 222 h for moxifloxacin, 241 h for gatifloxacin, and 244 h for ciprofloxacin), these values might (moxifloxacin) or might not (other three quinolones) be achieved at their usual clinical doses.

The quinolone-independent AUC_{24}/MIC relationship with resistance (as expressed by increases in MIC) was reflected by a bell-shaped curve with a maximum at the AUC_{24}/MIC value of 43 h (Fig. 6). This curve could be transformed into a sigmoid curve by plotting the ratios of elevated MICs to the initial values, i.e., MIC_{72}/MIC_0 against T_{MSW} (Fig. 6). The MIC_{72}/MIC_0 ratio correlated with T_{MSW} regardless of whether quinolone concentrations were above or below the MPCs. Similar curves have been reported for another strain of *S. aureus* exposed to gatifloxacin in a study that simulated normal and impaired quinolone elimination (Firsov et al., 42nd ICAAC). Moreover, the Gaussian function (equation 1) also fits reported resistance data on levofloxacin- and trovafloxacin-exposed *B. fragilis* (25) (Fig. 9). This leads to the assumption that the described pattern of the AUC_{24}/MIC-resistance curve may be quite general. Indirectly, this impression is supported by our analysis of resistance frequencies reported in a study of *S. aureus* exposed to norfloxacin and ciprofloxacin (1). As seen in Fig. 10, these data are consistent with a bell-shaped curve, despite the use of different endpoints of resistance. The more pronounced resistance to norfloxacin at a relatively large AUC_{24}/MIC value (55 h) compared to a less pronounced resistance at a small AUC_{24}/MIC value (3 h) no longer seems "paradoxical." Also, the similar resistance frequencies at AUC_{24}/MIC values of ciprofloxacin that vary 16-fold are quite explainable. Indeed, these data fit the simple idea that selective

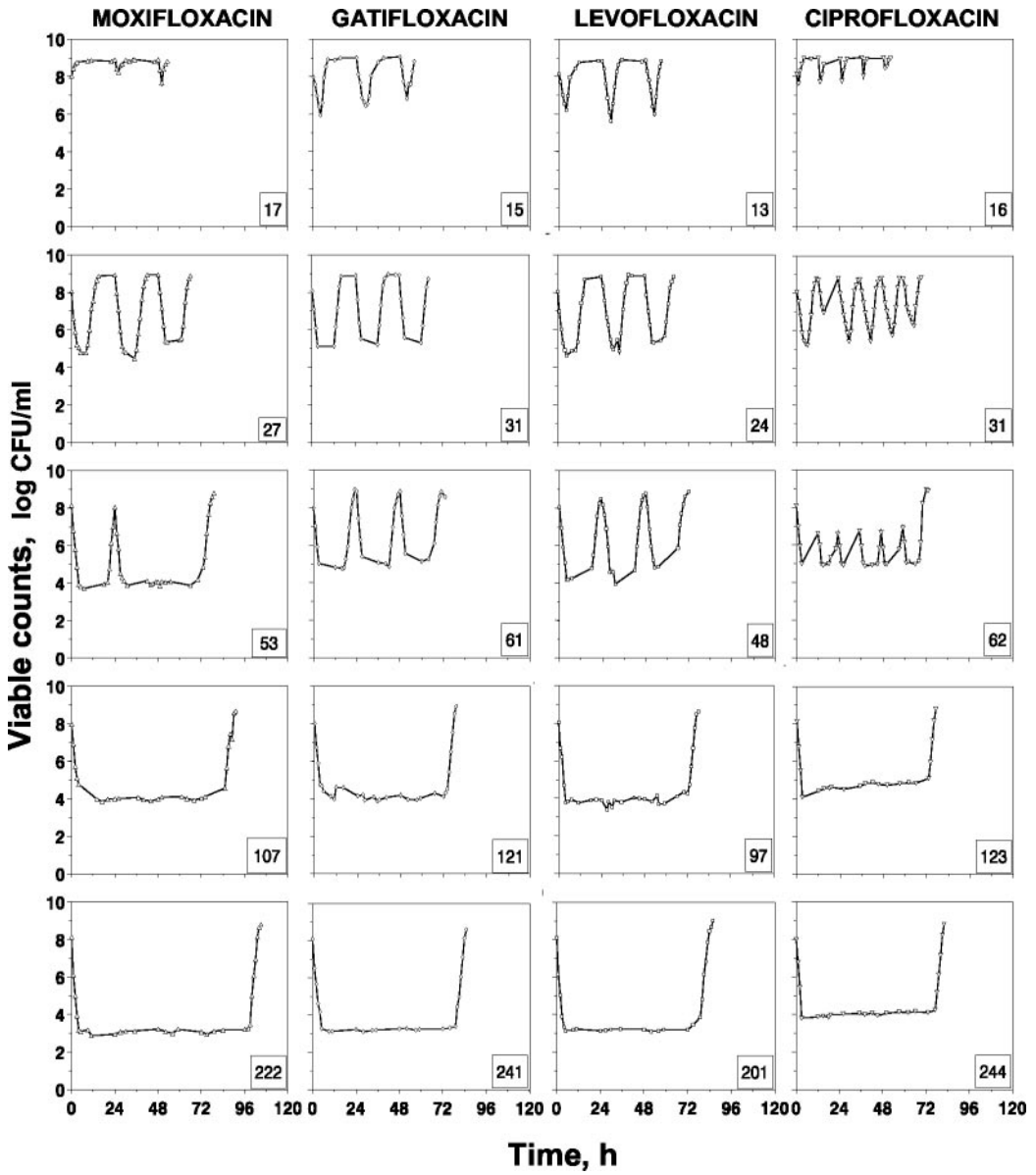

FIG. 7. Kinetics of killing and regrowth of *S. aureus* 201 exposed to 3-day courses of moxifloxacin, gatifloxacin, levofloxacin, and ciprofloxacin. Boxed numbers indicate the simulated AUC_{24}/MIC values (in hours).

pressure is absent below the MIC while rare double mutations are required for growth above the MPC (35).

Given the bell-shaped pattern of the AUC_{24}/MIC relationships with resistance, reported failures to correlate resistance with AUC/MIC and C_{max}/MIC values by using linear or log-linear regression are understandable. However, these failures as well as the contradictory estimates of reported "protective" AUC/MIC and C_{max}/MIC values might result from inadequate study design. Like traditional time-kill studies, most resistance studies exposed one strain (26, 30) or a few similarly susceptible strains (1, 7, 20, 34) to clinical quinolone doses. As a result, in these studies only one or two AUC_{24}/MIC values for each quinolone could be related to the observed resistance. Moreover, the majority of the simulated AUC_{24}/MIC values were high enough to completely sterilize the unit, and neither population analysis of antibiotic-exposed organisms nor repeated susceptibility testing was possible. For example, in experiments with *S. pneumoniae*, repeated MIC determinations could be made for only one or two of six fluoroquinolones (7, 34). Overall, only 30 to 50% of the observations in these studies provided useful information. It is fair to say that similar problems also were inherent in more rigorously designed dose (AUC/MIC)-ranging studies (17, 18, 25, 27, 33). For example, in studies where *S. pneumoniae* (17), *B. fragilis* (25), and *Bacteroides thetaiotamicron* (27) were exposed to wide ranges of quinolone AUC_{24}/MIC values, quantitative data could be obtained in only 10 to 66% of experiments. As a result, a "correspondence" between AUC/MIC values of $\leq$44 h (25) and AUC/MIC values of $<$100 h (29), which are associated with the selection of resistant mutants, was posited, adding further con-

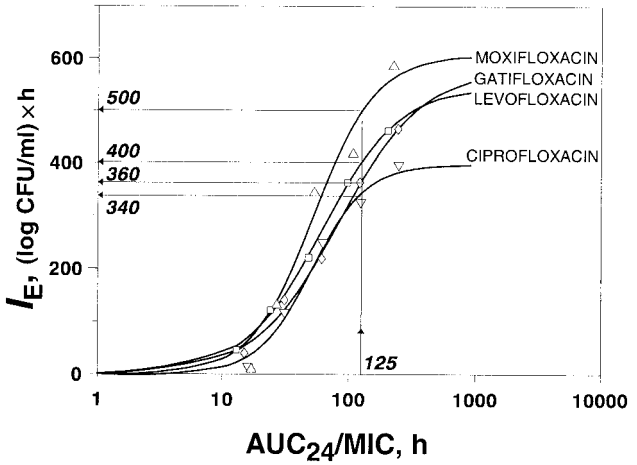

FIG. 8. AUC_{24}/MIC-dependent antistaphylococcal effects of fluoroquinolones fitted by equation 3. For moxifloxacin, $a = 4.1$, $b = 7.1$, $Y_{max} = 607$, and $x_{50} = 1.7$. For gatifloxacin, $a = 2.9$, $b = 5.7$, $Y_{max} = 595$, and $x_{50} = 2.0$. For levofloxacin, $a = 3.3$, $b = 6.1$, $Y_{max} = 550$, and $x_{50} = 1.8$. For ciprofloxacin, $a = 4.7$, $b = 8.1$, $Y_{max} = 398$, and $x_{50} = 1.7$.

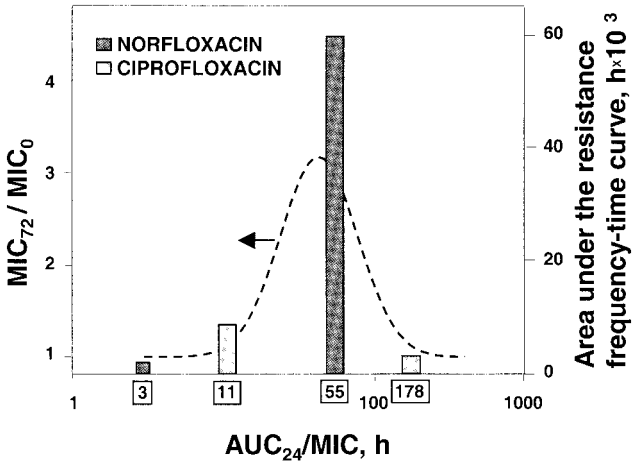

FIG. 10. AUC_{24}/MIC-dependent resistance frequency of *S. aureus* after a 48-h quinolone exposure. The bar graph presents data reconstructed from reference 1. Resistance is expressed as the area under the reported resistance frequency-time curve. Dotted curve, MIC_{72}/MIC_0-versus-AUC_{24}/MIC relationship obtained in the present study.

fusion to the picture. Given these limitations, reported "protective" AUC/MIC or C_{max}/MIC values (7, 25–27, 30, 34) should be considered cautiously.

Together with limited quantitative data, short-term observations (typically, 1-day [20, 25–27, 33] or 2-day [7, 17, 18, 34] courses) may contribute to the controversial results. As shown in our study, resistance of *S. aureus* was first observed on the third to fourth day of treatment and not earlier. A similar conclusion was drawn from a recent study with *S. pneumoniae* and *Pseudomonas aeruginosa* exposed to 3-day courses of moxifloxacin (A. MacGowan, and K. Bowker, 41st ICAAC): the longer the treatment, the greater the resistance. This unequivocal conclusion was possible due to the use of a novel index of resistance, the area under the population analysis profile-time curve.

The use of a relatively low starting inoculum—10^7 to 10^8 CFU (7, 18)—with few if any resistant mutants also might result in uncertain findings, because these inocula may contain only one resistant cell (35). It is not by chance that resistance data obtained in a study with moxifloxacin- and levofloxacin-exposed *S. aureus* at a starting inoculum of 10^6 CFU/ml in a 60-ml volume (6×10^7 CFU) (14) were less reproducible than those in the present study, where the starting inoculum was 6×10^9 CFU.

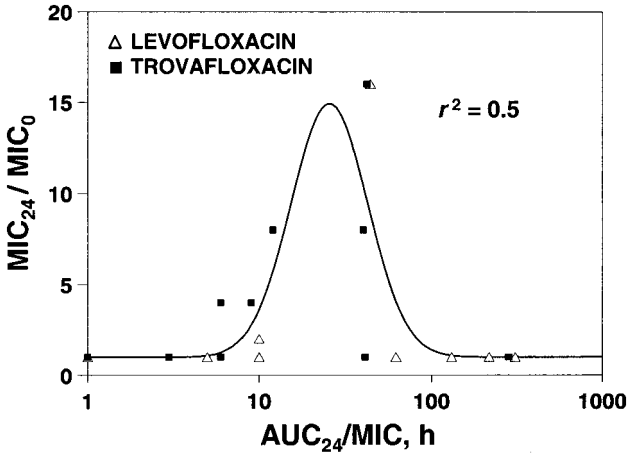

FIG. 9. AUC_{24}/MIC-dependent resistance of *B. fragilis* to levofloxacin and trovafloxacin fitted by equation 1 ($a = 10.1$, $b = 0.12$, $x_c = 1.4$). The graph presents combined data reconstructed from reference 25. Resistance is expressed as the ratio of MIC_{24} to MIC_0.

Pharmacodynamics. As in a previous pharmacodynamic study of moxifloxacin and levofloxacin against a less-susceptible strain of *S. aureus* at a lower starting inoculum (14), a specific AUC_{24}/MIC relationship with I_E was inherent for each of the four quinolones studied. This resulted in different antimicrobial effects of the quinolones at a given AUC_{24}/MIC ratio. These differences were primarily seen at the high simulated AUC_{24}/MIC ratios, whereas at lower AUC_τ/MIC ratios, no differences were detected among the curves. Similar patterns of the I_E-versus-log AUC/MIC relationships were reported in a previous single-dose study with gemifloxacin and ciprofloxacin against *S. aureus* (12).

Overall, the data obtained in this study are consistent with the concept that resistant mutants are selectively enriched when antibiotic concentrations fall inside the MSW. They also suggest that in vitro dynamic models can be used to predict the relative abilities of fluoroquinolones to prevent mutant selection, although further studies with other organisms are needed.

ACKNOWLEDGMENTS

This study was supported in part by a grant from the Bayer Corporation.

We are thankful to Xilin Zhao, who provided a detailed description of the MPC testing procedure.

REFERENCES

1. **Aeschlimann, J. R., G. W. Kaatz, and M. J. Rybak.** 1999. The effects of NorA inhibition on the activities of levofloxacin, ciprofloxacin and norfloxacin against two genetically related strains of *Staphylococcus aureus* in an in-vitro infection model. J. Antimicrob. Chemother. **44:**343–349.
2. **Bergan, T., and S. B. Thorsteinsson.** 1986. Pharmacokinetics and bioavailability of ciprofloxacin, p. 111–121. *In* H. C. Neu and H. Weuta (ed.),

Proceedings of the 1st International Ciprofloxacin Workshop. Current Clinical Practice series 34. Elsevier Science Publishers B.V. (Excerpta Medica), Amsterdam, The Netherlands.
3. **Blaser, J., B. B. Stone, M. C. Groner, and S. H. Zinner.** 1987. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine the importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. Antimicrob. Agents Chemother. **31:**1054–1060.
4. **Chien, S.-C., A. T. Chow, J. Natarajan, R. R. Williams, F. Wong, M. C. Rogge, and R. K. Nayak.** 1997. Absence of age and gender effects on the pharmacokinetics of a single 500-milligram oral dose of levofloxacin in healthy subjects. Antimicrob. Agents Chemother. **41:**1562–1565.
5. **Chien, S.-C., A. T. Chow, M. C. Rogge, R. R. Williams, and C. W. Hendrix.** 1997. Pharmacokinetics and safety of oral levofloxacin in human immunodeficiency virus-infected individuals receiving concomitant zidovudine. Antimicrob. Agents Chemother. **41:**1765–1769.
6. **Chien, S.-C., M. C. Rogge, L. G. Gisclon, C. Curtin, F. Wong, J. Natarajan, R. R. Williams, C. L. Fowler, W. K. Cheung, and A. T. Chow.** 1997. Pharmacokinetic profile of levofloxacin following once-daily 500-milligram oral or intravenous doses. Antimicrob. Agents Chemother. **41:**2256–2260.
7. **Coyle, E. A., G. W. Kaatz, and M. J. Rybak.** 2001. Activities of newer fluoroquinolones against ciprofloxacin-resistant *Streptococcus pneumoniae*. Antimicrob. Agents Chemother. **45:**1654–1659.
8. **Dudley, M. N., H. D. Mandler, D. Gilbert, J. Ericson, K. H. Mayer, and S. H. Zinner.** 1987. Pharmacokinetics and pharmacodynamics of intravenous ciprofloxacin. Studies in vivo and in an in-vitro model. Am. J. Med. **82**(Suppl. 4A)**:**363–368.
9. **Firsov, A. A., V. M. Chernykh, and S. M. Navashin.** 1991. Quantitative analysis of antimicrobial effect kinetics in an in vitro dynamic model. Antimicrob. Agents Chemother. **34:**1312–1317.
10. **Firsov, A. A., I. Y. Lubenko, S. N. Vostrov, O. V. Kononenko, S. H. Zinner, and Y. A. Portnoy.** 2000. Comparative pharmacodynamics of moxifloxacin and levofloxacin in an in vitro dynamic model: prediction of the equivalent AUC/MIC breakpoints and equiefficient doses. J. Antimicrob. Chemother. **46:**725–732.
11. **Firsov, A. A., A. A. Shevchenko, S. N. Vostrov, and S. H. Zinner.** 1998. Inter- and intraquinolone predictors of antimicrobial effect in an in-vitro dynamic model: new insight into a widely used concept. Antimicrob. Agents Chemother. **42:**659–665.
12. **Firsov, A. A., S. N. Vostrov, I. Y. Lubenko, O. V. Kononenko, S. H. Zinner, and G. Cornaglia.** 1999. A comparison of the AUC/MIC-response plots of gemifloxacin and ciprofloxacin: critical value of the AUC/MIC ranges simulated in an in vitro dynamic model. J. Antimicrob. Chemother. **44**(Suppl. A)**:**130.
13. **Firsov, A. A., S. N. Vostrov, A. A. Shevchenko, and G. Cornaglia.** 1997. Parameters of bacterial killing and regrowth kinetics and antimicrobial effect examined in terms of area under the concentration-time curve relationships: action of ciprofloxacin against *Escherichia coli* in an in vitro dynamic model. Antimicrob. Agents Chemother. **41:**1281–1287.
14. **Firsov, A. A., S. H. Zinner, S. N. Vostrov, Y. A. Portnoy, and I. Y. Lubenko.** 2002. AUC/MIC relationships to different endpoints of the antimicrobial effect: multiple-dose in vitro simulations with moxifloxacin and levofloxacin. J. Antimicrob. Chemother. **50:**533–539.
15. **Hoffken, G., H. Lode, C. Prinzing, K. Borner, and P. Koeppe.** 1985. Pharmacokinetics of ciprofloxacin after oral and parenteral administration. Antimicrob. Agents Chemother. **27:**375–379.
16. **Hyatt, J. M., D. E. Nix, and J. J. Schentag.** 1994. Pharmacokinetics 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.
17. **Klepser, M. E., E. J. Ernst, C. R. Petzold, P. Rhomberg, and G. V. Doern.** 2001. Comparative bactericidal activities of ciprofloxacin, levofloxacin, moxifloxacin, and trovafloxacin against *Streptococcus pneumoniae* in a dynamic in vitro model. Antimicrob. Agents Chemother. **45:**673–678.
18. **Lacy, M. K., W. Lu, X. Xu, P. R. Tessier, D. P. Nicolau, R. Quintiliani, and C. H. Nightingale.** 1999. Pharmacodynamic comparisons of levofloxacin, ciprofloxacin, and ampicillin against *Streptococcus pneumoniae* in an in vitro model of infection. Antimicrob. Agents Chemother. **43:**672–677.
19. **Lee, L.-J., B. Hafkin, I.-D. Lee, J. Hoh, and R. Dix.** 1997. Effects of food and sucralfate on a single oral dose of 500 milligrams of levofloxacin in healthy subjects. Antimicrob. Agents Chemother. **41:**2196–2200.
20. **Madaras-Kelly, K. J., and T. A. Demasters.** 2000. In vitro characterization of fluoroquinolone concentration/MIC antimicrobial activity and resistance while simulating clinical pharmacokinetics of levofloxacin, ofloxacin, or ciprofloxacin against *Streptococcus pneumoniae*. Diagn. Microbiol. Infect. Dis. **37:**253–260.
21. **Madaras-Kelly, K. J., A. J. Larsson, and J. C. Rotschafer.** 1996. A pharmacodynamic evaluation of ciprofloxacin and ofloxacin against two strains of *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **37:**703–710.
22. **Madaras-Kelly, K. J., B. E. Ostergaard, L. B. Hovde, and J. C. Rotschafer.** 1996. 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. Antimicrob. Agents Chemother. **40:**627–632.
23. **Marchbanks, C. R., J. R. McKiel, D. H. Gilbert, N. J. Robillard, B. Painter, S. H. Zinner, and M. N. Dudley.** 1993. Dose ranging and fractionation of intravenous ciprofloxacin against *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an in vitro model of infection. Antimicrob. Agents Chemother. **37:**1756–1763.
24. **Nakashima, M., T. Uematsu, K. Kosuge, H. Kusajima, T. Ooie, Y. Masuda, R. Ishida, and H. Uchida.** 1995. Single- and multiple-dose pharmacokinetics of AM-1155, a new 6-fluoro-8-methoxy quinolone, in humans. Antimicrob. Agents Chemother. **39:**2635–2640.
25. **Peterson, M. L., L. B. Hovde, D. H. Wright, G. H. Brown, A. D. Hoang, and J. C. Rotschafer.** 2002. Pharmacodynamics of trovafloxacin and levofloxacin against *Bacteroides fragilis* in an in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **46:**203–210.
26. **Peterson, M. L., L. B. Hovde, D. H. Wright, A. D. Hoang, J. K. Raddatz, P. J. Boysen, and J. C. Rotschafer.** 1999. Fluoroquinolone resistance in *Bacteroides fragilis* following sparfloxacin exposure. Antimicrob. Agents Chemother. **43:**2251–2255.
27. **Ross, G. H., D. H. Wright, L. B. Hovde, M. L. Peterson, and J. C. Rotschafer.** 2001. Fluoroquinolone resistance in anaerobic bacteria following exposure to levofloxacin, trovafloxacin, and sparfloxacin in an in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **45:**2136–2140.
28. **Stass, H. H., A. Dalhoff, D. Kubitza, and U. Schuhly.** 1998. Pharmacokinetics, safety, and tolerability of ascending single doses of moxifloxacin, a new 8-methoxy quinolone, administered to healthy subjects. Antimicrob. Agents Chemother. **42:**2060–2065.
29. **Thomas, J. K., A. Forrest, S. M. Bhavnani, J. M. Hyatt, A. Cheng, C. H. Ballow, and J. J. Schentag.** 1998. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. Antimicrob. Agents Chemother. **42:**521–527.
30. **Thorburn, C. E., and D. I. Edwards.** 2001. The effect of pharmacokinetics on the bactericidal activity of ciprofloxacin and sparfloxacin against *Streptococcus pneumoniae* and the emergence of resistance. J. Antimicrob. Chemother. **48:**15–22.
31. **Vostrov, S. N., O. V. Kononenko, I. Y. Lubenko, S. H. Zinner, and A. A. Firsov.** 2000. Comparative pharmacodynamics of gatifloxacin and ciprofloxacin in an in vitro dynamic model: prediction of equiefficient doses and the breakpoints of the area under the curve/MIC ratio. Antimicrob. Agents Chemother. **44:**879–884.
32. **Wise, R., D. Lister, C. A. M. McNulty, D. Griggs, and J. M. Andrews.** 1986. The comparative pharmacokinetics of five quinolones. J. Antimicrob. Chemother. **18**(Suppl. D)**:**71–81.
33. **Wright, D. H., S. M. Gunderson, L. B. Hovde, G. H. Ross, A. S. Ibrahim, and J. C. Rotschafer.** 2002. Comparative pharmacodynamics of three newer fluoroquinolones versus six strains of staphylococci in an in vitro model under aerobic and anaerobic conditions. Antimicrob. Agents Chemother. **46:**1561–1563.
34. **Zhanel, G. G., M. Walters, N. Laing, and D. J. Hoban.** 2001. In vitro pharmacodynamic modelling simulating free serum concentrations of fluoroquinolones against multidrug-resistant *Streptococcus pneumoniae*. J. Antimicrob. Chemother. **47:**435–440.
35. **Zhao, X., and K. Drlica.** 2001. Restricting the selection of antibiotic-resistant mutants: a general strategy derived from fluoroquinolone studies. Clin. Infect. Dis. **33**(Suppl. 3)**:**S147–S156.