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Comparison of the Postantibiotic and Postantibiotic Sub-MIC Effects of Levofloxacin and Ciprofloxacin on *Staphylococcus aureus* and *Streptococcus pneumoniae*

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The postantibiotic subminimum inhibitory concentration effect (PA SME) may simulate in vivo drug exposure more accurately than the postantibiotic effect (PAE) since subinhibitory concentrations of drug persist between antibiotic dosings. In this study, we compared the PAEs and PA SMEs of levofloxacin and ciprofloxacin for clinical isolates of fluoroquinolone-susceptible *Staphylococcus aureus* and *Streptococcus pneumoniae*. At two times the MIC, PAEs of levofloxacin were an average of 0.6 h longer than the PAEs obtained for ciprofloxacin for methicillin-susceptible and methicillin-resistant *S. aureus* strains. The PAEs of levofloxacin and ciprofloxacin ranged from 1.8 to 3.1 and 1.1 to 2.4 h, respectively. Continued exposure of the methicillin-resistant strain to 1/16, 1/8, and 1/4 the MIC resulted in PA SMEs of 6.5, 15.3, and >22.3 h, respectively, for levofloxacin and 3.8, 8.0, and 12.3 h, respectively, for ciprofloxacin. For isolates of *S. pneumoniae*, at two times the MIC of both fluoroquinolones, the average PAEs of levofloxacin and ciprofloxacin were equivalent: 1.3 h for the penicillin-susceptible isolate and 0.6 h for the penicillin-resistant isolate. Continued exposure of the penicillin-susceptible *S. pneumoniae* strain to 1/16, 1/8, and 1/4 the MIC resulted in average PA SMEs of 1.0, 1.4, and 2.8 h, respectively, for levofloxacin and 1.8, 2.0, and 2.5 h, respectively, for ciprofloxacin. Continued exposure of penicillin-resistant *S. pneumoniae* to 1/16, 1/8, and 1/4 the MIC of the same fluoroquinolones resulted in average PA SMEs of 0.6, 1.1, and 2.9 h, respectively, for levofloxacin and 0.6, 1.1, and 1.5 h, respectively, for ciprofloxacin. The PA SMEs observed demonstrate the superior activity of levofloxacin against methicillin-susceptible or methicillin-resistant *S. aureus*. Although PAEs were similar for the penicillin-susceptible and penicillin-resistant *S. pneumoniae* strains, the PA SME of levofloxacin at one-fourth the MIC was longer for penicillin-resistant *S. pneumoniae*.

Pharmacodynamic variables such as postantibiotic effect (PAE), subminimum inhibitory effect (SME), and postantibiotic subminimum inhibitory concentration effect (PA SME) have increasingly become the focus of investigations designed to determine optimal dosage regimens for antimicrobial agents. The PAE has been characterized as the period of time required for a microorganism to resume normal growth after the removal of an antimicrobial agent (3). Following a brief exposure to an antibiotic, bacteria in the postantibiotic phase have been shown to be sensitive to antibacterial agents subsequently added at levels below the MIC (12). SMEs reflect serum drug levels that may exist between antibiotic dosing intervals and therefore may reflect more accurately the in vivo condition (10). Investigators such as Cars and Odenholt-Tornqvist (2) have shown that when pneumococci were exposed to antimicrobial concentrations greater than the MIC and subsequently reexposed to an antimicrobial agent at concentrations less than the MIC, the microorganisms had growth rates different than those when the organisms were exposed to agents at sub-MICs alone. Earlier, Odenholt and colleagues (19) treated streptococci in the postantibiotic (PA) phase to sub-MICs of penicillin at 0.2 and 0.3 times the MIC, an approach which resulted in the attainment of significant PA SMEs. Although the exact mechanism of the PA SME was not determined, the investigators hypothesized that following exposure to a β-lactam at a supra-MIC, only a small amount of the β-lactam is needed to bind the additional penicillin-binding proteins produced (2, 19). Similarly, after a supra-MIC exposure to antimicrobial agents that bind DNA gyrase, such as the fluoroquinolones, only a small quantity of that antimicrobial agent may be required to inhibit bacterial growth. Prior to these studies, the same group of investigators had postulated that following antibiotic exposure and removal of the antibiotic, the resulting slowly growing bacteria would be less susceptible to the action of the same antimicrobial agent. This assumption may have been the reason that antibiotics were not tested with bacteria in the PA phase (18). Significant PAEs and PA SMEs suggest that the dosing intervals for these agents can be prolonged, with advantages that include not only a lowered cost of antimicrobial therapy and a reduced risk of toxicity but also the possibility of an enhanced efficacy with subsequent dosing (15).

Numerous studies have examined the in vitro PAEs of various fluoroquinolones against pathogenic organisms (8, 9, 14, 23–25). Pastor and coworkers (24) recognized that the PAEs of sparfloxacin and ciprofloxacin for *Pseudomonas aeruginosa* and *Enterococcus faecalis* increased as a function of both the drug concentration and the exposure time. A study with aminoglycosides also confirmed that the PAE is dependent on both the drug concentration and the organism tested (27). Recently, ciprofloxacin was tested against *Staphylococcus aureus* (MIC, 0.25 μg/ml) at one, two, and four times the MIC, with results that included average PAEs of 1.4, 1.5, and 3.1 h, respectively. When tested against *P. aeruginosa* (MIC, 0.25 μg/ml) at one, two, and four times the MIC, ciprofloxacin induced average PAEs of 1.0, 3.3, and 3.4 h, respectively (6). Previous studies conducted in our laboratory demonstrated that levofloxacin

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produced a significant PAE against many gram-positive organisms, including *S. aureus*, *Staphylococcus epidermidis*, and *E. faecalis*; longer PAEs were observed for levofloxacin than for ciprofloxacin for *S. aureus* and *S. epidermidis* (4). Houston and Jones (7) reported PAEs of 1.0 to 2.9 h against strains of *Escherichia coli* and *S. aureus.*

In contrast, relatively few studies have examined the sensitivity of bacteria in the PA phase to sub-MICs of fluoroquinolones. The PAEs and PA SMEs of sparfloxacin for both gram-negative and gram-positive microorganisms have been studied by Odenholt-Tornqvist and colleagues (20, 21). In one study, PAEs were shown by sparfloxacin for tested strains of *E. coli*, *P. aeruginosa*, and *Klebsiella pneumoniae*, with the longest PA SMEs observed for those same strains demonstrating the longest PAEs (20). In the second report (21), continued exposure to sparfloxacin at 0.1, 0.2, and 0.3 times the MIC resulted in the continued suppression of growth, or a longer PA SME than PAE, for *Streptococcus pneumoniae.*

Our current study is one of the first to compare the PA SMEs of ciprofloxacin with those of levofloxacin, a newly marketed fluoroquinolone. Levofloxacin, the *l* isomer of the racemic mixture ofloxacin, has already demonstrated an expanded spectrum of in vitro and in vivo activity against both aerobic and anaerobic bacteria compared to that of the parent compound (4, 5, 27). In the present study, we compared the PAEs induced by levofloxacin and ciprofloxacin for clinical isolates of *S. aureus* and *S. pneumoniae* following initial exposure to two times the MIC of either drug. In addition, the PA SMEs for PA-phase *S. aureus* and *S. pneumoniae* were determined after exposure to 1/4, 1/8, and 1/16 the MICs of levofloxacin and ciprofloxacin.

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MATERIALS AND METHODS

Bacterial strains and media. The *S. aureus* and *S. pneumoniae* strains tested were clinical specimens received from clinical laboratories throughout the United States. The isolates were identified by using the Vitek system (bioMerieux Vitek, Hazlewood, Mo.) and were subsequently stored at −70°C in cryovials (Microbank; Pro-Lab Diagnostics, Austin, Tex.) prior to use. *S. aureus* was recovered on prepared Trypticase soy agar plates (BBL, Becton Dickinson Microbiology Systems, Cockeysville, Md.), and *S. pneumoniae* was recovered on prepared Trypticase soy agar plates containing 5% sheep blood (BBL) and was grown at 35°C to ensure viability and purity. *S. pneumoniae* was grown under 5% CO_2. Cation-adjusted Mueller-Hinton broth (CAMHB; BBL) was used as the culture medium for *S. aureus* and *S. pneumoniae*. For studies with *S. pneumoniae*, the broth was supplemented with lysed, defibrinated horse blood (BBL).

Antimicrobial agents. Levofloxacin was supplied by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan), ciprofloxacin was obtained from Miles Inc., Diagnostics Division (Kankakee, Ill.), and benzylpenicillin (penicillin G) and methicillin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Stock concentrations were prepared in sterile water, aliquoted, and stored at −70°C until use.

MIC determinations. MIC tests were performed by using agar dilution techniques according to the approved standard guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) (16). The lowest concentration of antimicrobial agent that prevented visible growth was determined to be the MIC.

Inoculum. *S. aureus* strains were grown for 16 to 18 h in 50 ml of CAMHB. *S. aureus* cultures were adjusted to a 0.5 McFarland standard, diluted 1:300, and grown in fresh CAMHB at 35°C for 1 h in a water bath with agitation at 100 rpm to produce organisms in a logarithmic growth phase at a concentration of approximately 5×10^6 CFU/ml for use in PAE experiments.

S. pneumoniae strains were grown for 16 to 18 h in 50 ml of CAMHB supplemented with 5% lysed horse blood. Cultures were diluted 1:300 with CAMHB containing 5% lysed horse blood and were incubated at 35°C for 1 h in a water bath with agitation at 100 rpm to produce organisms in a logarithmic growth phase at a concentration of approximately 5×10^6 CFU/ml for use in PAE experiments.

PAE. *S. aureus* strains were exposed to levofloxacin and ciprofloxacin at two times the MIC for 1 h, whereas *S. pneumoniae* strains were exposed to levofloxacin and ciprofloxacin at two times the MIC for 2 h. Following exposure to levofloxacin and ciprofloxacin, the bacteria were removed from the fluoroquinolones by pelleting the cells by centrifugation at 3,446 × *g* at 35°C for 10 min. The bacteria were washed in fresh, drug-free CAMHB, pelleted by centrifugation, and suspended again in fresh, drug-free CAMHB. Fresh CAMHB plus 5% lysed horse blood was used for the *S. pneumoniae* studies. All CAMHB was warmed to 35°C prior to use. Control organisms were not exposed to either fluoroquinolone but were treated similarly.

Concentrations of bacteria before and after drug removal were confirmed by removing a 1-ml aliquot, performing serial dilutions, and determining the number of CFU of the sample per milliliter on cation-adjusted Mueller Hinton agar (CAMHA). To prevent any artifacts caused by drug carryover, the lowest dilution used regularly for determining viable cell counts was 1:100.

Following drug removal, the fluoroquinolone-exposed and control cultures were placed in fresh medium and were incubated in a water bath at 35°C with agitation (100 rpm). Growth was monitored hourly for 5 h by removing 1-ml samples, performing serial dilutions, and determining the number of CFU of the sample per milliliter on CAMHA. A test of final colony counts was performed at 24 h to allow for the sufficient growth of all samples. The PAE was measured by using the equation PAE (in hours) = $T - C$, where T is the time required for the treated organisms to grow 1 log unit and C is the time needed for the organisms with no drug exposure to grow 1 log unit, as described previously (1).

PA SME. Immediately following drug removal (see PAE above), the PA-phase *S. pneumoniae* or *S. aureus* organisms were exposed to different sub-MICs (1/4, 1/8, and 1/16 the MIC) of levofloxacin or ciprofloxacin. A 1-ml sample of the appropriate fluoroquinolone at the sub-MIC was added to a flask with 24 ml of PA-phase bacteria (suspended previously in fresh culture medium) to yield final concentrations of 1/16, 1/8, and 1/4 the MIC. A 25-ml sample of PA-phase bacteria to which no drug was added served as the control. All samples and controls were incubated in a water bath at 35°C with agitation at 100 rpm, and the growth of all cultures was monitored by determining viable cell counts several times for the first 8 h and again at 24 h. The PA SME was calculated by using the equation PA SME (in hours) = $T_{PA} - C$, where T_{PA} is the time required for sub-MIC-treated PA-phase organisms to grow 1 log unit and C is the time required for control organisms to grow 1 log unit, as described previously (17).

Analysis of data. For all experiments, $\log_{10}$ CFU/ml was plotted as a function of time (in hours). Displayed graphically, the −2 h and −1 h time points indicate the interval of exposure to a fluoroquinolone for *S. pneumoniae* and *S. aureus*, respectively. The 0 h time point indicates the period in which the antibiotic was removed by centrifugation and washing. T, T_{PA}, and C values were determined from the plots and were used in the PAE and PA SME equations described above.

RESULTS

The susceptibilities of the *S. pneumoniae* isolates to penicillin and the susceptibilities of the *S. aureus* isolates to methicillin were determined by the agar dilution method (NCCLS) prior to the PAE and PA SME studies. The interpretive criteria established by NCCLS were used to determine susceptibility. The penicillin MIC for *S. pneumoniae* 9132 was ≤0.06 μg/ml, and that for *S. pneumoniae* 3035 was 2 μg/ml. The methicillin MIC for *S. aureus* 9039 was 4 μg/ml, and that for *S. aureus* 2878 was >128 μg/ml. The MICs, average PAEs, and average PA SMEs of levofloxacin and ciprofloxacin for methicillin-susceptible *S. aureus* (MSSA strain 9039), methicillin-resistant *S. aureus* (MRSA strain 2878), penicillin-susceptible *S. pneumoniae* (strain 9132), and penicillin-resistant *S. pneumoniae* (strain 3035) strains are summarized in Table 1. Longer PAEs and PA SMEs were observed following exposure of *S. aureus* isolates to levofloxacin than following exposure of the isolates to ciprofloxacin at the same levels. The average PAE of levofloxacin for both isolates was 0.6 h longer than the average PAE of ciprofloxacin. Continued exposure of *S. aureus* 9039 to levofloxacin at 1/16, 1/8, and 1/4 the MIC resulted in PA SMEs longer by 3.8, 9, and 9.8 h, respectively, than those calculated for ciprofloxacin. Similarly, a pronounced difference was observed with the continued exposure of *S. aureus* 2878 to levofloxacin at 1/16, 1/8, and 1/4 the MIC. The resulting PA SMEs were longer than those induced by ciprofloxacin by 2.8, 7.3, and >10 h, respectively. Following exposure to levofloxacin and ciprofloxacin at two times the MICs for 2 h, equivalent PAEs for *S. pneumoniae* 9132 and *S. pneumoniae* 3035 were observed. However, the average PAE determined for levofloxacin and ciprofloxacin for the *S. pneumoniae* 9132 isolate was twice the average PAE observed for both fluoroquinolones for *S. pneumoniae* 3035. As observed with the *S. aureus* iso-

TABLE 1. PAEs and PA SMEs of levofloxacin and ciprofloxacin for isolates of *S. pneumoniae* and *S. aureus*

Organism and antibiotic	MIC (μg/ml)	PAE (h)[a]	PA SME (h)[b]		
			1/16 the MIC	1/8 the MIC	1/4 the MIC
S. pneumoniae 9132					
Levofloxacin	1.0	1.27 (0.50–2.30)	1.00 (0.50–1.50)	1.38 (0.50–2.25)	2.75 (2.25–3.25)
Ciprofloxacin	2.0	1.27 (0.75–2.30)	1.75 (1.25–2.25)	2.00 (1.50–2.50)	2.50 (1.75–3.25)
S. pneumoniae 3035[c]					
Levofloxacin	1.0	0.65 (0.50–0.80)	0.63 (0.50–0.75)	1.12 (0.75–1.50)	2.88 (2.50–3.25)
Ciprofloxacin	1.0	0.73 (0.50–1.25)	0.63 (0.50–0.75)	1.12 (1.00–1.25)	1.50 (1.25–1.75)
S. aureus 9039					
Levofloxacin	0.5	1.75 (0.75–2.75)	5.00 (4.75–5.25)	13.75 (11.25–16.25)	16.25 (14.75–17.75)
Ciprofloxacin	0.5	1.13 (0.75–1.75)	1.25 (1.25)	4.75 (4.25–5.25)	6.50 (5.57–7.25)
S. aureus 2878[d]					
Levofloxacin	0.5	3.06 (1.75–4.25)	6.50 (5.75–7.25)	15.25 (13.25–17.25)	>22.25 (>22.25)
Ciprofloxacin	0.5	2.44 (2.25–2.75)	3.75 (2.75–4.75)	8.00 (7.25–8.75)	12.25 (12.25)

[a] The organisms were exposed to two times the MIC. Values are averages of four to seven individual experiments; ranges are given in parentheses.
[b] Values are averages of two individual experiments; ranges are given in parentheses.
[c] Penicillin-resistant strain.
[d] Methicillin-resistant strain.

lates, the PA SMEs for both strains of *S. pneumoniae* (strains 9132 and 3035) induced by subsequent exposure to either levofloxacin or ciprofloxacin at sub-MICs were all concentration dependent. The PAE of levofloxacin and ciprofloxacin for *S. pneumoniae* 9132 was 1.3 h. Following continued exposure to 1/16, 1/8, and 1/4 the MIC, the resulting PA SMEs were observed at 1.0, 1.4, and 2.8 h, respectively, for levofloxacin and 1.8, 2.0, and 2.5 h, respectively, for ciprofloxacin. Exposure to either fluoroquinolone at two times the MIC resulted in a PAE of 0.7 h for *S. pneumoniae* 3035. Continued exposure to one-eighth and one-fourth the MIC resulted in PA SMEs of 1.1 and 2.9 h, respectively, for levofloxacin compared to 1.1 and 1.5 h, respectively, for ciprofloxacin. At 1/16 the MIC, no PA SME was observed for *S. pneumoniae* 3035 following subsequent exposure to either levofloxacin or ciprofloxacin. Although the PAEs and PA SMEs of levofloxacin and ciprofloxacin were similar for *S. pneumoniae* 3035, the PA SME due to continued exposure to levofloxacin at one-fourth the MIC resulted in a PA SME that was longer by 1.4 h than the PA SME due to ciprofloxacin at the same sub-MIC.

Typical results, as represented by individual experiments, that show the effect of exposure of bacterial cultures to levofloxacin or ciprofloxacin at two times the MIC as well as to subsequent exposure to sub-MICs (1/16, 1/8, and 1/4 the MIC) over time are shown for *S. pneumoniae* 9132 and 3035 (Fig. 1 and 2, respectively) and for *S. aureus* 9039 and 2878 (Fig. 3 and 4, respectively). In addition, the growth of the bacterial cultures for 1 h after the dilution step is depicted from −3 to −2 h in Fig. 1 and 2 for *S. pneumoniae* strains and from −2 to −1 h in Fig. 3 and 4 for *S. aureus* strains. The effect of exposure to two times the MIC of each fluoroquinolone is depicted from −2 to 0 h (Fig. 1 and 2) for *S. pneumoniae* and from −1 to 0 h for *S. aureus* (Fig. 3 and 4). For *S. pneumoniae* 9132, equivalent PAEs of approximately 0.8 h can be estimated from the growth curves for levofloxacin (Fig. 1a) and ciprofloxacin (Fig. 1b) following exposure of the culture to two times the MIC for 2 h. For the same strain, estimated PA SMEs of 1.5, 2.3, and 3.3 h were found for levofloxacin and PA SMEs of 2.3, 2.5, and 3.3 h were found for ciprofloxacin when tested at sub-MICs of 1/16, 1/8, and 1/4 the MIC, respectively. For *S. pneumoniae* 3035, equivalent PAEs of 0.5 h for both levofloxacin (Fig. 2a) and

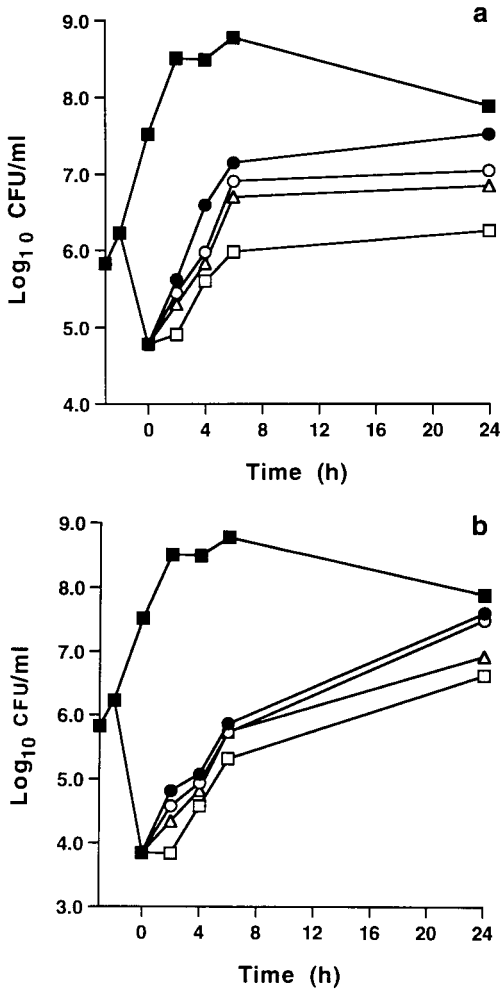

FIG. 1. PAE and PA SMEs of levofloxacin (a) and ciprofloxacin (b) for penicillin-susceptible *S. pneumoniae* 9132. The PAE (●) was induced with two times the MIC (1 μg/ml) of levofloxacin (a) and two times the MIC (2 μg/ml) of ciprofloxacin (b), and the PA SME was induced with subsequent exposure to 1/16 the MIC (○), 1/8 the MIC (△), and 1/4 the MIC (□) of levofloxacin (a) or ciprofloxacin (b). The growth control (■) contained no fluoroquinolone.

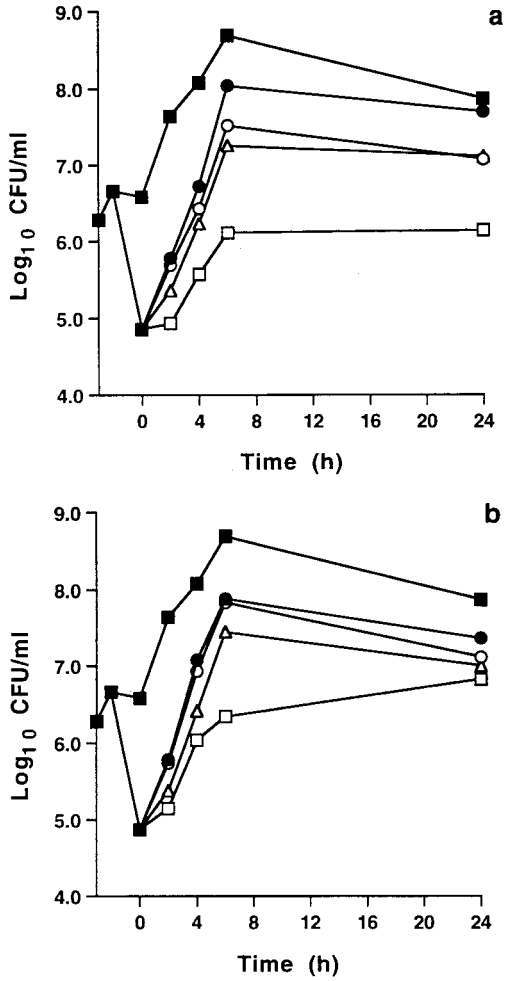

FIG. 2. PAE and PA SMEs of levofloxacin (a) and ciprofloxacin (b) for penicillin-resistant *S. pneumoniae* 3035. The PAE (●) was induced with two times the MIC for both drugs (1 μg/ml), and the PA SME was induced with subsequent exposure to 1/16 the MIC (○), 1/8 the MIC (△), and 1/4 the MIC (□) of levofloxacin (a) or ciprofloxacin (b). The growth control (■) contained no fluoroquinolone.

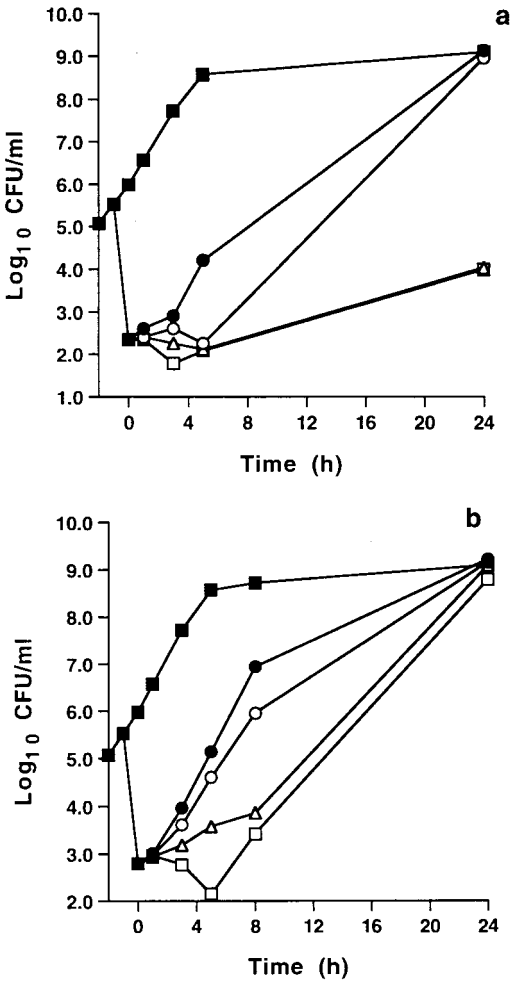

FIG. 3. PAE and PA SMEs of levofloxacin (a) and ciprofloxacin (b) for methicillin-susceptible *S. aureus* 9039. The PAE (●) was induced with two times the MIC for both drugs (0.5 μg/ml), and the PA SME was induced with subsequent exposure to 1/16 the MIC (○), 1/8 the MIC (△), and 1/4 the MIC (□) of levofloxacin (a) or ciprofloxacin (b). The growth control (■) contained no fluoroquinolone.

ciprofloxacin (Fig. 2b) induced by two times the MIC were observed, with estimated PA SMEs of 0.8, 1.5, and 3.3 h observed for levofloxacin and PA SMEs of 0.5, 1.3, and 1.8 h observed for ciprofloxacin following exposure to levels of 1/16, 1/8, and 1/4 the MIC, respectively. As shown in Fig. 3a, profound increases in PA SMEs were observed for *S. aureus* 9039 following exposure to sub-MICs of levofloxacin after the induction of a PAE (two times the MIC for 1 h). A PAE of 1.8 h was determined for levofloxacin, whereas estimated PA SMEs of 4.8, 16.3, and 17.8 h were observed with subsequent exposure to 1/16, 1/8, and 1/4 the MIC, respectively. A significant but less profound effect was determined for ciprofloxacin (Fig. 3b). The estimated PAE was 0.8 h, and the PA SMEs were 1.3, 5.3, and 8.3 h following exposure to 1/16, 1/8, and 1/4 the MIC, respectively. A similar duration of effects on growth was observed for *S. aureus* 2878 (Fig. 4a and b). The PAE determined for levofloxacin (Fig. 4a) was 0.5 h longer than the PAE determined for ciprofloxacin (Fig. 4b) for *S. aureus* 2878. PA SMEs that were greater by 1.0, 8.5, and >10.0 h were observed for levofloxacin (Fig. 4a) over ciprofloxacin (Fig. 4b) following subsequent exposure of isolate 2878 to 1/16, 1/8, and 1/4 the MIC of each fluoroquinolone, respectively.

DISCUSSION

In general, the pharmacodynamic activities of antimicrobial agents can differ significantly in variables other than just their MICs for specific pathogens. The PAE and PA SME are two such examples of variables possessing differences and similarities that may have an important impact on the future determination of dosage regimens, particularly in light of the emergence of microorganisms resistant to conventionally used agents. In previous studies, Fu and colleagues (4) compared the PAEs of levofloxacin and ciprofloxacin and observed that levofloxacin induced a 1.3 h-longer PAE for MSSA and a 0.8 h-longer PAE for MRSA when levofloxacin (4 μg/ml) and ciprofloxacin (2 μg/ml) were tested at concentrations readily achievable in human serum. Houston and Jones (7) reported PAEs that ranged from 1.0 to 2.9 h for *E. coli* and *S. aureus* strains. In the studies reported here, we observed a PAE that was 0.6 h longer for levofloxacin than for ciprofloxacin for both the MSSA and MRSA strains tested. Dramatic effects and

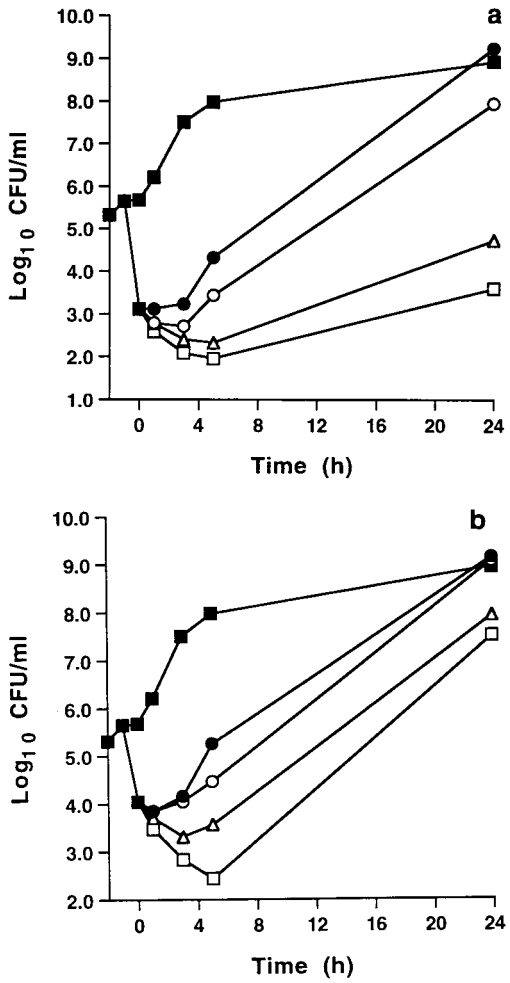

FIG. 4. PAE and PA SMEs of levofloxacin (a) and ciprofloxacin (b) for methicillin-resistant *S. aureus* 2878. The PAE (●) was induced with two times the MIC for both drugs (0.5 μg/ml), and the PA SME was induced with subsequent exposure to 1/16 the MIC (○), 1/8 the MIC (△), and 1/4 the MIC (□) of levofloxacin (a) or ciprofloxacin (b). The growth control (■) contained no fluoroquinolone.

notable differences were seen in the observed PA SMEs of levofloxacin and ciprofloxacin for these *S. aureus* strains. Although a pronounced suppression of bacterial growth extending to 4.8 and 6.5 h was seen with exposure to ciprofloxacin at one-eighth and one-fourth the MIC of ciprofloxacin for *S. aureus* 9039, respectively, even longer average PA SMEs of 13.8 and 16.3 h, respectively, were observed for levofloxacin at the same concentrations. PA SMEs of even longer duration were also observed for levofloxacin compared to those for ciprofloxacin for MRSA strain 2878. Treatment with levofloxacin at 1/16, 1/8, and 1/4 the MIC resulted in the suppression of growth that was twice as long as the effects observed for ciprofloxacin at identical concentrations.

PA SMEs have also been observed and compared elsewhere for other fluoroquinolones against *S. pneumoniae*. Odenholt-Tornqvist et al. (21) reported PA SMEs of 3.1, 3.8, and 6.0 h for sparfloxacin at 0.1, 0.2, and 0.3 times the MIC, respectively, when tested against an *S. pneumoniae* strain for which the average PAE was 2.5 h. In the studies presented here, the PA SMEs of levofloxacin and ciprofloxacin at one-fourth the MIC for *S. pneumoniae* 9132 were 2.8 and 2.5 h, respectively, despite PAEs of 1.3 h for both fluoroquinolones. Similarly, the exposure of *S. pneumoniae* 3035 to one-fourth the MICs resulted in a PA SME that was 1.4 h longer for levofloxacin than for ciprofloxacin, even though both fluoroquinolones had equivalent PAEs of 0.6 h.

The results of these studies, as well as previously reported PAE and PA SME data, indicate that continued exposure to sub-MICs of drugs after exposure to suprainhibitory concentrations allows for a much greater suppression of growth in vitro (13). The PA SME may be of importance for the prevention of bacterial growth of gram-positive bacteria between dosing and may be one factor to be considered, along with other pharmacodynamic parameters, in explaining the success of intermittent dosing (3). Additional studies are warranted to determine the precise mechanism of the PAEs and PA SMEs produced by both levofloxacin and ciprofloxacin exposure. Fluoroquinolones prevent the synthesis of bacterial DNA by inhibiting DNA gyrase, and the PAEs and PA SMEs induced by these antibiotics may represent the time required for the fluoroquinolones to disassociate from the receptor binding sites and to diffuse out of the bacterium (11). However, Gottfredsson and colleagues (6) observed no predictable pattern of DNA synthesis within drug classes when studying *S. aureus* and *E. coli* exposed to various antimicrobial agents, including ciprofloxacin. Therefore, distinct DNA synthesis patterns and PAE mechanisms may be dependent on the organism and the antibiotic being investigated. The relationship would have to be examined for each new combination. Nonetheless, an investigation of bacterial metabolic mechanisms, including DNA, RNA, and protein syntheses, could provide needed information on the successful mechanisms of the PAE and PA SME of levofloxacin.

Levofloxacin has been shown to be two times more active than ciprofloxacin against *S. pneumoniae* and two to four times more active than ciprofloxacin against *S. aureus* (5). Successful pharmacodynamic parameters observed in vitro, such as the rate of bacterial killing, the PAE, and the PA SME, are now providing a more accurate description of the antimicrobial activity of fluoroquinolones than is given by MIC observations alone (13). Indeed, higher fluoroquinolone concentrations in serum have been associated with longer PAEs and increased bactericidal activity. Orally administered levofloxacin is nearly 100% bioavailable, and therefore it achieves higher levels in serum and tissue than ciprofloxacin (5). Previously, in vivo PAE models have been used to identify further and clarify the relationship between in vitro results and clinical conditions. Oshida et al. (22) found shorter aspoxicillin PAEs for *S. aureus* in vitro than observed in an in vivo murine thigh infection model. Compared to the in vitro effects, the longer PAEs observed in in vivo *S. aureus* models may be explained by the PA SME.

Observed PA SME differences between levofloxacin and ciprofloxacin were detected for such clinically important pathogens as MRSA and penicillin-resistant *S. pneumoniae. S. aureus* is a major community-acquired and nosocomial pathogen, and therapeutic options for MRSA strains are rapidly becoming limited. Similarly, the numbers of infections caused by penicillin-resistant *S. pneumoniae* strains have increased significantly over the past 5 years (26). In this study, the superior PAE and PA SME data observed for levofloxacin compared to those observed for ciprofloxacin, along with the excellent pharmacokinetic profile of levofloxacin, illustrate the exceptional dosing possibilities and the excellent performance potential of this new fluoroquinolone in treating MRSA and penicillin-resistant *S. pneumoniae* infections.

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