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Postantibiotic Effect and Postantibiotic Sub-MIC Effect of Levofloxacin Compared to Those of Ofloxacin, Ciprofloxacin, Erythromycin, Azithromycin, and Clarithromycin against 20 Pneumococci

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The postantibiotic effect (PAE) (10 times the MIC of quinolones, 5 times the MIC of macrolides) and postantibiotic sub-MIC effect (PAE-SME) at 0.125, 0.25, and 0.5 times the MIC were determined for levofloxacin, ciprofloxacin, ofloxacin, erythromycin, azithromycin, and clarithromycin against 20 pneumococci. Quinolone PAEs ranged between 0.5 and 6.5 h, and macrolide PAEs ranged between 1 and 6 h. Measurable PAE-SMEs (in hours) at the three concentrations were 1 to 5, 1 to 8, and 1 to 8, respectively, for quinolones and 1 to 8, 1 to 8, and 1 to 6, respectively, for macrolides.

The past two decades, and in particular the past 5 years, have witnessed a dramatic increase worldwide in the incidence of pneumococci which are resistant to penicillin G and other antimicrobials (1). In a recent study from the United States, 23.6% of pneumococci showed lowered penicillin susceptibility, with 14.1% intermediately resistant and 9.5% resistant; erythromycin resistance rates of 20 and 49% were found in penicillin intermediately resistant and penicillin-resistant strains, respectively (7). In Europe, erythromycin resistance rates are higher (2).

There is an urgent need for antimicrobials which can be used for oral therapy of respiratory tract infections caused by penicillin- and macrolide-resistant pneumococci (1, 4, 8, 11, 12). Quinolone activity against pneumococci is independent of that of β-lactams and macrolides (7, 20, 21, 23). Levofloxacin, the *l*-isomer of ofloxacin, has MICs against *Streptococcus pneumoniae* which are 1 to 2 dilutions lower than those of ofloxacin and ciprofloxacin (3, 9, 16, 20), as well as good kill kinetics (23).

Postantibiotic effect (PAE) is the term used to describe suppression of bacterial growth that persists after brief exposure of organisms to antimicrobials. The PAE may have a clinical impact on antimicrobial dosing regimens. For example, drugs with no PAEs may require more frequent administration than those that demonstrate PAEs (6). However, the PAE alone may not fully explain the effectiveness of intermittent dosing, since the sum of the time concentrations of most antimicrobials are above the MIC and the time period of the PAE does not cover the entire dosing interval. Odenholt-Tornqvist et al. (17–19) have reported a long period of growth inhibition when some bacteria in the postantibiotic phase were exposed to 0.3 times the MIC of antibiotic and proposed the postantibiotic sub-MIC (PAE-SME) phenomenon in order to at least partially explain the latter discrepancy. Other explanations may be concentration-dependent killing, as seen with fluoroquinolones, and the postantibiotic leukocyte effect.

Fuursted et al. (10) tested the PAEs of macrolides against 10 pneumococcal strains and Licata et al. (13) tested the PAEs and PAE-SMEs of ciprofloxacin and levofloxacin against 2 pneumococcal strains. To simultaneously test both drug classes against a larger number of strains, we examined the PAEs and PAE-SMEs of levofloxacin, ofloxacin, ciprofloxacin, erythromycin, azithromycin, and clarithromycin against 20 pneumococci.

Twenty strains of pneumococci, for which the levofloxacin MICs were 0.5 to 2.0 μg/ml and the penicillin and macrolide MICs varied, were selected (see Table 1). A standard broth microdilution methodology, using Mueller-Hinton broth (Difco) with added lysed horse blood, was employed (15). The PAE was determined by the viable plate count method as described previously (22). For quinolones, antibiotic exposure for 1 h was at 10 times the MIC; for macrolides, for reasons of solubilization, 5 times the MIC was used. Cultures were diluted 1:1,000 to remove antibiotic. The PAE was defined (6) by the equation PAE = $T - C$, where T is the time required for viability counts of an antibiotic-exposed culture to increase by 1 $\log_{10}$ above the counts observed immediately after dilution and C is the corresponding time for the growth control.

The PAE-SME (17–19) was measured at 0.125, 0.25, and 0.5 times the MIC as described previously (22) and defined according to Odenhalt-Tornqvist and coworkers (17–19) by the equation PAE-SME = $T_{pa} - C$, where T_{pa} is the time for cultures previously exposed to antibiotic and then reexposed to different sub-MICs to increase by 1 $\log_{10}$ above the counts immediately after dilution and C is the corresponding time for the unexposed control.

Viability counts for PAE and PAE-SME tests were determined before exposure and immediately after dilution (0 h) and then every 2 h until the turbidity of the tube reached a no. 1 McFarland standard, for a total of 8 h. Recovery plates were incubated for up to 48 h. Colony counts were done only for plates with 100 to 300 CFU/ml. All results were the means of two separate assays.

Microbroth MICs for the 20 strains tested are presented in Table 1. As can be seen, levofloxacin MICs ranged between 0.5 and 2 μg/ml. Ten strains were macrolide susceptible (MIC, 0.016 to 0.25 μg/ml) and nine were highly macrolide resistant (MIC, ≥512 μg/ml); the macrolide MIC for one strain was 0.5 μg/ml. Five strains were penicillin susceptible, six were penicillin intermediately resistant, and nine were penicillin resistant. Ciprofloxacin and ofloxacin MICs ranged between 0.5 and 4 μg/ml.

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TABLE 1. MICs of individual strains

Strain no.	MIC (μg/ml)						
	Penicillin	Levofloxacin	Ofloxacin	Ciprofloxacin	Erythromycin	Azithromycin	Clarithromycin
153	0.06	1.0	2.0	2.0	0.06	0.06	0.03
118	1.0	1.0	2.0	1.0	0.03	0.06	0.016
357	0.25	1.0	2.0	1.0	0.03	0.03	0.03
227	0.125	1.0	2.0	1.0	0.03	0.03	0.03
158	2.0	1.0	2.0	1.0	0.06	0.06	0.03
167	2.0	1.0	1.0	2.0	0.03	0.06	0.06
18	0.016	1.0	2.0	1.0	512.0	512.0	512.0
21	0.03	1.0	2.0	4.0	512.0	512.0	512.0
114	0.03	1.0	2.0	2.0	512.0	512.0	512.0
681	4.0	1.0	4.0	2.0	512.0	512.0	512.0
149	0.06	2.0	4.0	4.0	0.016	0.016	0.016
135	2.0	2.0	1.0	2.0	0.06	0.125	0.03
683	2.0	2.0	4.0	1.0	0.125	0.25	0.125
525	2.0	0.5	1.0	0.5	0.016	0.03	0.016
24	4.0	1.0	2.0	1.0	512.0	512.0	512.0
471	0.125	2.0	4.0	4.0	1,024.0	1,024.0	1,024.0
455	2.0	2.0	2.0	1.0	0.5	0.5	0.5
184	2.0	1.0	4.0	1.0	512.0	512.0	512.0
425	0.125	1.0	1.0	1.0	1,024.0	1,024.0	>1,024.0
433	1.0	1.0	2.0	1.0	>1,024.0	>1,024.0	>1,024.0

In all cases, exposure to antibiotics at 0.01 times the MIC did not yield bacteriostatic activity. Growth controls did not autolyse through 8 h. PAE and PAE-SME results are presented in Table 2. Macrolide PAEs and PAE-SMEs could not be determined for highly macrolide-resistant strains. In many cases, PAE-SMEs could not be quantitated, since drugs sometimes produced complete killing, especially at higher sub-MICs. No relationship between quinolone PAE or PAE-SME and penicillin susceptibility was ascertained.

Levofloxacin MICs relative to those of ciprofloxacin were similar to those reported previously (3, 9, 20, 23). Levofloxacin breakpoints of ≤2 μg/ml (susceptible), 4 μg/ml (intermediately resistant), and ≥8 μg/ml (resistant) have been approved by the National Committee for Clinical Laboratory Standards (15). Relative activity of macrolides against erythromycin-susceptible strains and cross-resistance of all three compounds against strains with erythromycin MICs of ≥512 μg/ml have been reported previously (7, 11, 14, 21–23).

TABLE 2. PAEs and PAE-SMEs of compounds[a]

Drug	PAE (h)		PAE-SME[b] (h)	
	Range	Mean	Range	Mean
Levofloxacin	0.5–4.5	2.5	1–5/1–6/2–8[c]	2.8/3.5/5.4
Ofloxacin	1–4.5	2.4	1–5/1–5/2–8[d]	2.6/3.3/5.0
Ciprofloxacin	0.5–6.5	2.3	1–4.5/1–8/1–7[e]	2.7/3.6/3.9
Erythromycin	1–5	2.5	1–8/1–8/1–6[f]	3.0/3.5/3.3
Azithromycin	1–4	1.9	1–4/1–5/1–5[g]	2.2/2.3/2.2
Clarithromycin	1–6	2.3	1–7/1–8/1–5[h]	3.5/4.0/3.3

[a] Range of values (arithmetic mean of two assays for each strain) and arithmetic mean of values for all strains tested are given. PAEs and PAE-SMEs were not determined for nine strains for which the macrolide MICs were ≥512.0 μg/ml.

[b] PAE-SME values at sub-MICs of 0.125/0.25/0.5 times the MIC.

[c] One, 2, and 12 strains had rapid bactericidal activity at 0.125, 0.25, and 0.5 times the MIC, respectively.

[d] One, 2, and 12 strains had rapid bactericidal activity at 0.125, 0.25, and 0.5 times the MIC, respectively.

[e] Two, 3, and 11 strains had rapid bactericidal activity at 0.125, 0.25, and 0.5 times the MIC, respectively.

[f] Two strains had rapid bactericidal activity at 0.5 times the MIC.

[g] One, one, and two strains had rapid bactericidal activity at 0.125, 0.25, and 0.5 times the MIC, respectively.

[h] Two strains had rapid bactericidal activity at 0.5 times the MIC.

Results of the present study show a significant PAE for all quinolones and macrolides tested. Fuursted and coworkers (10), using pneumococci with different macrolide and penicillin susceptibilities and exposure to antibiotics at 10 times the MIC, obtained macrolide PAEs similar to those reported in the present study, and Licata et al. (13) obtained similar results for ciprofloxacin and levofloxacin with one penicillin-susceptible and one penicillin-resistant pneumococcus.

PAE-SMEs were usually longer than PAEs. Complete killing of organisms in PAE-SME experiments, especially at higher subinhibitory concentrations, could have been due to drug-induced lysis; alternately, PAE-SMEs could have been longer than the 8-h period tested. In every case, drug-free controls yielded growth, and the results of separate duplicate testing were identical. More studies are required to elucidate this finding (22).

Peak concentrations of levofloxacin in serum from human volunteers have been reported as 6.55 ± 1.84 μg/ml with an oral dose of 500 mg every 24 h (5). Single oral doses of levofloxacin of 50 to 1,000 mg produce a mean maximum concentration of drug in serum and area under the concentration-time curve ranging from 0.6 to 9.4 μg/ml and 4.7 to 108 mg · h/liter, respectively, but increasing linearly in a dose-proportionate fashion (5).

Results of the present study, together with the above breakpoint and pharmacokinetic analyses, point to clinical use of levofloxacin for pneumococcal infections, irrespective of the penicillin or macrolide susceptibility status of the strains.

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