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Postantibiotic Effects of Grepafloxacin Compared to Those of Five Other Agents against 12 Gram-Positive and -Negative Bacteria

SHEILA K. SPANGLER,[1]† SARALEE BAJAKSOUZIAN,[2] MICHAEL R. JACOBS,[2] AND PETER C. APPELBAUM[1]*

Departments of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] and Department of Pathology (Clinical Microbiology), Case Western Reserve University, Cleveland, Ohio 44106[2]

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The postantibiotic effect (PAE) (10× the MIC) and the postantibiotic sub-MIC effects (0.125, 0.25, and 0.5× the MIC) were determined for six compounds against 12 strains. Measurable PAEs ranged between 0 and 1.8 h for grepafloxacin, 0 and 2.2 h for ciprofloxacin, 0 and 3.1 h for levofloxacin, 0 and 2.2 h for sparfloxacin, 0 and 2.4 h for amoxicillin-clavulanate and 0 and 4.8 h for clarithromycin. Reexposure to subinhibitory concentrations increased the PAEs against some strains.

The past decade has witnessed a dramatic worldwide increase in the incidence of pneumococci and other respiratory pathogens such as *Haemophilus influenzae* which are resistant to β-lactam and non-β-lactam agents (4, 5, 10). Newer agents are required to treat these infections (7, 10).

Grepafloxacin is a broad-spectrum quinolone with improved activity against pneumococci and is also very active against *Haemophilus influenzae*, *Moraxella catarrhalis*, and other organisms responsible for community-acquired respiratory tract infections, such as chlamydia, *Mycoplasma pneumoniae*, and *Legionella* (9, 12, 15, 18).

Postantibiotic effects (PAEs) and postantibiotic sub-MIC effects (PAE-SMEs) are pharmacodynamic phenomena which, if present, may influence antimicrobial dosing regimens (3, 14). We examined the PAEs and PAE-SMEs of grepafloxacin, ciprofloxacin, levofloxacin, sparfloxacin, amoxicillin-clavulanate, and clarithromycin against two strains each of methicillin-susceptible *Staphylococcus aureus*; penicillin-susceptible, -intermediate, and -resistant pneumococci; *H. influenzae*; and *Klebsiella pneumoniae*.

Microdilution MICs were determined according to standard recommendations (13) with freshly made *Haemophilus* test medium (HTM), which was used within 2 weeks of preparation, for *H. influenzae*.

The PAE was determined by the viable plate method (3, 16, 17) with Mueller-Hinton broth supplemented with 5% lysed horse blood for pneumococci; for *H. influenzae*, freshly prepared HTM was used. Bacterial inocula were prepared by suspending growth from an overnight agar plate (blood agar plates for gram-positive organisms and *K. pneumoniae*; chocolate agar plates for *H. influenzae*) in broth. The broth was incubated at 35°C for 2 to 4 h in a shaking water bath until the turbidity matched that of a no. 1 McFarland standard (approximately 3×10^8 CFU/ml). An additional control culture containing bacteria and antibiotic at a concentration of 0.01× the MIC was prepared to confirm that after dilution the antibiotic was no longer bacteriostatic (3, 16, 17).

Viability counts (3, 16, 17) were determined before exposure and immediately after dilution (0 h) and then every 2 h until the turbidity of the tube reached that of a no. 1 McFarland standard (maximum of 8 h). The PAE was defined as described by Craig and Gudmundsson (3). For each experiment, viability counts, expressed as $\log_{10}$ CFU per milliliter, were plotted against time. Results were expressed as the mean of two separate assays.

In cultures designated for PAE-SME determination (14), the PAE was induced, and experiments for determination of PAE-SMEs were performed as described previously (16, 17) with subinhibitory concentrations of 0.125, 0.25, and 0.5× the MIC. Viability counts were determined before exposure, immediately after dilution, and then every 2 h (maximum 8 h) until the turbidity reached that of a no. 1 McFarland standard, as described above for the PAE. Experiments (14) for determination of sub-MIC effects (SMEs; exposure of strains to subinhibitory drug concentrations) were not performed. The PAE-SME was defined as described by Odenholt-Tornqvist (14) and others (16, 17). Results were plotted (as described above for PAE) and are expressed as the arithmetic means of two separate assays.

Grepafloxacin MICs were all ≤0.25 μg/ml (range, 0.004 to 0.25 μg/ml). The MICs of ciprofloxacin, levofloxacin, and sparfloxacin ranged between 0.016 and 8.0, 0.016 and 1.0, and 0.004 and 0.5 μg/ml, respectively. Amoxicillin-clavulanate inhibited all strains at ≤8.0 μg/ml (range, 0.06 to 8.0 μg/ml), and clarithromycin inhibited all strains at ≤16.0 μg/ml (range, 0.03 to 16.0 μg/ml). For all *H. influenzae* strains tested, quinolone MICs were ≤0.016 μg/ml (Table 1).

The results of tests for PAEs and PAE-SMEs are presented in Table 1. Exposure of bacteria to antibiotics at 0.01× the MIC did not lead to bacteriostatic activity. Grepafloxacin PAEs range from 0 to 18 h, with this compound having no PAEs against one methicillin-susceptible *S. aureus* strain and one *K. pneumoniae* strain. The ranges of PAEs of the other quinolones were similar to those of grepafloxacin. Ciprofloxacin showed no PAE against one methicillin-susceptible *S. aureus* strain and both *K. pneumoniae* strains tested, and levofloxacin showed no PAE against one methicillin-susceptible *S. aureus* strain and one penicillin-resistant pneumococcal strain. Sparfloxacin had no PAE against one methicillin-susceptible *S. aureus* strain and one penicillin-intermediate pneumococcal

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, 500 University Dr., Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-7953. E-mail: pappelbaum@psghs.edu.
† Deceased.

TABLE 1. PAEs of inhibitory and subinhibitory concentrations of compounds[a]

Organism and drug	MIC (μg/ml)	PAE (h)[b]	PAE-SME (h)[c]		
			0.125× the MIC	0.25× the MIC	0.5× the MIC
Methicillin-susceptible *S. aureus*					
Grepafloxacin	0.12	1.3	3.1	6.5	NM[d]
Ciprofloxacin	1	0	0	0	0
Levofloxacin	1	0	4.2	NM	NM
Sparfloxacin	0.25	0	0.4	NM	NM
Amoxicillin-clavulanate	8	0.3	0.3	0.6	0.8
Clarithromycin	0.25	0	0	0	0
Methicillin-susceptible *S. aureus*					
Grepafloxacin	0.06	0	0.2	2.0	NM
Ciprofloxacin	8	0.5	1.7	1.7	NM
Levofloxacin	1	2.4	2.5	NM	NM
Sparfloxacin	0.25	1.3	1.6	1.8	NM
Amoxicillin-clavulanate	1	0	0.2	0.7	0.7
Clarithromycin	0.016	0.9	0.9	1.6	2.7
Penicillin-susceptible *Streptococcus pneumoniae*					
Grepafloxacin	0.25	1.2	3.6	NM	NM
Ciprofloxacin	2	1.8	3.9	NM	NM
Levofloxacin	1	1.7	2.3	3.0	NM
Sparfloxacin	0.25	1.5	1.7	2.8	6.4
Amoxicillin-clavulanate	0.016	1.3	1.7	2.1	3.9
Clarithromycin	0.06	3.9	NM	NM	NM
Penicillin-susceptible *S. pneumoniae*					
Grepafloxacin	0.25	1.7	5.9	NM	NM
Ciprofloxacin	2	2.1	3.9	NM	NM
Levofloxacin	1	1.3	1.5	2.2	6.0
Sparfloxacin	0.25	1.5	2.0	3.5	NM
Amoxicillin-clavulanate	0.06	2.2	2.2	4.0	6.2
Clarithromycin	0.12	4.8	NM	NM	NM
Penicillin-intermediate *S. pneumoniae*					
Grepafloxacin	0.25	0.6	0.6	2.5	NM
Ciprofloxacin	1	1.0	1.0	1.3	1.7
Levofloxacin	1	3.1	3.3	5.0	6.5
Sparfloxacin	0.25	0	0	1.0	6.4
Amoxicillin-clavulanate	0.12	2.4	2.4	4.9	NM
Clarithromycin	0.25	2.9	5.0	NM	NM
Penicillin-intermediate *S. pneumoniae*					
Grepafloxacin	0.12	0.7	1.7	3.3	NM
Ciprofloxacin	1	1.9	2.6	5.2	NM
Levofloxacin	1	1.5	1.5	2.7	NM
Sparfloxacin	0.5	1.3	3.1	7.5	NM
Amoxicillin-clavulanate	0.06	1.2	3.8	NM	NM
Clarithromycin	0.03	4.2	NM	NM	NM
Penicillin-resistant *S. pneumoniae*					
Grepafloxacin	0.12	1.3	1.7	1.7	3.3
Ciprofloxacin	2	0.5	0.5	0.5	0.5
Levofloxacin	1	0	0	0.9	1.8
Sparfloxacin	0.25	0.2	0.5	1.5	3.4
Amoxicillin-clavulanate	2	0.2	0.5	0.5	0.5
Clarithromycin	0.12	3.8	NM	NM	NM
Penicillin-resistant *S. pneumoniae*					
Grepafloxacin	0.25	1.8	6.2	NM	NM
Ciprofloxacin	2	2.2	4.6	NM	NM
Levofloxacin	1	2.2	2.6	3.4	NM
Sparfloxacin	0.5	2.2	5.1	NM	NM
Amoxicillin-clavulanate	1	1.0	3.2	3.7	NM
Clarithromycin	4	NM	NM	NM	NM
H. influenzae					
Grepafloxacin	0.004	NM	NM	NM	NM
Ciprofloxacin	0.016	NM	NM	NM	NM

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TABLE 1—*Continued*

Organism and drug	MIC (μg/ml)	PAE (h)[b]	PAE-SME (h)[c]		
			0.125× the MIC	0.25× the MIC	0.5× the MIC
Levofloxacin	0.016	NM	NM	NM	NM
Sparfloxacin	0.004	NM	NM	NM	NM
Amoxicillin-clavulanate	1	0.8	0.8	NM	NM
Clarithromycin	8	NM	NM	NM	NM
H. influenzae					
Grepafloxacin	0.008	NM	NM	NM	NM
Ciprofloxacin	0.016	NM	NM	NM	NM
Levofloxacin	0.016	NM	NM	NM	NM
Sparfloxacin	0.008	NM	NM	NM	NM
Amoxicillin-clavulanate	1	0.2	0.2	NM	NM
Clarithromycin	8	NM	NM	NM	NM
K. pneumoniae					
Grepafloxacin	0.06	0	0	1.0	1.7
Ciprofloxacin	0.5	0	0.04	0.3	1.3
Levofloxacin	0.5	1.8	2.1	7.8	NM
Sparfloxacin	0.12	0.7	1.0	1.7	2.0
Amoxicillin-clavulanate	8	0	0	0	0
Clarithromycin	16	0.6	1.0	1.3	1.3
K. pneumoniae					
Grepafloxacin	0.12	0.3	0.8	NM	NM
Ciprofloxacin	0.5	0	0	0.5	1.4
Levofloxacin	0.5	2.6	2.0	NM	NM
Sparfloxacin	0.25	1.3	NM	NM	NM
Amoxicillin-clavulanate	4	0	0	0	0
Clarithromycin	8	0.2	0.2	0.4	0.7

[a] Values are the means of two experiments.
[b] Exposure for 1 h to 10× the MIC; drug was removed by 1:1,000 dilution.
[c] Strains were exposed to 10× the MIC for 1 h, drug was removed (as for the PAE experiments), and then the strains were exposed to 0.125, 0.25, and 0.5× the MIC.
[d] NM, not measurable.

strain. Quinolone PAEs against *H. influenzae* could not be measured owing to rapid bactericidal activity. Amoxicillin-clavulanate had no PAEs against one methicillin-susceptible *S. aureus* strain and both *K. pneumoniae* strains tested, with PAEs ranging between 0 and 2.4 h. Clarithromycin PAEs ranged between 0 and 4.8 h, with no PAE against one methicillin-susceptible *S. aureus* and rapid bactericidal activity against both *H. influenzae* strains. The differences in PAEs in the two separate assays were between 0.1 and 0.5 h for all drugs tested, and no statistical difference between the results could be shown.

PAE-SMEs were generally longer than PAEs, especially at higher sub-MICs. PAE-SMEs at 0.125, 0.25, and 0.5× the MICs ranged between 0 and 6.2, 1.0 and 6.5, and 1.7 and 3.3 h, respectively, for grepafloxacin; 0 and 4.6, 0 and 5.2, and 0 and 1.7 h, respectively, for ciprofloxacin; 0 and 4.2, 0.9 and 7.8, and 1.8 and 6.5 h, respectively, for levofloxacin; 0 and 5.1, 1.0 and 7.5, and 2.0 and 6.4 h, respectively, for sparfloxacin; 0 and 3.8, 0 and 4.9, and 0 and 6.2 h, respectively, for amoxicillin-clavulanate; and 0 and 5.0, 0 and 1.6, and 0 and 2.7 h, respectively, for clarithromycin. The PAE-SMEs of all drugs except amoxicillin-clavulanate at 0.125× the MIC could not be measured against *H. influenzae* because of rapid bactericidal activity. With the exception of ciprofloxacin against one strain of *S. aureus*, amoxicillin-clavulanate against both *K. pneumoniae* strains tested, and clarithromycin against one *S. aureus* strain, the drugs which did not have PAEs had PAE-SMEs, especially at higher subinhibitory concentrations.

The microdilution MICs of grepafloxacin were similar to those reported by us and other workers (9, 12, 15). The PAE-SMEs were generally longer than the PAEs. Complete killing of strains in PAE-SME tests, 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 which was tested (16, 17). However, the SMEs (14) of the drugs against the strains were not tested. Thus, it is not possible to say whether the longer PAE-SMEs were due to the subinhibitory concentration itself or the subinhibitory concentration following prior exposure.

A single 400-mg oral dose of grepafloxacin yields a maximum concentration in serum of 1.5 ± 0.3 μg/ml, with an area under the curve value of 12.4 ± 2.4 mg · h/liter (1). Trough levels increase significantly over the first 3 days, reaching approximately 80% of the steady-state levels. The mean plasma elimination half-life is 12 h (6). Serum grepafloxacin concentrations are significantly exceeded in bronchial mucosa (mean ratio, 3.13), epithelial lining fluid (mean ratio, 12.21), and macrophages (mean ratio, 194.52) (2). Despite the latter increased concentrations in tissue and fluid, it is recognized that the exposure concentrations for organisms such as pneumococci were higher than the achievable concentrations in serum. In the past, many workers have used 10× the MIC as the PAE exposure concentration, and exposure time and inoculum density differ (3, 16, 17); these require standardization. However, with different exposure concentrations and exposure periods, the PAEs of different quinolones and also macrolides against pneumococci are very similar (3, 8, 11, 15, 16).

The results of MIC and pharmacokinetic tests suggest that

grepafloxacin should be used clinically as treatment for infections caused by susceptible strains. This is particularly the case for empiric therapy of community-acquired respiratory tract infections. Results of PAE testing support once-daily dosing.

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