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Postantibiotic Effects of Garenoxacin (BMS-284756) against 12 Gram-Positive or -Negative Organisms

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Conventional in vitro methods were used to determine the postantibiotic effects (PAEs), sub-MIC effects (SMEs), and postantibiotic sub-MIC effects (PA-SMEs) of garenoxacin for a range of organisms. The mean PAEs of garenoxacin for pneumococci, staphylococci, and enterococci were 0.3 to 2.2 h. For *Escherichia coli* and *Pseudomonas aeruginosa*, the PAEs were 0.9 to 1.6 h. The mean PA-SMEs (0.4 times the MIC) for pneumococci, staphylococci, and enterococci were 3.0 to >10 h, 1.8 to >10.7 h, and 5.8 h, respectively, while those for *E. coli* and *P. aeruginosa* were 7.6 and 4.4 h, respectively.

The postantibiotic effect (PAE) is a pharmacodynamic parameter that may be considered when choosing antibiotic dosing regimens. It is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic (2–4, 7). Odenholt-Tornqvist and coworkers have suggested that during intermittent dosage regimens, suprainhibitory levels of antibiotic are followed by subinhibitory levels that persist between doses, and they have hypothesized that persistent subinhibitory levels could extend the PAE. The effect of sub-MIC concentrations on growth during the PAE period has been defined as the postantibiotic sub-MIC effect (PA-SME), representing the time period that includes the PAE plus the additional time during which growth is suppressed by sub-MIC concentrations. In contrast to the PA-SME, the sub-MIC effect (SME) is a measure of the direct effect of subinhibitory levels on cultures which have not been previously exposed to antibiotics (10, 11).

We examined the PAEs, PA-SMEs, and SMEs of garenoxacin (BMS-284756; T-3811), a broad-spectrum quinolone lacking the C-6 fluorine of some other fluoroquinolone agents (1, 5, 6, 12, 13). We studied two strains each of penicillin-susceptible, -intermediate, and -resistant *Streptococcus pneumoniae* (all quinolone susceptible with levofloxacin MICs of ≤2.0 μg/ml); one strain of *Enterococcus faecalis*; one methicillin-susceptible and one methicillin-resistant *Staphylococcus aureus* strain (both with garenoxacin MICs of ≤1.0 μg/ml, but one strain being susceptible and one resistant to ciprofloxacin); one *Escherichia coli* strain; and one *Pseudomonas aeruginosa* strain. Organisms were identified by standard methods (8).

Garenoxacin MICs were determined by macrodilution procedures (9). The garenoxacin MICs were as follows: 0.03 to 0.06 μg/ml for pneumococci, 0.03 to 0.5 μg/ml for staphylococci, 0.25 μg/ml for *E. faecalis*, 0.06 μg/ml for *E. coli*, and 1.0 μg/ml for *P. aeruginosa*. The garenoxacin MIC for the ciprofloxacin-susceptible *S. aureus* strain was 0.03 μg/ml, and that for the ciprofloxacin-resistant *S. aureus* strain was 0.5 μg/ml.

The PAE was determined by the viable plate count method (4) by using Mueller-Hinton broth supplemented with 5% lysed horse blood when pneumococci were tested. The PAE was induced by exposure to 10 times the MIC of garenoxacin for 1 h, except in experiments with the *P. aeruginosa* isolate, where six times the MIC was used. The latter two concentrations were chosen so as not to exceed the approximate maximally achievable total serum levels of 6.9 to 7.8 μg/ml for all strains (14; C. Stewart, D. Gajjar, A. Bello, L. Christopher, D. Hollenbaugh, Z. Ge, and D. Grasela, Abstr. 41st Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-46, 2001).

For PAE testing, tubes containing 5 ml of broth with antibiotic were inoculated with approximately 5×10^6 CFU/ml. Inocula were prepared by suspending growth from an overnight blood agar plate in broth. Growth controls with inoculum but no antibiotic were included with each experiment. Inoculated test tubes were placed in a shaking water bath at 35°C for an exposure period of 1 h. At the end of the exposure period, cultures were diluted 1:1,000 in prewarmed broth to remove the antibiotic. Antibiotic removal was confirmed by comparison of growth curves of a control culture containing no antibiotic with those of another containing garenoxacin at 0.001 times the exposure concentration.

Viable counts 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. The PAE was defined as follows: PAE = $T - C$, where T is the time required for the viable counts of an antibiotic-exposed culture to increase by 1 $\log_{10}$ unit above the counts observed immediately after dilution and C is the corresponding time for the growth control (4).

In cultures designated for PA-SME experiments, the PAE was induced as described above after exposure to garenoxacin at 6 or 10 times the MIC (see above). Following 1:1,000 dilution, cultures were divided into four tubes. Garenoxacin was added to three tubes to produce final subinhibitory concentrations of 0.2, 0.3, and 0.4 times the MIC. The fourth tube did not receive antibiotic. Viable counts were determined before exposure, immediately after dilution, and then every 2 h until the culture turbidity of the tubes reached a no. 1 McFarland standard. SME experiments were performed in the same manner

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TABLE 1. PAEs, SMEs, and PA-SMEs of garenoxacin against 12 strains

Strain[a]	Mean effect (h)[b]						
	PAE	0.2× MIC		0.3× MIC		0.4× MIC	
		SME[c]	PA-SME[d]	SME	PA-SME	SME	PA-SME
Pen-S *S. pneumoniae*	1.2 (0.8–1.6)	1.0 (0–2.0)	3.4 (2.2–4.5)	1.2 (0.5–2.0)	4.7 (4.3–5.1)	1.8 (1.1–2.4)	5.0 (4.3–5.6)
Pen-S *S. pneumoniae*	2.2 (2.0–2.5)	2.6 (2.4–2.9)	3.2 (2.5–3.9)	3.3 (3.3–3.3)	≥8.4 (6.9–>9.8)	4.2 (3.5–4.8)	>9.4 (>9.4–>9.4)
Pen-I *S. pneumoniae*	1.6 (1.5–1.8)	1.8 (1.5–2.1)	2.2 (1.9–2.6)	1.6 (1.3–1.8)	2.4 (1.9–2.8)	2.0 (1.6–2.3)	3.0 (2.9–3.1)
Pen-I *S. pneumoniae*	1.2 (0.8–1.6)	0.8 (0.4–1.1)	1.1 (0.8–1.4)	1.3 (1.3–1.3)	2.2 (2.2–2.3)	2.4 (2.4–2.5)	3.1 (3.0–3.1)
Pen-R *S. pneumoniae*	0.8 (0.5–1.0)	1.7 (1.5–1.9)	2.2 (1.8–2.6)	3.7 (3.5–3.9)	6.2 (5.8–6.6)	≥10 (10–≥10.2)	>10 (>10–>10.2)
Pen-R *S. pneumoniae*	0.3 (0–0.6)	0.4 (0.3–0.5)	0.7 (0.3–1.1)	1.2 (1.1–1.3)	1.4 (1.1–1.6)	1.2 (1.1–1.3)	5.2 (5.1–5.3)
E. faecalis	1.0 (0.3–1.6)	0.7 (0.6–0.8)	2.4 (1.3–3.4)	2.0 (1.9–2.0)	3.4 (2.7–4.0)	3.8 (3.8–3.8)	5.8 (4.8–6.9)
Meth-S *S. aureus*	0.8 (0.7–0.8)	1.3 (1.0–1.6)	2.2 (1.9–2.6)	2.9 (2.1–3.7)	7.7 (6.7–8.7)	>10.7 (>10.7–>10.7)	>10.7 (>10.7–>10.7)
Meth-R, cipro-S *S. aureus*	0.6 (0.6–0.7)	0.3 (0.3–0.3)	1.3 (1.2–1.4)	0.6 (0.5–0.8)	2.2 (1.5–2.8)	0.9 (0.5–1.3)	4.3 (4.0–4.6)
Meth-R, cipro-R *S. aureus*	0.6 (0.1–1.2)	0.3 (0.2–0.4)	1.0 (0.9–1.2)	0.3 (0.2–0.4)	1.2 (1.1–1.2)	1.1 (0.4–1.8)	1.8 (1.3–2.2)
E. coli	0.9 (0.8–1.0)	1.1 (0.7–1.5)	2.8 (2.4–3.3)	2.2 (2.0–2.5)	4.6 (4.2–5.1)	4.5 (4.0–5.0)	7.6 (7.5–7.8)
P. aeruginosa	1.6 (1.2–2.1)	1.6 (1.2–1.9)	2.7 (2.5–2.9)	2.2 (2.0–2.3)	4.0 (4.0–4.0)	3.8 (3.2–4.4)	4.4 (4.0–4.9)

[a] Pen-S, Pen-I, and Pen-R, penicillin-susceptible, -intermediate, and -resistant, respectively; Meth-S and Meth-R, methicillin-susceptible and -resistant, respectively; cipro-S and cipro-R, ciprofloxacin-susceptible and -resistant, respectively.

[b] Values are means of two separate experiments. Values in parentheses are ranges. Strains were exposed to 6 to 10 times the MIC of garenoxacin (see text) for 1 h at 35°C. The drug was removed by 1:1,000 dilution.

[c] Strains were not previously exposed to garenoxacin.

[d] Strains were previously exposed to garenoxacin.

as for PA-SME experiments, except that the PAE was not induced in cultures designated for SME experiments.

The PA-SME was defined as follows: PA-SME = $T_{pa} - C$, where T_{pa} is the time required for the viable counts of cultures previously exposed to antibiotic and then reexposed to different sub-MIC concentrations to increase by 1 $\log_{10}$ unit above the counts observed immediately after dilution and C is the corresponding time for the unexposed control (10, 11). The SME was defined as follows: SME = $T_s - C$, where T_s is the time required for the viable counts of cultures exposed only to sub-MIC concentrations to increase by 1 $\log_{10}$ unit above the counts seen immediately after dilution and C is the corresponding time for the unexposed control. The PA-SME and SME (10, 11) were measured in two separate experiments. For each experiment, viable counts ($\log_{10}$ CFU per milliliter) were plotted against time and the results are expressed as the mean of two separate assays.

The results are presented in Table 1. The PA-SMEs were longer than the PAEs for all strains tested and increased with the subinhibitory concentration of garenoxacin. For the six pneumococci, the mean PAE was 1.2 h and PAEs ranged between 0.8 and 2.2 h. At 0.4 times the MIC, the PA-SMEs for the two penicillin-susceptible strains were 5.0 and >9.4 h and that for one penicillin-resistant strain was 5.2 h. These PA-SMEs were greater than the sums of the corresponding PAEs and SMEs for these strains. For the remaining three strains, at 0.4 times the MIC, the PA-SMEs were 3.0 to >10 h, with PA-SME values being similar to the PAE plus SME values.

PAEs for staphylococci were 0.6 to 0.8 h, with a mean of 0.7 h. These PAEs did not differ greatly (0.6 to 0.8 h) between methicillin-susceptible and -resistant *S. aureus* strains. For the ciprofloxacin-susceptible, methicillin-resistant strain, the PA-SME at 0.4 times the MIC (4.3 h) was longer than the sum of the PAE and SME. For the ciprofloxacin-resistant, methicillin-resistant strain, the PA-SME was not found to be increased compared with the SME (1.8 and 1.1 h, respectively) at 0.4 times the MIC. For the methicillin-susceptible strain, a very long PA-SME of >10.7 h at 0.4 times the MIC corresponded to an equally long SME. For *E. faecalis*, the garenoxacin PAE was 1.0 h and the PA-SME at 0.4 times the MIC was 5.8 h.

For *E. coli* and *P. aeruginosa*, the PAEs were 0.9 and 1.6 h, respectively. For *E. coli*, the PA-SMEs were generally greater than the sums of the PAEs and SMEs at all subinhibitory concentrations. For the strain of *P. aeruginosa*, the PA-SME at 0.4 times the MIC was 4.4 h, which was not greater than the sum of the PAE and SME (5.4 h).

The garenoxacin MICs were similar to those described previously (1, 5, 6, 12, 13). Garenoxacin, like other quinolones, exhibits rapid concentration-dependent bactericidal activity. Longer intervals between doses may be possible when an antibiotic has a long half-life as well as a prolonged PAE and PA-SME, because regrowth continues to be prevented when serum and tissue levels fall below the MICs (2, 4, 10, 11).

In this study, the sums of the PAEs and SMEs were less than the PA-SMEs at 0.4 times the MIC for two penicillin-susceptible and one penicillin-resistant pneumococcal strain and one methicillin-resistant, ciprofloxacin-susceptible *S. aureus* strain, indicating that for these organisms (and not the two penicillin-intermediate pneumococcal strains, the ciprofloxacin-resistant, methicillin-resistant *S. aureus* strain, and the *P. aeruginosa* strain), the sub-MIC concentrations of garenoxacin had a greater effect on preexposed cultures than on unexposed cultures. The PAE and PA-SME would only be important for organisms for which the MICs are high, where serum levels (at least of free drug) would fall below the MIC. This would not occur with pneumococci and staphylococci for which the MICs are low and could be a problem for strains such as our ciprofloxacin-resistant, methicillin-resistant *S. aureus* and *P. aeruginosa* strains which had relatively short PAE and PA-SME values. With these caveats in mind, our results support once daily dosing of garenoxacin.

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