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Postantibiotic Effect of Trovafloxacin against Gram-Positive and -Negative Organisms

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Trovafloxacin pneumococcal and staphylococcal postantibiotic effects (PAEs) were 0.7 to 1.8 and 0.7 to 2.4 h, respectively. For *Escherichia coli* and *Pseudomonas aeruginosa*, PAEs were 2.4 to 4.4 h. Pneumococcal and staphylococcal postantibiotic sub-MIC effects (PA-SMEs) (0.4 times the MIC) were 2.3 to 3.7 and 2.4 to >9.2 h, respectively, and *E. coli* PA-SMEs (0.3 times the MIC) were 6.8 to >12.0 h. For one *P. aeruginosa* strain, the PA-SME (0.4 times the MIC) was >10 h; in the other, rapid bactericidal activity precluded measurement.

The postantibiotic effect (PAE) is a pharmacodynamic parameter contributing to antibiotic dosing schedules. It is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic (4, 7). Odenholt-Tornqvist and coworkers (2, 11, 12) have suggested that during intermittent dosage regimens, suprainhibitory antibiotic levels are followed by subinhibitory levels that persist between doses and have hypothesized that persistent sub-MICs could extend the PAE. The effect of sub-MICs on growth during the PAE period has been defined as the postantibiotic sub-MIC effect (PA-SME), representing the time interval that includes the PAE plus the additional time during which growth is suppressed by sub-MICs. In contrast to the PA-SME, the sub-MIC effect (SME) measures the direct effect of sub-MICs on cultures which have not been previously exposed to antibiotics (2, 11, 12).

We examined the PAE, PA-SME, and SME of trovafloxacin, a fluoroquinolone with a wide spectrum of activity (5, 6, 10, 13, 14), against two penicillin-susceptible, two intermediately penicillin-susceptible and two penicillin-resistant pneumococcal strains; two methicillin-susceptible and two methicillin-resistant *Staphylococcus aureus* strains; two *Escherichia coli* strains; and two *Pseudomonas aeruginosa* strains. Organisms were identified by standard methods (8).

Standard broth microdilution MIC-determining methodology (9) was used. The PAE was determined by the viable plate count method (4) using Mueller-Hinton broth supplemented with 5% lysed horse blood when testing pneumococci. The PAE was induced by exposure to a drug concentration equivalent to 10 times the MIC for 1 h. Tubes containing 5 ml of broth with antibiotic were inoculated with approximately 5×10^6 CFU/ml. Growth controls with an inoculum but not antibiotic were included with each experiment. Tubes were placed in a shaking water bath at 35°C for 1 h. At the end of the exposure period, cultures were diluted 1:1,000 to remove the antibiotic. A control containing bacteria pre-exposed to the antibiotic at a concentration of 0.01 times the MIC was also prepared.

Viability counts (16) were determined before exposure, immediately after dilution (0 h), and then every 2 h until tube turbidity reached a no. 1 McFarland standard. Inocula were prepared by suspending growth from an overnight blood agar plate in broth. The broth was incubated at 35°C for 2 to 4 h in a shaking water bath until the turbidity matched a no. 1 McFarland standard and checked for viability by plate counts (16).

The PAE was defined (4) as 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 immediately after dilution and C is the corresponding time for the growth control.

The PA-SME and SME (12) were measured in two separate experiments. In cultures designated for PA-SME determination, the PAE was induced as described above, after exposure to a drug concentration of 10 times the MIC. Following 1:1,000 dilution, cultures were divided into five tubes. To four tubes, trovafloxacin was added to make final subinhibitory concentrations of 0.1, 0.2, 0.3, and 0.4 times the MIC. The fifth tube received no antibiotic. Viability counts were determined before exposure, immediately after dilution, and then every 2 h until the turbidity reached a no. 1 McFarland standard. The PAE was not induced in cultures designated for SME determination.

The PA-SME was defined (12) as PA-SME = $T_{pa} - C$, where T_{pa} is the time for cultures previously exposed to an antibiotic and then reexposed to different sub-MICs to increase by 1 $\log_{10}$ above the counts determined immediately after dilution and C is the corresponding time for the unexposed control. The SME (12) was defined as SME = $T_s - C$, where T_s is the time for the cultures exposed only to sub-MICs to increase 1 $\log_{10}$ above the counts determined immediately after dilution and C is the corresponding time for the unexposed control. For each experiment, viability counts ($\log_{10}$ CFU per milliliter) were plotted against time and results were expressed as the mean of two separate assays ± the standard deviation.

Pneumococcal trovafloxacin MICs were all 0.06 μg/ml. Staphylococcal trovafloxacin MICs were 0.016 μg/ml for the methicillin-susceptible strains and 1.0 μg/ml for the methicillin-resistant strains. The *E. coli* trovafloxacin MICs were 0.03 μg/ml, and those for *P. aeruginosa* were 0.25 μg/ml.

Results are presented in Table 1. The antibiotic at 0.01 times the MIC had no activity. The mean PAE for the six pneumococci was 1.3 h, ranging from 0.7 to 1.8 h. Pneumococcal PA-SMEs were slightly longer than PAEs. At 0.4 times the MIC, PA-SMEs were 2.3 to 3.7 h, with a mean of 3.2 h. Pneumococcal PA-SMEs approximated the sum of the PAE

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TABLE 1. PAE, SME, and PA-SME of trovafloxacin against 14 strains[a]

Strain (drug susceptibility)[b]	Mean (range) effect (h)									
	PAE[c]	0.1 × MIC		0.2 × MIC		0.3 × MIC		0.4 × MIC	
		SME[d]	PA-SME[e]	SME	PA-SME	SME	PA-SME	SME	PA-SME
Pneumococcus (pen S)	1.0 (0.2–1.8)	0.2 (0–0.3)	1.4 (1.0–1.8)	0.2 (0–0.5)	1.5 (1.2–1.8)	0.8 (0.7–0.8)	1.4 (0.9–1.8)	1.1 (0.9–1.4)	2.3 (2.3–2.5)
Pneumococcus (pen S)	1.3 (1.1–1.5)	0.3 (0–0.6)	1.3 (1.1–1.5)	0.4 (0–0.9)	1.9 (1.6–2.2)	0.4 (0–0.9)	2.4 (2.4)	1.9 (1.6–2.2)	3.1 (2.4–3.8)
Pneumococcus (pen I)	1.8 (1.7–1.8)	0.2 (0.2)	1.9 (1.8–2.0)	0.4 (0.1–0.7)	2.0 (2.0–2.1)	0.6 (0.6–0.7)	2.0 (2.0–2.1)	1.0 (0.6–1.3)	3.4 (2.5–4.2)
Pneumococcus (pen I)	1.4 (1.4–1.5)	0.8 (0.7–0.8)	1.6 (1.5–1.6)	0.4 (0.4–0.5)	1.7 (1.5–1.9)	0.4 (0.4–0.5)	2.4 (2.4–2.5)	1.1 (1.1–1.2)	3.4 (1.9–4.8)
Pneumococcus (pen R)	0.7 (0.6–0.8)	0.3 (0.2–0.3)	1.0 (1.0)	0.5 (0.2–0.8)	1.2 (1.0–1.4)	0.1 (0–0.2)	2.0 (2.0)	1.4 (1.3–1.6)	3.7 (3.5–3.8)
Pneumococcus (pen R)	1.4 (1.4–1.5)	0	1.3 (1.1–1.5)	0.2 (0–0.3)	2.1 (2.0–2.2)	0.2 (0–0.3)	2.5 (2.5)	1.4 (1.4)	3.5 (3.5–3.6)
S. aureus (methicillin S)	2.4 (2.3–2.5)	0.1 (0–0.2)	2.6 (2.5–2.7)	0.1 (0–0.2)	3.9 (3.7–4.0)	0.6 (0.4–0.7)	4.0 (3.9–4.0)	1.6 (1.5–1.6)	6.2 (5.9–6.4)
S. aureus (methicillin S)	0.9 (0.9)	0.2 (0–0.5)	0.3 (0.1–0.5)	0.2 (0.2–0.3)	0.5 (0.5)	0.4 (0.3–0.5)	2.0 (1.8–2.1)	1.0 (0.9–1.1)	2.4 (2.3–2.4)
S. aureus (methicillin R)	1.1 (1.0–1.2)	0.1 (0–0.1)	0.8 (0.7–1.0)	0	0.9 (0.6–1.1)	1.4 (1.2–1.6)	2.8 (2.1–3.5)	>9.2 (>9.2)	>9.2 (>9.2)
S. aureus (methicillin R)	0.7 (0.4–1.0)	0.4 (0.1–0.7)	1.0 (0.6–1.3)	0.4 (0.4)	1.3 (1.1–1.5)	0.6 (0.4–0.7)	2.0 (1.9–2.0)	1.1 (1.0–1.1)	>5.7 (5.7–>8.1)
Escherichia coli	2.4 (1.7–3.2)	0	3.2 (2.9–3.6)	0.7 (0.2–1.2)	5.2 (4.2–6.2)	2.0 (1.2–2.7)	6.8 (5.2–8.4)	5.1 (3.2–7.0)	9.2 (6.7–11.7)
Escherichia coli	4.4 (4.0–4.7)	0.5 (0.3–0.7)	5.4 (5.1–5.7)	0.6 (0.5–0.7)	6.2 (6.2–6.3)	2.2 (2.0–2.3)	>12.0 (>12.0)	11.0 (11.0)	>12.0 (>12.0)
Pseudomonas aeruginosa	3.6 (3.5–3.6)	1.0 (0.6–1.3)	3.8 (3.5–4.1)	1.6 (0.9–1.5)	4.6 (4.1–5.2)	1.4 (0.9–2.0)	5.6 (4.3–6.9)	2.0 (1.9–2.0)	>10.0 (7.1–>12.0)
Pseudomonas aeruginosa	RBE[f]	RBE	RBE	RBE	RBE	RBE	RBE	RBE	RBE

[a] Each value is for two separate experiments, unless otherwise noted.
[b] Pen, penicillin; S, susceptible; I, intermediately susceptible; R, resistant.
[c] Exposure to 10 times the MIC for 1 h at 35°C. The drug was removed by 1,000-fold dilution.
[d] SME, strains not previously exposed to trovafloxacin at 10 times the MIC.
[e] PA-SME, strains previously exposed to trovafloxacin at 10 times the MIC.
[f] RBE, rapid bactericidal effect within 1 h.

and the SME, indicating that sub-MICs alone accounted for the slightly longer PA-SMEs.

Staphylococcal PAEs were 0.7 to 2.4 h, with a mean of 1.3 h. PA-SMEs were longer than PAEs. PA-SMEs at 0.4 times the MIC ranged from 2.4 to >9.2 h. At 0.4 times the MIC, the PA-SMEs of two staphylococci were >2 h longer than the PAE plus the SME. This indicates that, for these two strains, sub-MICs delayed regrowth.

For both *E. coli* strains and one *P. aeruginosa* strain, PAEs were 2.4 to 4.4 h, with a mean of 3.5 h. The PA-SMEs of both *E. coli* strains were longer than the PAE plus the SME. At 0.3 times the MIC, the PA-SME was >2 h longer than the PAE plus the SME. For one strain of *P. aeruginosa*, the PA-SME at 0.4 times the MIC was >2 h longer than the sum of the PAE and the SME, indicating that sub-MICs suppressed the regrowth of these strains when they were pre-exposed to trovafloxacin in the PAE phase. Rapid bactericidal activity against one *P. aeruginosa* strain precluded measurement of the PAE or the PA-SME.

Trovafloxacin MICs were similar to those described previously (5, 6, 10). Trovafloxacin, like other quinolones, exhibits rapid, concentration-dependent bactericidal activity (3, 16). 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 drug levels in serum and tissue fall below the MIC. In this study, PAEs against gram-negative strains were generally longer than those against gram-positive strains, indicating possible differences between the rates at which trovafloxacin killed these organisms.

Boswell et al. (1) reported trovafloxacin PAEs between 0.3 and 2.3 h for four *P. aeruginosa* strains. The former study also reported, as we have, that the PAE against some *P. aeruginosa* strains could not be determined because of rapid killing.

PA-SMEs exceeded the sum of the PAE and the SME for two staphylococcal, one *P. aeruginosa*, and two *E. coli* strains, indicating that for these strains, trovafloxacin at sub-MICs had a greater effect on pre-exposed than on unexposed cultures. Therefore, a longer PAE can be achieved by trovafloxacin at sub-MICs when they follow a suprainhibitory level.

In this study, pre-exposure at 10 times the MIC was below clinically achievable trovafloxacin levels (15) for all strains except the two methicillin-resistant *S. aureus* strains. For the other strains, the MICs were so low that concentrations in serum would exceed the MIC for the entire recommended 24-h dosing interval (15). Our results suggest that a longer dosing interval may be possible for the latter strains with a PAE and a PA-SME, because bacterial regrowth would be prevented when drug levels in serum fall below the MIC.

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REFERENCES

1. **Boswell, F. J., J. M. Andrews, and R. Wise.** 1997. Postantibiotic effect of trovafloxacin on *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **39:** 811–814.
2. **Cars, O., and I. Odenholt-Tornqvist.** 1993. The postantibiotic subMIC effect *in vitro* and *in vivo*. J. Antimicrob. Chemother. **31:**159–166.
3. **Craig, W.** 1993. Pharmacodynamics of antimicrobial agents as a basis for determining dosage regimens. Eur. J. Clin. Microbiol. Infect. Dis. **12**(Suppl. 1)**:**6–8.
4. **Craig, W. A., and S. Gudmundsson.** 1996. Postantibiotic effect, p. 296–329. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore, Md.
5. **Eliopoulos, G. M., K. Klimm, C. T. Eliopoulos, M. J. Ferraro, and R. C. Moellering, Jr.** 1993. In vitro activity of CP-99,219, a new fluoroquinolone, against clinical isolates of gram-positive bacteria. J. Antimicrob. Chemother. **37:**366–370.

6. **Fass, R. J., J. Barnishan, M. C. Solomon, and L. W. Ayers.** 1996. In vitro activities of quinolones, β-lactams, tobramycin, and trimethoprim-sulfamethoxazole against nonfermentative gram-negative bacilli. Antimicrob. Agents Chemother. **40:**1412–1418.
7. **MacKenzie, F. M., and I. M. Gould.** 1993. The post-antibiotic effect. J. Antimicrob. Chemother. **32:**519–537.
8. **Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.).** 1995. Manual of clinical microbiology. ASM Press, Washington, D.C.
9. **National Committee for Clinical Laboratory Standards.** 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Neu, H. C., and N.-X. Chin.** 1994. In vitro activity of the new fluoroquinolone CP-99,219. Antimicrob. Agents Chemother. **38:**2615–2622.
11. **Odenholt-Tornqvist, I.** 1993. Studies on the postantibiotic effect and the postantibiotic sub-MIC effect of meropenem. J. Antimicrob. Chemother. **31:**881–892.
12. **Odenholt-Tornqvist, I., E. Löwdin, and O. Cars.** 1992. Postantibiotic sub-MIC effects of vancomycin, roxithromycin, sparfloxacin, and amikacin. Antimicrob. Agents Chemother. **36:**1852–1858.
13. **Pankuch, G. A., M. R. Jacobs, and P. C. Appelbaum.** 1995. Activity of CP 99,219 compared to DU-6859a, ciprofloxacin, ofloxacin, levofloxacin, lomefloxacin, tosufloxacin, sparfloxacin and grepafloxacin against penicillin-susceptible and -resistant pneumococci. J. Antimicrob. Chemother. **35:**230–232.
14. **Spangler, S. K., M. R. Jacobs, and P. C. Appelbaum.** 1994. Activity of CP 99,219 compared with those of ciprofloxacin, grepafloxacin, metronidazole, cefoxitin, piperacillin, and piperacillin-tazobactam against 489 anaerobes. Antimicrob. Agents Chemother. **38:**2471–2476.
15. **Teng, R., S. C. Harris, D. E. Nix, J. J. Schentag, G. Foulds, and T. E. Liston.** 1996. Pharmacokinetics and safety of trovafloxacin (CP-99,19), a new quinolone antibiotic, following administration of single oral doses to healthy male volunteers. J. Antimicrob. Chemother. **36:**385–394.
16. **Visalli, M. A., M. R. Jacobs, and P. C. Appelbaum.** 1996. Activity of CP 99,219 (trovafloxacin) compared with ciprofloxacin, sparfloxacin, clinafloxacin, lomefloxacin, and cefotaxime against ten penicillin-susceptible and penicillin-resistant pneumococci by time-kill methodology. J. Antimicrob. Chemother. **37:**77–84.