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Postantibiotic Effect of Ceftaroline against Gram-Positive Organisms[▽]

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The postantibiotic effects (PAEs), postantibiotic sub-MIC effects (PA-SMEs), and sub-MIC effects (SMEs) of ceftaroline, a novel injectable cephalosporin, were determined for 15 gram-positive organisms. The pneumococcal, staphylococcal, and enterococcal PAEs were 0.8 to 1.8 h, 0.7 to 2.2 h, and 0.2 to 1.1 h, respectively. The corresponding PA-SMEs (0.4 times the MIC) were 2.5 to 6.7 h, 2.9 to >0.0 h, and 7.9 to >10.3 h, respectively. The PA-SMEs were longer than the PAEs, suggesting that sub-MIC levels extend the PAE of ceftaroline against gram-positive cocci.

The postantibiotic effect (PAE) is a pharmacodynamic parameter that may be considered in choosing antibiotic dosing regimens. It is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic (5, 6). Cars and Odenholt-Tornqvist (2) have suggested that during intermittent dosage regimens, suprainhibitory levels of antibiotic are followed by subinhibitory levels that persist between doses and 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 interval 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) measures the direct effect of subinhibitory levels on cultures that have not previously been exposed to antibiotics (2, 10).

We examined the PAE, PA-SME, and SME of ceftaroline, a new broad-spectrum injectable cephalosporin with bactericidal activity against gram-positive organisms, including multidrug-resistant *Streptococcus pneumoniae* and methicillin (meticillin)-resistant *Staphylococcus aureus* as well as common gram-negative organisms (7, 9, 14, 15). We studied two penicillin-susceptible strains (penicillin MICs, 0.016 and 0.5 μg/ml), one penicillin-intermediate strain (penicillin MIC, 4.0 μg/ml), and one penicillin-erythromycin-resistant strain (penicillin MIC, 8.0 μg/ml; erythromycin MIC, >32 μg/ml) of *S. pneumoniae*; two methicillin-susceptible *S. aureus* strains; four methicillin-resistant *S. aureus* strains (one vancomycin susceptible, one vancomycin hetero-intermediate, one vancomycin intermediate, and one vancomycin resistant); three *Enterococcus faecalis* strains (one vancomycin sensitive and two vancomycin resistant); and two *Enterococcus faecium* strains (both vancomycin sensitive). The susceptibility breakpoints were obtained from CLSI M100-S19 (4). For *S. pneumoniae*, the latest CLSI parenteral (nonmeningitis) penicillin G susceptibility breakpoints were used. Organisms were identified by standard methods (11). Ceftaroline powder (lot no. FMD-CEF-019) was obtained from Forest Laboratories, Inc., New York, NY.

Ceftaroline MICs were determined by standard macrodilution procedures (3). The PAE was determined by the viable-plate-count method using freshly prepared 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 ceftaroline for 1 h.

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 reciprocal shaking water bath (60 rpm) 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 by dilution. Antibiotic removal was confirmed by comparing the growth curve of a control culture containing no antibiotic to that of one containing ceftaroline at 0.001 times the exposure concentration (10 times the MIC).

Viability counts were determined before exposure, immediately after dilution (zero hour), and then every 2 h until the turbidity of the tube reached a 1 McFarland standard. The PAE was defined as $\text{PAE} = T - C$, where T represents the time required for the viability count of an antibiotic-exposed culture to increase by 1 $\log_{10}$ above the count obtained immediately after dilution and C represents the corresponding time for the growth control (13).

For measurement of PA-SME, the PAE was induced as described above after exposure to 10× MIC of ceftaroline. Following 1:1,000 dilution, cultures were divided into four tubes. To three tubes, ceftaroline was added to produce final subinhibitory concentrations of 0.2×, 0.3×, and 0.4× MIC. The fourth tube did not receive antibiotic. Viability counts were determined before exposure, immediately after dilution, and then every 2 h until their culture turbidity reached a 1 McFarland standard. Cultures designated for SME were treated the same as for PA-SME testing, except no prior antibiotic treatment was employed.

The PA-SME was defined as $\text{PA-SME} = T_{\text{pa}} - C$, where T_{pa} represents the time required for cultures previously exposed to antibiotic and then reexposed to different sub-MIC concentra-

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TABLE 1. PAEs of ceftaroline against 15 strains

Organism and strain description (no.)	MIC (μg/ml)	PAE (h)	Effect (h) at[a]:					
			0.2 × MIC		0.3 × MIC		0.4 × MIC	
			SME[c]	PA-SME[d]	SME	PA-SME	SME	PA-SME
Streptococcus pneumoniae[b]								
Penicillin sensitive (s502)	0.008	1.8, 1.8	0.0, 0.0	2.7, 3.5	0.3, 0.7	3.5, 4.7	2.5, 2.7	4.5, 6.7
Penicillin sensitive (ATCC 49619)	0.016	1.8, 1.6	0.2, 0.8	4.1, 4.7	0.7, 1.1	5.9, 6.1	0.8, 1.4	7.2, 8.4
Penicillin intermediate (s1234)	0.12	0.8, 0.7	0.0, 0.3	1.4, 1.7	0.0, 0.5	1.8, 2.3	0.6, 0.7	2.5, 3.3
Penicillin and erythromycin resistant (37)	0.25	1.5, 1.5	0.5, 0.7	1.9, 2.2	0.5, 1.1	3.0, 4.4	0.5, 2.2	3.7, 4.7
Staphylococcus aureus								
Methicillin sensitive								
Vancomycin sensitive (ATCC 29213)	0.25	1.0, 1.1	0.2, 0.3	2.5, 2.6	0.8, 1.1	4.5, 4.8	2.8, 4.5	>10.5, 10.8
Vancomycin sensitive (sox1)	0.25	0.8, 1.6	0.2, 0.6	2.2, 2.4	0.9, 1.2	3.6, 4.2	1.6, 1.8	6.2, 7.6
Methicillin resistant								
Vancomycin sensitive (vs1)	0.5	1.2, 1.3	0.4, 0.7	2.7, 3.7	0.7, 1.0	4.7, 5.5	1.2, 1.5	6.7, 7.8
Vancomycin heterointermediate (618)	2.0	0.7, 2.1	0.0, 0.0	2.2, 3.2	0.0, 0.2	2.7, 3.8	0, 0.7	2.9, 4.3
Vancomycin intermediate (770)	1.0	1.3, 2.0	0.3, 0.9	1.5, 2.0	0.5, 1.0	5.0, 5.2	0.6, 1.5	7.1, 8.0
Vancomycin resistant (510)	0.5	1.4, 2.2	0.0, 0.5	5.4, 6.7	0.7, 1.8	9.1, >10.0	1.3, 2.0	>10.0, >10.4
Enterococcus faecium								
Vancomycin sensitive (10)	0.5	0.5, 0.6	0.8, 1.2	3.2, 4.5	1.5, 1.9	6.6, 7.0	1.8, 2.3	8.5, 10.6
Vancomycin sensitive (20)	0.5	0.5, 0.9	0.9, 1.6	4.1, 4.5	1.3, 2.0	8.6, 9.3	3.6, 4.2	10.5, 10.6
Enterococcus faecalis								
Vancomycin sensitive (ATCC 29212)	1.0	0.8, 0.9	3.1, 3.2	7.6, 8.6	3.6, 4.6	>10.6, >10.6	10.0, 10.8	>10.6, >10.6
Vancomycin resistant (266)	2.0	0.9, 1.1	1.4, 1.8	8.3, 8.6	1.8, 2.0	>10.3, >10.6	9.5, 10.6	>10.3, >10.6
Vancomycin resistant (609)	1.0	0.2, 1.0	1.4, 1.4	3.0, 3.4	1.4, 1.7	3.3, 4.9	2.2, 2.5	7.9, 7.9

[a] Values were obtained in two separate experiments. Strains were exposed to 10 times the MIC of ceftaroline (see the text) for 1 h at 35°C. The drug's activity was removed by 1:1,000 dilution.
[b] Parenteral (nonmeningitis) pneumococcal penicillin G breakpoint isolates (4).
[c] The strains had not previously been exposed to ceftaroline.
[d] The strains had previously been exposed to ceftaroline.

tions to increase by 1 $\log_{10}$ above the count obtained immediately after dilution and C represents the corresponding time for the unexposed control (13). The SME was defined as SME = $T_s - C$, where T_s represents the time required for the cultures exposed only to sub-MIC concentrations to increase by 1 $\log_{10}$ above the count obtained immediately after dilution and C represents the corresponding time for the unexposed control. PA-SME and SME (13) were measured in two separate experiments. For each experiment, viability counts ($\log_{10}$ CFU/ml) were plotted against time and the results expressed as the means for two separate assays. The ceftaroline MIC ranges were as follows: for pneumococci, 0.008 to 0.25 μg/ml; for *S. aureus*, 0.25 to 2.0 μg/ml; for *E. faecium*, 0.5 μg/ml; and for *E. faecalis*, 1.0 to 2.0 μg/ml (Table 1).

PA-SMEs were longer than PAEs for all strains tested and increased with increasing subinhibitory concentrations of ceftaroline. At each subinhibitory level (0.2×, 0.3×, and 0.4× MIC), the PA-SMEs exceeded the sum of PAEs and SMEs. For the four pneumococci, the mean PAE was 1.4 h, ranging from 0.7 to 1.8 h. At 0.4× MIC, the mean PA-SME was 5.1 h and ranged from 2.5 to 8.4 h (Table 1).

The staphylococcal PAEs were 0.7 to 2.2 h, with a mean of 1.4 h. Staphylococcal PAEs did not differ greatly in methicillin-susceptible (0.8 to 1.6 h) and -resistant (0.7 to 2.2 h) strains. The PA-SMEs at 0.4× MIC ranged from 2.9 to >10.0 h (Table 1).

The mean PAE of the two *E. faecium* strains was 0.6 h, with a mean PA-SME (0.4× MIC) of 10.0 h (Table 1). The three *E. faecalis* strains had a mean PAE of 0.8 h. At 0.4× MIC, the PA-SME values were 7.9 to >10.3 h.

In vivo postantibiotic effects produced by ceftaroline in a murine thigh infection model have previously been studied (1). In that study, the in vivo PAEs for one *S. aureus* and one *S. pneumoniae* strain were reported to be 0.8 to 7.2 h and −1.9 to 1.5 h, respectively, depending on the dose administered. One of these strains, *S. aureus* ATCC 29213, was also used in the current study. For this strain, we found the PAEs to be 1 to 1.1 h and the PA-SMEs at 0.2×, 0.3×, and 0.4× MIC to be 2.5 to 2.6 h, 4.5 to 4.8 h, and >10.5 h, respectively. In general, in vivo PAEs tend to be longer than those found in vitro and usually correspond better to the PA-SME (2, 5).

Cephalosporins generally produce low to moderate in vitro PAEs of approximately 0.5 to 4.0 h against gram-positive strains (10, 12, 13, 16). Previous studies have also shown that the PAEs of ceftriaxone and ceftobiprole may be extended by subinhibitory levels of these antibiotics. Odenholt et al. reported PAEs of 0.7 to 1.0 h and 2.3 to 2.9 h produced by ceftriaxone against *S. aureus* ATCC 29213 and an isolate of *S.*

pneumoniae, respectively (12). After reexposing these cultures to ceftriaxone at 0.2×, 0.3×, and 0.4× MIC, they found that the duration of the PAE was extended, producing PA-SMEs of 3.7 to 5.6 h, 5.7 to 8.5 h, and >14.3 h, respectively. In a recent study of 12 gram-positive strains, we found that ceftobiprole produced PAEs of between 0 and 3.1 h (13). After reexposing cultures to 0.2×, 0.3×, and 0.4× MIC, we found PA-SMEs of 0 to 5.3 h, 0.3 to >10.3 h, and 1.5 to >10.3 h, respectively. In our previous study of ceftobiprole, we used three of the same strains that are included in the current study, with similar results (13).

Ceftaroline is administered as the prodrug ceftaroline fosamil, a derivative that is rapidly converted in vivo to the microbiologically active form, ceftaroline. Phase 1 studies of ceftaroline determined that a dosing regimen of 600 mg intravenously every 12 h for 14 days produced a maximum concentration of drug in serum of approximately 21 μg/ml and a half-life of 2.6 h (8). In the current study, we produced PAEs and PA-SMEs by exposure to ceftaroline within clinically achievable peak serum levels. PA-SMEs generally exceeded the sum of the PAE and the SME, suggesting a greater effect of sub-MIC treatment on preexposed cultures (PAE phase) than on unexposed cultures. This is clinically important because subinhibitory levels may persist between doses when an intermittent dosing regimen is used. The PAE and PA-SME would be important only for organisms for which the ceftaroline serum levels (free levels) fall below the MIC. Our results suggest that the long PAE and PA-SME found in this study for gram-positive organisms could prevent bacterial regrowth when ceftaroline levels in serum fall below the MIC.

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