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Postantibiotic Effects of Daptomycin against 14 Staphylococcal and Pneumococcal Clinical Isolates

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Daptomycin mean staphylococcal postantibiotic effects (PAEs) were 1.1 to 6.2 h, with a mean of 2.5 h. The mean pneumococcal PAEs were 1.7 h, ranging between 1.0 and 2.5 h. The staphylococcal and pneumococcal postantibiotic sub-MIC effects at 0.4 times the MIC ranged from 3.0 to >12.0 h and 1.9 to >12.0 h, respectively.

The postantibiotic effect (PAE) is a pharmacodynamic parameter contributing to the choosing of antibiotic dosing regimens. It is defined as the length of time that bacterial growth is suppressed following brief exposure to an antibiotic (4, 5, 10). 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 those authors have hypothesized that persistent subinhibitory levels could extend the PAE (3, 13–15). 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 SME measures the direct effect of subinhibitory levels on cultures which have not been previously exposed to antibiotics (13–15).

We examined the PAE, PA-SME, and SME of daptomycin, a lipopeptide active against gram-positive organisms (1, 2, 6, 8, 16–18). We studied two clinical strains each of methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *S. aureus*, methicillin-susceptible coagulase-negative staphylococci, methicillin-resistant coagulase-negative staphylococci, and penicillin-susceptible, -intermediate, and -resistant *Streptococcus pneumoniae*. Organisms were identified by standard methods (11). No attempt was made to identify coagulase-negative staphylococci by species. Daptomycin MICs were determined by macrodilution procedures (12). Mueller-Hinton broth was adjusted to contain 50 mg of calcium per liter for testing daptomycin, as recommended by the NCCLS (12).

The PAE was determined by the viable plate count method (5), using Mueller-Hinton broth supplemented with calcium as described above and 5% lysed horse blood when testing pneumococci. The PAE was induced by exposure to 10 times the MIC of daptomycin for 1 h. Because the protein binding of daptomycin is 90 to 92%, this corresponds to total serum drug concentrations of approximately 100 times the MIC and approximates serum free drug levels at peak concentrations only (8, 18).

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 by dilution. Antibiotic removal was confirmed by comparing growth curves of a control culture containing no antibiotic to another containing daptomycin at 0.01 times the exposure concentration.

Viability counts were determined before exposure and immediately after dilution (0 h) and then every 2 h for up to 12 h or until turbidity of the tube reached 1 McFarland standard. The PAE was defined as $PAE = T - C$; T is the time required for viability counts of an antibiotic-exposed culture to increase by 1 $\log_{10}$ above counts immediately after dilution, and C is the corresponding time for growth control (5).

In cultures designated for PA-SME determinations, the PAE was induced as described above after exposure to 6 times or 10 times the MIC (see above). Following 1:1,000 dilution, cultures were divided into four tubes. To three tubes, daptomycin was added to produce final subinhibitory concentrations of 0.2, 0.3, and 0.4 times the MIC. The fourth tube did not receive antibiotic. Viability counts were determined before exposure, immediately after dilution, and then every 2 h for up to 12 h or until their culture turbidity reached 1 McFarland standard. SME experiments were performed as for PA-SME experiments; however, cultures designated for SME determinations were treated the same as for PA-SME testing except that PAE was not induced. SME cultures were not exposed during a PAE phase and were simply exposed after each culture dilution and were under the constant influence of 0.2, 0.3, or 0.4 times the corresponding sub-MIC.

The PA-SME was defined as $PA\text{-}SME = T_{pa} - C$; 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 counts immediately after dilution, and C is the corresponding time for the unexposed control (13–15). The SME was defined as $SME = T_s - C$; T_s is the time for the cultures exposed only to sub-MICs to increase 1 $\log_{10}$ above counts immediately after dilution, and C is the corresponding time for the unexposed control. The PA-SME and SME (13–15) were measured in two

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separate experiments. For each experiment, viability counts ($\log_{10}$ CFU/ml) were plotted against time, and results are the mean of two separate experiments.

The daptomycin MICs (in micrograms per milliliter) were as follows: staphylococci, 0.25 to 0.5; pneumococci, 0.12 to 0.5. PAE, SME, and PA-SME results are presented in Table 1. As expected, the PA-SMEs were longer than the PAEs for all the strains tested and increased with subinhibitory concentrations of daptomycin. For some strains, a long (>12 h) PA-SME corresponded to an equally long SME, but in others, at specific time periods, the PA-SME was longer than the PAE plus the SME.

Staphylococcal PAEs were 1.1 to 6.2 h, with a mean of 2.5 h. Mean PAEs of 2.4 h and 4.1 h were found for methicillin-sensitive and -resistant *S. aureus* strains, respectively. One methicillin-resistant *S. aureus* strain had a PAE of 7.6 h. At 0.2 times the MIC, but not at 0.3 or 0.4 times the MIC, the PA-SME for this strain, >12 h, was greater than the sum of the PAE and SME.

Methicillin-sensitive and -resistant coagulase-negative staphylococci mean PAEs were 1.3 and 2.2 h, respectively. At 0.4 times the MIC, the PA-SMEs of the two methicillin-sensitive coagulase-negative strains were >9 h, while the PA-SMEs of the two methicillin-resistant strains were both approximately 3 h.

For the six pneumococcal isolates, the mean PAE was 1.7 h, ranging between 1.0 and 2.5 h. The PA-SMEs at 0.4 times the MIC ranged from 1.9 to >12 h and approximated the sum of the PAE and SME.

Daptomycin MICs were similar to those described previously (1, 6, 16). Daptomycin has been shown to be rapidly bactericidal against staphylococci and pneumococci. Time-kill studies of daptomycin against staphylococci have shown a mean time to achieve a >3 $\log_{10}$ reduction for 25 staphylococcal strains with daptomycin to be 2.3 to 8.0 h (6, 17). We have recently reported a rapid bactericidal effect of daptomycin on pneumococci that was similar to that of quinupristin-dalfopristin (G. Pankuch, M. Jacobs, and P. Appelbaum, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-1445, 2002). The bactericidal activity of daptomycin is reported to be concentration dependent and is influenced by the pH and ionized calcium concentration (8).

In this study, long PA-SMEs (>12 h) were found for some strains. For some of these strains an equally long SME was also present, indicating that sub-MICs of daptomycin alone slowed the rate of growth. Sub-MIC antimicrobial concentrations have long been known to cause a variety of effects on bacteria cultures, including slowing their growth rate (7). It is not surprising that daptomycin, with its known rapid bactericidal effects at or just above the MIC (6, 17), would affect the growth rate of cultures at concentrations just below the MIC. However, it is surprising that the growth of some strains was delayed beyond 12 h, even at 0.2 times the MIC. The mechanism underlying this SME of daptomycin, as well as that underlying differing SMEs at 0.2 times the MIC compared with 0.3 and 0.4 times the MIC observed in some strains, is unclear. Some studies have reported equally high SMEs at low sub-MICs when using different strains and antibiotics (13). There was higher variability (2 to 3 h) in the length of the PAEs, SMEs, and PA-SMEs obtained from three of the four *S. aureus* strains

TABLE 1. PAE of daptomycin against 14 strains

Strain	Mean (range) effect (h)[a,b]							
	PAE	0.2× MIC		0.3× MIC		0.4× MIC		
		SME[c]	PA-SME[d]	SME[c]	PA-SME[d]	SME[c]	PA-SME[d]	
Met[s] *S. aureus* (SA1)	2.7 (1.2–4.2)	1.0 (0.7–1.2)	2.8 (1.2–4.3)	≥9.7 (7.3–>12)	≥11.1 (10.1–>12)	≥11.1 (10.2–>12)	≥11.3 (10.6–>12)	
Met[s] *S. aureus* (SA2)	2.0 (0.8–3.1)	0.2 (0–0.3)	2.7 (1.2–4.1)	0.2 (0.1–0.2)	3.9 (2.5–5.2)	≥10.9 (9.9–>12)	>12 (>12)	
Met[s] *S. aureus* (SA3)	6.2 (4.8–7.6)	2.7 (1.5–3.9)	>12 (>12)	>12 (>12)	>12 (>12)	>12 (>12)	>12 (>12)	
Met[s] *S. aureus* (SA4)	2.0 (1.3–2.6)	0.5 (0.5–0.6)	3.3 (3.1–3.4)	3.0 (2.7–3.3)	8.8 (8.2–9.4)	>12 (>12)	>12 (>12)	
Met[s] coagulase[−] staphylococci (CNS1)	1.5 (1.1–1.9)	0.4 (0–0.8)	2.7 (2.5–2.8)	0.8 (0–1.5)	4.5 (3.3–5.6)	4.3 (4.1–4.5)	≥9.7 (7.3–>12)	
Met[s] coagulase[−] staphylococci (CNS2)	1.1 (0.9–1.3)	0.1 (0–0.2)	1.8 (1.5–2.0)	1.5 (0.5–2.4)	2.3 (1.6–2.9)	≥8.6 (5.2–>12)	≥9.3 (6.5–>12)	
Met[r] coagulase[−] staphylococci (CNS3)	2.0 (1.2–2.7)	0.7 (0.5–0.9)	2.0 (1.8–2.1)	1.2 (0.5–1.8)	2.9 (2.1–3.6)	0.8 (1.0–0.5)	3.0 (2.1–3.9)	
Met[r] coagulase[−] staphylococci (CNS4)	2.4 (2.0–2.7)	0.2 (0–0.4)	2.7 (2.5–2.9)	0.8 (0.4–1.1)	2.9 (2.5–3.3)	0.9 (0.5–1.2)	3.2 (3.1–3)	
S. pneumoniae (SP1) Pen[s]	1.2 (1.6–0.7)	0.1 (0–0.2)	0.9 (0–1.7)	0 (0)	1.4 (1.1–1.6)	0.4 (0–0.8)	1.9 (1.8–2.0)	
S. pneumoniae (SP2) Pen[s]	2.2 (1.5–2.8)	1.0 (0.2–1.8)	7.1 (6.0–8.1)	8.2 (7.3–9.0)	>12 (>12)	>12 (>12)	>12 (>12)	
S. pneumoniae (SP3)[e] Pen[i]	1.0 (0.7–1.3)	0.2 (0.1–0.3)	1.8 (1.7–1.8)	0.3 (0–0.5)	1.9 (1.8–2.0)	0.5 (0.2–0.7)	2.3 (2.1–2.5)	
S. pneumoniae (SP4) Pen[i]	1.6 (1.5–1.6)	0.1 (0–0.1)	2.4 (1.6–3.2)	0.9 (0.7–1)	2.4 (1.6–3.1)	1.4 (1–1.8)	4.2 (2.6–5.7)	
S. pneumoniae (SP5) Pen[r]	2.5 (2.2–2.8)	0.2 (0–0.3)	3.8 (3.8–3.8)	5.8 (5.7–5.9)	>12 (>12)	>12 (>12)	>12 (>12)	
S. pneumoniae (SP6) Pen[r]	1.4 (1.0–1.7)	9.5 (9.4–9.6)	>12 (>12)	>12 (>12)	>12 (>12)	>12 (>12)	>12 (>12)	

[a] Mean of two separate experiments.
[b] Exposure to 10 times the MIC (see text) for 1 h at 35°C. Drug was removed by 1,000-fold dilution.
[c] Strains not previously exposed to daptomycin.
[d] Strains previously exposed to daptomycin.
[e] Pen[i], penicillin intermediate susceptibility.

compared to the other strains in this study. This same degree of variability has been reported before for levofloxacin PAEs, PA-SMEs, and SMEs using *S. aureus* (9).

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 drug levels fall below the MICs (3, 5, 14, 15). Previous studies have reported daptomycin PAEs for *S. aureus* ranging from 2.4 to 6.3 h (2, 8). Our mean PAE results for *S. aureus* (2.0 to 6.2 h) were similar. In this study we tested the PAE and PA-SMEs by using daptomycin within clinically achievable free peak serum drug levels. The PA-SMEs were generally longer than the PAEs for all of the strains tested, indicating that sub-MIC levels of daptomycin extend the PAE. Therefore, a longer PAE can be achieved by sub-MIC daptomycin concentrations when they follow a suprainhibitory level (14, 15). The half-life of daptomycin in plasma is approximately 7 h (18). This together with the long PAEs and PA-SMEs found in this study supports once-daily dosing of daptomycin.

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