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In Vitro Postantibiotic Effect of Daptomycin (LY146032) against *Enterococcus faecalis* and Methicillin-Susceptible and Methicillin-Resistant *Staphylococcus aureus* Strains

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The suppression of bacterial growth that persists after brief exposure to antimicrobial agents has been termed the postantibiotic effect (PAE). This pharmacodynamic interaction varies for each microorganism-antimicrobial agent combination. Daptomycin (LY146032) is a new lipopeptide antibiotic with activity against gram-positive organisms. We studied the in vitro bactericidal activities and PAEs of the following drugs: daptomycin compared with penicillin G and vancomycin, without and with gentamicin against *Enterococcus faecalis* strains; daptomycin compared with nafcillin and vancomycin against methicillin-susceptible *Staphylococcus aureus* strains; and daptomycin compared with vancomycin against methicillin-resistant *S. aureus* strains. Daptomycin, alone and when used in combination with gentamicin, exhibited greater bactericidal activity and in general produced a longer PAE than standard effective regimens against the organism strains studied.

The continued suppression of bacterial growth after brief exposure of organisms to antimicrobial agents has been termed the postantibiotic effect (PAE) (2, 7). The presence or duration of a PAE can differ significantly for various microorganism-antimicrobial agent combinations and has been demonstrated in vitro for virtually all antimicrobial agents (2, 17), with in vivo correlation for many (18). The PAE is a pharmacodynamic parameter which provides information concerning the interaction between an antimicrobial agent and a microorganism and may influence antimicrobial therapy in clinical practice.

Daptomycin (LY146032) is a cyclic lipopeptide antibiotic which exerts its antibacterial effect by inhibiting cell wall synthesis of aerobic, facultative and anaerobic gram-positive bacteria (10, 11). Although the spectrum of antibacterial activity of daptomycin is similar to that of the glycopeptide antibiotics teicoplanin and vancomycin, it differs from the latter two in structure and mechanism of action.

The purpose of this study was to determine the in vitro PAEs and bactericidal activities of daptomycin for *Enterococcus faecalis* and methicillin-susceptible and -resistant *Staphylococcus aureus* (MSSA and MRSA, respectively) and to compare them with the PAEs and bactericidal activities of the antibiotics routinely used in clinical practice to treat infections caused by these pathogens.

MATERIALS AND METHODS

Organism and susceptibility testing. The strains of *E. faecalis*, MSSA, and MRSA used in this study were clinical isolates from patients with bacteremia. The initial identification of the organisms was performed by the hospital clinical microbiology laboratory. Methicillin susceptibility of the *S. aureus* strains was determined by standard methods (5). Stock cultures were made by incubating the organisms in Mueller-Hinton broth (MHB) at 37°C for 24 h and storing 1-ml samples at −20°C. Solutions of antimicrobial agents were freshly prepared from the standard powders obtained from the various manufacturers (daptomycin, vancomycin, and penicillin G from Lilly Research Laboratories; nafcillin from Bristol-Myers Co.; and gentamicin from Schering Corp.), and concentrations were diluted in the appropriate medium so that the desired concentration was achieved for each experiment.

The MICs and MBCs of daptomycin, vancomycin, penicillin G, nafcillin, and gentamicin were determined with an inoculum of 10^6 CFU/ml of cation-supplemented MHB in logarithmic phase of growth as previously described (12, 16). The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after incubation for 24 h at 37°C. The MBC was defined as the lowest concentration of antimicrobial agent that killed at least 99.9% of the organisms within 24 h, as determined by plating 0.1-ml portions of the MIC dilutions.

PAE experiment. Suspensions of 10^5 to 10^6 CFU/ml of each organism studied in logarithmic phase of growth were tested for the presence and duration of a PAE after a 2-h exposure to each of the antimicrobial agents, using the technique of rapid drug removal by repeated washings as described by Craig and Gudmundsson (6). The concentrations of antimicrobial agents used were chosen to mimic the expected peak drug concentrations in human serum samples; for gentamicin, a concentration known to cause enhanced bactericidal activity against *E. faecalis* when used in combination with penicillin G or vancomycin, was used. The antimicrobial agents and concentrations used (in micrograms per milliliter) were: daptomycin, 15; vancomycin, 30; penicillin G, 15; nafcillin, 100; and gentamicin, 4.

Figure 1 is a graphic representation of the PAE experiment. Cultures in MHB of each microorganism-antibiotic combination and controls in glass tubes were incubated at 37°C for 2 h. At the end of this period, the extent of bactericidal activity was calculated by determining the number of CFU/ml in the tubes by serial dilution and plating techniques (4). The tubes were then centrifuged at 2,500 rpm

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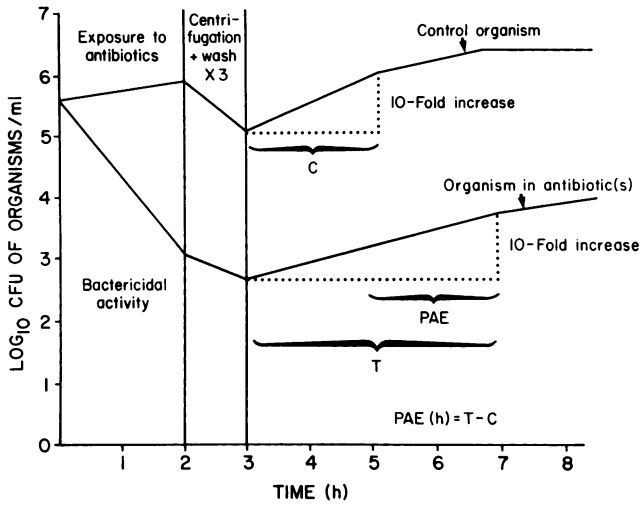

FIG. 1. Graphic representation of the PAE experiment. See the text for details.

for 10 min, followed by aspiration of the supernatant and suspension of the pellet in equal volumes of MHB. This wash procedure, designed to remove the antibiotic, was repeated two more times. After the final wash, the suspended microorganisms were poured into sterile glass tubes, thereby eliminating any residual antibiotic which may have adhered to the glass tubes. Samples from each tube were then removed and tested for the presence of antibacterial activity by an agar diffusion method using paper disks with *Bacillus subtilis* as the indicator organism (1) to confirm that the PAE was not a result of residual antibiotic. (For daptomycin, the indicator organism used was a strain of *Sarcinia lutea* supplied by Lilly Research Laboratories.) The number of CFU per milliliter for each sample was determined by serial dilution and plating techniques (4) at the end of the three wash procedures and again at each succeeding hour until visible growth was observed. The suspensions were incubated at 37°C throughout the experiment.

The duration of the PAE was calculated by using the equation PAE = $T - C$, where T is the time required for the CFU count in the test culture to increase 1 $\log_{10}$ above the count observed immediately after antibiotic removal and C is the time required for the CFU count in an untreated control culture to increase 1 $\log_{10}$ above the count observed immediately after the same procedure used on the test culture for antibiotic removal.

RESULTS

Table 1 shows the MIC and MBC of each antibiotic against each strain of bacteria used in this study. Daptomycin exhibited the greatest potency of all the antibiotics used.

Table 2 shows the extent of bactericidal activity at 2 h and the duration of the PAE for each microorganism-antibiotic combination, and for *E. faecalis*, for each cell wall-active antibiotic in combination with gentamicin. Daptomycin exhibited the highest bactericidal activity at 2 h against each strain of bacteria studied. The addition of gentamicin to daptomycin resulted in enhanced bactericidal activity against the two strains of *E. faecalis* tested. The net killing effect of this combination was greater than the enhanced bactericidal activity of gentamicin used in combination with penicillin G or vancomycin.

The PAE produced after exposure of daptomycin to each strain of bacteria tested was greater than that produced after exposure of these bacteria to the other antibiotics studied, except for one strain of MSSA, (MSSA-1), in which nafcillin and vancomycin produced a longer PAE. Although the PAE produced by the latter two drugs was longer than that of daptomycin, the bactericidal activity of daptomycin at 2 h against this particular strain, MSSA-1, was many times greater than that of nafcillin or vancomycin.

Combining gentamicin with each cell wall-active antibiotic produced a more prolonged PAE against the enterococcal isolates studied, except for the combination of gentamicin plus vancomycin against strain 1 of *E. faecalis*. The longest PAEs occurred when daptomycin was the cell wall-active antibiotic.

DISCUSSION

The PAE has been demonstrated to be a characteristic of almost every antimicrobial agent since the observation by Eagle and Musselman (9) that various gram-positive cocci did not resume normal growth for some time after brief exposure to penicillin. Although the exact mechanism leading to the PAE has not been defined, this persistent effect is thought to involve drug-induced nonlethal damage or limited persistence of the drug at the antibacterial site of action (2). Factors which have been shown to affect the PAE include the type of organism, the class and concentration of the antimicrobial agent and duration of exposure to the antimicrobial agent.

Clinically, the relevance of the PAE pertains to its effect on the dosing regimen of antimicrobial agents. Those agents with prolonged PAEs may allow the drug concentrations in sera and tissue to fall below the MIC for significant periods of time without hindering the overall efficacy of the drug. When used in conjunction with MIC and MBC data, information on the PAE of an antimicrobial agent may result in the more efficient use of that antimicrobial agent.

The results of our study are not unlike those observed in other PAE studies in which *S. aureus* and *E. faecalis* were exposed to known bactericidal antimicrobial agents or combinations of agents (14; M. Trexler-Hessen, P. G. Pitsakis,

TABLE 1. MICs and MBCs of each antimicrobial agent for each microorganism studied

Antimicrobial agent	MIC/MBC (μg/ml) for strain:					
	E. faecalis isolate 1	*E. faecalis* isolate 2	MSSA-1	MSSA-2	MRSA-1	MRSA-2
Daptomycin	1.6/6.3	1.6/1.6	0.4/1.6	0.4/3.1	0.4/0.8	0.2/0.4
Vancomycin	1.6/>50	3.1/>50	0.8/3.1	3.1/6.3	1.6/1.6	1.6/3.1
Penicillin G	3.1/6.3	1.6/3.1				
Nafcillin			0.4/3.1	0.2/0.4		
Gentamicin	3.1/6.3	6.3/12.5				

TABLE 2. Bactericidal activities at 2 h and PAEs of each antimicrobial agent, alone and combined, for each microorganism studied

Antimicrobial agent(s)	Bactericidal activity (log_{10} CFU/ml)/PAE (h) against strain:					
	E. faecalis isolate 1	*E. faecalis* isolate 2	MSSA-1	MSSA-2	MRSA-1	MRSA-2
Daptomycin	−1.0/3.9	−4.1/3.5	−5.0/3.1	−1.1/5.3	−1.6/2.4	−2.4/4.0
Vancomycin	−0.1/3.3	0.0/1.9	−0.1/4.9	−0.2/4.0	−0.2/1.4	−0.1/3.0
Penicillin G	−0.5/1.3	−0.5/1.9				
Nafcillin			−0.1/6.0	−0.1/5.2		
Gentamicin	0.0/0.3	0.0/0.0				
Daptomycin + gentamicin	−2.2/6.1	−5.0/4.8				
Vancomycin + gentamicin	−0.9/3.3	−2.2/2.0				
Penicillin G + gentamicin	−1.4/1.9	−4.1/3.1				

and M. E. Levison, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 584, 1986). However, information on the PAE of daptomycin and comparing it with standard antimicrobial agents against these microorganisms have not been reported.

In our study, exposure of the organisms to daptomycin consistently resulted in a longer PAE compared with exposure to the other antimicrobial agents tested, with the exception of MSSA-1. It is possible that the very rapid bactericidal action of daptomycin against this strain of MSSA made it difficult to quantitate accurately the PAE. A similar observation has been made in an in vitro PAE experiment with *S. aureus* and gentamicin (14).

Preliminary studies of daptomycin suggest that this lipopeptide antibiotic may prove to be a valuable antimicrobial agent when used to treat infections involving gram-positive bacteria. Its rapid bactericidal activity, intramuscular as well as intravenous routes of administration, extended elimination half-life (approximately 8 h [unpublished data, Lilly Research Laboratories]), together with our demonstration of prolonged PAEs for enterococci and staphylococci, are characteristics of daptomycin which make it a potentially important antibiotic.

It is important to stress that results of in vitro studies may not accurately reflect those which would be observed under in vivo conditions. In particular, antibiotics which exhibit high protein binding may have significantly less antimicrobial activity (than predicted from in vitro susceptibility results) in human sera or at the actual site of infection, where the amount of unbound active drug is only a small fraction of the total drug concentration. Although the effect, if any, of protein binding on the clinical efficacy of an antimicrobial agent is controversial (8), studies have suggested that this pharmacokinetic property alters the pharmacodynamics of highly protein-bound antimicrobial agents (13, 15). Bush and colleagues (3) have postulated that the discrepancy between the in vitro bactericidal activity of daptomycin against *E. faecalis* and the absence of the same activity in their experimental model of enterococcal endocarditis was due to the high protein binding of daptomycin (over 90%), resulting in insufficient concentrations of free drug to significantly affect the bacterial counts in vegetations.

One may propose that if a concentration of daptomycin which allowed for high free drug levels in serum were achieved, then the observed bactericidal activity and PAE of this antibiotic in our study would also occur in vivo.

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