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Daptomycin (LY146032) for Prevention and Treatment of Experimental Aortic Valve Endocarditis in Rabbits

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The efficacy of daptomycin (LY146032), a vancomycinlike lipopeptide antibiotic, was compared with that of antibiotics commonly in use for prevention and treatment of experimental aortic valve endocarditis in rabbits. Strains of *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus sanguis*, and *Enterococcus faecalis* were used to establish endocarditis. A single 10-mg/kg dose of daptomycin and a single 25-mg/kg dose of vancomycin were both effective in prevention of endocarditis produced by strains of *S. aureus* and *S. sanguis*. Daptomycin was more effective than vancomycin for prevention of endocarditis caused by the strain of *S. epidermidis*. A single dose of daptomycin also was more effective in prevention of staphylococcal and enterococcal endocarditis than were single-dose regimens of cefazolin (100 mg/kg) and the combination of ampicillin (30 mg/kg) plus gentamicin (3 mg/kg), respectively. For treatment of endocarditis, daptomycin (10 mg/kg) as a single daily dose was as effective as regimens of either vancomycin or beta-lactam antibiotics for staphylococcal and enterococcal endocarditis. Daptomycin, however, was not as effective as a single daily dose of 600,000 U of procaine penicillin for endocarditis caused by the strain of *S. sanguis*.

Daptomycin (LY146032) is a lipopeptide antibiotic active against gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci (4). Because of its long half-life (2), daptomycin may be effective as a single daily dose. The efficacy of a once-daily dose of daptomycin was compared with that of standard regimens for prevention and therapy of aortic valve endocarditis in a rabbit model.

MATERIALS AND METHODS

Experimental strains. The following strains, all of which were clinical isolates, were used to establish endocarditis: methicillin-susceptible strain *S. aureus* 1-63, methicillin-resistant strain *S. aureus* 67-0, methicillin-resistant strain *Staphylococcus epidermidis* 313, *Streptococcus sanguis* Poise, and *Enterococcus faecalis* Cordero.

Susceptibility tests. MICs were determined by the broth dilution method in calcium- and magnesium-supplemented Mueller-Hinton broth (as recommended by the manufacturer for susceptibility testing of daptomycin) at an inoculum of 2×10^5 CFU/ml, incubated at 37°C, and interpreted at 24 h (5). The MIC was defined as the lowest concentration of antibiotic that prevented turbidity in the test tube after 24 h of incubation at 37°C. The MBC, defined as a 99.9% reduction in number of viable counts, was determined by quantitative subculture of 0.01 ml from each clear broth tube onto blood agar plates, which were then incubated for 24 h.

Experimental endocarditis protocol. Endocarditis of the aortic valve was established in 2-kg New Zealand White rabbits. At 24 h before infection, a catheter was positioned across the aortic valve and left in place (6). The infection was established by an intravenous (i.v.) inoculation of a number of organisms that produced infection in 90 to 100% of untreated rabbits. In the prophylaxis experiments, a single dose of drug was administered 30 min before infection. Drugs, doses, and routes of administration for the various standard antibiotic regimens were vancomycin (25 mg/kg i.v.), cefazolin (100 mg/kg i.v.), ampicillin (30 mg/kg intramuscularly [i.m.]), gentamicin (3 mg/kg i.m.) (3), procaine penicillin (600,000 U i.m.), and streptomycin (15 mg/kg i.v.). Daptomycin was given as a single 10-mg/kg i.v. dose. Control rabbits were sacrificed 24 h after infection. Treated rabbits were sacrificed 72 to 240 h after infection.

In the treatment experiments, treatment began 24 h after infection, except for enterococcus and *S. sanguis*, for which treatment began at 12 and 48 h, respectively. A 12-h infection has been used before in studies of single-drug therapy of enterococcal endocarditis (7). A 48-h infection was used for the strain of *S. sanguis* because this model gives more reproducible results than those achieved when a shorter pretreatment period is used.

Untreated rabbits were sacrificed 24 h (12 h for the enterococcus and 48 h for *S. sanguis*) after infection. Treated rabbits were sacrificed either 12 or 24 h after the last dose of drug. Treatment regimens to which daptomycin (10 mg/kg i.v. every 24 h) was compared were as follows: for methicillin-susceptible *S. aureus* 1-63, nafcillin (100 mg/kg every 8 h i.m. for 4 days); for methicillin-resistant *S. aureus* 67-0, vancomycin (25 mg/kg every 12 h i.v. for 4 days); for *S. epidermidis* 313, vancomycin (25 mg/kg every 12 h i.v. for 2 days); for the enterococcal strain Cordero, penicillin (600,000 U every 24 h i.m. for 3 days); and for *S. sanguis* Poise, penicillin (600,000 U every 24 h i.m. for 2 days). These times of sacrifice were chosen based on previous experience with these infections, so that with standard therapies vegetation counts of CFU would be reduced compared with those in controls, yet a sufficient number of CFU still would be present to permit quantification (i.e., most vegetations would not be sterile). Peak levels in serum were drawn 1 h after the drug was administered, and the trough levels in serum were drawn at the time of sacrifice.

At the completion of the study, rabbits were euthanized by an i.v. injection of pentobarbital. The heart was then aseptically excised and examined to determine whether the catheter was in position. The aortic valve and its vegetations were aseptically removed, weighed, and then put into 0.5 ml

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TABLE 1. In vitro susceptibilities

Strain	Drug	MIC (μg/ml)	MBC (μg/ml)
Staphylococcus epidermidis 313	Daptomycin	0.5	8.0
	Vancomycin	4.0	4.0
Staphylococcus aureus 67-0	Daptomycin	0.25	2.0
	Vancomycin	2.0	8.0
Staphylococcus aureus 1-63	Daptomycin	0.25	1.0
	Vancomycin	1.0	4.0
	Cefazolin	1.0	8.0
	Nafcillin	0.5	4.0
Enterococcus faecalis Cordero	Daptomycin	0.5	8.0
	Ampicillin	1.0	8.0
	Gentamicin	16.0	32.0
	Penicillin	≤0.25	64.0
Streptococcus sanguis Poise	Daptomycin	1.0	64.0
	Vancomycin	1.0	1.0
	Penicillin	1.0	1.0
	Streptomycin	32.0	32.0

of sterile 0.9% saline in a 5-ml plastic tube. The vegetation was homogenized for 30 to 45 s with a polytron homogenizer, and a serial 10-fold dilution of it was cultured onto blood agar plates. After incubation for 48 h at 37°C, colonies growing on the agar were counted, and the result was expressed as the vegetation titer, defined as $\log_{10}$ CFU per gram of vegetation. Because any remaining undiluted vegetation homogenate was cultured, as few as 5 to 10 CFU/g of vegetation could be detected. Sterile vegetations were assigned a value of 0 $\log_{10}$ CFU/g (corresponding to 1 CFU/g).

Antibiotic assay. Concentrations of antibiotic in serum were measured by the agar well diffusion method (1). A strain of *Sarcina lutea* was used to assay daptomycin. A strain of *Bacillus subtilis* was used to assay all other antibiotics. Daptomycin was assayed in Ca^{2+}- and Mg^{2+}-supplemented Penassay medium, as directed by the manufacturer. Cefazolin was assayed in Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.). Other antibiotics were assayed in antibiotic medium no. 11 (Difco Laboratories, Detroit, Mich.). Standard curves were determined by using pooled rabbit serum.

Statistical analysis. Parametric data are reported as the mean ± standard deviation, and statistical significance was determined by Student's unpaired *t* test. For the prophylaxis experiments, the Fisher exact test was used to determine *P* values for rates of infection in treated rabbits. Bonferroni correction was used to determine *P* values for more than two comparisons. A *P* of <0.05 was considered significant.

RESULTS

Susceptibility tests. All strains were susceptible to daptomycin in vitro (Table 1). The MICs and MBCs of daptomycin were equal to or lower than those of the beta-lactam antibiotics and vancomycin for each strain, with the exception of the Poise strain of *S. sanguis*, for which the daptomycin MBC was 64 μg/ml, compared with 1 μg/ml for either penicillin or vancomycin.

Drug concentrations in serum. Mean peak concentrations (Table 2) exceeded the MBCs of all drugs except gentamicin, penicillin, and ampicillin for the enterococcal strain and daptomycin and streptomycin for the strain of *S. sanguis*.

TABLE 2. Drug concentrations in serum

Drug (mg/kg)	Route	Dosing interval (h)	Concn, μg/ml (*n*)	
			1 h after dosing	Trough time[a]
Daptomycin (10)	i.v.	24	49 ± 12 (31)	3.1 ± 2.0 (5)
Vancomycin (25)	i.v.	12	43 ± 12 (38)	1.2 ± 1.7 (6)
Nafcillin (100)	i.m.	8	16 ± 6.8 (6)	0.7 ± 0.7 (6)
Penicillin (600,000 U)	i.m.	24	13 ± 5 (9)	3.4 ± 3.0 (13)
Streptomycin (15)	i.m.[b]		15 ± 6.0 (18)	
Cefazolin (100)	i.v.[b]		58 ± 25 (4)	
Ampicillin (30)	i.m.[b]		6.9 ± 3.0 (9)	
Gentamicin (3)	i.m.[b]		6.0 ± 3.4 (13)	

[a] Trough samples were obtained at the following times after the last dose: 24 h for daptomycin, 12 h for vancomycin, 8 h for nafcillin, and 24 h for penicillin.
[b] Prophylaxis regimens.

Mean daptomycin trough concentrations were above the MIC for all strains in the treatment experiments.

Prophylaxis of endocarditis. Vancomycin and daptomycin were equally effective in the prevention of endocarditis caused by methicillin-resistant and methicillin-susceptible *S. aureus* and by *S. sanguis* (Table 3). Daptomycin was significantly better than vancomycin against the methicillin-resistant *S. epidermidis* isolate and better than ampicillin plus gentamicin against the enterococcal strain. Daptomycin was also more active than cefazolin against either strain of *S. aureus*, although the *P* value for the comparison of daptomycin to cefazolin for the susceptible strain did not achieve statistical significance after Bonferroni correction (uncorrected $P = 0.034$).

Treatment of endocarditis. Daptomycin was equally effective or better than standard regimens for the treatment of endocarditis caused by all strains except *S. sanguis* (Table 4). Penicillin-treated rabbits infected with the strain of *S. sanguis* had mean vegetation titers of 2.0 $\log_{10}$ CFU/g, whereas daptomycin-treated rabbits had a titer of 6.6 $\log_{10}$ CFU/g. The strain of *S. sanguis* differed from all other strains in susceptibility to daptomycin. Its daptomycin MBC of 64 μg/ml was the highest of all strains and exceeded the peak concentration in serum achieved during therapy.

DISCUSSION

In these studies of prophylaxis and treatment of experimental aortic valve endocarditis in rabbits, daptomycin given as a single daily dose of 10 mg/kg was generally as effective as or more effective than antibiotics now commonly used to prevent or treat endocarditis in humans. For prevention of endocarditis, daptomycin was more effective than single-dose vancomycin against a strain of methicillin-resistant *S. epidermidis* or single-dose ampicillin-gentamicin against the enterococcus. It was more effective than cefazolin for both susceptible and methicillin-resistant strains of *S. aureus*. These results suggest that daptomycin may be a particularly effective agent for prevention of endocarditis caused by strains that commonly are encountered clinically. The long half-life of daptomycin (approximately 6 h in rabbits) and, therefore, its sustained effect may account in part for its superiority to standard regimens using single doses of drugs with relatively short half-lives.

For treatment of experimental staphylococcal endocarditis, daptomycin equalled or exceeded the efficacy of vancomycin (for the methicillin-resistant strain) and nafcillin (for the susceptible strain). In vivo, the mean daptomycin concentration was 49 μg/ml at 1 h after dosing and 3.1 μg/ml at

TABLE 3. Single-dose prophylaxis of daptomycin compared with standard regimens against experimental gram-positive endocarditis

Strain[a]	No. sterile/total (%)					
	No drug	Daptomycin	Vancomycin	Cefazolin	Ampicillin-gentamicin	Penicillin-streptomycin
1-63 (MSSA)	0/21 (0)	19/21 (90)[b]	8/11 (73)[b]	4/8 (50)[c]		
67-0 (MRSA)	0/16 (0)	15/18 (83)[b]	9/15 (60)[b]	0/4 (0)[d]		
313 (MRSE)	0/12 (0)	10/10 (100)[b]	2/9 (22)[e]			
Cordero (*E. faecalis*)	0/15 (0)	12/17 (70)[b]			2/15 (13)[e]	
Poise (*S. sanguis*)	0/20 (0)	10/11 (91)[b]	11/17 (65)[b]			15/18 (83)[b]

[a] MSSA, methicillin-susceptible *S. aureus*; MRSA, methicillin-resistant *S. aureus*; MRSE, methicillin-resistant *S. epidermidis*.
[b] $P < 0.001$ versus the control.
[c] $P < 0.025$ versus the control.
[d] $P < 0.025$ versus daptomycin regimen.
[e] $P < 0.005$ versus daptomycin regimen.

the trough time. If daptomycin were 80 to 90% protein bound, as has been reported for rabbit serum (2), then the free drug concentrations would be 5 to 10 and 0.3 to 0.6 μg/ml, respectively, approximately equalling or exceeding MICs for the staphylococcal strains throughout the 24-h dosing interval. Trough concentrations of vancomycin were near or below MICs for the methicillin-resistant staphylococcal strains, and possibly vancomycin would have been more efficacious if higher trough levels could have been achieved. Daptomycin as a single daily dose of 10 mg/kg achieved sustained antibacterial activity against the staphylococcal strains.

For enterococcal endocarditis daptomycin as a single agent was no more effective than penicillin alone despite apparent bactericidal activity of daptomycin against enterococci and the much lower MBC of daptomycin than for penicillin. Other investigators have reported that daptomycin was less effective than penicillin in vivo (2). Differences in method between our studies and those of others could account for these different results. Therapy began 12 h after infection in our studies versus 24 h in the other studies. A 12-h period was chosen because this has been successfully used to examine single drug therapy of enterococcal endocarditis (7). Although single drug regimens have not been useful clinically in treating enterococcal endocarditis, our purpose was to determine whether daptomycin might be uniquely active as a single agent. Delay in therapy after infection may result in a higher density of organisms in the vegetation and make their eradication more difficult; therefore, differences in efficacy between two antibiotics might be more apparent. In addition, differences in strains and doses of drugs between our study and others could also affect results. Further studies are needed to determine the role of daptomycin for enterococcal infections. It does not seem, however, to possess unique bactericidal activity against enterococci.

The only strain for which daptomycin was less effective than standard therapy was the strain of *S. sanguis*. The MBC of daptomycin for this strain was 64 μg/ml, which may account for the lack of efficacy of daptomycin. At 1 h after the administration of daptomycin, the mean concentration in serum was 49 μg/ml, and full drug concentrations were undoubtedly much less. Thus, a bactericidal concentration was not achieved in vivo.

Daptomycin given as single daily dose of 10 mg/kg was effective for endocarditis when concentrations in serum achieved the MBC for the infecting strain for at least part of the dosing interval. However, a single daily dose of daptomycin was ineffective for endocarditis caused by *S. sanguis*, for which the daptomycin MBC is relatively high in vitro. Doses larger than 10 mg/kg and more than a single daily dose may be needed for effective therapy of endocarditis caused by strains requiring relatively high concentrations of daptomycin before killing occurs. Although not examined in this study, because daptomycin reportedly is so highly protein bound, concentrations in vivo may have to exceed by severalfold the bactericidal concentrations measured in vitro for daptomycin to be effective for endocarditis.

TABLE 4. Mean vegetation titers in treatment of experimental endocarditis

Strain[a]	Regimen (*n*)	Vegetation titer (mean $\log_{10}$ CFU/g ± SD)
1-63 (MSSA)	No treatment (10)	6.7 ± 3.2
	Nafcillin (18)	2.7 ± 2.3
	Daptomycin (12)	0.7 ± 0.9[b]
67-0 (MRSA)	No treatment (13)	8.8 ± 0.6
	Vancomycin (15)	3.5 ± 1.8
	Daptomycin (13)	3.9 ± 2.4
313 (MRSE)	No treatment (12)	6.9 ± 1.0
	Vancomycin (15)	2.6 ± 2.8
	Daptomycin (21)	1.8 ± 1.9
Cordero (*E. faecalis*)	No treatment (15)	6.9 ± 1.0
	Penicillin (9)	2.9 ± 2.0
	Daptomycin (11)	2.1 ± 2.1
Poise (*S. sanguis*)	No treatment (20)	8.3 ± 0.7
	Penicillin (10)	2.0 ± 1.5
	Daptomycin (15)	6.6 ± 2.2[b]

[a] See footnote *a* of Table 3.
[b] $P < 0.001$ versus nafcillin or penicillin regimen.

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