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Daptomycin (LY146032) Treatment of Experimental Enterococcal Endocarditis

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This study compared daptomycin (LY146032) with penicillin G procaine and vancomycin without and with gentamicin for treatment of experimental enterococcal endocarditis. The strain of *Streptococcus* (*Enterococcus*) *faecalis* used in this study was killed by daptomycin in vitro in broth but not in serum. In rabbits treated for 3 days, daptomycin significantly reduced bacterial counts of vegetations compared with no therapy but was significantly less effective than penicillin G procaine or vancomycin. Daptomycin-gentamicin significantly reduced bacterial counts of vegetations compared with daptomycin alone but was significantly less effective than vancomycin plus gentamicin. The efficacy of daptomycin-gentamicin did not differ significantly from that of penicillin G procaine-gentamicin. The lack of enterococcal killing by daptomycin alone in serum and in experimental endocarditis is probably related to the high protein binding of the agent.

Enterococci are the third most common cause of infective endocarditis and are responsible for about 10% of infective endocarditis cases (8). Unlike most other streptococcal species, enterococci demonstrate resistance to a wide range of antimicrobial agents, and rarely is a single agent bactericidal against the organisms in vitro (15). To achieve in vitro bactericidal activity against enterococci, an aminoglycoside must be added to penicillin G, ampicillin, or vancomycin (16, 21). Although controlled trials are lacking, the recommended treatment of serious enterococcal infections, e.g., endocarditis, involves a combination of penicillin G or vancomycin plus an aminoglycoside (9, 11).

The emergence of clinical enterococcal isolates which demonstrate high-level streptomycin resistance (16) and, more recently, strains with high-level gentamicin resistance (12, 23) threatens our ability to provide bactericidal antimicrobial therapy in cases of enterococcal endocarditis. In vitro experiments (12) and animal studies (2) have shown a loss of bactericidal activity with penicillin G plus an aminoglycoside against enterococcal isolates which demonstrate high-level aminoglycoside resistance. These developments, together with the recent demonstration of β-lactamase production by isolates of *Streptococcus* (*Enterococcus*) *faecalis* (17), illustrate the need for alternative antimicrobial therapy for serious enterococcal infections.

Daptomycin (LY146032) is a cyclic lipopeptide compound that belongs to a class of antibiotics produced by *Streptomyces roseosporus* (4). It has a spectrum of activity against aerobic, facultative, and anaerobic gram-positive bacteria. Although its spectrum of activity is similar to those of the glycopeptide antibiotics vancomycin and teicoplanin, daptomycin differs from these antibiotics in structure and mechanism of action. Another characteristic of daptomycin which distinguishes it from the glycopeptide antibiotics is the in vitro demonstration of bactericidal activity against enterococci at concentrations near the MIC (4, 19). These features suggest that daptomycin may be effective as a single agent in the treatment of enterococcal endocarditis.

The endocarditis model in rabbits is a severe test of the efficacy of antimicrobial agents for deep-seated infections. The purpose of this study was to compare the therapeutic efficacy of daptomycin with those of penicillin G procaine and vancomycin without and with gentamicin for the treatment of experimental enterococcal endocarditis.

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MATERIALS AND METHODS

Organism. The enterococcus (*S. faecalis*) isolate used in this study was a clinical isolate from a patient with bacteremia. Stock cultures were made by incubating the organism in Mueller-Hinton broth (MHB) at 37°C for 24 h and storing 1-ml samples at −20°C. For each experiment, a sample was subcultured into MHB and incubated at 37°C for 18 h.

In vitro studies. MICs and MBCs of daptomycin, penicillin G procaine, vancomycin, and gentamicin were determined with an inoculum of 5×10^5 CFU of *S. faecalis* per ml of cation-supplemented MHB as previously described (7). The MIC was defined as the lowest concentration of the antimicrobial agent that prevented turbidity after incubation for 24 h at 37°C. The MBC was defined as the lowest concentration of the antimicrobial agent that killed at least 99.9% of the organisms within 24 h, as determined by plating of 0.1-ml portions of the MIC dilutions. MBCs of each antmicrobial agent were also determined by using the same inoculum of the enterococcal isolate in 100% rabbit serum (GIBCO Laboratories, Grand Island, N.Y.). Determination of MICs of each antimicrobial agent in 100% rabbit serum was precluded by the inability to evaluate turbidity in the rabbit serum.

Survival of *S. faecalis* was studied in flasks with cation-supplemented MHB alone and in flasks containing daptomycin (15 μg/ml), penicillin G procaine (15 μg/ml) or vancomycin (30 μg/ml) without or with gentamicin (4 μg/ml). The concentrations of antimicrobial agents chosen were based on achievable levels in serum in humans. An inoculum of *S. faecalis* was added to result in 4×10^5 CFU/ml. The flasks were incubated at 37°C. Samples were removed at 0, 3, 6, 24, and 48 h. The numbers of CFU/ml in the flasks were determined by serial dilution and plating techniques (2). Survival of *S. faecalis* was also studied in flasks with 100% rabbit serum alone or also containing daptomycin (15 μg/ml), penicillin G procaine (15 μg/ml), or vancomycin (30 μg/ml) without or with gentamicin (4 μg/ml).

Animal experiments. Female New Zealand White rabbits (Ace Animals, Boyertown, Pa.) ranging from 2 to 2.5 kg in weight were anesthetized, and the right carotid artery of each animal was cannulated, with advancement of the catheter across the aortic valve (3). Each rabbit was inoculated 24 h later by ear vein with 10^8 CFU of *S. faecalis* in 1 ml of MHB. This inoculum produced endocarditis in all rabbits which had properly placed catheters. The catheter was left in place throughout the experiment.

At 24 h after inoculation, rabbits were randomly assigned to an untreated control group and the following six treatment groups: daptomycin, penicillin G procaine, vancomycin, daptomycin-gentamicin, penicillin G procaine-gentamicin, and vancomycin-gentamicin. The following dosage regimens for the antimicrobial agents were used: daptomycin at 10 mg/kg subcutaneously (s.c.) every 12 h, penicillin G procaine at 1.2×10^6 U intramuscularly (i.m.) divided into two sites every 12 h, vancomycin at 75 mg/kg i.m. every 12 h, and gentamicin at 5 mg/kg i.m. every 12 h. All six treatment groups received therapy for 72 h. After 3 days of therapy, the rabbits were randomly killed with an intravenous injection of pentobarbital sodium 12 h after the last doses of antimicrobial agents.

All aortic valve vegetations from each rabbit were excised, pooled, and weighed. The vegetations form each rabbit weighed a total of 14 to 436 mg. After a 1:10 suspension of each vegetation pool in MHB was homogenized, the number of CFU/g was determined by serial dilution and plating techniques (2). In sterile vegetations, the number of CFU was recorded as 2 $\log_{10}$ CFU/g, because the largest weight of vegetations plated in some rabbits was 10 mg.

Antimicrobial agent levels and half-lives in serum. Blood was taken from the ear veins of uninfected rabbits 0.5, 1, 2, 4, and 6 h after one injection of daptomycin at 10 mg/kg administered s.c., after penicillin G procaine at 1.2×10^6 U given i.m. divided into two sites, and after one i.m. injection of vancomycin at 75 mg/kg or gentamicin at 5 mg/kg. Serum was separated from blood samples and stored at −20°C until assay. Concentrations of penicillin G procaine, vancomycin, and gentamicin were measured by an agar diffusion method using paper disks with *Bacillus subtilis* as the indicator organism (1). Concentrations of daptomycin were determined by the same method, but the indicator organism used was a strain of *Sarcina lutea* (supplied by Lilly Research Laboratories, Indianapolis, Ind.). The half-lives of elimination of the antimicrobial agents other than penicillin G procaine from serum were calculated by the method of least squares (10). The half-life of elimination of penicillin G procaine from serum could not be calculated.

Statistical analysis. A one-way, seven-level Kruskal-Wallis analysis of variance by ranks, followed by the Mann-Whitney U rank test, was used to determine significant differences among bacterial counts of vegetations. With the Bonferroni correction, a P value of <0.00625 was required for significance.

RESULTS

In vitro studies. Table 1 shows the respective MICs and MBCs of each antimicrobial agent for an inoculum of 5×10^5 CFU of the strain of *S. faecalis* used per ml when measured in cation-supplemented MHB. Similar results were observed when an inoculum of 10^7 CFU of the same strain per ml was used. The MBCs of each antimicrobial agent for the same inoculum of the same organism when measured in 100% rabbit serum are also shown in Table 1.

TABLE 1. MICs and MBCs of each antimicrobial agent

Antimicrobial agent	Effective concn (μg/ml) in cation-supplemented MHB		MBC (μg/ml) in 100% rabbit serum
	MIC	MBC	
Daptomycin	1.6	6.3	>50
Penicillin G procaine	3.1	6.3	25
Vancomycin	1.6	>50	>50
Gentamicin	3.1	6.3	12.5

Figure 1 shows the rate of decrease in numbers of *S. faecalis* organisms in cation-supplemented MHB containing daptomycin, penicillin G procaine, or vancomycin without and with gentamicin with an inoculum of 4×10^5 CFU/ml. Daptomycin was the single most effective antimicrobial agent, demonstrating a decrease in numbers of enterococci of 3 $\log_{10}$ CFU/ml at 24 h, while penicillin G procaine was the next most effective single agent with a decrease of 1.5 $\log_{10}$ CFU/ml at 24 h. With vancomycin and gentamicin, the numbers of enterococci actually increased or remained the same. Combining gentamicin with daptomycin, penicillin G procaine, or vancomycin resulted in enhanced bactericidal activity, with sterile cultures at 6 h.

Figure 2 shows the rate of decrease in numbers of *S. faecalis* organisms in 100% rabbit serum containing daptomycin, penicillin G procaine, or vancomycin without or with gentamicin with an inoculum of 4×10^5 CFU/ml. Penicillin G procaine was the most effective single agent at

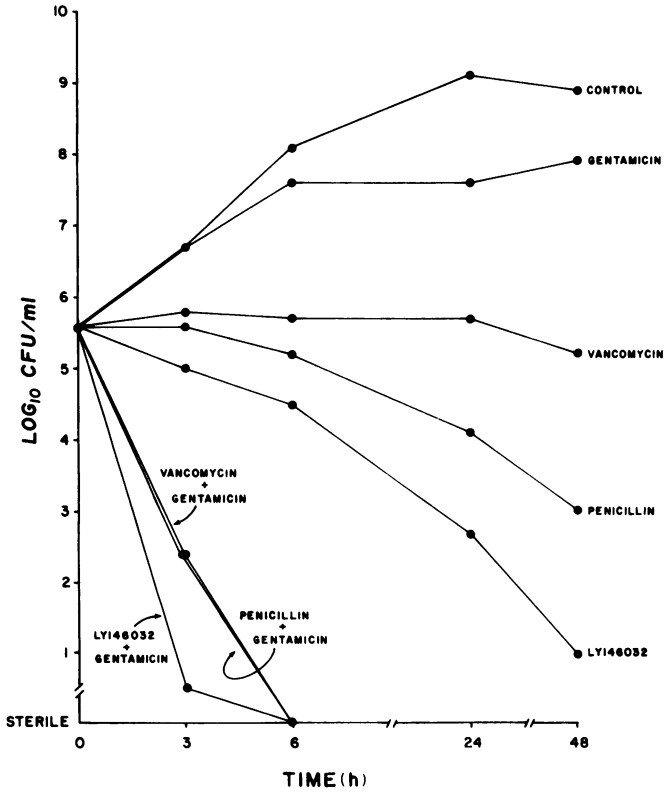

FIG. 1. Rate of decrease of numbers of *S. faecalis* organisms in cation-supplemented MHB alone (control) or containing daptomycin (15 μg/ml), penicillin G procaine (15 μg/ml), vancomycin (30 μg/ml), gentamicin (4 μg/ml), daptomycin plus gentamicin, penicillin G procaine-gentamicin, or vancomycin-gentamicin.

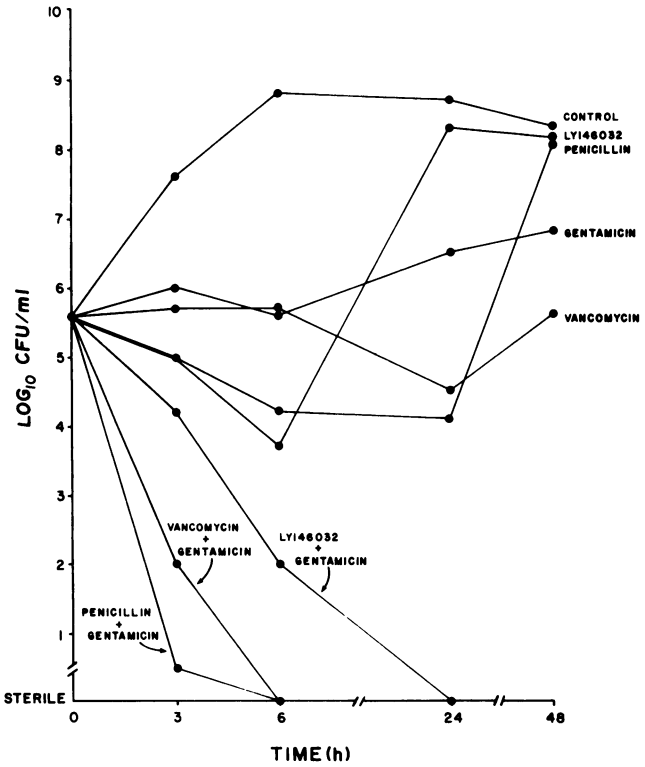

FIG. 2. Rate of decrease in numbers of *S. faecalis* organisms in 100% rabbit serum alone (control) or containing daptomycin (15 μg/ml), penicillin G procaine (15 μg/ml), vancomycin (30 μg/ml), gentamicin (4 μg/ml), daptomycin-gentamicin, penicillin G procaine-gentamicin, or vancomycin-gentamicin.

24 h, with a decrease of 1.4 $\log_{10}$ CFU/ml. Vancomycin was the next most effective single agent, with a decrease in numbers of organisms of 1.1 $\log_{10}$ CFU/ml at 24 h. Unlike the continued decline in numbers of organisms after 24 h observed in MHB with penicillin G procaine or vancomycin, the number of organisms increased in the time interval between 24 and 48 h in 100% rabbit serum with these two antimicrobial agents. Daptomycin decreased the numbers of organisms by 1.9 $\log_{10}$ CFU/ml at 6 h, but by 24 h the numbers of organisms had increased by 2.7 $\log_{10}$ CFU/ml over the initial inoculum. Gentamicin in 100% rabbit serum did not decrease the numbers of organisms. Combining gentamicin with daptomycin, penicillin G procaine, or vancomycin resulted in enhanced bactericidal activity, with sterile cultures at 6 to 24 h. However, this occurred less rapidly with daptomycin-gentamicin than with penicillin G procaine-gentamicin or vancomycin-gentamicin. Also, daptomycin-gentamicin sterilized less rapidly in 100% rabbit serum than in MHB.

Animal experiments. Table 2 shows the mean (± the standard error) counts of *S. faecalis* (as $\log_{10}$ CFU/g of vegetation) for the untreated control group of rabbits and for the six treatment groups after 3 days of therapy. A one-way seven-level Kruskal-Wallis analysis of variance by ranks revealed a significant overall effect of antimicrobial treatment on reducing bacterial counts of vegetations ($P < 0.0001$). The Mann-Whitney U rank test was then performed on a series of comparisons. With the Bonferroni correction, a P value of <0.00625 was required for significance. Daptomycin significantly reduced bacterial counts of vegetations compared with no treatment ($P = 0.0003$), but it was

TABLE 2. Counts of *S. faecalis* organisms in vegetations after 3 days of therapy

Treatment	Mean ± SE $\log_{10}$ CFU of vegetation/g (no. sterile/total)
None	9.0 ± 0.1 (0/8)
Daptomycin	7.4 ± 0.3 (0/10)
Penicillin G procaine	5.6 ± 0.2 (0/8)
Vancomycin	6.0 ± 0.2 (0/9)
Daptomycin-gentamicin	4.4 ± 0.5 (0/10)
Penicillin G procaine-gentamicin	3.7 ± 0.3 (1/9)
Vancomycin-gentamicin	3.2 ± 0.1 (0/9)

significantly less effective than penicillin G procaine ($P = 0.0004$), vancomycin ($P = 0.0008$), penicillin G procaine-gentamicin ($P = 0.0002$), vancomycin-gentamicin ($P = 0.0002$), or daptomycin-gentamicin ($P = 0.0005$). Daptomycin-gentamicin was significantly less effective than vancomycin-gentamicin ($P = 0.00047$), but it was not significantly less effective than penicillin G procaine-gentamicin ($P = 0.39$). No animals from either the control group or the treatment groups died during the study.

Antimicrobial agent levels and half-lives in serum. The mean (± the standard error) peak concentrations in serum and half-lives of elimination from serum after one injection of daptomycin at 10 mg/kg administered s.c., after penicillin G procaine at 1.2×10^6 U given i.m. and divided into two sites, and after single i.m. injections of vancomycin at 75 mg/kg and gentamicin at 5 mg/kg are shown in Table 3. Peak levels in serum occurred 2 h after administration of daptomycin, 1 h after administration of penicillin G procaine, 2 h after administration of vancomycin, and 30 min after administration of gentamicin. Daptomycin and vancomycin had much longer half-lives of elimination from serum than did gentamicin. The half-life of elimination of penicillin G procaine from serum could not be calculated.

DISCUSSION

This study compared the therapeutic efficacy of daptomycin with those of penicillin G procaine and vancomycin without and with gentamicin for treatment of experimental enterococcal endocarditis. Daptomycin was bactericidal for the strain of *S. faecalis* used at four times the MIC and was more bactericidal than penicillin G procaine or vancomycin in time-kill studies in broth. Other investigators have demonstrated this in vitro bactericidal action of daptomycin against various strains of enterococci (4–6, 19, 20). However, in 100% rabbit serum the MBC increased at least sevenfold for daptomycin, and no bactericidal activity was present in time-kill studies by 24 h. Adding gentamicin to daptomycin, penicillin G procaine, or vancomycin led to

TABLE 3. Peak concentrations of antimicrobial agents in serum and half-lives of elimination of antimicrobial agents from serum

Drug, dose, and route (no. of rabbits)	Mean ± SE peak concn (μg/ml)	Mean ± SE half-life (h)
Daptomycin, 10 mg/kg, s.c. (3)	43.6 ± 1.5	5.8 ± 0.7
Penicillin G procaine, 1.2×10^6 U, i.m. (4)	15.2 ± 2.0	—[a]
Vancomycin, 75 mg/kg, i.m. (3)	59.5 ± 5.8	3.5 ± 0.6
Gentamicin, 5 mg/kg, i.m. (4)	14.8 ± 2.4	1.0 ± 0.1

[a] —, Not possible to calculate.

rapid bactericidal action, with sterile cultures in broth by 6 h, confirming similar results in previous studies (2, 22). In serum, similar bactericidal activity occurred, but daptomycin-gentamicin was less rapidly bactericidal than penicillin G procaine-gentamicin, vancomycin-gentamicin, or daptomycin-gentamicin in broth.

In rabbits with enterococcal endocarditis treated for 3 days, daptomycin significantly reduced bacterial counts of vegetations compared with no treatment. However, daptomycin alone was found to be significantly less effective than either penicillin G procaine or vancomycin without or with gentamicin. Daptomycin-gentamicin significantly reduced bacterial counts of vegetations compared with daptomycin alone but was significantly less effective than vancomycin-gentamicin. Daptomycin-gentamicin was less effective than penicillin G procaine-gentamicin, but not significantly so. In only one animal (that received penicillin G procain-gentamicin) were the vegetations sterilized. This is not an unexpected observation, since it would be unusual to sterilize vegetations infected with enterococci after only 3 days of antimicrobial therapy with the antimicrobial regimens studied to date.

Daptomycin did not prove to be a very effective antimicrobial agent for reducing the bacterial counts of vegetations. The superior activity of this antimicrobial agent against the enterococcus in time-kill studies (performed in cation-supplemented MHB) compared with penicillin G procaine and vancomycin was not observed in serum or in vivo. The probable explanation for this is that daptomycin is highly protein bound in serum. Preliminary data indicate that it is 90 to 93% protein bound in human serum (Lilly Research Laboratories, unpublished data) and is reported to be 82% protein bound in rabbit serum (Robert B. Kammer, Lilly Research Laboratories, personal communication). However, in our laboratory the protein binding in rabbit serum appeared to be over 90% (unpublished data). It has been postulated that only the unbound free portion of an antimicrobial agent in serum was biological activity, and this has been supported by both in vitro (18) and in vivo (13) studies. Therefore, the actual amount of free daptomycin in serum would seem to be less than optimal for treating infective endocarditis when the bacterial counts in vegetations are known to approximate 10^8 CFU/g and the infection is in an area which does not allow the natural host defenses to operate optimally.

One can argue that greater doses of daptomycin should have been administered to the rabbits to achieve higher peak levels, and therefore higher free drug levels, in serum. However, in uninfected rabbits single doses of daptomycin at 10 mg/kg administered s.c. achieved a mean peak concentration in serum of about 44 μg/ml. This was much greater than the mean peak level achieved in serum in humans of about 16 μg/ml after i.v. administration of single doses of daptomycin at 1 mg/kg; this is the dose currently being considered for human use (Lilly Research Laboratories, unpublished data). One can also argue that if an enterococcal isolate that was more susceptible to daptomycin had been used, resulting in free daptomycin levels in serum that were greater than the MBC, the outcome of the studies might have been different. However, in using animal models in which, for technical reasons, few strains of bacteria can be studied, negative results are extremely important. If inferior results occur in even as few as one of five bacterial isolates compared with standard effective therapeutic regimens, it could have serious implications for widespread application to human disease.

In the only other published animal model of enterococcal endocarditis using daptomycin as an antimicrobial agent (4), daptomycin appeared to have in vivo bactericidal activity equivalent to that of vancomycin, conflicting with other observations. The major differences between that study and ours are that in that study a different animal model was used; daptomycin was compared only with vancomycin, the animals were treated for 5 days, and they received the antimicrobial agents via continuous infusion at a much greater total daily dose in order to achieve mean concentrations in serum of four to eight times the MIC of each antimicrobial agent. In an experimental enterococcal pyelonephritis study, daptomycin was bactericidal in vitro but proved less effective than ampicillin in vivo (14). The difference between the in vitro and in vivo activities of daptomycin against enterococci in that study was similar to that in ours.

Daptomycin alone does not appear to be an alternative antimicrobial agent for treatment of enterococcal endocarditis. However, in combination with an aminoglycoside it may have a role in the therapy of enterococcal endocarditis, especially with highly susceptible strains. Daptomycin may be appropriate therapy for other enterococcal infections, such as in the urinary tract and infections caused by other, more susceptible gram-positive bacteria.

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