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Treatment of Experimental Endocarditis Caused by a β-Lactamase-Producing Strain of *Enterococcus faecalis* with High-Level Resistance to Gentamicin

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Several antimicrobial regimens were evaluated in the treatment of experimental enterococcal endocarditis due to a β-lactamase-producing, highly gentamicin-resistant strain of *Enterococcus faecalis*. Ampicillin alone cleared bacteremia in the majority of rats and reduced titers of bacteria within vegetations (6.84 versus 8.80 $\log_{10}$ CFU/g in controls) but did not sterilize valves. Ampicillin-sulbactam combinations, vancomycin, daptomycin, and imipenem each reduced residual bacterial titers within vegetations to 4.01 $\log_{10}$ CFU/g or less; in 26 to 43% of animals receiving 5 days of therapy, titers of bacteria were reduced to undetectable levels. In a separate experiment, rats received ampicillin-sulbactam, daptomycin, or vancomycin for 10 days and were then observed for 10 days after termination of therapy for evidence of relapse. In surviving rats, valves remained sterile in four of five rats treated with ampicillin-sulbactam, in five of seven treated with daptomycin, but in only one of eight receiving vancomycin.

Because enterococci are characteristically tolerant to the bactericidal effects of cell wall-active antibiotics, synergistic combinations of cell wall-active agents and aminoglycosides have been employed as the treatment of choice for enterococcal endocarditis (8). First reported in 1979, plasmid-borne enzymatically mediated high-level resistance to gentamicin (and other aminoglycosides) which precludes such bactericidal synergism has now emerged widely among clinical isolates of *Enterococcus faecalis* (20). Optimal antimicrobial therapy of endocarditis and other serious infections caused by such organisms has yet to be determined. More recently, strains of *E. faecalis* which produce β-lactamase in addition to aminoglycoside-modifying enzymes have now been encountered in several U.S. cities (12, 14).

The present study was undertaken to compare the efficacies of several antimicrobial regimens in the treatment of serious infections due to a β-lactamase-producing, highly gentamicin-resistant clinical isolate of *E. faecalis*. For this purpose, we employed a rat model of aortic valve endocarditis in which antibiotics were administered by continuous intravenous infusion.

MATERIALS AND METHODS

Bacterial strain. *E. faecalis* HH22 is a β-lactamase-producing, highly gentamicin-resistant clinical isolate initially described in 1983 (12) and extensively characterized in subsequent studies (10, 11, 13).

Antimicrobial susceptibility testing. Susceptibility of the test strain to agents used in this study was determined in glucose phosphate broth by using a macrodilution technique (7). The medium was supplemented with calcium (50 mg/liter) chloride when daptomycin was tested (4). Inocula of approximately 5×10^5 CFU/ml (or as specified) were prepared from overnight broth cultures. MBCs were determined by the method of Pearson et al. (15), which is based on the transfer of 0.01-ml samples to antibiotic-free plates. The influence of serum on drug activity was determined by microdilution in cation-supplemented Mueller-Hinton broth supplemented with 50% serum from rat, rabbit, or human. Time-kill studies were performed as previously described (9). Antimicrobial agents were generous gifts as follows: daptomycin (LY146032) and vancomycin, Eli Lilly & Co., Indianapolis, Ind.; ampicillin and sulbactam, Pfizer Inc., Groton, Conn.; imipenem and imipenem-cilastatin, Merck Sharp & Dohme Co., Rahway, N.J. Gentamicin was obtained from the Schering Corp., Bloomfield, N.J.

Experimental infection. Aortic valve endocarditis was established in male Sprague-Dawley rats by the method of Santoro and Levison (16) as previously described (19). With the rats under anesthesia, a polyethylene catheter (PE10 Intramedic tubing) was introduced via the right carotid artery and advanced across the aortic valve. After 30 min, 5×10^5 CFU of *E. faecalis* HH22 was injected through the catheter, which was then heat sealed and left in place for the duration of the experiment. Blood cultures were obtained 24 h after inoculation; only animals with positive cultures at this point were included in the evaluation.

Antimicrobial therapy. Treatment was begun 24 h after bacterial challenge. Antimicrobial agents were administered by continuous infusion through an indwelling central venous catheter inserted through the left jugular vein as previously described (19). Infusions were precisely controlled with syringe pumps (Orion Research, Inc., Cambridge, Mass.). Drug doses were selected to achieve levels in serum in rats comparable to mean concentrations in serum attainable in humans treated with standard doses of antibiotics. Doses (milligrams per kilogram of body weight per day) were as follows: ampicillin, 400; sulbactam, 100; vancomycin, 200; imipenem, 300; daptomycin, 50 and 25; and gentamicin, 30. Treatment was administered for 5 days. Each experiment included a control group of untreated animals.

In a separate set of studies, we assessed the efficacies of ampicillin plus sulbactam (400 and 100 mg/kg per day), vancomycin (100 mg/kg per day), and daptomycin (25 mg/kg

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per day) in achieving a bacteriologic cure. In these experiments, animals received 10 days of therapy and were then observed for 10 days without treatment, after which survivors were sacrificed.

Monitoring of therapy and outcome. Levels of antibiotic in serum after 24 h of therapy were determined by microbiologic assay (1). On the last day of treatment, blood cultures were obtained, and the animals were sacrificed 2 h following discontinuation of therapy. Cardiac vegetations were aseptically excised, weighed, homogenized, and diluted for colony counting. This process reduced concentrations of antimicrobial agents which could be potentially carried over to $<0.1\times$ MIC before the sample was spread over the surface of an agar plate. The lower limit of detection of growth was approximately 2.3 $\log_{10}$ CFU/g of vegetation, a value which was assigned to valves from which no growth was obtained.

Statistical evaluation. The chi-square test with the Yates correction was used to compare nominal variables. Differences in residual bacterial titers in heart valves were examined by analysis of variance followed by the Student-Newman-Keuls method for multiple comparisons (5).

RESULTS

In vitro susceptibility of test strain. MICs (MBCs) of antimicrobial agents against *E. faecalis* HH22 were as follows (in micrograms per milliliter): gentamicin, 8,000 (8,000); sulbactam alone, 64 (>64); vancomycin, 2 (>128); daptomycin, 4 (32); and imipenem, 0.5 (4). Ampicillin MICs (MBCs) ranged from 1.0 μg/ml with an inoculum of 4×10^3 CFU/ml to 32 (>128) μg/ml with an inoculum of 1.5×10^6 CFU/ml. By checkerboard titrations, ampicillin-sulbactam combinations demonstrated bacteriostatic (fractional inhibitory concentration index, ≤0.07) and bactericidal (fractional bacterial concentration index, ≤0.02) synergism. At an inoculum of 10^6 CFU/ml, sulbactam at concentrations as low as 1.0 μg/ml exerted a near-maximal effect in augmenting the activity of ampicillin, lowering the MIC of the latter to 2 μg/ml. In cation-supplemented Mueller-Hinton broth, microdilution MICs of vancomycin (1.0 μg/ml) and daptomycin (0.5 μg/ml) were lower than those obtained by the broth macrodilution method. Supplementation of medium with 50% serum from rat, rabbit, or human increased the MIC of vancomycin to 4 μg/ml and that of daptomycin to 8 μg/ml. MBCs of vancomycin remained >128 μg/ml in the presence of any serum, while those of daptomycin remained in the range of 16 μg/ml (rabbit and human) to 64 μg/ml (rat).

By time-kill methods, ampicillin at 4 to 16 μg/ml resulted in approximately 10-CFU/ml killing at 4 h of incubation followed by complete regrowth to control levels by 24 h of incubation. In vitro bactericidal effects of ampicillin-sulbactam and other drugs at concentrations pertinent to the animal model are shown in Table 1. Addition of gentamicin (5 μg/ml) to ampicillin (10 or 20 μg/ml) plus sulbactam (1.0 μg/ml) or to imipenem (8 μg/ml) did not affect the rate or extent of killing by the β-lactams.

Experimental infection. (i) Five-day treatment regimens. Results of therapy are shown in Table 2. Ampicillin alone sterilized some blood cultures ($P < 0.001$) and did reduce bacterial titers in heart valves ($P < 0.05$) compared with titers in controls, but this drug did not sterilize any heart valves. Ampicillin-sulbactam was superior to ampicillin in sterilizing blood cultures ($P = 0.03$), in reducing titers in vegetations ($P < 0.05$), and in sterilizing valves ($P = 0.03$). Addition of gentamicin to either ampicillin or ampicillin-sulbactam failed to improve outcome as measured by these criteria.

TABLE 1. In vitro bactericidal activities of antimicrobial agents against *E. faecalis* HH22 at concentrations achieved in rat serum during treatment of experimental endocarditis

Drug	Concn (μg/ml)	Reduction in viable organisms ($\log_{10}$ CFU/ml)[a] at:	
		4 h	24 h
Ampicillin + sulbactam	16		
	1.0	1.1	2.8
Vancomycin	15	0.4	1.2
	30	0.1	1.2
Daptomycin	20	1.7	2.8
	40	2.5	3.6
Imipenem	8	1.5	2.8

[a] Relative to inoculum.

Ampicillin-sulbactam, imipenem, vancomycin, and both daptomycin regimens effectively cleared bacteremia ($P < 0.001$ compared with controls). Low-dose daptomycin was less effective than the other regimens (except imipenem) in sterilizing blood cultures but was equal to the others in sterilizing valves. Each of these drugs reduced vegetation bacterial titers compared with control levels. The two daptomycin regimens were superior to the vancomycin and ampicillin-sulbactam regimens in reducing vegetation titers; however, while statistically significant, these differences were small. Ampicillin-sulbactam, vancomycin, and imipenem were equally effective in reducing bacterial titers in heart valves.

(ii) Ten-day treatment regimens. Mean levels in serum attained with drug doses employed were 15 μg/ml for ampicillin, 2 μg/ml for sulbactam, 11 μg/ml for vancomycin, and 18 μg/ml for daptomycin. In each group, 10 to 15 rats completed 10 days of therapy (Table 3). Of these, 5 (46%) of 11 treated with ampicillin-sulbactam, 6 (60%) of 10 treated with daptomycin, and 1 (7%) of 15 treated with vancomycin had sterile valves at sacrifice 10 days following completion of therapy or at autopsy if death occurred prior to that time. Daptomycin was superior to vancomycin ($P = 0.01$); ampicillin-sulbactam did not differ from daptomycin but was marginally better than vancomycin ($P = 0.07$) in producing sterile valves.

Of animals surviving a full 10 days beyond completion of 10 days of antibiotic treatment, four (80%) of five receiving ampicillin-sulbactam, five (71%) of seven receiving daptomycin, and one (13%) of eight receiving vancomycin had sterile valves at autopsy. The potentially superior efficacies of ampicillin-sulbactam and daptomycin compared with vancomycin in achieving a bacteriologic cure for endocarditis did not attain statistical significance ($P = 0.07$).

DISCUSSION

Although continuous infusion of ampicillin alone did clear bacteremia in some animals and did lead to a statistically significant reduction in viable organisms within cardiac vegetations, the magnitude of these effects was small, and in no case did ampicillin alone sterilize vegetations. As predicted by in vitro data, the addition of sulbactam markedly enhanced the activity of ampicillin against infection due to this β-lactamase-producing strain. The effectiveness of this combination against *E. faecalis* HH22 was comparable to

TABLE 2. Outcome of experimental endocarditis due to *E. faecalis* HH22

Regimen[a]	Mean (± SD) level in serum (μg/ml)	No. of rats surviving[b]/no. treated	No. of survivors with sterile:		Bacterial titer in vegetation (log_{10} CFU/g) (mean ± SEM)
			Blood	Vegetations	
Control		17/31	0	0	8.80 ± 0.23
Gentamicin	3.1 ± 1.3	11/18	0	0	9.22 ± 0.20
Ampicillin	15.4 ± 5.1	12/14	8	0	6.84 ± 0.45
Ampicillin + gentamicin		18/20	11	0	6.89 ± 0.50
Ampicillin + sulbactam	1.9 ± 0.5	19/23	19	8	3.87 ± 0.39
Ampicillin + sulbactam + gentamicin		20/20	20	12	3.13 ± 0.26
Vancomycin	24.1 ± 11.7	19/21	18	5	4.01 ± 0.28
Daptomycin, LD	18.5 ± 4.9	19/22	11	6	2.88 ± 0.17
Daptomycin, HD	45.3 ± 4.7	21/24	21	9	2.90 ± 0.21
Imipenem	9.7 ± 1.5	12/15	10	4	3.30 ± 0.32

[a] LD, Low dose (25 mg/kg per day); HD, high dose (50 mg/kg per day).
[b] Completing 5 days of treatment.

that of ampicillin alone against a non-β-lactamase-producing enterococcus which we have previously studied in this model (19).

Residual bacterial titers in cardiac vegetations after 5 days of ampicillin-sulbactam therapy (3.87 log_{10} CFU/g) were similar to those noted by Ingerman et al. (6) (4.3 log_{10} CFU/g), who used procaine penicillin plus clavulanic acid by intermittent injection in a rat model of endocarditis due to another β-lactamase-producing enterococcus. However, in that study, none of 6 rats treated for 5 days had sterile vegetation, while in the present study, ampicillin-sulbactam sterilized vegetations in 8 of 19 animals. The potential in vivo activity of this combination was further supported by results of our 10-day treatment study, in which at least 46% of animals appeared to have been cured.

Despite the weak in vitro bactericidal effect of vancomycin, this drug was as effective as any other in the 5-day treatment model. Residual bacterial titers in our vancomycin-treated animals (4.01 log_{10} CFU/g) were comparable to those observed by Ingerman et al. (6) with doses of 60 mg/kg twice daily (mean titers, 3.7 to 4.5 log_{10} CFU/g). However, our study also included observation of animals following 10 days of therapy, and we noted a higher relapse rate among vancomycin-treated animals than among those treated with other regimens. Judged by these findings, vancomycin might not be an optimal choice for treatment of such infections, at least at the dosing schedule described here.

Imipenem demonstrated an almost 99.9% bactericidal effect over 24 h in vitro and was effective in vivo in reducing bacterial titers within vegetations. Nevertheless, only one-third of the animals had sterile vegetations after 5 days of therapy with this agent. Previous models of enterococcal (not β-lactamase-producing or highly gentamicin-resistant strains) endocarditis in rabbits have yielded unimpressive results with this agent alone. In one study, the drug was no better than penicillin alone over 3 days of therapy (2). In another, although imipenem reduced bacterial titers to levels seen with penicillin-gentamicin combinations over 5 days of therapy, bacteriologic relapse following cessation of therapy occurred in 80% of imipenem-treated animals in contrast to 28% receiving combination therapy (18).

TABLE 3. Outcome of 10-day treatment courses for experimental enterococcal endocarditis

Outcome category[a]	No. of sterile valves/no. of rats examined (%)		
	Ampicillin-sulbactam	Vancomycin	Daptomycin
Completed 10-day therapy	5/11 (46)	1/15 (7)	6/10 (60)
Died, day 11–20	1/6	0/7	1/3
Sacrificed on day 21	4/5 (80)	1/8 (13)	5/7 (71)

[a] After 10 days of therapy, rats were observed without treatment during days 11 to 20. Rats surviving this observation period were sacrificed on day 21.

The relative success of daptomycin in this study stands in contrast to results of previous studies using this drug in the treatment of enterococcal pyelonephritis (17) or endocarditis (3). Possible reasons for differences between our results and those of Bush et al. (3) include differences in duration of treatment (3 versus 5 days), biological properties of the individual strains used, and the fact that in the rabbit model, daptomycin was administered by injection twice daily. Our mean levels in serum with doses of 25 mg/kg per day (approximately 20 μg/ml) were within the range of levels in serum attained in the other study (peak level, 44 μg/ml; serum half-life, 5.8 h).

In our relapse model, approximately one-half of animals receiving at least a 10-day course of treatment with ampicillin-sulbactam or daptomycin had no evidence of residual infection. Of animals surviving a full 10 days beyond completion of this extended treatment regimen, 70 to 80% had sterile values. Although numbers of animals surviving for this period are relatively small, these results are particularly promising in light of the fact that the transvalvular catheter remained in place for the duration of the experiment. We emphasize, in addition, that these results were derived by using only one β-lactamase-producing, highly gentamicin-resistant enterococcal isolate. It is conceivable that other strains would have responded differently to the regimens employed here; thus, appropriate caution should be exercised in applying these results in a clinical setting.

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