

Chemoprophylactic Efficacy against Experimental Endocarditis Caused by β -Lactamase-Producing, Aminoglycoside-Resistant Enterococci Is Associated with Prolonged Serum Inhibitory Activity

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We studied the prevention of experimental aortic endocarditis caused by a β -lactamase-producing, aminoglycoside-resistant strain of *Enterococcus faecalis* (HH22) in 146 catheterized rabbits. Both vancomycin and ampicillin-sulbactam readily killed this resistant enterococcus strain in vitro. At a challenge inoculum of $\sim 10^9$ CFU, vancomycin (40 mg/kg intravenously [i.v.]), ampicillin (40 mg/kg i.v.), or a combination of ampicillin plus a β -lactamase inhibitor, sulbactam (20 mg/kg, i.v.), did not prevent the development of endocarditis in any of the animals, although mean intravegetation bacterial densities were significantly lower in animals that received vancomycin than they were in animals that received other therapies ($P < 0.001$). At a challenge inoculum of 10^6 CFU, vancomycin was 100% effective in preventing enterococcal endocarditis compared with ampicillin (29%; $P < 0.00001$) and ampicillin-sulbactam (65%; $P < 0.01$). Factors associated with the superior prophylactic efficacy of vancomycin in this model included prolonged serum inhibitory activity and time above MICs. Factors not associated with the antienterococcal prophylactic efficacy of vancomycin included the duration of the in vitro postantibiotic effect of the drug and the magnitude of the ability of this drug to enhance enterococcal in vitro opsonophagocytic killing by polymorphonuclear leukocytes. The superior prophylactic efficacy of vancomycin in this endocarditis model related to the superior pharmacokinetic profile of the drug when it was given intermittently at dose intervals of every 6 h.

Until the early 1970s, most enterococcal isolates were both moderately susceptible to penicillin and were able to be killed synergistically by combinations of penicillin plus aminoglycosides (13). Plasmid-mediated aminoglycoside resistance has emerged widely among clinical enterococcal isolates, precluding a bactericidal synergism between cell wall-active antibiotics and aminoglycosides against such strains (4, 12). More recently, investigators from diverse geographic areas have reported the isolation of enterococcal strains with plasmid-mediated production of both aminoglycoside-modifying enzymes as well as β -lactamase (15). These strains have been involved in nosocomial colonization and clinical infectious syndromes, including endocarditis (17; E. Rhinehart, C. Wennersten, E. Gorss, G. Eliopoulos, R. C. Moellering, N. Smith, and D. Goldmann, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1073, 1988). The optimal therapy for human enterococcal infections caused by aminoglycoside-resistant, β -lactamase-producing strains is not known; similarly, the recommended prophylaxis for endocarditis caused by such strains remains undefined. Preliminary experiences in experimental endocarditis models suggest that either vancomycin or combinations of penicillin or ampicillin plus a β -lactamase inhibitor (e.g., sulbactam or clavulanate) afford adequate efficacy against aminoglycoside-resistant, β -lactamase-producing enterococci (7).

The current study was designed to (i) determine the role of vancomycin and the combination of ampicillin-sulbactam in preventing experimental aortic enterococcal endocarditis caused by an aminoglycoside-resistant, β -lactamase-producing strain and (ii) define the mechanism(s) involved in the

prophylactic efficacy against this form of experimental enterococcal endocarditis.

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MATERIALS AND METHODS

Organism. The enterococcal strain used in this investigation, *Enterococcus faecalis* HH22 (kindly supplied by Barbara Murray, Houston, Tex.), has been well characterized in detail (15). It is an *E. faecalis* isolate which exhibits high-level aminoglycoside and penicillin resistance by virtue of its plasmid-mediated production of modifying and hydrolyzing enzymes, respectively (16).

Antibiotics. Vancomycin was supplied by Eli Lilly Research (Indianapolis, Ind.), while ampicillin and sulbactam were gifts from Pfizer Pharmaceuticals (New York, N.Y.). Stock solutions of each agent (1,000 μ g/ml) were kept at -70°C until they were thawed on the day of in vitro testing. For use in animal treatments, antibiotics were reconstituted just prior to administration.

Antibiotic susceptibility testing. The MICs and MBCs of vancomycin, sulbactam, and ampicillin for strain HH22 were determined in brain heart infusion (BHI) broth by a macrodilution technique (18) by using logarithmic-growth-phase inocula of HH22. MICs and MBCs were determined by both $\sim 10^5$ and 10^8 CFU of inocula per ml to encompass the intravegetation bacterial counts observed in established experimental enterococcal endocarditis (7); in addition, β -lactamase production by HH22 is maximal at the higher inocula in vitro (15). Moreover, we wanted to evaluate the relative antienterococcal efficacy of vancomycin versus those of ampicillin with and without sulbactam in vitro in an inoculum dose-response fashion. The range of final concen-

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trations of vancomycin, ampicillin, and sulbactam tested were 0.25 to 512 $\mu\text{g/ml}$. The MIC of each agent was the lowest antibiotic concentration resulting in no visible turbidity after 24 h of incubation at 37°C. The MBC of each antibiotic was determined at 24 h by spreading and subculturing 10 μl from each clear MIC tube with a sterile glass rod onto antibiotic-free BHI agar plates and then incubating the plates for 48 h at 37°C. The MBC was defined as the lowest drug concentration that caused $\geq 99.9\%$ reduction of the initial inoculum. For MBC testing at 10^5 CFU of inoculum per ml, the rejection value involved a single surviving colony; thus, we were at the sensitivity limits of this technique. In parallel experiments, MICs and MBCs were determined as described above by using 50% normal rabbit serum as the diluent in lieu of BHI broth.

The *in vitro* bactericidal effects of ampicillin-sulbactam against strain HH22 were compared with those of ampicillin, sulbactam, and vancomycin alone by the time-kill curve technique. A final inoculum of $\sim 10^7$ logarithmic-phase HH22 cells per ml was incubated with (final antibiotic concentrations) vancomycin (10 $\mu\text{g/ml}$), ampicillin (32 $\mu\text{g/ml}$), sulbactam (20 $\mu\text{g/ml}$), or ampicillin-sulbactam. The high HH22 inoculum represented bacterial densities that are readily achieved within cardiac vegetations *in vivo* (8). The antibiotic concentrations used encompassed peak levels in serum that were achieved in pilot pharmacokinetic studies by using those dose regimens that were subsequently used in the experimental endocarditis investigation.

The fall in CFU/ml of $\geq 2 \log_{10}$ units below that effected by ampicillin or sulbactam alone after 24 h of exposure to ampicillin-sulbactam was defined as evidence of an enhanced bactericidal effect of the drug combination (13).

***In vitro* PAE.** The presence of a persistent, sublethal postantibiotic effect (PAE) was determined for vancomycin, ampicillin, and the combination of ampicillin-sulbactam by a modification of the technique of McDonald et al. (10). HH22 in the late logarithmic growth phase in BHI broth was diluted 1:10 in fresh BHI containing no antibiotics, vancomycin, ampicillin, or ampicillin-sulbactam to achieve (i) a final inoculum of 10^7 CFU/ml, (ii) final vancomycin and ampicillin concentrations 10 times the respective MICs of the drugs, and (iii) a final sulbactam concentration of 20 $\mu\text{g/ml}$. After incubation of these tubes for 2 h at 37°C, the antibiotics were removed by centrifugation of the organism at $15,000 \times g$ for 10 min and subsequent washing of the cell pellets twice with normal saline solution (1). After washing, the cell pellets were suspended in fresh, antibiotic-free BHI broth to the original volume (20 ml). Quantitative cultures were obtained from each tube at 0 h (preincubation), 2 and 3 h (before and after antibiotic removal, respectively), hourly for 5 h after antibiotic removal, and 15 h after antibiotic removal. Curves were then constructed relating time to viable HH22 counts. The PAE duration (in hours) was defined as the difference in the time required for the antibiotic-exposed and unexposed HH22 cells to increase in counts by $1 \log_{10}$ CFU/ml above the number present just after the centrifugation-washing-resuspension step (1). For each antibiotic regimen tested, four separate PAE experiments were performed to generate a mean \pm standard error of the mean PAE.

Postantibiotic leukocyte enhancement. We examined the susceptibilities of strain HH22 cells to polymorphonuclear leukocyte (PMN)-mediated opsonophagocytosis *in vitro* following brief exposure of the growing organisms to vancomycin, ampicillin, or ampicillin-sulbactam by the method of McDonald et al. (11). Blood from healthy volunteers who were not taking any medications was drawn into heparinized

tubes. After thorough mixing, the heparinized blood was added to tubes containing Ficoll-Hypaque (Mono-Poly Resolving Media; Flow Laboratories, Inc., McLean, Va.). Tubes were then centrifuged at $300 \times g$ for 60 min. The PMN-rich layer was then drawn off into 50-ml polypropylene tubes. Contaminating erythrocytes were removed via a lysing buffer-centrifugation step. PMNs were washed twice and then suspended in Eagle minimal essential medium (MEM) with 0.1% gelatin. The PMN suspension was adjusted to a concentration of $\sim 10^7$ cells per ml in a hemacytometer counting chamber. PMN viability was determined by trypan blue exclusion; preparations exhibiting $>95\%$ viability were used in these studies. For each experiment, freshly collected PMNs from a single donor were used.

Pooled normal human serum, which was used for opsonization of strain HH22, from five healthy donors was prepared and stored at -20°C . Pooled normal human serum was thawed on the day of use and was used at a final dilution of 10% in opsonophagocytic assays.

For assessment of postantibiotic leukocyte enhancement (PAE), HH22 was grown to the logarithmic phase, washed twice in normal saline, and suspended in MEM-gelatin to a concentration of $\sim 10^9$ CFU/ml (optical density at 600 nm, 1.0); dilution to a final concentration of $\sim 10^7$ CFU/ml was then performed. All washing and dilution steps were carried out at 37°C. To the bacterial-containing suspension (0.5 ml) was added 0.5 ml of either antibiotic-containing or antibiotic-free MEM-gelatin for 10 min at 37°C; final antibiotic concentrations were identical to those described above for use in the PAE determinations. Previous studies have indicated that antibiotic-induced bacterial sensitization is effective by 10 min of exposure (11), while it still allows for the presence of adequate numbers of surviving organisms for assessment of PAE.

At the end of the 10-min incubation period, tubes were microcentrifuged at 10,000 rpm ($22,500 \times g$), and the resulting cell pellet was washed twice in phosphate-buffered saline to remove residual antibiotics (dilution factor of the antibiotic[s], $>1,000$). Portions (0.1 ml) of antibiotic-sensitized or unsensitized HH22 cells ($\sim 10^7$ CFU/ml) were added to 0.9-ml mixtures in MEM of PMN alone, pooled normal human serum alone, MEM alone (positive growth control), or PMN-pooled normal human serum. The final ratio of bacterial cells:PMNs in the reaction mixtures was $\sim 5:1$. Assessments of the PAEs of vancomycin, ampicillin, and ampicillin-sulbactam were performed at different experimental sessions. At 0, 60, 120, and 180 min of incubation at 37°C on a rotator (10 rpm), 50- μl portions from all reaction tubes were added to sterile distilled water to lyse PMNs. At postlysis, portions were quantitatively cultured onto sheep blood agar plates. After incubation at 37°C for 48 h, surviving HH22 colonies were counted; survival curves were then constructed in which the mean \log_{10} CFU/ml of surviving HH22 cells in the various reaction mixtures were compared over time. For each antibiotic regimen tested, four to six separate PAE experiments were performed.

Experimental endocarditis. The experimental rabbit model was used to assess the prophylactic efficacies of the drugs against strain HH22 aortic endocarditis. One-hundred forty-six New Zealand White rabbits underwent transaortic catheterization as described previously (19), and then 24 h later they were randomly assigned to receive either no therapy or their first prophylactic antibiotic dose. Thirty minutes after they received the first prophylactic antibiotic dose, animals were challenged intravenously (*i.v.*) with an inoculum of HH22 cells of either 10^6 or 10^9 CFU. It is unlikely that

humans with underlying valvular heart disease experience bacteremic inocula of 10^9 CFU during invasive medical-surgical procedures. However, we challenged catheterized rabbits with enterococcal inocula of both 10^6 and 10^9 CFU in order to evaluate the various prophylactic drug regimens in a dose-response fashion, as in the *in vitro* studies described above. In parallel experiments, animals received either single-dose or multiple-dose prophylactic antibiotic regimens. During individual experimental sessions, animals were given either the 10^6 or the 10^9 enterococcal inoculum to ensure uniformity of the challenge inoculum in all prophylactic groups. Antibiotic dose strategies included ampicillin or vancomycin (40 mg/kg *i.v.* infusion for either one dose or every 6 h for four doses); or ampicillin (40 mg/kg)-sulbactam (20 mg/kg) given as either one dose or four doses every 6 h by *i.v.* infusion. Intermittent 6-h dose interval strategies were designed to reflect those in current clinical use, as recommended by the American Heart Association (22). The vancomycin dose regimen was designed to achieve peak levels of drug in serum in excess of the MBC for HH22; the ampicillin and sulbactam dose regimens were designed to achieve peak levels of each agent in serum in excess of those required to produce *in vitro* bactericidal synergy. A prophylactic treatment group with sulbactam alone was not included in this study because the β -lactamase inhibitor exhibits no intrinsic antibacterial activity against HH22 *in vitro*.

For assessment of prophylactic efficacy, all animals were sacrificed by *i.v.* sodium pentobarbital overdosage 48 h following the last drug dose to minimize antibiotic carry-over effects. At the time of sacrifice, the heart was removed, and the chambers on the left side were examined for confirmation of both catheter position and macroscopic vegetative endocarditis on the aortic valve and in the left ventricle. All vegetations from a single animal were removed, pooled, weighed, homogenized, and quantitatively cultured as described previously by Carrizosa and Kaye (5); for calculation of the mean bacterial densities per gram of vegetation, culture-negative vegetations were considered to contain $2 \log_{10}$ CFU/g (5).

Antibiotic pharmacokinetics in serum. The serum pharmacokinetics of vancomycin, ampicillin, sulbactam, and ampicillin-sulbactam were determined in catheterized animals following the first prophylactic antibiotic dose *i.v.* Samples were obtained at 15, 30, 60, 120, 180, 240, 300, and 360 min and at 24 h postdose and analyzed for the pertinent drug levels by high-pressure liquid chromatography (courtesy of Roger Bawdon, Houston, Tex.). The assay for each agent had <10% day-to-day variability. The β half-life ($t_{1/2}$) was determined by the least-squares method, while the area under the concentration-time curve (AUC) was calculated by the linear trapezoidal rule method.

Serum inhibitory and bactericidal activities. The serum samples obtained for the drug level determinations described above were also used for determination of serum inhibitory and bactericidal titers (SITs and SBTs, respectively), as determined by the microdilution method of Prober et al. (20). Heat-inactivated (56°C, 30 min), freshly pooled normal rabbit serum was used as the diluent. The strain HH22 inoculum was $\sim 10^5$ CFU/ml. The SIT was defined as the highest serum dilution that visually inhibited turbidity after 24 h of incubation at 37°C. After determination of the SITs, 25 μ l was subcultured from each clear well onto blood agar plates, which were incubated for 24 h for determination of the SBT. SBT was defined as the highest serum dilution that killed $\geq 99.9\%$ of the initial HH22 inoculum (20).

Statistical analysis. The Fisher exact test was used for

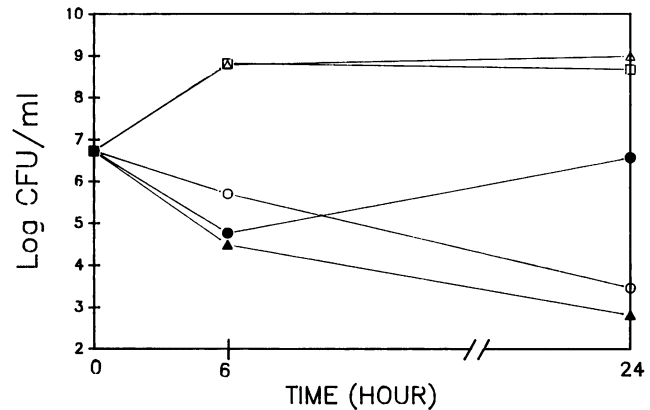


FIG. 1. Time-kill curve of ampicillin with and without sulbactam versus that of vancomycin against enterococcal strain HH22. Symbols: Δ , sulbactam; \square , control; \bullet , ampicillin; \circ , vancomycin; \blacktriangle , ampicillin-sulbactam.

comparing proportional data, while analysis of variance was used for comparing the differences between continuous data (e.g., the \log_{10} number of CFU per gram of vegetations and geometric mean reciprocal SITs and SBTs). The Bonferroni test was used, when appropriate, for pairwise comparisons, while the nonparametric Kruskal-Wallis test was used to evaluate results of the analysis of variance.

RESULTS

Antibiotic susceptibility testing. The MICs and MBCs (in micrograms per milliliter) of the three study antibiotics tested at $\sim 10^5$ CFU of inoculum per ml were as follows: ampicillin, 2 and 32; sulbactam, >512 and >512; and vancomycin 2 and 32, respectively. When tested at $\sim 10^8$ CFU of inoculum per ml, the MICs and MBCs were as follows: ampicillin, 256 and >512; sulbactam, >512 and >512; and vancomycin, 128 and 256, respectively. The use of 50% rabbit serum as a diluent instead of BHI broth in these determinations did not change the MICs and MBCs significantly.

Kill curves (Fig. 1) demonstrated and enhanced *in vitro* bactericidal effect of ampicillin-sulbactam against strain HH22.

***In vitro* PAE.** Vancomycin, ampicillin, and ampicillin-sulbactam did not exert a bactericidal effect against HH22 during the first 2 h of incubation (Fig. 2). There were no significant differences between the PAEs determined for ampicillin, vancomycin, or ampicillin-sulbactam ranging from 1.5 to 1.7 h.

PAE. Strain HH22, in the absence of antibiotic sensitization, was efficiently killed by PMNs via opsonophagocytic mechanisms, with a mean survival at 180 min of $\sim 40\%$ versus the number of organisms present at 0-h inoculum; in contrast, HH22 cells were refractory to nonopsonic PMN-mediated killing. Irrespective of the regimen that was used, antibiotic pretreatment resulted in an enhanced susceptibility of HH22 cells to PMN-induced opsonophagocytic killing compared with that of antibiotic-unsensitized cells (Fig. 3 and 4). Reductions in the percentage of HH22 cells that survived opsonophagocytic killing by PMNs at 180 min in antibiotic-pretreated versus those in untreated regimens were 49% for ampicillin, 40% for ampicillin-sulbactam, and 43% for vancomycin; there were no significant differences in these percent reductions in surviving HH22 counts in the

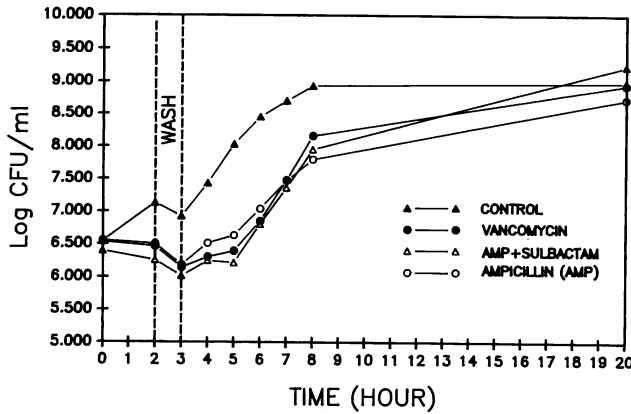


FIG. 2. PAE durations for ampicillin with and without sulbactam versus that for vancomycin against enterococcal strain HH22.

various antibiotic presensitization groups. Use of decomplexed human serum abrogated the ability of PMNs to kill HH22 cells opsonophagocytically, whether or not they were presensitized.

Antibiotic pharmacokinetics in serum. The pharmacokinetics of the three antibiotic study regimens in serum are given in Table 1. The AUCs and $t_{1/2}$ values were substantially greater for vancomycin than they were for ampicillin or sulbactam. Similarly, the mean time above MIC was substantially longer for vancomycin (9 h) than it was for either ampicillin or sulbactam ($P < 0.01$). Neither ampicillin nor sulbactam achieved supra-MBC levels postdose, while the mean time above MBC was 2 h postdose for vancomycin (Fig. 5). For ampicillin and sulbactam, the mean peak drug levels were seen at 15 min postdose and were 31 and 34 $\mu\text{g/ml}$, respectively. Neither drug was detectable at trough samplings (6 h postdose).

SITs and SBTs. A comparison of the SITs induced by the three antibiotic regimens are depicted in Fig. 6. For animals given vancomycin, SITs were $\geq 1:8$ for at least 4 h postdose, with a mean reciprocal geometric titer of 38.6 ± 3.9 over the 6-h postdose interval. The geometric mean reciprocal serum inhibitory activity induced by vancomycin was significantly greater than that observed with ampicillin (2.14 ± 1.23 ; $P < 0.0001$) or ampicillin-sulbactam (9.44 ± 4.1 ; $P < 0.001$).

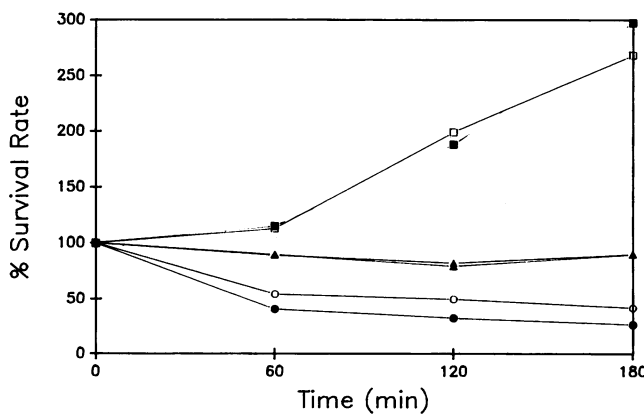


FIG. 3. PALE of PMN-mediated opsonophagocytosis of enterococcal strain HH22 following brief exposure (10 min) of bacterial cells to ampicillin-sulbactam. Symbols: ■, ampicillin-sulbactam-serum; □, serum alone; Δ, PMNs; ▲, ampicillin-sulbactam-PMNs; ○, PMNs-serum; ●, ampicillin-sulbactam-PMNs-serum.

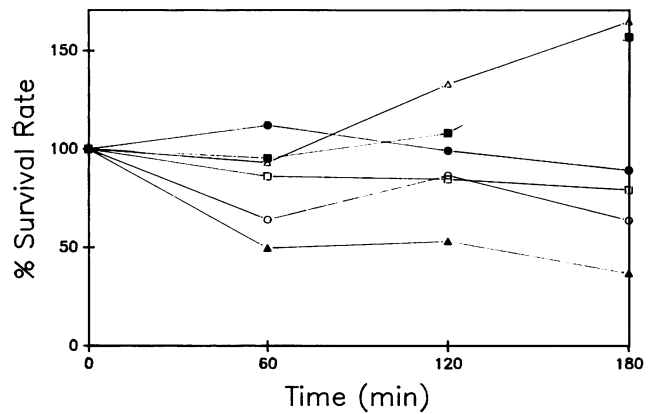


FIG. 4. PALE of PMN-mediated opsonophagocytosis of enterococcal strain HH22 following brief exposure (10 min) of bacterial cells to vancomycin. Symbols: Δ, serum; ■, vancomycin-serum; ●, PMNs; □, vancomycin-PMNs; ○, PMNs-serum; ▲, vancomycin-PMNs-serum.

Serum inhibitory activity induced by ampicillin-sulbactam never exceeded a 1:8 titer beyond 30 min postdose, while for ampicillin alone, the serum inhibitory activity was barely measurable above a 1:2 titer at any time postdose. Similar findings were noted for SBTs (data not shown). For vancomycin, SBTs of at least 1:8 were seen in all animals for at least 120 min postdose, while for animals given ampicillin-sulbactam, SBTs rarely exceeded 1:8, however, this difference in SBTs observed between the vancomycin and ampicillin-sulbactam regimens did not reach statistical significance. Ampicillin alone induced no measurable serum bactericidal activity postdose in any animals.

Prophylaxis of experimental enterococcal endocarditis. Table 2 summarizes the comparative results of the three antibiotic regimens in preventing experimental aortic enterococcal endocarditis caused by strain HH22. At the higher challenge inoculum in catheterized rabbits ($\sim 10^9$ CFU), neither vancomycin nor ampicillin alone or with sulbactam was able to prevent the development of HH22-induced enterococcal endocarditis in any animal whether single-dose or multiple-dose prophylactic strategies were used. Of interest, mean \log_{10} bacterial densities (\pm standard deviations) in vegetations from animals given vancomycin (5.24 ± 2.3) were significantly lower than those from control animals or those given either ampicillin or ampicillin-sulbactam (mean \log_{10} densities, >9.3 in each group; $P < 0.001$).

At strain HH22 challenge inocula of $\sim 10^6$ CFU, enterococcal endocarditis was prevented in significantly more animals given ampicillin-sulbactam by either single-dose or multiple-dose strategies (65%) than was prevented in animals given ampicillin alone (29%; $P < 0.05$). Vancomycin given as

TABLE 1. Pharmacokinetic and pharmacodynamic parameters of ampicillin, sulbactam, and vancomycin in serum in experimental enterococcal endocarditis prophylaxis

Agent (i.v. dose [mg/kg])	$t_{1/2}$ (h)	AUC ($\mu\text{g} \cdot \text{h/ml}$)	Time (h) above MIC/MBC
Ampicillin (40)	0.19	18.9	2/0
Sulbactam (20)	0.34	27.5	0/0
Vancomycin (40)	1.01	180.9 ^a	9 ^b /2

^a $P < 0.001$ versus other regimens.

^b $P < 0.01$ versus other regimens.

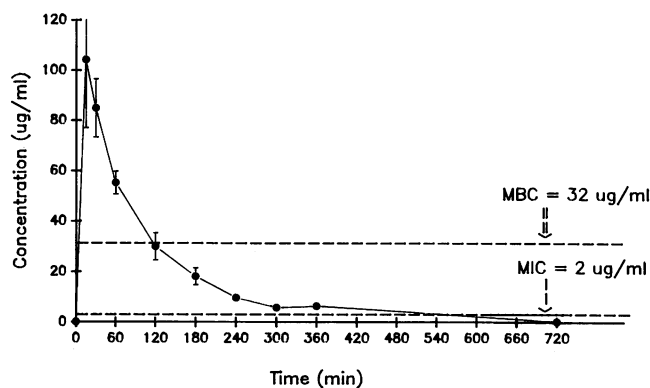


FIG. 5. Vancomycin time-concentration pharmacokinetic curve after a 40-mg/kg i.v. bolus dose was given to rabbits with experimental endocarditis caused by enterococcal strain HH22.

a single or multiple prophylactic dose regimen prevented enterococcal endocarditis in 100% of animals, a preventative rate that was significantly greater than that seen overall with either ampicillin regimen ($P < 0.01$).

DISCUSSION

Enterococci account for ~10 to 20% of all cases of endocarditis, most of which occur on the aortic or mitral valve (9). Among the cases of enterococcal endocarditis, ~40% can be linked to a previous invasive genitourinary or gastrointestinal procedure performed within the usual incubation period of endocarditis (~2 to 6 weeks [8, 23]). These observations have made enterococcal endocarditis a prime candidate for prophylactic antibiotic regimens in patients with underlying valvular heart disease who are undergoing invasive genitourinary or gastrointestinal procedures (22). The recommended regimens for preventing enterococcal endocarditis in such settings have been synergistic combinations of either parenteral ampicillin or vancomycin (for penicillin-allergic patients) plus gentamicin (22). However, the recent widespread nosocomial isolation of enterococci exhibiting plasmid-mediated, high-level resistance to aminoglycosides, with or without resistance to penicillins by virtue

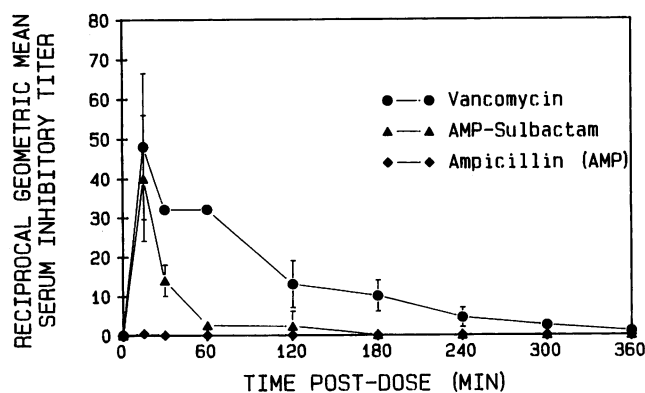


FIG. 6. Serum inhibitory activities induced by ampicillin with and without sulbactam versus that induced by vancomycin following an i.v. bolus dose of each drug regimen. Data are shown as the geometric mean \pm standard error of the mean of the reciprocal titers seen at each specified time point after administration of the antibiotic dose.

TABLE 2. Comparative prophylactic efficacy of vancomycin versus that of ampicillin with or without sulbactam against experimental aortic enterococcal endocarditis in rabbits

Regimen (i.v. dose [mg/kg])	Challenge inoculum (CFU)	Dose strategy	No. in which endocarditis prevented/total no. of challenged (% prophylactic efficacy)
Control	10^9		0/17 (0)
	10^6		0/10 (0)
Ampicillin (40)	10^9	Single	0/8 (0)
		Multiple	0/17 (0)
	10^6	Single	1/9 (12) ^a
		Multiple	4/9 (44) ^a
Ampicillin (40)-sulbactam (20)	10^9	Single	0/9 (0)
		Multiple	0/17 (0)
	10^6	Single	6/9 (67) ^b
		Multiple	5/8 (63) ^b
Vancomycin (40)	10^9	Single	0/8 (0)
		Multiple	0/8 (0)
	10^6	Single	9/9 (100) ^{a,b}
		Multiple	9/9 (100) ^{a,b}

^a $P < 0.00001$ for vancomycin versus ampicillin regimens.

^b $P < 0.01$ for vancomycin versus ampicillin-sulbactam regimens.

of β -lactamase production, has challenged traditional prophylactic antibiotic strategies for enterococcal endocarditis.

In the present study we examined the comparative roles of vancomycin versus a combination of a β -lactam (ampicillin) and a β -lactamase inhibitor (sulbactam) in preventing experimental aortic enterococcal endocarditis caused by an aminoglycoside-resistant, β -lactamase-producing strain. Several findings emanated from this investigation. Neither vancomycin nor ampicillin with or without sulbactam prevented the development of endocarditis in catheterized animals challenged with a high enterococcal inoculum (10^9 CFU). This is in concert with this organism's in vitro inoculum effect showing increased MICs and MBCs above levels that are achievable in serum for the three antibiotics studied. It is noteworthy that, although it did not prevent the development of enterococcal endocarditis following a high-inoculum challenge, vancomycin prophylaxis was associated with a significant reduction in intravascular bacterial densities compared with ampicillin-sulbactam regimens. This was most likely due to either a vancomycin-induced intravascular killing of enterococci contained within the initial challenge or by virtue of the prolonged serum $t_{1/2}$ and serum inhibitory activity associated with vancomycin prophylaxis in comparison with those of the other antibiotic regimens that were evaluated. At a lower challenge inoculum (10^6 CFU), the addition of sulbactam to ampicillin significantly improved the prophylactic efficacy over that seen with ampicillin alone. However, vancomycin was universally effective at preventing enterococcal endocarditis caused by this multiresistant strain whether given by single-dose or multiple-dose strategies. These findings are in agreement with those of Ingerman et al. (7), who found that vancomycin and a β -lactam (penicillin)- β -lactamase inhibitor (clavulanate) combination were the most effective regimens in treating established enterococcal aortic endocarditis in rats caused by a multiresistant strain similar to the one we used.

The mechanism(s) by which vancomycin exerted its superior prophylactic efficacy compared with that of ampicillin-sulbactam in the current study was also investigated. Glauser and colleagues (2, 6, 14) and Scheld et al. (21) have

characterized three possible steps that are involved in the prophylactic efficacy of antibiotics against endocarditis, including initial intravascular killing of challenge inocula at supra-MBC levels in serum, antiadherence effects exerted on the challenge organisms that prevent initial vegetation surface lodgement, and prolonged growth inhibition of vegetation surface-adherent organisms. Elegant studies by Moreillon et al. (14) suggested that the ability of the antibiotic regimens to be present at supra-MICs in serum while inducing prolonged serum inhibitory activity correlates best with prophylactic efficacy against experimental viridans group streptococcal endocarditis. Our results tend to support this concept. The best correlates of the superior prophylactic efficacy of vancomycin in the present study were its significantly greater AUC and serum inhibitory activity, as well as a significantly longer time above MIC, compared with those of ampicillin-sulbactam. Factors that are apparently unrelated to prophylactic efficacy in our endocarditis model were the duration of the PAE and the magnitude of the PALE effects, which were each of the same magnitude in vancomycin- and ampicillin-sulbactam-treated animals. We did not specifically examine the comparative effects of vancomycin versus ampicillin-sulbactam on enterococcal valvular adherence, since Moreillon et al. (14) have convincingly eliminated this factor as being of importance in streptococcal endocarditis prophylaxis. How does prolonged serum inhibitory activity render prophylactic efficacy? Initially, it was postulated that growth-inhibited surface organisms would be susceptible to *in vivo* postantibiotic leukocyte-enhanced opsonophagocytic killing mechanisms (11, 14). However, our data showing equivalent *in vitro* PALEs for the three prophylactic regimens do not support this hypothesis. Moreover, recent work by Berney and Francioli (3) in the neutropenic endocarditis model confirms a maintenance of prophylactic efficacy, even though antibiotics were administered after bacterial challenge (postadherence phase) in the absence of circulating PMNs. The factor(s) responsible for the eradication of growth-inhibited, vegetation surface-adherent organisms to render prophylactic efficacy in endocarditis remain undefined.

In the present study, the superior prophylactic efficacy of vancomycin compared with those of the ampicillin-sulbactam and ampicillin alone regimens seemed to be related to the prolonged serum inhibitory activities induced by this agent; the inferior serum inhibitory activities seen with ampicillin-sulbactam and ampicillin alone were, in turn, associated with the relatively rapid clearance of these drugs. If prolonged growth inhibition of valve surface-adherent bacteria is, in fact, the major determinant in rendering prophylactic efficacy against endocarditis, then optimal prophylactic dose intervals should not exceed the sum of the two pharmacodynamic parameters, time above MIC and PAE duration, for a particular agent. Based on the data generated in this study, this optimal dose interval for ampicillin-sulbactam should be ≤ 3.5 h (time above MIC, 2 h; PAE, 1.5 h). We are examining the value of such pharmacodynamic parameters in predicting prophylactic efficacy against experimental enterococcal endocarditis.

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