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Antimicrobial Therapy of Experimental Enterococcal Endocarditis

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The successful therapy of enterococcal endocarditis requires prolonged administration of synergistic antibiotic combinations. Controversy has arisen regarding optimal therapy (i) when the organism possesses high-level streptomycin resistance, and (ii) when the patient is allergic to penicillin. This study examines these questions in vitro and in a rabbit model of enterococcal endocarditis. The combination of penicillin with either streptomycin or gentamicin increased the rate of bacterial killing in vitro and in vivo when compared with penicillin alone ($P < 0.05$) when the test strain was relatively susceptible to streptomycin (minimal inhibitory concentration, 128 μg/ml). Only the combination of penicillin and gentamicin was consistently more effective than penicillin alone ($P < 0.01$) when the test strain was highly resistant to streptomycin (minimal inhibitory concentration > 150,000 μg/ml). The combination of vancomycin and streptomycin was more rapidly bactericidal than vancomycin alone in vitro and in the animal model against the streptomycin-susceptible strain ($P < 0.01$). The relative rate of in vitro bacterial killing by various antibiotics and combinations was predictive of the efficacy of these drugs in eradicating enterococci from cardiac vegetation in experimental endocarditis.

The antimicrobial therapy for enterococcal endocarditis, unlike endocarditis caused by penicillin-susceptible bacteria, remains a therapeutic challenge. The protection afforded bacteria by cardiac vegetations and the relative resistance of enterococci to penicillin make long-term, high-dose parenteral therapy with synergistic antibiotic combinations necessary for the successful treatment of this disease. The recommended therapy for enterococcal endocarditis has been high-dose penicillin G with streptomycin for a period of 6 weeks (6). The presence of high-level streptomycin resistance (minimal inhibitory concentration [MIC] > 2,000 μg/ml), however, has recently been reported in 13 to 40% of enterococcal isolates (8, 13). In vitro antibiotic synergism of penicillin plus streptomycin has not been demonstrated against these highly streptomycin-resistant enterococci. Synergism, however, could be demonstrated against these strains using penicillin plus gentamicin (13, 15). Based on these findings, the latter combination has been suggested as an alternative therapy in patients with enterococcal endocarditis caused by highly streptomycin-resistant organisms (13, 15).

The therapy of enterococcal endocarditis in the penicillin-allergic patient is also a difficult therapeutic problem. Based on in vitro studies, vancomycin alone or in combination with streptomycin has been recommended (7, 14, 17). However, only a limited number of patients treated in either manner have been reported to date (2, 17).

The purpose of this study was to evaluate the efficacy of the antimicrobial therapeutic regimens recommended for treatment of enterococcal endocarditis. Bacterial killing by antibiotics was examined in vitro and in rabbits with experimental enterococcal endocarditis. Penicillin alone and in combination with either streptomycin or gentamicin was studied against two strains of enterococci, one exhibiting high-level streptomycin resistance and the other a relatively streptomycin-susceptible strain. Vancomycin alone and in combination with streptomycin was also studied using the relatively susceptible strain.

MATERIALS AND METHODS

Test organisms. Two strains of enterococci isolated from patients with bacterial endocarditis were utilized in these studies. One strain exhibited high-level resistance to streptomycin (hereafter referred to as strain R), whereas the other strain was relatively susceptible (strain S). The MICs of antibiotics for these strains are shown in Table 1.

In vitro tests of antibiotic killing. Time-kill studies were performed using the antibiotics and combi-

TABLE 1. *MICs of antibiotics for strains R and S enterococci*

Antibiotic	MIC (μg/ml)	
	Strain S	Strain R
Penicillin G	2.0	2.0
Streptomycin	128	>150,000
Gentamicin	16	16
Vancomycin	4.0	

nations mentioned below. Approximately 10^7 enterococci were incubated in tubes containing designated concentrations of antibiotics in Trypticase soy broth containing 1% sheep erythrocytes. Enterococci were prepared by dilution of an overnight culture in Trypticase soy broth to the appropriate concentration immediately before the addition of antibiotics. Aliquots were removed after 8, 24, and 48 h of incubation. Tenfold dilutions were made in sterile saline and incorporated into pour plates of Trypticase soy agar. Concentrations of antibiotics employed were penicillin, 10 μg/ml; gentamicin, 5 μg/ml; streptomycin, 10 μg/ml; and vancomycin, 10 μg/ml.

Production of bacterial endocarditis. Nonbacterial thrombotic endocarditis was produced in 2-kg male New Zealand white rabbits utilizing methods previously reported (3, 9, 10). After anesthetization with 60 mg of sodium pentabarbital intravenously, the right carotid artery was exposed and ligated cephalad, and a sterile polyethylene catheter (Intramedic; Clay-Adams, PE-90) was inserted through a small incision in the arterial wall. The catheter was advanced toward the heart until pulsation and resistance indicated that it had reached the apex of the left ventricle. The catheter was then clamped and tied to the artery with 00 silk thread, and the skin incision was closed. Strict asepsis was not observed; however, local wound infection was not noted. At 6 to 24 h after catheterization, an intravenous injection of approximately 10^7 enterococci diluted from an overnight culture in broth was given. Criteria used to indicate the existence of infection were the presence of 10^2 or greater organisms/ml in blood and fever greater than 39.6 C (rectally). These parameters have previously been shown to accurately predict the existence of streptococcal endocarditis in this animal model (10).

Administration of antibiotics. At 24 to 48 h after establishment of infection, antibiotic therapy was initiated. Procaine penicillin G (Wyeth Labs), gentamicin (Schering Corporation), and streptomycin (Pfizer) were administered intramuscularly. Vancomycin (Eli Lilly & Co.) was administered intravenously. The following antibiotic schedules were used: penicillin, 1.2×10^6 U twice a day (BID); streptomycin, 40 mg BID; gentamicin, 10 mg BID or 15 mg three times a day (TID); and vancomycin, 125 mg BID. Serum levels of antibiotics, as determined by the agar well diffusion technique, are shown in Table 2.

Efficacy of antimicrobial therapy. Animals were treated for periods of 5 or 10 days and sacrificed 12 h after the last antibiotic dose. Cardiac vegetations were aseptically removed, weighed, suspended in 9.9 ml of sterile saline containing penicillinase, and homogenized in a tissue grinder. Serial dilutions of the homogenate were incorporated in pour plates of Trypticase soy agar. Plates were incubated for 24 h at 37 C, and titers were recorded as colonies per gram of vegetation.

RESULTS

In vitro studies. The rate of killing in broth of the streptomycin-susceptible strain (strain S) by penicillin was increased with the addition of either streptomycin or gentamicin (Fig. 1A). The initial concentration of 2.5×10^7 enterococci/ml was reduced to 3.2×10^5 and 4.7×10^2/ml, respectively, after 8 and 48 h of incubation with penicillin. Penicillin plus streptomycin reduced the same initial concentration of bacteria to 1.1×10^3/ml at 8 h and 11 organisms/ml after 48 h of incubation, whereas incubation with penicillin plus gentamicin reduced the concentrations similarly to 6.0×10^2 and 5 organisms/ml after the same period. Bacterial growth was not inhibited after incubation with either aminoglycoside alone.

As shown in Fig. 2A, the addition of streptomycin to vancomycin markedly increased the rate of bacterial killing of strain S when compared with vancomycin alone. After 48 h of incubation, vancomycin had reduced the initial concentration of 2.5×10^7 enterococci/ml to 1.3×10^6 bacteria/ml. Only 6.5×10^1 organisms/ml were recovered after incubation with vancomycin plus streptomycin for the same period.

The addition of streptomycin to penicillin failed to significantly alter the rate of killing of the streptomycin-resistant strain (strain R) (Fig. 3A). The rate of killing was markedly increased by the addition of gentamicin to peni-

TABLE 2. *Serum levels of antibiotics used*

Time (h)	Serum level (μg/ml)[a]				
	Penicillin (1.2×10^6 U)	Streptomycin (40 mg)	Gentamicin (10 mg)	Gentamicin (15 mg)	Vancomycin (125 mg)
1	15.2	12.0	12.0	15.4	54
2		8.9	7.95	7.9	
3	12.1				21
4		1.8	2.5	3.4	
6	10.5		0.86		11
8	8.9	0.7	0.49	0.38	
12	7.7	0.2	0		2

[a] Penicillin, streptomycin, and gentamicin were administered intramuscularly; vancomycin was administered intravenously.

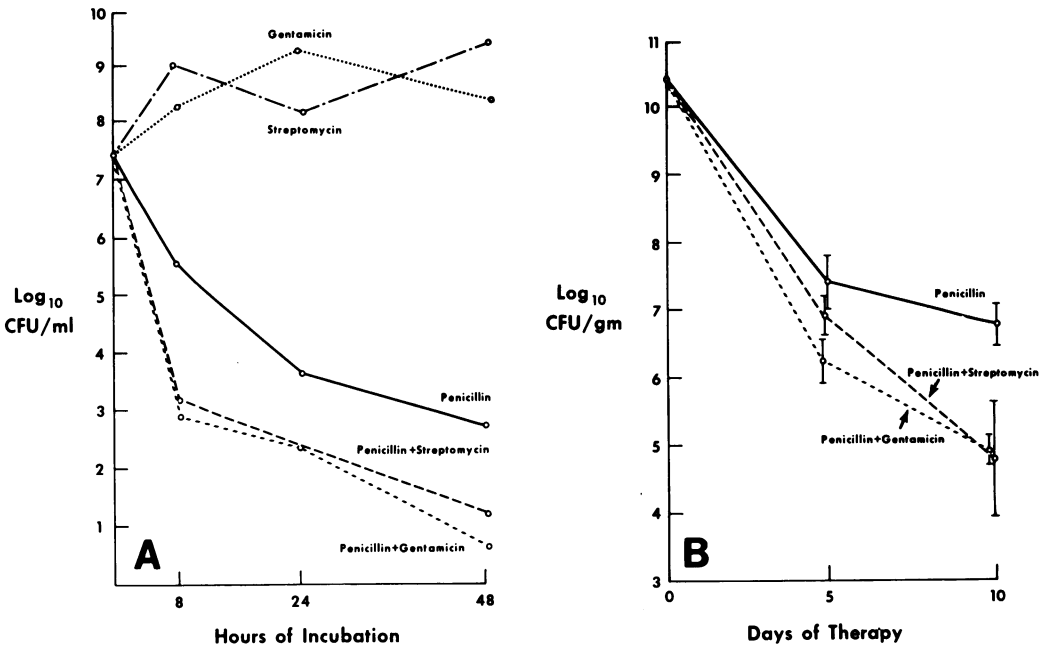

FIG. 1. *(A) Rate of killing of streptomycin-susceptible enterococci in broth by antibiotics. Concentration of antibiotics: penicillin, 10 μg/ml; gentamicin, 5 μg/ml; and streptomycin, 10 μg/ml. (B) Rate of eradication of streptomycin-susceptible enterococci from cardiac vegetations in experimental endocarditis after 5 and 10 days of antibiotic therapy (Φ represents geometric mean with standard error).*

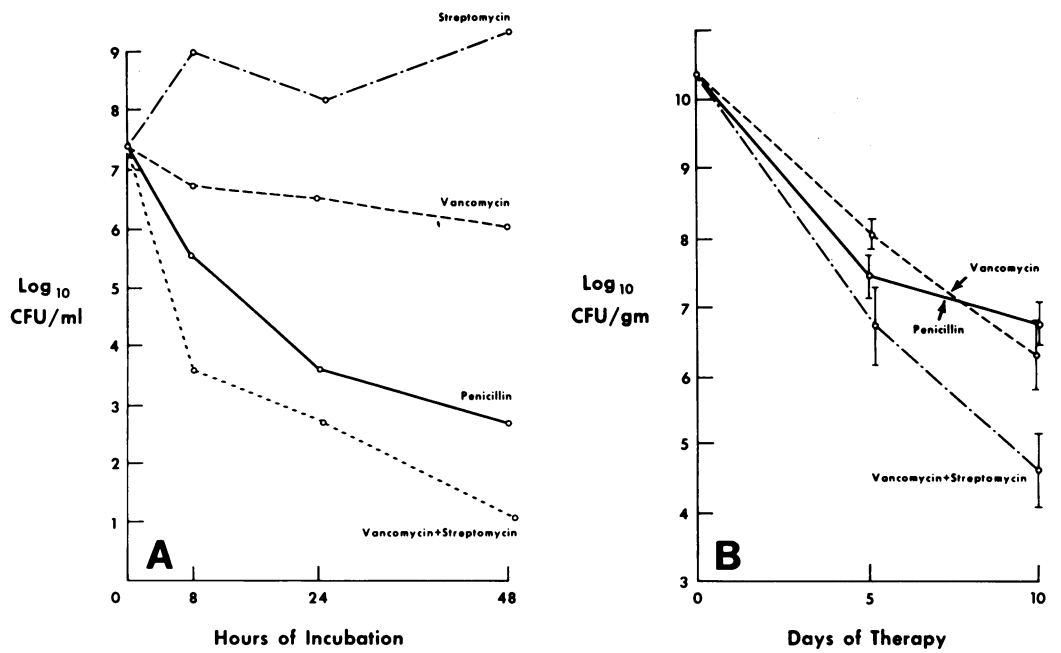

FIG. 2. *(A) Rate of killing of streptomycin-susceptible enterococci in broth by antibiotics. Concentration of antibiotics: vancomycin, 10 μg/ml; penicillin, 10 μg/ml; and streptomycin, 10 μg/ml. (B) Rate of eradication of streptomycin-susceptible enterococci from cardiac vegetations in experimental endocarditis after 5 and 10 days of therapy with antibiotics (Φ represents geometric mean with standard error).*

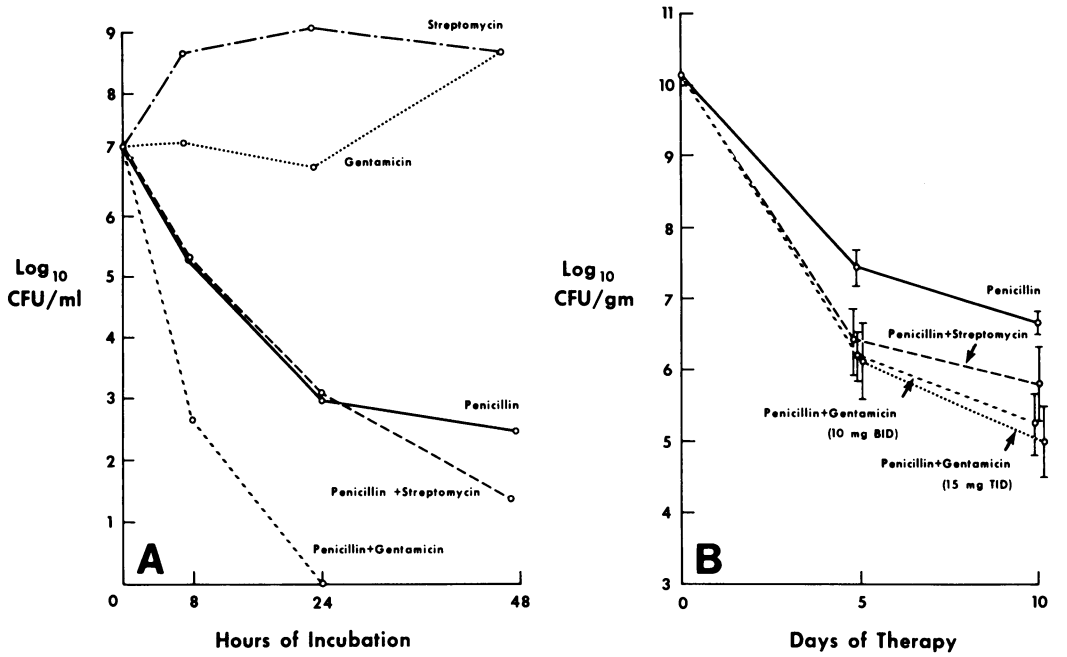

FIG. 3. *(A) Rate of killing of streptomycin-resistant enterococci in broth by antibiotics. Concentration of antibiotics: penicillin, 10 μg/ml; gentamicin, 5 μg/ml; and streptomycin, 10 μg/ml. (B) Rate of eradication of streptomycin-resistant enterococci from cardiac vegetations in experimental endocarditis after 5 and 10 days of therapy with antibiotics (Φ represents geometric mean with standard error).*

cillin. In tubes initially containing 1.8×10^7 enterococci/ml, incubation with penicillin and penicillin plus streptomycin for 8 h reduced the number of viable organisms to 1.5×10^5 and 1.6×10^5/ml, respectively. By 48 h, 4.9×10^2 and 2.2×10^1 bacteria/ml were isolated. In contrast, only 8×10^2 bacteria/ml were present at 8 h, and cultures were sterile at 24 h after incubation with penicillin plus gentamicin.

In vivo studies. Three rabbits with bacterial endocarditis caused by strain S and four rabbits with endocarditis caused by strain R were followed without antibiotic therapy. All untreated animals died within 4 days. The mean bacterial concentrations present in vegetations were in excess of 10^{10}/g of vegetation (Table 3).

Twenty-nine rabbits were infected with strain S and treated with penicillin and an aminoglycoside (Table 3, Fig. 1B). In rabbits treated with penicillin for 5 days a mean concentration of $10^{7.4}$ enterococci/g of vegetation was recovered, whereas after 10 days a mean concentration of $10^{6.8}$ organisms/g was present. Viable enterococci were recovered in mean concentrations of $10^{6.9}$ and $10^{4.8}$/g of vegetation in rabbits receiving penicillin plus streptomycin for 5 and 10 days, respectively. Similarly, from rabbits treated with penicillin plus gentamicin mean vegetation concentrations of $10^{6.2}$ bacteria/g at 5 days and $10^{4.9}$ bacteria/g at 10 days were isolated. The difference in the concentrations of organisms recovered from the vegetations of animals receiving penicillin alone between 5 and 10 days was significant ($P < 0.05$ [Student's t test]). The addition of either aminoglycoside to penicillin significantly increased the rate of bacterial killing of the streptomycin-susceptible strain at 5 and 10 days when compared with penicillin alone ($P < 0.05$). There was no significant difference in the bactericidal action of the combination of penicillin and streptomycin as compared to penicillin and gentamicin at 5 or 10 days of therapy ($P > 0.05$).

Twenty rabbits with endocarditis caused by strain S were treated with vancomycin alone or vancomycin plus streptomycin (Table 3, Fig. 2B). Rabbits receiving vancomycin had mean concentrations of $10^{8.0}$ enterococci/g of vegetation after 5 days of therapy and $10^{6.3}$ organisms/g after 10 days. In rabbits given vancomycin and streptomycin, mean concentrations were significantly reduced in comparison to vancomycin alone after 5 and 10 days of therapy to $10^{6.7}$ and $10^{4.5}$ bacteria/g of vegetation, respectively ($P < 0.01$). Furthermore, after 5 and 10 days of therapy, there was no significant differ-

TABLE 3. *Results of antibiotic therapy of experimental enterococcal endocarditis*

Therapeutic regimen	No. of animals	Days of therapy	Log_{10} CFU/g of vegetation ± SD[a]	
			Streptomycin-susceptible strain (S)	Streptomycin-resistant strain (R)
Control	7	0	10.4 ± 0.7	10.2 ± 0.7
Penicillin	9	5	7.4 ± 0.8	7.4 ± 0.5
	10	10	6.8 ± 0.7	6.6 ± 0.3
Penicillin + streptomycin	10	5	6.9 ± 0.6	6.2 ± 0.7
	10	10	4.8 ± 1.9	5.8 ± 1.2
Penicillin + gentamicin (10 mg BID)	10	5	6.2 ± 0.7	6.4 ± 0.9
	10	10	4.9 ± 0.4	5.2 ± 1.0
Penicillin + gentamicin (15 mg TID)	5	5		6.1 ± 1.2
	4	10		5.0 ± 1.0
Vancomycin	5	5	8.0 ± 0.4	
	5	10	6.3 ± 1.1	
Vancomycin + streptomycin	5	5	6.7 ± 1.3	
	5	10	4.5 ± 1.2	

[a] CFU, Colony-forming units; SD, standard deviation.

ence between animals that received penicillin plus streptomycin when compared to the group of animals that received vancomycin plus streptomycin ($P = 0.4$).

Thirty-nine rabbits with enterococcal endocarditis due to strain R were treated with penicillin or penicillin plus an aminoglycoside (Table 3, Fib. 3B). The vegetations of rabbits receiving penicillin therapy contained mean concentrations of $10^{7.4}$ and $10^{6.6}$ bacteria/g at 5 and 10 days, respectively. These findings are similar to those employing strain S. The mean concentrations of enterococci recovered from vegetations at 5 and 10 days in rabbits treated with penicillin and streptomycin were $10^{6.2}$ and $10^{5.8}$, respectively. Two dosage schedules of gentamicin were used in combination with penicillin. Animals receiving penicillin and 10 mg of gentamicin BID had mean vegetation concentrations of $10^{6.4}$ and $10^{5.2}$ organisms/g at 5 and 10 days of therapy, respectively. Similarly, rabbits treated for 5 and 10 days with penicillin plus 15 mg of gentamicin TID had concentrations of $10^{6.1}$ and $10^{5.0}$ viable bacteria/g of vegetation, respectively. Ten days of treatment with penicillin alone significantly reduced the mean concentration of recoverable organisms in comparison to the mean concentration after 5 days of penicillin therapy ($P < 0.01$). The addition of either gentamicin regimen to penicillin significantly reduced the concentration of enterococci recovered when compared with penicillin alone after 5 and 10 days of therapy ($P < 0.05$). Surprisingly, streptomycin plus penicillin also significantly reduced the bacterial concentration in vegetations versus penicillin alone after 5 days ($P < 0.03$). However, after 10 days of therapy the difference was no longer significant.

DISCUSSION

The antibiotic therapy of enterococcal endocarditis, particularly in those instances where the disease occurs in a penicillin-allergic patient or is caused by highly streptomycin-resistant bacteria, presents the physician with a difficult therapeutic problem (2, 4, 5, 7). The efficacy of antimicrobial therapy in this disease has not been previously considered with comparative clinical trials or with in vivo experimental studies.

The rabbit model of bacterial endocarditis provides a means of examining the therapy of enterococcal endocarditis in controlled, in vivo experiments. This model has been shown in previous studies to closely parallel many aspects of bacterial endocarditis in humans and to be useful in the evaluation of various antibiotics in the therapy of endocarditis (1, 10).

A substantial portion of cases of enterococcal endocarditis may be caused by highly streptomycin-resistant (MIC > 2,000 μg/ml) enterococci. Three of twelve cases (25%) of enterococcal endocarditis seen at the New York and University of Virginia hospitals between 1971 and 1975 were caused by highly streptomycin-resistant enterococci (E.W. Hook, R.B. Roberts, and M.A. Sande, unpublished observation). This finding supports previous reports by Moellering et al. that up to 40% of enterococcal isolates from blood are highly streptomycin resistant (8). At the present time, the suggested therapy for all patients with enterococcal endocarditis is long term (4 to 6 weeks), high-dose penicillin in combination with streptomycin (6). Use of this

regimen is based largely on the in vitro synergistic activity of penicillin-streptomycin combinations and observation of the effectiveness of this combination in treating patients with enterococcal endocarditis. Unlike relatively streptomycin-susceptible enterococci, the majority of highly streptomycin-resistant enterococci are not killed synergistically by in vitro combinations of penicillin and streptomycin (8, 11, 13), whereas penicillin-gentamicin combinations do act synergistically in vitro (13, 15). The in vivo efficacy of these penicillin-aminoglycoside combinations against highly streptomycin-resistant enterococci have not been previously published. Interestingly, 5 days of treatment with penicillin plus either streptomycin or gentamicin reduced concentrations of viable highly streptomycin-resistant enterococci in rabbit cardiac vegetations with equal effectiveness, either combination being significantly more effective than penicillin alone ($P < 0.03$). After 10 days of therapy, however, only the penicillin-gentamicin combination remained significantly more effective than penicillin alone in reducing the numbers of viable highly streptomycin-resistant enterococci in vegetations. Although the trend of increased bactericidal activity of the penicillin-streptomycin combination over penicillin alone established at 5 days was still present after 10 days of therapy, it was no longer statistically significant. Thus, only penicillin plus gentamicin was significantly more effective than penicillin alone in reducing the concentrations of highly streptomycin-resistant enterococci in cardiac vegetations at 5 and 10 days of therapy. Although a case of enterococcal endocarditis caused by highly streptomycin-resistant enterococci and successfully treated with penicillin plus streptomycin has been reported (12), these studies indicate that the combination of penicillin and gentamicin may be more effective than penicillin and streptomycin in treating enterococcal endocarditis caused by highly streptomycin-resistant enterococci.

The addition of either 10 mg of gentamicin BID or 15 mg TID to the standard penicillin dosage (1.2×10^6 U BID) increased bacterial killing with equal effectiveness. With both dosage schedules, serum levels of gentamicin fell to less than 4 μg/ml with 4 h of drug administration. Enterococci were eliminated from vegetations of rabbits receiving the lower gentamicin dose at effectively the same rate (Fig. 3B) as animals receiving higher doses on a schedule which maintained detectable serum levels of gentamicin throughout the period of therapy. Thus, it would appear that gentamicin need not be present in the serum at all times to cause significant in vivo synergistic effects. In vivo antibiotic synergism may not be directly dose-related phenomenon requiring the presence of detectable serum levels throughout the period of therapy.

Alternatives to penicillin therapy for use in the penicillin-allergic patient with enterococcal endocarditis were also considered. The suggested antibiotic therapy of enterococcal endocarditis in up to 10% of patients allergic to penicillin is hyposensitization of the patient (16) followed by long-term intravenous therapy with penicillin plus streptomycin (6). Vancomycin, however, alone or in combination with streptomycin has been recommended as alternative therapy in these patients on the basis of in vitro experimental studies (7, 14) and the successful therapy of enterococcal endocarditis using vancomycin alone or in combination with streptomycin in four and one patients, respectively (2, 17). In these studies, rates of in vitro and in vivo bacterial killing of a relatively streptomycin-susceptible enterococcus by vancomycin or penicillin alone or in combination with streptomycin were compared. In vitro bacterial killing by penicillin alone was significantly increased by the addition of streptomycin or gentamicin. In vitro killing by vancomycin plus streptomycin effectively equaled the rate achieved by penicillin-aminoglycoside combinations. Vancomycin alone, however, was not effective in killing the relatively streptomycin-susceptible enterococcus in vitro. In vivo studies using the rabbit model of endocarditis demonstrated similar results. The elimination of viable relatively streptomycin-susceptible enterococci from vegetations by penicillin or vancomycin was significantly increased with the addition of aminoglycoside antibiotics ($P < 0.002$ at 10 days). After 5 and 10 days of therapy, no significant differences could be demonstrated in the rates of bacterial eradication in animals treated with penicillin plus streptomycin or gentamicin and those animals receiving the vancomycin-streptomycin combination ($P > 0.35$). Thus, the combination of vancomycin plus streptomycin is superior to vancomycin alone both in vitro and in the therapy of experimental enterococcal endocarditis caused by relatively streptomycin-susceptible enterococci and is as effective as the penicillin-aminoglycoside combinations in treating experimental enterococcal endocarditis. Thus, these findings provide in vivo experimental evidence supporting previous in vitro studies (7, 14) which have concluded that the combination of vancomycin plus streptomycin may be a useful alternative to penicillin plus streptomycin in the therapy of penicillin-allergic patients with enterococcal endocarditis.

These studies, therefore, indicate that (i) penicillin plus gentamicin may be the drugs of choice for enterococcal endocarditis caused by highly streptomycin-resistant enterococci, and (ii) that vancomycin plus streptomycin may be a useful alternative to penicillin plus streptomycin for therapy of enterococcal endocarditis in penicillin-allergic patients.

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