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Synergism of Vancomycin-Gentamicin and Vancomycin-Streptomycin Against Enterococci

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The in vitro activity of vancomycin and combinations of vancomycin-gentamicin and vancomycin-streptomycin against enterococci was investigated. The minimal inhibitory concentration of vancomycin for 99 of 100 enterococcal strains isolated clinically was 3.12 μg or less/ml. When cultures of eight strains were incubated with vancomycin, regardless of the inoculum size (10^6, 10^5, or 10^4) and concentration of vancomycin (10 or 20 μg/ml), there was no significant reduction in the number of viable enterococci at 6, 24, and 48 h. Gentamicin and streptomycin in concentrations attainable clinically were not effective against enterococci. Vancomycin combined with gentamicin or streptomycin was tested against 41 enterococcal strains. With the combination of vancomycin at 10 μg/ml and gentamicin at 4 μg/ml or vancomycin at 5 μg/ml and gentamicin at 4 μg/ml, synergism was demonstrated against all 41 strains at 6 h. The combination of vancomycin at 10 μg/ml and streptomycin at 10 μg/ml was only synergistic against 25 of 41 strains at 6 h, and only 22 of 41 strains were affected synergistically at 6 h by vancomycin at 5 μg/ml with streptomycin at 10 μg/ml. With few exceptions, the enhanced killing was more pronounced at 24 and 48 h. The combination of vancomycin and gentamicin or vancomycin and streptomycin (where in vitro studies demonstrate synergism) may be a useful alternate therapy in enterococcal endocarditis.

The standard recommended therapy for enterococcal endocarditis is the combination of penicillin and streptomycin (9). Penicillin in combination with gentamicin has recently been shown to be highly synergistic in vitro against enterococci and therefore shows potential as an effective regimen for treatment of this serious disease (12, 17, 19). However, there are significant numbers of patients with endocarditis who have a history of severe hypersensitivity reactions to penicillin. Although "desensitization" to penicillin can be performed prior to the intravenous administration of large doses of penicillin, there are still certain numbers of patients who develop severe cutaneous hypersensitivity reactions even after "desensitization" (4). Antihistamines and adrenal cortical steroids are not consistently effective in suppressing the hypersensitivity reactions (15). An alternate mode of therapy with antibiotics unrelated to penicillin becomes desirable in situations where the use of penicillin is to be avoided.

Vancomycin has significant in vitro activity against enterococci (16). It has been used successfully in the treatment of four patients with enterococcal endocarditis, but only one of these four patients received vancomycin as the sole antibiotic during the course of the disease (3). The combination of vancomycin and streptomycin has been reported to be synergistic against some enterococcal strains (11, 18, 19), and a patient with enterococcal endocarditis was cured with such a combination (18).

The present study was undertaken to examine the in vitro activity of vancomycin and the combination of vancomycin with gentamicin or streptomycin against enterococci.

MATERIALS AND METHODS

Enterococci. The enterococci used in this study were isolated from clinical materials and included 16 strains isolated from blood cultures of patients with endocarditis. Only one isolate per patient was studied. All isolates were gram-positive cocci which grew in chains and as nonhemolytic or alpha-hemolytic colonies on sheep blood agar. On bile-esculin agar (Pfizer Selective Enterococcus Agar, Pfizer Co., Inc., Brooklyn, N.Y.), all strains grew as colonies sur-

rounded by black zones (1, 5). All strains grew in 6.5% NaCl brain heart influsion broth (Difco). Blood agar slants were used to maintain the enterococcal strains.

Antibiotic susceptibility tests. The minimal inhibitory concentration (MIC) of vancomycin hydrochloride (Eli Lilly & Co., Indianapolis, Ind.), streptomycin sulfate (Premo Pharmaceutical Laboratories, Inc., South Hackensack, N.J.), and gentamicin sulfate (Schering Corp., Bloomfield, N.J.) was determined by a standard method of twofold dilutions in broth as previously described (13).

Studies of bacterial killing by vancomycin. Eight enterococcal strains isolated from patients with endocarditis were used to study the effect of vancomycin on the viability of these microorganisms at time intervals. A culture grown overnight in Trypticase soy broth (TSB, BBL) was diluted with TSB to give approximately 10^6, 10^5, and 10^4 colony-forming units per ml. Vancomycin was added to each culture in final concentrations of 10 and 20 μg/ml. The vancomycin-enterococcus mixture and proper controls were incubated in a water bath at 37 C. At 6, 24, and 48 h of incubation, viable colony-forming units were enumerated by the pour-plate technique with the use of Trypticase soy agar (TSA). Colonies on TSA were counted after incubation at 37 C for 48 h.

Studies for synergism. Forty-one enterococcal strains were tested for synergism of antibiotic combinations by a method described previously (17). Briefly, a culture grown overnight in TSB was diluted with TSB to give approximately 10^5 to 10^6 colony-forming units per ml and incubated with different antibiotics in a water bath at 37 C. The final concentrations of antibiotics were as follows: streptomycin, 10 μg/ml; gentamicin, 4 μg/ml; vancomycin, 5 μg/ml; vancomycin, 10 μg/ml; vancomycin, 10 μg/ml, with gentamicin, 4 μg/ml; vancomycin, 5 μg/ml, with gentamicin, 4 μg/ml; vancomycin, 10 μg/ml, with streptomycin, 10 μg/ml; and vancomycin, 5 μg/ml, with streptomycin, 10 μg/ml. At 6, 24, and 48 h of incubation, viable colony-forming units were enumerated by the pour-plate technique with the use of TSA. Colonies on TSA were counted after incubation at 37 C for 48 h.

An antibiotic combination was considered synergistic when it killed more enterococci (by a factor of at least $1 \times \log_{10}$) than the same concentration of vancomycin alone at a given time.

RESULTS

MIC of vancomycin. The MIC of vancomycin for 100 enterococcal strains is shown in Fig. 1. All strains were inhibited by vancomycin at a concentration of 6.25 μg or less/ml, and the MIC for 92% of the strains was between 0.78 and 3.12 μg/ml.

Bacterial killing by vancomycin. The MIC of vancomycin for the eight enterococcal strains used in studies of bacterial killing was 1.56 μg/ml for two strains, 3.12 μg/ml for five strains, and 6.25 μg/ml for one strain. The ranges with the means of the number of viable colony-forming units of the eight strains at different time intervals for all three inocula incubated with 10 μg of vancomycin/ml are presented in Fig. 2. Regardless of the size of the inoculum, there was no significant reduction in the viable colony-forming units at 6, 24, and 48 h. Identical results were obtained with 20 μg of vancomycin/ml.

Synergism. The MIC of vancomycin for 40 of the 41 strains used for studies of synergism ranged from 0.78 to 3.12 μg/ml; the MIC for 1 strain was 6.25 μg/ml. The MIC of gentamicin for 39 of the 41 strains was between 6.25 and 50 μg/ml; 2 strains were inhibited by gentamicin at 1.56 and 3.12 μg/ml. The MIC of streptomycin ranged from 39 to >50,000 μg/ml.

With the combination of vancomycin at 5 or 10 μg/ml and gentamicin at 4 μg/ml, synergism was demonstrated against all 41 strains at 6 h. With few exceptions, the enhanced killing was more pronounced at 24 and 48 h. However, with the combination of vancomycin at 10 μg/ml and gentamicin at 4 μg/ml, the enhanced killing for two strains at 24 h was less than $1 \times \log_{10}$ and therefore was not considered synergistic; at 48 h, the enhanced killing for one of these two strains was more than $1 \times \log_{10}$ and was again considered synergistic (Table 1). Results with the combination of vancomycin at 5 μg/ml and gentamicin at 4 μg/ml were similar. When vancomycin-gentamicin was tested against the two strains for which the MIC of gentamicin was 3.12 and 1.56 μg/ml, the combination killed 3 to $5 \times \log_{10}$ more enterococci than vancomycin or gentamicin alone.

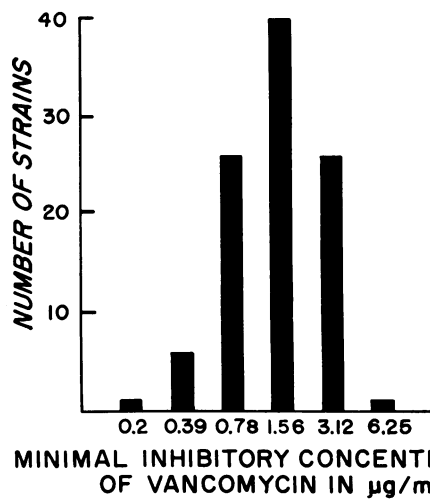

FIG. 1. *Minimal inhibitory concentration of vancomycin for 100 enterococcal strains.*

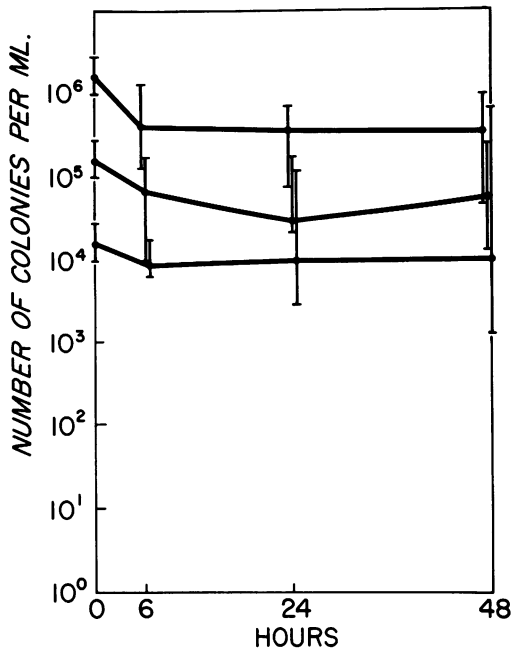

FIG. 2. *Effect of vancomycin (10 μg/ml) on the viability of eight enterococcal strains at time intervals. Three different inocula and the ranges with the means of the number of viable colony-forming units of the eight strains are shown.*

The combination of vancomycin at 10 μg/ml and streptomycin at 10 μg/ml was synergistic against only 25 of 41 strains at 6 h. With vancomycin at 5 μg/ml and streptomycin at 10 μg/ml, the enhanced killing of 3 of these 25 strains was less than $1 \times \log_{10}$ and was considered not synergistic. However, synergism was again demonstrated against these three strains at 24 and 48 h (Table 1). Similar to the findings with vancomycin-gentamicin combinations, the enhanced killing of the strain susceptible to synergism of vancomycin-streptomycin was also more pronounced at 24 and 48 h (Table 1).

Figures 3 and 4 show typical results. Figure 3 illustrates the synergistic effects of vancomycin combined with gentamicin and vancomycin combined with streptomycin against an enterococcal strain. Figure 4 shows the synergistic effect of vancomycin with gentamicin and the lack of synergism of vancomycin with streptomycin.

Combination of vancomycin and other antibiotics. We also investigated the effect of combinations of vancomycin with rifampin (Ciba Pharmaceutical Co., Summit, N.J.), clindamycin phosphate (The Upjohn Co., Kalamazoo, Mich.), and erythromycin ethylsuccinate (Abbott Laboratories, North Chicago, Ill.) on five enterococcal strains recovered from patients with endocarditis.

The MIC of rifampin ranged from 3.12 to >50 μg/ml; of clindamycin, from 25 to >400 μg/ml; and of erythromycin, from 0.19 to 0.78 μg/ml. Rifampin (5 μg/ml) or clindamycin (4 μg/ml) alone had no inhibitory effect. Erythromycin (1 μg/ml) exerted inhibitory but no killing effect. No synergism was demonstrated against any of the strains by the combination of vancomycin and rifampin, clindamycin, or erythromycin.

DISCUSSION

If bacterial killing by an antibiotic is considered essential in the treatment of infectious endocarditis, the effectiveness of vancomycin alone in the therapy of enterococcal endocarditis is questionable. Although the growth of enterococci was inhibited by relatively small amounts of vancomycin, the viable colony-forming units were not reduced even in the presence of 20 μg of vancomycin/ml, which is the peak concentration achieved in serum after intravenous administration of 1 g of vancomycin (8). These data confirm the previous reports of the inability of vancomycin to kill enterococci in vitro (2, 18). Gentamicin alone or streptomycin alone is ineffective against enterococci, but the combination of vancomycin and gentamicin consistently showed enhanced bacterial killing. As for the combination of vancomycin and streptomycin, synergism was demonstrated against only some strains, confirming reports by other investigators (11, 18, 19). This also demonstrated the necessity of actual laboratory testing when antibiotic combinations are to be used clinically for expected synergism against a particular infecting organism (6). In patients who have enterococcal endocarditis with a history of severe life-threatening hypersensitivity reactions to penicillin or patients who develop uncontrollable severe cutaneous hypersensitivity to penicillin during therapy, the combination of vancomycin-gentamicin or vancomycin-streptomycin (where in vitro studies demonstrate synergism) may be a useful alternate therapy in this serious infectious disease. The efficacy of these treatment regimens remains to be tested clinically.

Both vancomycin and the aminoglycoside antibiotics have potential ototoxicity and nephrotoxicity, and the reluctance of clinicians to use these combinations of drugs is understandable. However, combinations of antibiotics with lesser toxic potentials, such as vancomycin with rifampin, clindamycin, or erythromycin, have proved not to be synergistic in vitro against

TABLE 1. *Enhanced killing by combinations of antibiotics as compared with vancomycin alone*

Antibiotic combination	Time (h)	Synergism demonstrated/no. of strains tested	Differential decrease in no. of colonies ($\times \log_{10}$)			
			1-2	2-3	3-4	>4
Vancomycin, 10 μg/ml	6	41/41	8	26	7	0
+	24	39/41	8	13	10	8
Gentamicin, 4 μg/ml	48	40/41	19	6	7	8
Vancomycin, 5 μg/ml	6	41/41	10	28	3	0
+	24	38/41	13	17	4	4
Gentamicin, 4 μg/ml	48	39/41	13	11	7	8
Vancomycin, 10 μg/ml	6	25/41	17	6	2	0
+	24	25/41	6	13	3	3
Streptomycin, 10 μg/ml	48	27/41	8	12	3	4
Vancomycin, 5 μg/ml	6	22/41	15	6	1	0
+	24	25/41	11	10	3	1
Streptomycin, 10 μg/ml	48	26/41	10	8	3	5

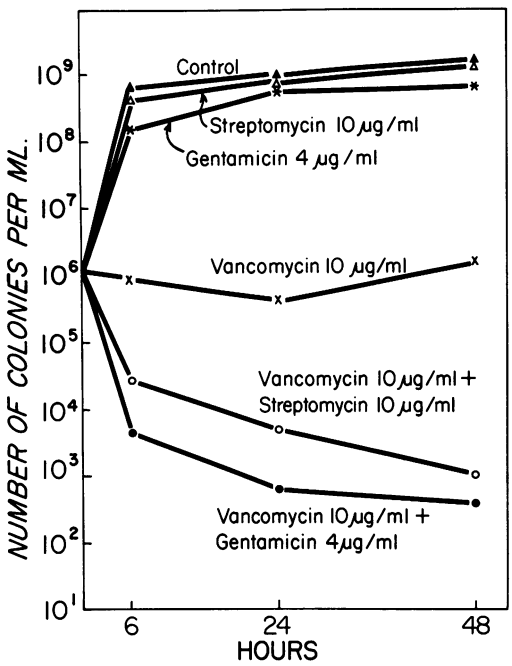

FIG. 3. *Synergistic effect of vancomycin-gentamicin and vancomycin-streptomycin against an enterococcal strain.*

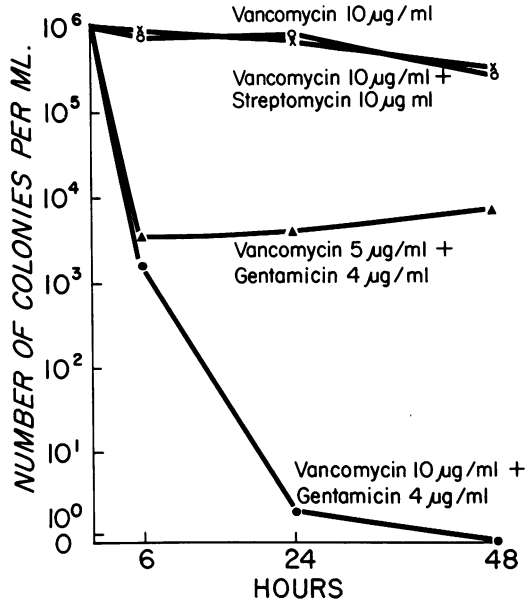

FIG. 4. *Synergistic effect of vancomycin-gentamicin and the lack of synergism of vancomycin-streptomycin against an enterococcal strain. The growth curves of enterococci in the control culture and cultures with gentamicin or streptomycin alone were similar to Fig. 3 and therefore are not shown.*

enterococci. The combination of cephalothin and streptomycin has been shown to be ineffective in the treatment of enterococcal endocarditis (14). The concentrations of vancomycin, gentamicin, and streptomycin required to produce a synergistic effect in vitro against enterococci are obtainable in the serum after the administration of less than maximally safe therapeutic doses of these antibiotics. A patient with enterococcal endocarditis was cured with 500 mg of vancomycin intravenously every 8 h and 500 mg of streptomycin intramuscularly every 12 h for 6 weeks without toxic side effects (18). The doses of antibiotics should be adjusted by following serum bactericidal titers against the infecting organism (7, 10). Serial testing of the vestibular, auditory, and renal function may be used to detect any evidence of toxicity. Adjust-

ment of antibiotic dosage may be indicated in the face of toxic reactions, and discontinuation of therapy may be warranted in certain situations. However, the difficulties in the antibiotic therapy of such a serious disease as enterococcal endocarditis and the potential risk of fatal hypersensitivity reactions to penicillin may dictate the use of the best available alternate regimen of antibiotics, notwithstanding the potential toxicity of such antibiotic combinations.

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