ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 1980, p. 944-947
0066-4804/80/12-0944/04$02.00/0

Vol. 18, No. 6

Synergy of Penicillin and Decreasing Concentrations of Aminoglycosides Against Enterococci from Patients with Infective Endocarditis

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To determine whether low concentrations of aminoglycosides in combination with penicillin could effectively kill enterococci in vitro, we tested penicillin (20 μg/ml) in combination with decreasing concentrations of either streptomycin (20, 10, 5, and 1 μg/ml) or gentamicin (5, 3, 1, and 0.5 μg/ml) against 13 strains of streptomycin-susceptible and 7 strains of streptomycin-resistant enterococci isolated from patients with infective endocarditis. At 24 h, penicillin plus each increment in streptomycin concentration resulted in a statistically significant increase in killing of streptomycin-susceptible enterococci, compared with the next lower streptomycin concentration ($P < 0.01$). At 24 h, against streptomycin-susceptible and streptomycin-resistant enterococci, there were no statistically significant differences in killing between combinations containing 5 μg of gentamicin per ml and those containing 3 μg/ml. Against streptomycin-susceptible enterococci, there were statistically significant differences in killing between combinations containing 3 μg of gentamicin per ml and those containing 1 μg/ml. Against streptomycin-resistant enterococci, statistically significant differences in killing were detected with combinations containing 5 μg of gentamicin per ml and those containing 1 μg/ml.

The antibiotic treatment currently recommended for enterococcal endocarditis consists of penicillin combined with either streptomycin or gentamicin administered during a period of 4 to 6 weeks. The usual dosages result in relatively high serum concentrations of aminoglycoside and are frequently complicated by serious toxicity. In one study, 22% of patients with enterococcal endocarditis who were treated with streptomycin in a dosage of 2 g per day for 2 or 3 weeks suffered vestibular disturbances (2). An added complication of aminoglycoside therapy is that approximately 40% of strains of enterococci have high-level resistance to streptomycin (minimal inhibitory concentration [MIC], >2,000 μg/ml) (3, 7) and are resistant in vitro to combinations of penicillin and streptomycin. Because penicillin and gentamicin act synergistically in vitro against these strains, this combination has been recommended for therapy of infective endocarditis caused by these strains (5). Nephrotoxicity frequently complicates the use of gentamicin and was observed in 12 (71%) of 17 patients with enterococcal infective endocarditis treated for 4 weeks with dosages of 3 to 5 mg/kg per day (W. R. Wilson, C. J. Wilkowske, R. L. Thompson, and J. E. Geraci, Program Abstr. Int. Cong. Chemother. 11th Intersci. Conf. Antimicrob. Agents Chemother., 19th, Boston, Mass., abstr. no. 1063, 1979).

A safer and equally effective antimicrobial regimen for enterococcal endocarditis would be desirable. Such a regimen might be achieved by lowering the dosages and, therefore, lowering the serum concentrations of aminoglycosides from those currently used. Fekety and Weiss (1) reported that most strains of enterococci were killed synergistically in vitro by a combination of penicillin and 20 μg of streptomycin per ml. When the concentration of streptomycin was reduced to ≤10 μg/ml, the percentage of strains affected synergistically declined. However, none of these strains of enterococci was tested for streptomycin resistance, and the lack of a synergistic effect with the combination of penicillin and low concentrations of streptomycin may have been attributable to streptomycin resistance.

To determine whether low concentrations of aminoglycosides in combination with penicillin could effectively kill enterococci in vitro, we tested penicillin (20 μg/ml) in combination with decreasing concentrations of either streptomycin (20, 10, 5, and 1 μg/ml) or gentamicin (5, 3, 1, and 0.5 μg/ml) against 13 strains of streptomycin-susceptible and 7 strains of streptomycin-

resistant enterococci isolated from patients with infective endocarditis.

MATERIALS AND METHODS

Bacteria. Twenty strains of enterococci isolated from patients with infective endocarditis were tested. Growth in 6.5% NaCl and hydrolysis of esculin were initially used to characterize the isolates. All isolates were serologically grouped, identified as to species, and then frozen until tested.

Antibiotics. Stock solutions of penicillin G potassium (Eli Lilly & Co.) and streptomycin sulfate (Eli Lilly & Co.) and gentamicin (Schering Corp.) were prepared and stored at −20°C.

Determination of MIC and minimal bactericidal concentration. An overnight broth culture of the test strain was diluted 1:1,000 in Mueller-Hinton broth (Difco Laboratories); 0.5 ml of this dilution (1×10^5 to 6×10^5 colony-forming units) was added to tubes containing serial dilutions of antibiotic in 0.5 ml of Mueller-Hinton broth. After incubation for 18 to 24 h at 37°C, the tubes were inspected for signs of visible growth. The lowest concentration of drug which prevented the appearance of visible turbidity was defined as the MIC. From each clear tube and from the lowest concentration showing turbidity, 0.05 ml was subcultured to and mixed in 10 ml of fluid thioglycolate medium. The minimal bactericidal concentration was defined as the lowest concentration of drug subculture which yielded 10 or fewer colonies after 72 h of incubation. Enterococci with MICs of <2,000 μg/ml were classified as streptomycin susceptible; those with MICs of ≥2,000 μg/ml were considered to be streptomycin resistant.

Test of synergy. A 0.5-ml sample from an overnight broth culture of the test strain was inoculated into 9.5 ml of Mueller-Hinton broth containing antibiotics singly or in combination. After 0, 4, and 24 h of incubation at 37°C, 0.2-ml samples were removed and diluted 10-fold in broth. A 0.1-ml sample from each dilution was spread over the surface of a tryptic soy agar plate (Difco), pH 7.35. Penicillinase (Bacto-penase concentrate; Difco) was incorporated in the agar of plates receiving undiluted broth. Colony counts were performed after incubation for 48 h. Synergy was defined as an increase of at least 100-fold in killing at 24 h by a combination of drugs compared with the most effective drug alone (penicillin in all cases) (4, 5).

Statistical analysis. To test whether the decreasing concentrations of aminoglycosides were associated with significant decreases in the $\log_{10}$ colony-forming units per milliliter, a repeated measures analysis of variance was performed. Pair differences and colony counts were evaluated by the Student t test and Hotelling's T^2 (6).

RESULTS

The susceptibilities of the 20 strains of enterococci to penicillin, gentamicin, and streptomycin are depicted in Fig. 1. Penicillin inhibited all strains at a concentration of ≤8 μg/ml; killing, however, required ≥500 μg/ml. For gentamicin, the MICs were ≥8 μg/ml, and the minimal bactericidal concentrations were ≥16 μg/ml for all 20 strains. Of the 20 strains, 13 were susceptible to streptomycin and 7 strains were resistant to streptomycin (MIC, >2,000 μg/ml).

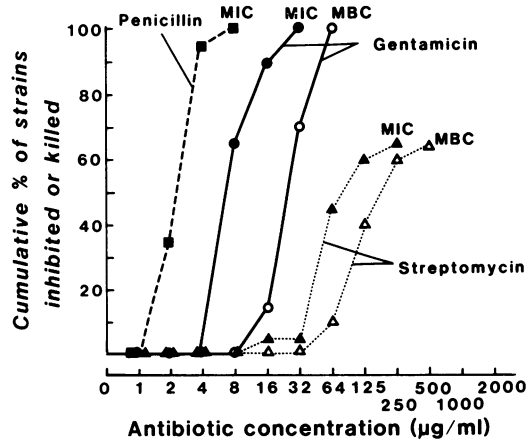

FIG. 1. *Distribution of MIC and minimal bactericidal concentration (MBC) values for penicillin, gentamicin, and streptomycin against 20 strains of enterococci.*

The 13 streptomycin-susceptible strains were studied for synergy by testing 20 μg of penicillin per ml in combination with decreasing concentrations of streptomycin (20, 10, 5, and 1 μg/ml).

Penicillin in combination with the highest concentration of streptomycin tested (20 μg/ml) synergistically killed 12 of the 13 strains. As the concentration of streptomycin was decreased to 10, 5, and 1 μg/ml, synergy occurred in 10, 6, and 2 of the 13 strains, respectively.

The mean $\log_{10}$ values of colony-forming units per milliliter at 0, 4, and 24 h were computed and analyzed by repeated measures using Hotelling's T^2 and by Student's t test as another means of comparing the effectiveness of the combinations (Fig. 2). At 24 h, each increment in streptomycin concentration resulted in a significant increase in killing compared with the next lower concentration ($P < 0.01$).

All 20 organisms were tested for synergy with penicillin in combination with decreasing concentrations of gentamicin (5, 3, 1, and 0.5 μg/ml).

When penicillin was combined with 5, 3, 1, and 0.5 μg of gentamicin per ml, synergy occurred in 11, 12, 6, and 6 of the 13 streptomycin-susceptible strains, respectively. There was no statistically significant difference in killing at 24 h between the combinations of penicillin plus 3 μg of gentamicin per ml and penicillin plus 5 μg of gentamicin per ml (Fig. 3). Statistically significant differences in killing were demonstrated

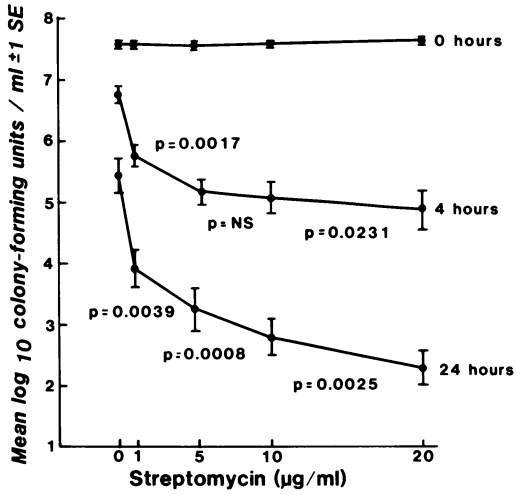

FIG. 2. *Penicillin (20 μg/ml) and streptomycin synergy against 13 strains of streptomycin-susceptible enterococci.*

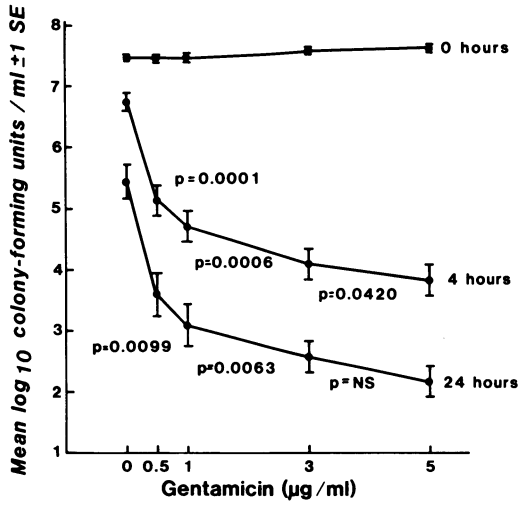

FIG. 3. *Penicillin (20 μg/ml) and gentamicin synergy against 13 strains of streptomycin-susceptible enterococci.*

between combinations of penicillin with 3 μg and 1 μg of gentamicin per ml and between combinations of penicillin with 1 μg and 0.5 μg of gentamicin per ml at 4 and 24 h. Synergy occurred in six of seven of the streptomycin-resistant strains with penicillin plus 5 μg of gentamicin per ml. The decrease in concentrations of gentamicin (3, 1, and 0.5 μg/ml) resulted in synergy in five, five, and two of the seven strains, respectively. Penicillin (20 μg/ml) combined with streptomycin (20 μg/ml) did not act synergistically against any of the seven strains.

The mean $\log_{10}$ values of colony-forming units per milliliter of these streptomycin-resistant organisms increased as the concentration of gentamicin in the combinations was decreased (Fig. 4). At 4 h, significant differences in killing were detected between 0.5 and 1 μg of gentamicin per ml and between 1 and 3 μg of gentamicin per ml. There were no statistically significant differences in killing between these concentrations, however, at 24 h. No statistically significant difference could be shown between combinations containing 5 μg of gentamicin per ml and those containing 3 μg/ml at either 4 or 24 h. The difference in killing between combinations containing 5 and 1 μg of gentamicin per ml was significant at both 4 and 24 h ($P = 0.037$ and $P = 0.007$, respectively).

DISCUSSION

The number of strains of streptomycin-susceptible enterococci affected synergistically by antibiotic combinations containing 20 and 10 μg of streptomycin per ml was similar. The magnitude of killing, however, was greater with combinations containing 20 μg of streptomycin per ml. To our knowledge, no data have been reported comparing the results with patients who had enterococcal infective endocarditis treated with streptomycin dosages designed to achieve peak serum concentrations of 20 μg/ml with those designed to achieve peak serum concentrations of 10 μg/ml or lower. Based on our in vitro data, the use of streptomycin dosages designed to achieve a peak serum concentration of 20 μg/ml appears to be preferable to the use of lower dosages. In patients who are at high risk

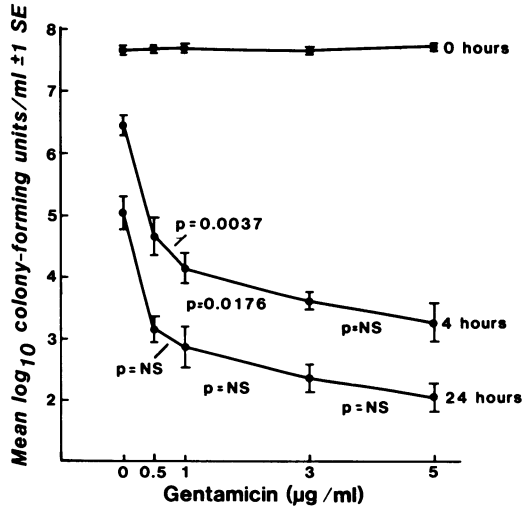

FIG. 4. *Penicillin (20 μg/ml) and gentamicin synergy against seven strains of streptomycin-resistant enterococci.*

of developing streptomycin-associated vestibular toxicity (patients over 70 years old or those with abnormal renal function or preexisting vestibular dysfunction), dosages of streptomycin designed to achieve a peak serum concentration of 10 μg/ml instead of 20 μg/ml may be desirable. However, this assertion has not been confirmed by the results of clinical trials.

Previous in vitro studies have shown that synergy of penicillin and gentamicin against enterococci occurs consistently when the penicillin concentration is ≥5 μg/ml (5, 8). In our experience, penicillin in combination with 3 or 5 μg of gentamicin per ml had similar in vitro activity against both streptomycin-susceptible and streptomycin-resistant enterococci. Penicillin in combination with 3 or 5 μg of gentamicin per ml produced synergistic killing against 85% of the 20 strains tested. Reducing the concentration of gentamicin from 5 to 3 μg/ml showed no cumulative decrease in the number of strains affected synergistically. Nor was there a statistically significant difference in reduction of the mean $\log_{10}$ colony-forming units per milliliter.

Our in vitro observations with streptomycin-resistant strains indicate that a reduction in the serum concentration of gentamicin from 5 to 3 μg/ml might not adversely affect patients with enterococcal endocarditis treated with penicillin plus gentamicin, but could substantially reduce the frequency of nephrotoxicity associated with prolonged treatment with gentamicin. In a study of 17 patients who were treated for enterococcal endocarditis, elevations of serum creatinine levels were noted in 2 of 9 cases treated with <3 (mean, 2.67) mg of gentamicin per kg per day and in all 11 cases treated with ≥3 (mean, 4.4) mg of gentamicin per kg per day (Wilson et al., Program Abstr. 11th ICC, 19th ICAAC, abstr. no. 1063).

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