Comparison of the Action of Ampicillin and Benzylpenicillin on Enterococci In Vitro

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The bactericidal activity of benzylpenicillin and ampicillin on 21 strains of enterococci was evaluated and compared to the activity of these drugs in combination with streptomycin (20 μg/ml). On a weight basis, ampicillin was about twice as effective as benzylpenicillin. Neither of the drugs was rapidly and completely bactericidal for any of the 21 strains of enterococci when used alone. The addition of streptomycin greatly enhanced the early bactericidal rate achieved with any given amount of either penicillin and permitted the elimination of viable organisms in vitro. These results suggest that, for the time being, combined antibiotic therapy might be desirable in enterococcus endocarditis and that ampicillin, although more effective than benzylpenicillin, should not be relied upon as a single drug in that disease.

Enterococcus (*Streptococcus faecalis*) can be an important pathogen in such diseases as bacterial endocarditis and urinary tract infections. These organisms are peculiar in their reactions to antibiotics. Most strains can be inhibited by 0.3 to 6.0 μg of benzylpenicillin per ml, but the bactericidal effect is limited and never as complete as with viridans streptococci. In addition, enterococci are virtually never inhibited in vitro by 20 μg of streptomycin per ml, but the addition of this amount of streptomycin to an inhibitory quantity of penicillin strikingly enhances the bactericidal effect. This is a well-studied example of antibiotic synergism in vitro. Treatment of enterococcal endocarditis with combinations of benzylpenicillin plus streptomycin or benzylpenicillin plus kanamycin has resulted in a very high proportion of cures, whereas treatment with penicillin alone has often failed (3, 5). It is probably the most widely accepted example of synergism in vivo.

It was reported that ampicillin is more active against enterococci than benzylpenicillin and that ampicillin is markedly bactericidal against these organisms (1, 6, 8). On the basis of this information, a few patients with enterococcal endocarditis were treated with ampicillin alone, and some initial bacteriological or clinical successes were recorded (1, 2, 6, 7, 9, 10). In view of the undesirable side effects associated at times with the administration of streptomycin or kanamycin, the potential curative ability of ampicillin used alone must be given serious consideration. We therefore decided to evaluate the bacteriostatic and

bactericidal activity of ampicillin alone, and in combination with streptomycin, on 21 recently isolated strains of enterococci and to compare the results quantitatively with the action of benzylpenicillin alone, and in combination with streptomycin.

MATERIALS AND METHODS

Bacterial isolates. Twenty-one recent isolates from urine and blood cultures were used. All were typical enterococci by colony form on blood-agar, growth in 6.5% NaCl broth, in 0.1% methylene blue milk, and at 41 C. The strains were preserved during the study in the form of broth cultures stored at −20 C.

Drugs. Potassium benzylpenicillin and ampicillin trihydrate were purchased commercially in sterile vials suitable for intravenous administration. The dried drug was dissolved in sterile saline to give solutions of suitable concentration. A new vial of each drug was employed for the tests performed on a given day.

Evaluation of bactericidal activity of drugs. The methods used were described earlier (4, 5). In brief, Tryptose Phosphate Broth (Difco) in 10-ml tubes containing various concentrations of drugs was inoculated with a sufficient amount of 18-hr culture of enterococci to yield a concentration of approximately 10^5 colony-forming units per ml upon plate count. The tubes were incubated at 37 C. At intervals, the contents were mixed and samples of 0.5 ml were withdrawn. The samples were suitably diluted and numbers of viable organisms were estimated by plate counts. The results are reported as "viable bacteria per milliliter" over periods of 4 to 7 days. In addition, all strains were also tested by a second

technique which estimates the number of survivors after various periods of incubation in 0.02 ml-samples (5). This method indicates the minimum amount of a penicillin, alone or in combination with streptomycin, which kills 99.9% of the viable organisms present in the initial inoculum.

RESULTS

The reduction of the viable population of enterococci by at least 99.9% (from 10^5/ml to less than 10^2/ml) in 24 hr at 37 C was compared for benzylpenicillin and ampicillin (Fig. 1). Of the 21 strains of enterococci tested, 20 required 3.0 μg of ampicillin or less per ml, whereas 19 of the 21 strains required 6.0 μg of benzylpenicillin or less per ml for bactericidal action. By this criterion, ampicillin was approximately twice as effective against enterococci as was benzylpenicillin. There was a similar ratio of activity of each penicillin in the presence of 20 μg of streptomycin per ml. Ampicillin (1.5 μg/ml) plus streptomycin (20 μg/ml) was bactericidal for 20 of the 21 strains, and benzylpenicillin (3 μg/ml) plus streptomycin (20 μg/ml) had a similar effect on 17 strains. If this ratio were to apply in vivo as well, ampicillin might be preferred over benzylpenicillin in the treatment of enterococcal infections, when used alone or in combination with streptomycin.

Exposure of enterococci to benzylpenicillin resulted in partial bactericidal effects, but always permitted the survival of some "persisters." In vitro these persisters resumed multiplication when the drug concentration fell below the inhibitory level in the course of incubation (5). Before considering treatment with ampicillin alone, one must ask whether ampicillin is more effectively bactericidal than benzylpenicillin and whether it can eliminate most persisters. Viable counts at frequent intervals were therefore performed on enterococcal populations exposed to a penicillin alone, or in combination with streptomycin (Fig. 2).

Streptomycin (20 μg/ml) alone produced no measurable inhibition of growth. Minimal effective concentrations of each penicillin lowered the viable count for 12 to 24 hr, but then permitted almost unrestricted growth as thermal inactivation of penicillin took place. These same minimal effective concentrations of either penicillin in the presence of streptomycin resulted in apparent complete killing of the exposed enterococcal
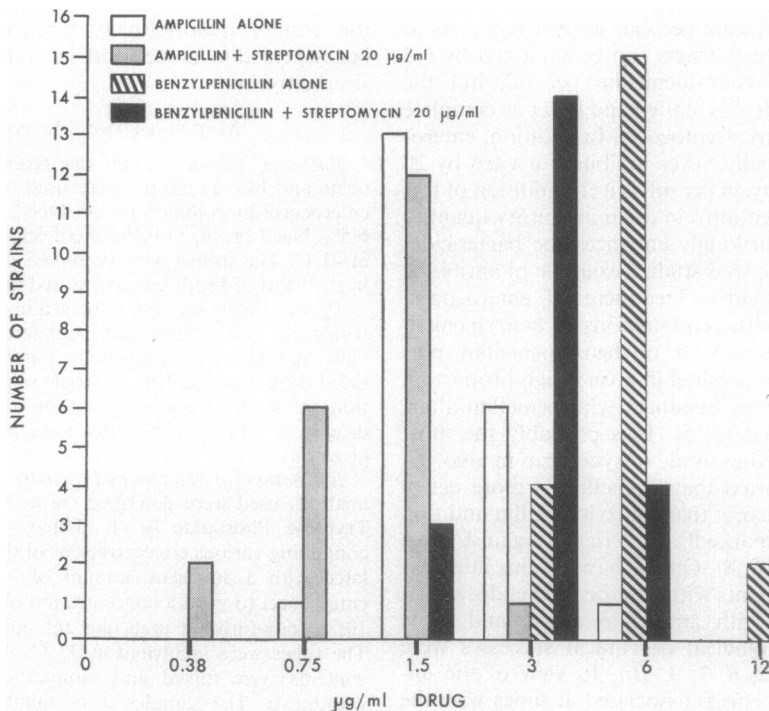

FIG. 1. *Distribution of susceptibility to penicillin or ampicillin, alone and in combination with streptomycin (20 μg/ml), among 21 strains of enterococci. "Susceptibility" is defined here as the minimal amount of drug required to reduce the number of viable organisms by 99.9% in 24 hr.*
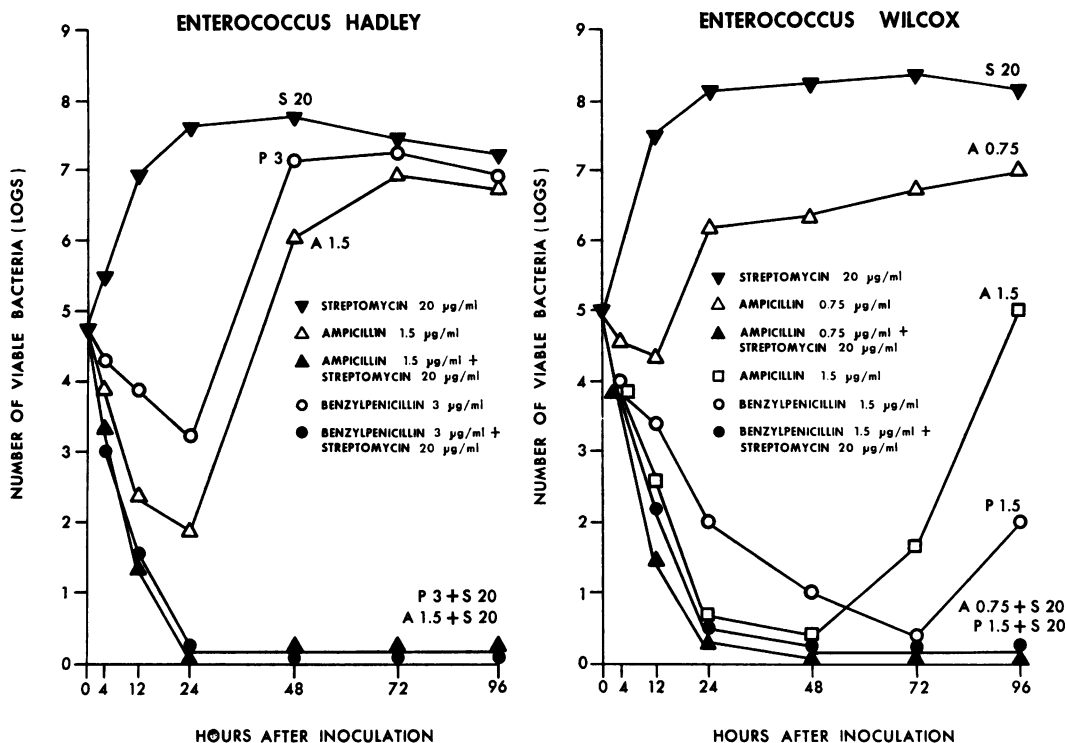
FIG. 2. *Effect of penicillin or ampicillin, alone or in combination with streptomycin, on the number of viable enterococci of two strains, during incubation at 37 C for 4 days.*

populations. This "synergistic" action was similar with ampicillin and with benzylpenicillin, although only half of the concentration of ampicillin was required for satisfactory bactericidal effect. The early bactericidal rate was usually faster with the combination than with ampicillin alone. Bactericidal synergism occurred with concentrations of each penicillin which were temporarily inhibitory (Enterococcus Hadley), as well as with concentrations which permitted no recovery of viable organisms for 48 to 72 hr (Enterococcus Wilcox). With the latter organism, a minimal effective concentration of ampicillin (0.75 μg/ml) became completely bactericidal upon addition of streptomycin, whereas an increase in ampicillin concentration alone (to 1.5 μg/ml) merely delayed the emergence and growth of persisters. It is possible that a much larger increase in ampicillin concentration might result not only in prolonged suppression but in complete bactericidal action. However, the experience with benzylpenicillin suggests that in those strains where persisters emerge in vitro, and clinical relapse occurs in vivo, an increase in penicillin concentration only prolongs suppression but does not affect the ultimate outcome (3, 5). We did not examine exhaustively the effects of very high concentrations of ampicillin alone, because the pattern of response in 21 enterococcus strains to ampicillin alone and in combination with streptomycin was entirely comparable to the pattern observed with benzylpenicillin (5). Sixteen strains required the drug combination for apparently complete bactericidal action in vitro. Six of the 21 strains seemed to be killed in vitro as well by ampicillin alone (albeit in a 4 to 20 times higher concentration) as by the combination of ampicillin with streptomycin.

DISCUSSION

Bacterial endocarditis is one of the human diseases in which curative action of antimicrobial drugs is directly related to bactericidal efficacy in vitro, particularly rapid early bactericidal rate, and lack of late emergence of persisters. When these criteria were applied to ampicillin alone as compared to ampicillin combined with streptomycin, the combination was unequivocally more effective in a majority of the 21 strains of enterococci tested. On the basis of such evidence, ampicillin alone cannot be recommended for the treatment of enterococcal endocarditis at this

time. Ampicillin in combination with streptomycin (or kanamycin) probably has advantages over benzylpenicillin in these combinations.

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