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Synergistic Effects of Ampicillin-Aminoglycoside Combinations on Group B Streptococci

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The in vitro activity of gentamicin, tobramycin, kanamycin, and amikacin in combination with ampicillin was determined against aminoglycoside-resistant group B streptococci. Synergy in each combination was determined by quantitative kill curves and demonstrated in all the combinations tested.

In recent years, the group B streptococcus has become one of the most common agents in neonatal infections. This fact has stirred interest in the dynamics of in vitro activity of antimicrobial agents alone and in combination against this pathogen. In cases of suspected neonatal sepsis, empirical therapy usually consisting of a combination of an aminoglycoside and penicillins or ampicillin is begun (3). If the culture yields group B streptococci, customary practice is to discontinue the aminoglycoside and continue treatment with penicillin or ampicillin alone (4). An in vivo synergistic effect was observed by Broughton et al. by using a combination of kanamycin and penicillin (1). Scauf et al. reported an in vitro synergistic effect by using gentamicin and ampicillin (6), and Deveikis et al. reported in vivo synergism in a mouse model (2). To date, only gentamicin in combination with ampicillin has shown both in vitro and in vivo synergy (2, 6).

All organisms used in this study were clinical isolates and identified as group B streptococci by Gram-stain reaction and morphology, positive test for hippurate hydrolysis, negative test on bile esculin agar, positive CAMP test, and positive capillary precipitation with Lancefield group B grouping sera. The organisms were grown in tryptose phosphate broth. They were shown to be aminoglycoside resistant by broth tube dilution and Kirby-Bauer disc diffusion. The mean aminoglycoside minimal inhibitory concentration of the six resistant strains was: gentamicin, 45 μg/ml; tobramycin, 75 μg/ml; amikacin 170 μg/ml; and kanamycin 150 μg/ml. The mean aminoglycoside minimal bactericidal concentration of the strains was gentamicin, 85 μg/ml; tobramycin, 150 μg/ml; amikacin, 213 μg/ml; and kanamycin, 234 μg/ml. The highest concentration of the aminoglycosides used was: gentamicin, 13 μg/ml; tobramycin, 10 μg/ml; amikacin, 40 μg/ml; and kanamycin, 40 μg/ml.

Tobramycin (Eli Lilly & Co.), gentamicin (Schering Corp.), kanamycin (Bristol Laboratories), amikacin (Bristol Laboratories), and ampicillin (Parke, Davis & Co.) were reconstituted with sterile water to a concentration of 2 mg/ml stock solution and either incorporated into the test media or stored at −70°C.

Tryptose phosphate broth (Difco), containing each drug alone or various combinations of ampicillin with one of the aminoglycosides, was inoculated with bacteria (10^5 colony-forming units per ml) from an overnight culture and incubated at 37°C. At 4, 8, and 24 h, a 0.1-ml sample was removed and the number of colony-forming units per milliliter was determined by streak plate counts using Mueller-Hinton agar (Difco).

Synergy was defined as a decrease of 100-fold or more in the number of viable organism as a result of the combination as compared with the most effective drug when tested alone (5).

The series of experiments performed with the six strains to determine the kinetics of killing by the aminoglycoside or ampicillin or in combination is shown in Fig. 1. There was no significant difference in the rate of killing produced by 1 or 10 μg of ampicillin. Combinations of ampicillin and gentamicin or tobramycin resulted in synergy against all six strains at 24 h. Amikacin or kanamycin combinations with ampicillin demonstrated synergy in five of six strains at 24 h.

The rate of killing with the ampicillin-gentamicin combination was dramatic (Fig. 1A). After 8 h of incubation, two of the three concentrations were synergistic. All concentrations of the combination were bactericidal at 24 h, whereas 10 μg of ampicillin alone was not. Only the highest concentration of the ampicillin-amikacin combination showed synergy at 8 h (Fig. 1B). Again, ampicillin alone (10 μg/ml) was not bactericidal

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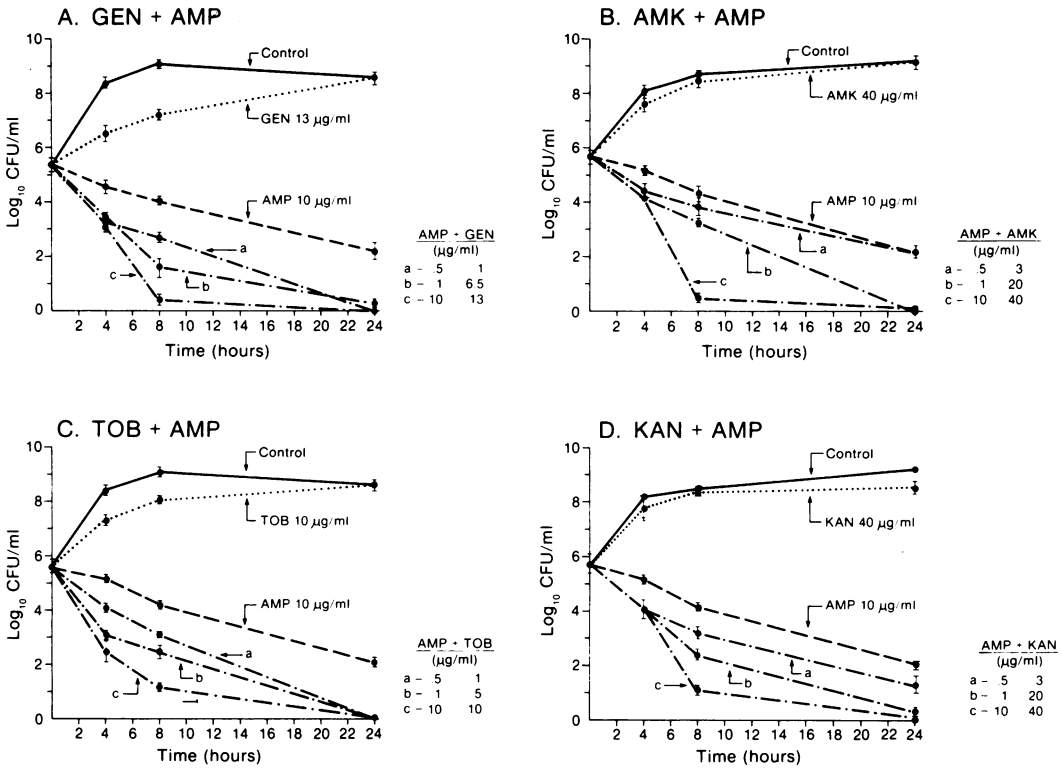

FIG. 1. *Kinetics of killing of group B streptococci by ampicillin-aminoglycoside combinations. Each point represents the mean of six isolates (± standard error).*

but, when combined with amikacin, two of the three combinations showed synergy at 24 h. Figure 1C shows the results of the ampicillin-tobramycin combinations. Concentrations of 1 μg of ampicillin with 5 μg of tobramycin per ml and of 10 μg of ampicillin and 10 μg of tobramycin per ml resulted in synergy at 8 h. All combinations tested were bactericidal at 24 h, whereas 10 μg of ampicillin alone was not. Figure 1D shows the result of ampicillin-kanamycin combinations. Combinations of 1 μg of ampicillin and 20 μg of kanamycin per ml and 10 μg of ampicillin and 40 μg of kanamycin per ml showed synergy after 8 and 24 h of incubation.

The results indicate that combinations of ampicillin with gentamicin, tobramycin, amikacin, or kanamycin demonstrate in vitro synergy against aminoglycoside-resistant group B streptococci. The lowest concentrations of the combinations were not synergistic, but the higher concentrations were.

The kinetic studies of the ampicillin-aminoglycoside combinations indicate that rapid killing can be achieved by a mixture which contains each antibiotic at a concentration insufficient to be bactericidal alone. The early initiation of killing at 8 h by the ampicillin-aminoglycoside combination compared with activity of ampicillin alone indicates that the aminoglycoside rather than ampicillin is responsible for the early bactericidal death.

These data confirm the observation of Schauf et al. (6) that gentamicin and ampicillin act synergistically against group B streptococci and extend the information on such synergism to include tobramycin, amikacin, gentamicin, and kanamycin. They also support the in vivo murine data of Deveikis et al. (2), which showed increased rates of clearance of group B streptococci from mice with ampicillin-aminoglycoside combinations.

Our data indicate that all of the aminoglycoside combinations tested are synergistic in vitro, and no single combination is more effective than any of the others tested. Some combinations may have clinical application.

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