Bactericidal Activity of Combinations of Gentamicin with Penicillin or Clindamycin Against Streptococcus mutans

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Received for publication 29 October 1974

Data derived from testing the bactericidal activity of combinations of penicillin with gentamicin or streptomycin and of clindamycin with gentamicin on nine isolates of Streptococcus mutans were analyzed by preparing isobolograms to determine the presence of additive, synergistic, or antagonistic effects. Synergy with penicillin-aminoglycoside combinations was found in two strains; additive effects occurred in seven instances with penicillin-gentamicin combinations; and antagonism occurred in eight instances with clindamycin-gentamicin combinations.

Endocarditis caused by the viridans streptococci is usually treated with penicillin and streptomycin, a combination that has been reported recently by Wolfe and Johnson (9) to act synergistically in vitro against this group of organisms. Moreover, penicillin-aminoglycoside synergy has been reported by Sande and Irvin (6) in experimental endocarditis produced in rabbits by a single strain of a viridans streptococcus. To our knowledge, no studies have been reported on the possible synergism of gentamicin with penicillin or clindamycin against one of the species of viridans streptococci, Streptococcus mutans. To evaluate this possibility, we examined the effects of combinations of (1) gentamicin and penicillin or clindamycin and (2) streptomycin and penicillin against strains of S. mutans isolated from nine patients with endocarditis.

MATERIALS AND METHODS

Bactericidal activity was measured by a two-dimensional broth dilution checkerboard technique (7, 8) in Trypticase soy broth (BBL). Each tube was inoculated with 10⁶ colony-forming units per ml. After 16 h of incubation at 35 C, 0.06 ml from each tube was transferred into a tube containing 15 ml of thioglycolate medium without indicator-135C (BBL). The small amount of agar normally present in thioglycolate medium permits enumeration of small numbers of discrete colonies. After incubation for 72 h at 35 C, these subcultures were recorded as demonstrating either complete absence of growth, a specified number of discrete colonies, or confluent growth. Bactericidal activity was considered to have occurred at a particular concentration of one or both antibiotics when there was either a complete lack of growth or fewer than 10 colonies (representing at least 99.9% killing of the original inoculum) in the subculture.

The data were analyzed, for determination of synergistic, antagonistic, or additive effects, by preparing isobolograms as described by Loewe (4) and simplified by Lacey (3) and Sabath (5). In such a plot, a line bowing away from the coordinates represents antagonism, a straight line indicates an additive effect, and a line that bows toward the coordinates represents a synergistic effect.

RESULTS

In the studies with penicillin and gentamicin, the minimal bactericidal concentrations (MBC) of penicillin were 3.1 μg/ml for one strain, 6.2 μg/ml for three strains, and 12.5 μg/ml for five strains, 50 μg/ml for one strain, and >100 μg/ml for five strains, 12.5 μg/ml for five strains, 25 μg/ml for two strains, 50 μg/ml for one strain, and >100 μg/ml for one strain. There was only one instance of synergy between the two antibiotics; in another seven strains there was an additive effect, as illustrated for one strain in Fig. 1. With the ninth strain, no conclusions could be drawn because the gentamicin MBC value remained greater than that of the highest concentration tested.

In the studies with penicillin and streptomycin, the MBC values of streptomycin were 50 μg/ml for two strains and >100 μg/ml for the seven other strains. There was evidence of synergy in one instance and of antagonism in one. The strain affected synergistically by penicillin and streptomycin was not the same one that was similarly affected by penicillin and gentamicin. With the seven other strains, no conclusions could be drawn because the MBC values of streptomycin remained >100 μg/ml.

In the studies with clindamycin and gentamicin, the MBC values of clindamycin were 6.2
and Irvin cillin-aminoglycoside combinations, such as those of seven studies, activity of additively by the penicillin-gentamicin affected eradicated from were (6).

Irvin more rapidly by a penicillin-aminoglycoside experimental endocarditis in rabbits due to a viridans streptococcus reported by Sande and section of combination in demonstrated additive.

However, it may be reasonable to assume that an additive effect of this combination of antibiotics is helpful clinically, particularly in view of the results of studies with experimental endocarditis in rabbits due to a viridans streptococcus reported by Sande and Irvin (6). In this experimental model, bacteria were eradicated from the cardiac vegetations more rapidly by a penicillin-aminoglycoside combination than by penicillin alone. In our studies, seven of the isolates of S. mutans were affected additively by the penicillin-gentamicin combination.

Data derived from time-kill curves with penicillin-aminoglycoside combinations, such as those of Wolfe and Johnson (9) and of Sande and Irvin (6), are not completely comparable to our own because the species of viridans streptococci tested in those studies were not defined and there are insufficient data regarding possible species differences in antimicrobial susceptibility of viridans streptococci. Moreover, the diminution in colony-forming units in the time-kill curves reported by Wolfe and Johnson (9) and by Sande and Irvin (6) may represent effects that could be interpreted as being additive in an isobologram rather than synergistic. In reporting synergy of penicillin and streptomycin in 47 of 48 strains of viridans streptococci, Wolfe and Johnson (9) used two different methods to test synergy; by either method alone, approximately 77% of their strains were stated to be affected synergistically by the antibiotic combination tested.

As noted by Jawetz (2), there are neither standardized methods to determine summation of antimicrobial action in vitro nor universally accepted definitions to separate additive from synergistic actions. One method, however, of making the distinction between these two types of drug interaction was discussed by Loewe (4) with reference to drugs other than antimicrobics and has been applied to interactions between antimicrobics by Lacey (3) and, more recently, by Sabath (5). According to Loewe, an isobologram bowing away from the coordinates indicates antagonism and one bowing toward the coordinates indicates synergy. A straight line is indicative of an additive effect; the validity of this interpretation may be checked by plotting an isobologram in which the drug plotted on the ordinate is in fact the same as is plotted on the abscissa. Killing curves can demonstrate antagonistic or indifferent drug interactions; however, they do not provide the means of distin-

**DISCUSSION**

Because synergy with a combination of penicillin and an aminoglycoside was demonstrated in vitro with only two stains of S. mutans, it may appear to be unnecessary to use this combination in the treatment of S. mutans endocarditis. However, it may be reasonable to assume that an additive effect of this combination of antibiotics is helpful clinically, particularly in view of the results of studies with experimental endocarditis in rabbits due to a viridans streptococcus reported by Sande and Irvin (6). In this experimental model, bacteria were eradicated from the cardiac vegetations more rapidly by a penicillin-aminoglycoside combination than by penicillin alone. In our studies, seven of the isolates of S. mutans were affected additively by the penicillin-gentamicin combination.

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guishing between additive and synergistic interactions. How important this distinction between additive and synergistic antimicrobial interactions is clinically is not known; however, it seems likely that it is relatively unimportant in the treatment of viridans streptococcal endocarditis with a combination of a penicillin and an aminoglycoside.

It would seem that treatment of S. mutans endocarditis with a clindamycin-gentamicin combination may be contraindicated because of the mutually antagonistic effects of these drugs in vitro. In fact, recently reported data have shown that clindamycin interferes with the early bactericidal activity of gentamicin against gram-negative bacilli and Staphylococcus aureus (L. Riff and D. Matulionis, Abstr. 291; S. H. Zinner, R. B. Provonchee, K. S. Elias, and G. Peter, Abstr. 283, 14th Intersci. Conf. Antimicrob. Agents Chemother., San Francisco, 1974).

Because all of our patients with S. mutans endocarditis have been successfully treated with a penicillin-streptomycin combination (1), we have no clinical data substantiating the efficacy of penicillin therapy alone or the inefficacy of therapy with clindamycin and gentamicin. The antagonistic effects of clindamycin and gentamicin have not been studied in other species of viridans streptococci.

LITERATURE CITED