SYNERGISM WITH AMINOGLYCOSIDES OF PENICILLIN, AMPICILLIN AND VANCOMYCIN AGAINST NON-ENTEROCOCCAL GROUP-D STREPTOCOCCI AND VIRIDANS STREPTOCOCCI

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THE non-enterococcal group-D streptococci, most of which belong to the species *Streptococcus bovis* (Facklam, 1972), have recently been recognised as an important cause of bacterial endocarditis (Ravreby, Bottone and Keusch, 1973; Haldane *et al.*, 1974; Hoppes and Lerner, 1974; Moellering, Watson and Kunz, 1974; Watanakunakorn, 1974). Their antibiotic susceptibility has been shown to be more similar to that of the penicillin-susceptible viridans streptococci than of the enterococcal group-D streptococci (Hoppes and Lerner, 1974; Thornsberry, Baker and Facklam, 1974). While the combination of penicillin and streptomycin is synergistic against the penicillin-susceptible viridans streptococci (Wolfe and Johnson, 1974), there is little information about antibiotic synergism against the non-enterococcal group-D streptococci (Wilkowske *et al.*, 1971; Watanakunakorn, 1974).

In the present study we (a) compared the antibiotic susceptibility of the non-enterococcal group-D streptococci with that of viridans streptococci, and (b) determined the effect of the combination of penicillin, ampicillin or vancomycin with streptomycin, kanamycin, gentamicin or tobramycin on the in-vitro killing of non-enterococcal group-D streptococci and of viridans streptococci.

MATERIALS AND METHODS

Streptococci. All streptococci used in this study were isolated from blood cultures of patients with bacteraemia and endocarditis. Only one isolate per patient was studied. Ten strains were non-enterococcal group-D streptococci that gave either α haemolysis or no haemolysis on blood agar, a positive reaction on bile-aesculin agar and failed to grow in Brain Heart Infusion (BHI) broth (Difco) to which 6.5% w/v of NaCl had been added (Facklam, 1972). Twenty strains were viridans streptococci that gave α haemolysis on blood agar, a negative reaction on bile-aesculin agar and failed to grow in 6.5% NaCl BHI broth.

Antibiotic susceptibility. The MIC of penicillin, ampicillin, oxacillin, nafcillin, cephalothin, vancomycin, erythromycin, clindamycin, streptomycin, kanamycin, gentamicin, tobramycin and sisomicin for the streptococci was determined by an agar-dilution method recommended by the International Collaborative Study (Ericsson and Sherris, 1971). Mueller-Hinton agar supplemented with 5% v/v of defibrinated human blood was used. The inoculum was 0.002 ml of a 10^{-2} dilution of an overnight culture (that had been incubated at 37°C in 95% air + 5% CO₂), and was delivered by a Steers replicator (Steers, Foltz and Graves, 1959).

Studies of synergism. All streptococci were tested for possible synergism of antibiotic combinations by the killing-curve method (Watanakunakorn, 1971 and 1974). The streptococcus was subcultured in BHI broth and incubated at 37°C in the presence of 5% CO₂ for 24 h. It was then diluted to give between 10^6 and 10^7 colony-forming units (c.f.u.) per ml of Mueller-Hinton broth supplemented with 5% v/v of defibrinated human blood. The following test systems with the indicated final concentrations of antibiotics added to the cultures were set up for each strain: (1) no antibiotic (control); (2) penicillin 2 µg per ml; (3) ampicillin 2 µg per ml; (4) vancomycin 2 µg per ml; (5) gentamicin 1 µg per ml; (6) tobramycin 1 µg per ml; (7) streptomycin 10 µg per ml; (8) kanamycin 10 µg per ml; (9) penicillin 2 µg per

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TABLE I

Antibiotic susceptibility of non-enterococcal group-D streptococci and viridans streptococci

Antibiotics	Range (and median) of MIC (μg per ml) of the stated antibiotic for	
	non-enterococcal group-D streptococci*	viridans streptococci†
Penicillin	0.0312-0.0625 (0.0468)	0.0156-0.125 (0.0625)
Ampicillin	0.25-1.0 (0.5)	0.125-1.0 (0.5)
Oxacillin	0.5-1.0 (1.0)	0.125-1.0 (0.25)
Nafcillin	0.25-0.5 (0.25)	0.0625-2.0 (0.25)
Cephalothin	0.125 (0.125)	0.0156-0.125 (0.0625)
Vancomycin	0.25-0.5 (0.5)	0.5 (0.5)
Erythromycin	0.25-0.5 (0.5)	0.0625-0.25 (0.125)
Clindamycin	0.0625-0.125 (0.125)	0.0625-0.125 (0.0625)
Streptomycin	64->64 (64)	16->64 (32)
Kanamycin	64->64 (>64)	64->64 (>64)
Gentamicin	8-32 (32)	32->64 (64)
Tobramycin	32-64 (64)	32->64 (64)
Sisomicin	8-16 (12)	16->64 (32)

* Ten strains tested.

† Twenty strains tested.

TABLE II

Synergism of antibiotic combinations against 10 strains of non-enterococcal group-D streptococci

Antibiotic combination	Number of strains against which the stated combination showed synergism at		
	6 h	24 h	48 h
Penicillin + gentamicin	9	10	10
Penicillin + tobramycin	2	10	10
Penicillin + streptomycin	9	10	10
Penicillin + kanamycin	8	10	10
Ampicillin + gentamicin	9	10	10
Ampicillin + tobramycin	4	9	10
Ampicillin + streptomycin	9	9	10
Ampicillin + kanamycin	9	9	10
Vancomycin + gentamicin	9	10	9
Vancomycin + tobramycin	8	10	10
Vancomycin + streptomycin	10	10	10
Vancomycin + kanamycin	9	10	10

ml+gentamicin 1 μg per ml; (10) penicillin 2 μg per ml+tobramycin 1 μg per ml; (11) penicillin 2 μg per ml+streptomycin 10 μg per ml; (12) penicillin 2 μg per ml+kanamycin 10 μg per ml; (13) ampicillin 2 μg per ml+gentamicin 1 μg per ml; (14) ampicillin 2 μg per ml+tobramycin 1 μg per ml; (15) ampicillin 2 μg per ml+streptomycin 10 μg per ml; (16) ampicillin 2 μg per ml+kanamycin 10 μg per ml; (17) vancomycin 2 μg per ml+gentamicin 1 μg per ml; (18) vancomycin 2 μg per ml+tobramycin 1 μg per ml; (19) vancomycin

TABLE III

Synergism of antibiotic combinations against 20 strains of viridans streptococci

Antibiotic combination	Number of strains against which the stated combination showed synergism at		
	6 h	24 h	48 h
Penicillin + gentamicin	9	17	15
Penicillin + tobramycin	4	14	15
Penicillin + streptomycin	17	16	15
Penicillin + kanamycin	11	17	17
Ampicillin + gentamicin	8	17	12
Ampicillin + tobramycin	6	12	15
Ampicillin + streptomycin	17	15	16
Ampicillin + kanamycin	10	18	17
Vancomycin + gentamicin	17	17	18
Vancomycin + tobramycin	9	16	18
Vancomycin + streptomycin	18	17	18
Vancomycin + kanamycin	16	18	18

2 µg per ml + streptomycin 10 µg per ml; (20) vancomycin 2 µg per ml + kanamycin 10 µg per ml. The streptococcus-antibiotic mixtures were then incubated at 37°C with added CO₂. After incubation for 6, 24 and 48 h, viable counts were made by serial 10-fold dilution and subculture on to Mueller-Hinton agar supplemented with 5% v/v of defibrinated human blood. Colonies were counted after incubation at 37°C with 5% added CO₂ for 48 h.

An antibiotic combination was considered synergistic at a given time when it killed at least 10 times more streptococci than did the most effective antibiotic alone.

RESULTS

Antibiotic susceptibility. The MIC of all the antibiotics tested for the 10 strains of non-enterococcal group-D streptococci and 20 strains of viridans streptococci is shown in table I. The antibiotic susceptibility of the two groups of streptococci was very similar; both groups were resistant to the aminoglycosides.

Synergism. For all the antibiotic combinations tested, synergism was demonstrated against all strains of non-enterococcal group D streptococci after one or more time-interval (table II). Data on the synergistic action of antibiotic combinations against viridans streptococci are presented in table III; some or all of the antibiotic combinations were synergistic against all strains after one or more time-interval. Figs. 1 and 2 are examples of the synergistic action of antibiotic combinations against a strain of non-enterococcal group-D streptococcus and a strain of viridans streptococcus respectively. All streptococci grew in the presence of aminoglycosides. Penicillin, ampicillin and vancomycin alone killed the streptococci slowly. In these two examples, synergism was demonstrated with all antibiotic combinations at all time-intervals.

DISCUSSION

Among the streptococci that are presumptively identified as members of group D by their growth on bile-aesculin agar, the non-enterococcal group-D streptococci can be separated from the enterococci by their failure to grow in 6.5% NaCl BHI broth (Facklam, 1972). This separation is clinically important, because the antibiotic susceptibility of the non-enterococcal group-D streptococci closely resembles that of viridans streptococci, as shown

by this and previous studies (Hoppes and Lerner, 1974; Thornsberry *et al.*, 1974). Moreover, we have demonstrated in this study that penicillin or other cell-wall active antibiotics in combination with an aminoglycoside are synergistic against the non-enterococcal group-D streptococci as against the viridans streptococci.

In experimental viridans-streptococcal endocarditis in rabbits, the combination of penicillin and streptomycin has been shown to sterilise valvular vegetations faster than penicillin alone (Durack, Pelletier and Petersdorf, 1974; Sande and Irwin, 1974). With the use of penicillin and streptomycin in combination, the duration of antibiotic therapy in penicillin-susceptible viridans-streptococcal endocarditis in man has been successfully shortened to 2 weeks (Tan *et al.*, 1970). Because the synergistic effect of the penicillin-streptomycin combination against the non-enterococcal group-D streptococci was similar to that against viridans streptococci, a 2-week course of penicillin and streptomycin will probably be equally effective in the treatment of endocarditis caused by penicillin-susceptible non-enterococcal group-D streptococci.

FIG. 1.—Antibiotic synergism against a strain of non-enterococcal group-D streptococcus: C = control; G = gentamicin 1 μ g per ml; K = kanamycin 10 μ g per ml; S = streptomycin 10 μ g per ml; T = tobramycin 1 μ g per ml; P = penicillin 2 μ g per ml; A = ampicillin 2 μ g per ml; and V = vancomycin 2 μ g per ml.

FIG. 2.—Antibiotic synergism against a strain of viridans streptococcus: C = control; G = gentamicin 1 μ g per ml; K = kanamycin 10 μ g per ml; S = streptomycin 10 μ g per ml; T = tobramycin 1 μ g per ml; P = penicillin 2 μ g per ml; A = ampicillin 2 μ g per ml; and V = vancomycin 2 μ g per ml.

In contrast to the situation with enterococci (Watanakunakorn, 1971; Watanakunakorn and Bakie, 1973), the newer aminoglycosides, gentamicin and tobramycin, offered no advantage over streptomycin in synergism with penicillin, ampicillin or vancomycin against non-enterococcal group-D streptococci or viridans streptococci. Ampicillin was much less effective than penicillin and offered no advantage over penicillin in synergism with the aminoglycosides. Vancomycin in combination with the aminoglycosides was much more active than vancomycin alone against both groups of streptococci.

SUMMARY

The antibiotic susceptibility of 10 strains of non-enterococcal group-D streptococci was compared with that of 20 strains of viridans streptococci. The minimal inhibitory concentrations of penicillin, ampicillin, oxacillin, nafcillin, cephalothin, vancomycin, erythromycin and clindamycin for the two groups of streptococci were very similar in range and median values. Both groups of streptococci were resistant to the aminoglycosides.

The effect of the combination of penicillin, ampicillin or vancomycin with streptomycin, kanamycin, gentamicin or tobramycin on the in-vitro killing of the two groups of streptococci was compared. For all the antibiotic combinations tested, synergism was demonstrated against all strains of non-enterococcal group-D streptococci after one or more of the time-intervals 6, 24 and 48 h. Some or all of the antibiotic combinations were synergistic against all strains of viridans streptococci after one or more of the same time-intervals. The other aminoglycosides (kanamycin, gentamicin and tobramycin) offered no advantage over streptomycin in synergism with penicillin, ampicillin or vancomycin against non-enterococcal group-D streptococci or viridans streptococci.

These results suggest that non-enterococcal group-D streptococcal endocarditis may be treated by the same regimen as endocarditis caused by the viridans streptococci.

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