Technical methods

A modified technique for the detection of antibiotic synergism

C. M. ANAND¹ and A. PAULL *Department of Clinical Bacteriology, University Hospital of Wales, Heath Park, Cardiff*

A suitable therapy for subacute bacterial endocarditis (SBE) caused by *Streptococcus faecalis* is a combination of a penicillin and an aminoglycoside (Garrod *et al*, 1973). This treatment has also been advocated for treating SBE caused by other streptococci, especially those with a higher resistance to penicillin (Garrod *et al*, 1973). Although most strains of *Str. faecalis* and *Str. viridans* are susceptible to this combined therapy, some strains are not affected. It is essential therefore to test *in vitro* for synergistic action of antibiotics against strains of streptococci implicated in SBE.

Methods available for studying the synergistic effect of an antibiotic combination are tedious and time-consuming, and some require considerable experience. We describe a simple technique which can be carried out in most routine laboratories.

Material, methods, and results

Nineteen strains of *Str. faecalis* freshly isolated from various clinical specimens were tested.

Plates containing 20 ml of Oxoid Diagnostic Sensitivity Test Agar with 7% horse blood were surface inoculated by flooding with a suspension of each strain containing about 10^7 organisms/ml. Any surplus fluid was removed with a Pasteur pipette. Sterile blotting paper strips, 0.5×5 cm, were immersed in a solution of either streptomycin (1000 µg/ml) or ampicillin (500 µg/ml). Excess fluid was removed by lightly blotting on sterile blotting paper, and the strips were placed at right-angles to one another on the surface of the medium. Plates were incubated for five hours at 37°C, and penicillinase solution (Burroughs Wellcome) containing 5000 iu/ml was sprayed on to the surface of the plate with a general purpose spray (Quikfit). Spraying for five seconds ensured sufficient coverage of the surface without gross wetting. After overnight incubation the plates were examined for synergistic action of the anti-

biotic. Figure 1 illustrates the presence of, and figure 2 the lack of, synergism between ampicillin and streptomycin against strains of *Str. faecalis*. Synergism is denoted by an area devoid of growth in the angle between the ampicillin and streptomycin strips.

The minimum inhibitory concentration (MIC) of streptomycin for each strain was determined by the method described by Standiford *et al* (1970).

Of the 19 strains of *Str. faecalis* tested, 15 displayed synergism. MICs of streptomycin for these organisms ranged between 32 µg/ml and 125 µg/ml.

The four remaining strains displayed a lack of synergy. The MICs of streptomycin for these organisms were greater than 2000 µg/ml.

Discussion

Several methods of testing for bacterial synergy between antibiotics have been described. These have included the tube dilution method (Garrod and Waterworth, 1962) and the Cellophane transfer technique (Chabbert and Waterworth, 1965). Both these methods are tedious and time consuming, the final results not being available for at least 48 hours. Chabbert's technique moreover requires special apparatus and some experience.

Methods using antibiotic-impregnated discs have been described recently (Lee and Komarmy, 1975; Yourassowsky *et al*, 1975). These are dependent upon exact positioning of the antibiotic disc in relation to one another. Because this position is determined by the knowledge of the size of the zones of inhibition of the organism by each disc, these techniques are more suitable for adoption by laboratories using Kirby-Baur techniques of antibiotic sensitivity testing. Since this method is not generally used in Britain, synergy testing by the use of antibiotic discs necessitates a preliminary step of determining precise zones around each of the antibiotic discs, and calls for experience in the use of the Kirby-Baur method. The technique described here eliminates this additional step.

Direct proof of bactericidal action of the combination of antibiotics in the zone of synergy is not provided by the test. This cannot be confirmed directly as it is not yet possible to inactivate ampicillin and streptomycin simultaneously. However, streptomycin by itself is only weakly inhibitory against streptococci and is bactericidal only when combined with penicillin. After inactivation of the penicillin

¹Present address: Provincial Laboratory of Public Health, PO Box 2490, Calgary, Alberta T2P 2 M7, Canada

Fig 1

Fig 1 Synergism is displayed by a clear area devoid of growth in the angle between ampicillin (vertical) and streptomycin (horizontal) strips.

Fig 2

Fig 2 Lack of synergism is displayed by growth of organism in the zone of inhibition.

and secondary incubation, any residual zone at the junction of the strips larger than that around the distal part of the streptomycin strip is likely to be caused by the previous synergistic bactericidal activity of the drug combination rather than the bacteriostatic activity of the unmodified streptomycin alone.

Demonstration of synergy correlated well with sensitivity to streptomycin. The 15 strains that displayed synergy by our technique had minimum inhibitory concentrations well below 1 mg/ml, and the four strains that showed lack of synergy all had an MIC to streptomycin greater than 2 mg/ml.

Our results demonstrate in all strains the synergy predicted for *Str. faecalis* by Standiford *et al* (1970) on the basis of whether the MIC for streptomycin was greater or less than 2 mg/ml. However, as this synergy was not shown invariably by Standiford *et al* (1970) when the MIC was less than 2 mg/ml, we believe that the direct demonstration of synergy by

this simple technique is advantageous.

References

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