Sensitivity of *Nocardia* to trimethoprim and sulphonamides *in vitro*

WILLIAM A. BLACK
with the technical assistance of DORA A. McNELLIS
*From the University Department of Bacteriology, Glasgow Royal Infirmary*

SYNOPSIS  Studies *in vitro* of the sensitivity to trimethoprim and sulphonamides of nine strains of *N. asteroides*, two strains of *N. caviae*, and one of *N. blackwellii* are presented. No unequivocal evidence of synergism was found. Despite this, the inclusion of trimethoprim in the drug regime when sulphonamides are used in the treatment of nocardiosis is suggested on empirical grounds.

In recent years it has been demonstrated that trimethoprim (2,4-diamino-5-(3,4,5-trimethoxybenzyl)-pyrimidine) reacts with sulphonamides in a manner which is strongly synergistic and that drugs, which are themselves bacteriostatic, achieve a bactericidal effect when used together. The pharmacology of this drug combination has been thoroughly evaluated *in vitro* and in laboratory animals by Bushby and Hitchings (1968). Darrell, Garrod, and Waterworth (1968) confirmed the *in vitro* findings of the above authors and reported favourably on the use of a sulphonamide-trimethoprim regime in successfully treating respiratory infections, urinary tract infection, and Gram-negative septicaemia. More recently, there have been further reports of success in the treatment of urinary tract infection (Grüneberg and Kolbe, 1969; Reeves, Faiers, Pursell, and Brumfitt, 1969), of neonatal meningitis due to *Escherichia coli* (Morzaria, Walton, and Pickering, 1969), and it has been found (Akinkugbe, Lewis, Montefiore, and Okubadejo, 1968) that a combination of sulphonamethoxazole and trimethoprim was equal in value to chloramphenicol in the treatment of typhoid fever.

There have, however, been no reports to date on the use of a sulphonamide-trimethoprim mixture in the treatment of *Nocardia* infections where, despite the advent of many potent new antibiotics, sulphadiazine remains the drug of choice (Strauss, Kligman, and Pillsbury, 1951; Neu, Silva, Hazen, and Rosenheim, 1967; Shuster, Klein, Pribor, and Kozub, 1967). Peabody and Seabury (1960), however, in an extensive review of the therapy of actinomycosis and nocardiosis have suggested that better results might be obtained if sulphadiazine were to be used along with another chemotherapeutic drug in the treatment of the latter condition. Trimethoprim would appear on theoretical grounds to be the obvious choice of drug for this purpose, and the following paper, in an attempt to substantiate this theory, presents an account of studies *in vitro* of the effect of sulphonamide-trimethoprim mixtures on nine strains of *Nocardia asteroides*, two of *N. caviae*, and one of *N. blackwellii*.

Materials and Methods

The strain N1 was isolated in this laboratory from a fatal case of nocardiosis, and the strain NHS was isolated by Dr H. Singh from a fatal case at another hospital in this area. The origin of the other species of *Nocardia* is given in Table I. *Staphylococcus aureus* (CN 491) and *Escherichia coli* (CN 314) were included as controls.

The trimethoprim, sulphonamethoxazole, and sulphadiazine were freshly prepared before each experiment.

Trimethoprim, available as trimethoprim lac-
William A. Strain

Minimum 1-25
NHS 25 1-25
N8595 2 5 1-25 1-25
N870 1-25 1-25 2-5 1-25 2-5
CN2470 1-25 1-25 2-5 1-25 2-5
RG874 1-25 1-25 2-5 1-25 2-5
RG886 1-25 1-25 2-5 1-25 2-5
W4 1-25 1-25 2-5 1-25 2-5
N1934 1-25 1-25 2-5 1-25 2-5
W2 1-25 1-25 2-5 1-25 2-5
N630 1-25 1-25 2-5 1-25 2-5
Staph. aureus
CN491 0-625 <0-312 <0-312 <0-312 <0-312 5
Esch. coli
CN314 0-625 0-625 <0-312 <0-312 <0-312 10

Table I Origin of Nocardia species

<table>
<thead>
<tr>
<th>Strain</th>
<th>Source</th>
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</thead>
<tbody>
<tr>
<td>N1</td>
<td>Fatal case of nocardiosis</td>
</tr>
<tr>
<td>NHS</td>
<td>Fatal case of nocardiosis</td>
</tr>
<tr>
<td>N8595</td>
<td>National Collection of Type Cultures</td>
</tr>
<tr>
<td>N870</td>
<td>National Collection of Type Cultures</td>
</tr>
<tr>
<td>CN2470</td>
<td>Mycology Reference Laboratory</td>
</tr>
<tr>
<td>RG874</td>
<td>Wellcome Bacterial Collection</td>
</tr>
<tr>
<td>RG886</td>
<td>Institute of Microbiology, New Jersey</td>
</tr>
<tr>
<td>W4</td>
<td>Institute of Microbiology, New Jersey</td>
</tr>
<tr>
<td>N1934</td>
<td>Westminster Medical School</td>
</tr>
<tr>
<td>W2</td>
<td>Westminster Medical School</td>
</tr>
<tr>
<td>N630</td>
<td>National Collection of Type Cultures</td>
</tr>
</tbody>
</table>

Table II Minimum inhibitory concentrations of sulphonamide, trimethoprim, and various combinations

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sulphamethoxazole + Trimethoprim</th>
<th>Trimethoprim</th>
<th>Sulphamethoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20:1</td>
<td>10:1</td>
<td>5:1</td>
</tr>
</tbody>
</table>

Results

The results, which were read after 48 hours’ incubation at 37°C, are detailed in Tables II and III.

From these it can be seen that the sulphonamide MICs are satisfactory and could be quite easily achieved in vivo by standard dosages. Raich, Casey, and Hall (1961), for example, showed that a blood sulphonamide level of 140 μg/ml could be maintained by a dosage of 6 to 8 g of sulphadiazine per day, and Garrod and O’Grady (1968) report a peak plasma level of 100 μg/ml after an initial dose of 4 g of sulphadiazine followed by 1 g four hourly. Stokes (1968) maintains that for most acute infections the average blood antibiotic level should exceed the MIC by a safety factor of 2 or 4, but for actinomycosis (and presumably nocardiosis) the safety factor should be higher. These criteria are satisfied by the present figures which show an average MIC of about 2-5 μg/ml with a potential blood level of 100 to 140 μg/ml, i.e., 20 to 30 times the MIC.

The sensitivity to trimethoprim is disappointing. In other series the average MIC for most of the bacteria tested was of the order of 1 μg/ml.
or less (Bushby and Hitchings, 1968; Darrell et al, 1968) whereas with the Nocardia in our series the MIC of trimethoprim was 50 μg/ml in eight out of the 12 strains tested, 25 μg/ml in three strains, and 10 μg/ml in the remaining strain. As Bushby and Hitchings (1968) were able to obtain peak serum levels of only 2 to 3 μg/ml of trimethoprim one hour after giving an oral dose of 250 mg, it must be concluded that the use of trimethoprim alone would be of no value in the treatment of nocardiosis.

In the case of Neisseria gonorrhoeae, it has been shown (Bushby and Hitchings, 1968) that there was a high degree of potentiation of sulphadiazine by trimethoprim despite the fact that these organisms are relatively resistant to the latter drug. The results shown in Tables II and III, however, indicate that this synergistic effect was not demonstrated unequivocally in any of the four sulphonamide:trimethoprim ratios tested. There did, however, seem to be some enhancement of the sulphonamide when this was used with an equal amount of trimethoprim but in only one or two cases was there more than a two-fold difference between the sulphonamide acting alone and the sulphonamide combined with trimethoprim.

Sulphamethoxazole is the sulphonamide of choice for use with trimethoprim as these drugs are absorbed and excreted at the same rate. We chose to test sulphadiazine as well as sulphamethoxazole because the former has been used most widely in the treatment of nocardiosis (Peabody and Seabury, 1960). Tested individually, the MIC of sulphadiazine is the same, or two-fold higher, than that of sulphamethoxazole, a finding which tallies with that of Neipp (1964) in his survey of comparative activities of sulphonamides in vitro. Sulphadiazine displayed the same lack of marked synergistic activity with trimethoprim as did sulphamethoxazole.

Discussion

At the time of publication, sulphadiazine remains the drug of choice in the treatment of nocardiosis (Strauss et al, 1951; Neu et al, 1967; Shuster et al, 1967). There has, however, been a recent report of the successful treatment of a case of cerebral nocardiosis using a combination of sulphonamides and cycloserine (Hoeprich, Brandt, and Parker, 1968), and Vasanish (1968) reported the successful treatment of a case of primary cutaneous nocardiosis using a combination of sulphadiazine, erythromycin, and sulfone. The fact that no unequivocal synergism with trimethoprim was seen in vitro was disappointing but might have been expected from the relatively high MIC of the latter for, in their study of urinary tract infection, Reeves et al (1969) showed that the failure of treatment in all species tested was associated with a relatively high resistance to trimethoprim.

On the other hand, Wright and Grimble (1969) found that the cure rate in gonorrhoea was only 30% when treated with sulphonamide-trimethoprim but that when this was combined with trimethoprim the cure rate rose to 80% even although the MIC of trimethoprim for the gonococcus has been shown to be as high in some cases as that which we found for Nocardia (Bushby and Hitchings, 1968). Again, Csonka and Knight (1967) have shown that gonorrhoea can be successfully treated with a sulphonamide-trimethoprim mixture even although trimethoprim itself is useless in treating this condition. It must be remembered, however, that a synergistic effect of sulphonamide and trimethoprim on the gonococcus has been demonstrated in vitro (Bushby and Hitchings, 1968) whereas we have failed to demonstrate this convincingly with Nocardia.

The question of therapy is not merely of academic interest as nocardiosis is a chronic, disabling condition which even if correctly diagnosed and treated is still attended by a high morbidity and mortality. Hoeprich et al (1968) in a review of 148 cases of nocardiosis report a fatality rate of about 87% in patients with cerebral involvement and 10% in patients with uncomplicated pulmonary disease who had received sulphonamide therapy. In dealing with a condition of this severity, the present findings cannot justify any reduction in what is accepted as a reasonable dose of sulphonamide, viz, 6-8 g/day, although the addition to this regimen of 1 g/day of trimethoprim (which is the maximum clinically acceptable dose) would appear worthy of trial, as our laboratory findings suggest that a 1:1 sulphonamide:trimethoprim ratio (and, to a lesser extent a 5:1 ratio) does give some minor degree of synergism. The choice of sulphonamide used in treating nocardiosis will depend on whether or not trimethoprim is to be added to the regime. If it is, then sulphamethoxazole would be preferable; if it is not, then sulphadiazine should be chosen because of its proven efficacy.

It is hoped that this report will stimulate further investigation of this topic in an effort to reduce the morbidity and mortality of nocardiosis to a more acceptable level.

We should like to thank Dr L. G. Petty of the Wellcome Foundation for supplying trimethoprim lactate, sulphamethoxazole, and the control strains of Staphylococcus aureus (CN 491) and Escherichia coli (CN 314). Our thanks are also due to Dr S. R. M. Bushby of the Wellcome Research Laboratories for helpful criticism and advice. May & Baker kindly supplied the sulphadiazine. Our grateful thanks are also due to the following who provided us with strains of Nocardia: Dr Ruth E. Gordon of the Institute of Microbiology, New Jersey; Professor B. W. Lacey of Westminster Medical School; Dr I.
Murray of the Mycological Reference Laboratory, London; Miss E. Jean Shelton of the National Collection of Type Cultures and the curator of the Wellcome Bacterial Collection.

References


Addendum

Since the completion of this work it has been observed that our original strain N1 is sensitive in vitro to 0-625 μg/ml of fucidin. At present we are in the process of investigating the comparative MICs of fucidin and sulphonamides for Nocardia.
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William A. Black and Dora A. McNellis

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