COMPARATIVE IN-VITRO SENSITIVITY OF NOCARDIA SPECIES TO FUSIDIC ACID AND SULPHONAMIDES

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Nocardiosis is a chronic granulomatous disorder caused by the aerobic actinomycete Nocardia asteroides. It is a remarkable fact that, despite the introduction of several potent new antimicrobial agents in recent years, the treatment of this condition is essentially unchanged with sulphadiazine remaining the drug of choice (Peabody and Seabury, 1960; Neu et al., 1967; Shuster et al., 1967). Therefore, when it was noticed that the strain of N. asteroides isolated from a fatal case of nocardiosis in this hospital was sensitive to very low concentrations of fusidic acid (Fucidin, Leo Laboratories), it was felt that this effect warranted further study as it had not previously been reported in the literature. In this report, we have compared the minimum inhibitory concentrations (MICs) of fusidic acid and two commonly used sulphonamides for various strains of the genus Nocardia.

MATERIALS AND METHODS

Organisms. Nine strains of N. asteroides, two of N. caviae and one of N. blackwellii were investigated (table). The strains N1 and NHS were isolated from fatal cases of nocardiosis, the former in this laboratory and the latter by Dr H. Singh at a neighbouring hospital. Strains no. 8595, 6761, 1934 and 630 were obtained from the National Collection of Type Cultures (NCTC). Strain no. 770 was obtained from Dr I. Murray, Mycology Reference Laboratory, London; strain no. CN2470 from the Wellcome Collection, London; strains no. RG874 and RG886 from Dr Ruth E. Gordon, Rutgers State University, New Jersey, USA, and strains no. W2 and W4 from Professor B. W. Lacey, Westminster Hospital Medical School, London.

Antimicrobial agents. Solutions of fusidic acid, sulphamethoxazole and sulphadiazine were freshly prepared before each experiment. Sulphadiazine is the sulphonamide used most widely in the treatment of nocardiosis (Peabody and Seabury; Hoeprich, Brandt and Parker, 1968), but it was decided to test another sulphonamide in parallel with it because of the description by Neipp (1964, cited by Garrod and O'Grady, 1968, p. 15) of variation in the in-vitro activity of sulphonamides. Fusidic acid is freely soluble, but the sulphonamides could be dissolved only at an alkaline pH, although once this had been achieved it was found possible to re-adjust the pH of the solution to 7.5 without precipitation occurring.

Media. For the estimation of MICs, sulphonamide inhibitors were neutralised by adding lysed horse blood to Diagnostic Sensitivity Test Agar (Oxoid—CM261) to a final concentration of 5 per cent. Lysed horse blood added to the fusidic acid plates doubled the MIC, an effect probably attributable to protein-binding of the drug and akin to that observed when sensitivity-testing to fusidic acid is carried out by the disk-diffusion method on blood-agar plates (Garrod and O'Grady, p. 436).

Estimation of MIC. Dilutions of the agents were made in nutrient broth (Oxoid CM1), 1 ml of each dilution being added to a volume of melted Diagnostic Sensitivity Test (DST) Agar (Oxoid—CM261) to give final concentrations ranging from 50 to 0.312 µg per ml. When sulphonamides were tested, lysed horse blood at a final concentration of 5 per cent. was added. Plates were then poured. An inoculum of approximately 10^5–10^6 organisms—prepared from a 6-hr mechanically agitated culture in 10 ml of nutrient broth containing 0.5 ml Tween 80—was added to every plate with a loop delivering 0.0025 ml. This inoculum was spread over an area of about 1 cm². The plates were incubated at 37°C for 48 hr and the MIC was read as the highest dilution of the antibiotic that inhibited obvious bacterial growth.

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RESULTS

These are shown in the table, where it can be seen that the MIC of fusidic acid was less than that of the sulphonamides in three instances, equal to that of the sulphonamides in three, and greater than that of the sulphonamides in six. If, however, *N. asteroides* alone is considered, the MIC of fusidic acid was less than that of the sulphonamides in three instances, equal to that of the sulphonamides in three, and greater than that of the sulphonamides in three. These experiments were repeated several times and, with the exception of strain no. NCTC6761 which was inhibited by varying concentrations of sulphadiazine, the results were remarkably consistent.

**Table**

*Minimum inhibitory concentrations of fusidin and two sulphonamides for strains of Nocardia*

<table>
<thead>
<tr>
<th>Strain no.</th>
<th>Species</th>
<th>MIC (µg per ml) of</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>fusidic acid</td>
<td>sulphamethoxazole</td>
</tr>
<tr>
<td>N1</td>
<td><em>N. asteroides</em></td>
<td>0.625</td>
<td>1.25</td>
</tr>
<tr>
<td>NHS</td>
<td><em>N. asteroides</em></td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>NCTC8595</td>
<td><em>N. asteroides</em></td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>NCTC6761</td>
<td><em>N. asteroides</em></td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>N770</td>
<td><em>N. asteroides</em></td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>CN2470</td>
<td><em>N. asteroides</em></td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>RG874</td>
<td><em>N. asteroides</em></td>
<td>2.5</td>
<td>0.525</td>
</tr>
<tr>
<td>RG886</td>
<td><em>N. asteroides</em></td>
<td>2.5</td>
<td>1.25</td>
</tr>
<tr>
<td>W4</td>
<td><em>N. asteroides</em></td>
<td>5</td>
<td>1.25</td>
</tr>
<tr>
<td>NCTC1934</td>
<td><em>N. caviae</em></td>
<td>20</td>
<td>2.5</td>
</tr>
<tr>
<td>W2</td>
<td><em>N. caviae</em></td>
<td>10</td>
<td>2.5</td>
</tr>
<tr>
<td>NCTC630</td>
<td><em>N. blackwellii</em></td>
<td>2.5</td>
<td>1.25</td>
</tr>
</tbody>
</table>

DISCUSSION

Although superficial skin lesions can occur, the characteristic clinical picture of nocardiosis is that of a recalcitrant, localised lung lesion that resembles pulmonary actinomycosis in some respects, but differs both in its histopathological appearances and also in displaying a striking predilection to metastasise to the brain.

There is still a very high fatality rate in this condition even when it is correctly diagnosed and treated. For example, in a recent survey of 148 cases, Hoeprich *et al.* (1968) reported a fatal outcome in about 87 per cent. of patients with cerebral involvement and 10 per cent. of patients with uncomplicated pulmonary nocardiosis who had been treated with sulphonamides. In view of these depressing results, it is interesting that two recent publications report successful treatment of nocardiosis with a combination of sulphonamides and other microbial agents as suggested by Peabody and Seabury (1960). Hoeprich *et al.* give details of successful therapy of cerebral nocardiosis with sulphonamides and cycloserine, and Vasarinsgh (1968) describes the successful outcome of a case of primary cutaneous nocardiosis treated with a combination of sulphadiazine, erythromycin and Sulfone. However, these reports and also the possible synergic effect of trimethoprim and sulphonamides on *Nocardia* species (Baikie, Macdonald and Mundy, 1970; Black and McNellis, 1970) still await further clinical evaluation. In the meantime, any new therapeutic agent that promises to be of value in the treatment of nocardiosis is obviously worth consideration.

The MICs of fusidic acid for *Nocardia asteroides* ranged from 0.625 µg per ml to 5.0 µg per ml, the value most frequently found being 2.5 µg per ml. Although the MIC first found
for strain N1 (0.625 μg per ml) was not found with any of the other strains studied, the median value of 2.5 μg per ml is still within the range of therapeutic usefulness. With oral fusidic acid in normal dosage, plasma concentrations of 11–41 μg per ml are obtainable after 24 hr and these rise to 30–144 μg per ml after 96 hours’ therapy as a result of accumulation of the drug (Garrod and O’Grady, 1968, p. 202). This means that the plasma concentration during treatment would exceed the MIC by a factor of between 12 and almost 60, a value considerably in excess of the usual factor of 2 or 4 considered satisfactory in the normal control of antibiotic therapy (Stokes, 1968).

Fusidic acid might be of value in treating the pulmonary lesions of nocardiosis, both by virtue of its excellent penetration into avascular areas (Stewart, 1964; Hierholzer et al., 1966) and also of its relative freedom from side-effects—an important consideration when the treatment of nocardiosis may need to extend over several months. It would, however, be of very limited value as the sole therapeutic agent in the treatment of cerebral nocardiosis because of its poor penetration into the central nervous system.

The correlation of in-vitro findings with the clinical situation in man is notoriously difficult, but these results justify consideration of fusidic acid, either alone or preferably in conjunction with sulphonamides, for dealing with the intractable lesions of nocardiosis. The superior in-vitro activity of sulphamethoxazole compared with that of sulphadiazine suggests that the former may be of some value in treating pulmonary nocardiosis. The latter, however, is preferable for the treatment of the cerebral lesions because of the high concentrations of drug that appear in the cerebrospinal fluid (Garrod and O’Grady, p. 38).

### Summary

The MICs of fusidic acid and two sulphonamides for nine strains of *Nocardia asteroides*, two strains of *N. caviae* and one strain of *N. blackwellii* were studied by means of a plate-dilution technique. The median value for the MIC of fusidic acid, 2.5 μg per ml, compared very favourably with those of the sulphonamides, and fusidic acid may well have a part to play in the treatment of nocardiosis.

We would like to acknowledge the generous supply of fusidic acid by Leo Laboratories Limited, sulphadiazine by May & Baker Limited and sulphamethoxazole by the Wellcome Foundation. We are also very grateful to those mentioned in the text who supplied us with strains of *Nocardia*. Finally we wish to thank Dr J. C. J. Ives for helpful criticisms and advice.

### References


