Spectinomycin: minimum inhibitory concentrations for Neisseria gonorrhoeae

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Actinospectacin, a broad-spectrum antibiotic derived from Streptomyces spectabilis, was released for clinical trials more than 10 years ago. Although it possesses activity against many micro-organisms, both Gram-positive and Gram-negative, its use has been limited mainly to the treatment of gonorrhoea. Its efficacy in the treatment of this disease has been reported by a number of investigators. Laird and Taylor (1962) claimed that a single injection of 2 g. actinospectacin sulphate effected a cure in 47 (94 per cent.) of fifty male patients. Willcox (1963) showed that, of 134 male patients similarly treated, only thirteen (9-7 per cent.) could be considered as treatment failures.

More recently, actinospectacin has been prepared as the hydrochloride and it is now available as spectinomycin dihydrochloride pentahydrate (Trobicin). This preparation is more soluble and less likely to cause discomfort on injection, which makes it particularly helpful in the treatment of the female patient for whom a single injection of 4 g. spectinomycin is the recommended dosage.

The success of spectinomycin hydrochloride in the treatment of gonorrhoea has been reported in a number of recent publications (Duncan, Holder, Roberts, and Knox, 1972; Reyn, Schmidt, Trier, and Bentzon, 1973).

Before the release of this new preparation for clinical trial in Great Britain in June, 1973, it seemed wise to ascertain the minimum inhibitory concentrations of spectinomycin for strains of N. gonorrhoeae routinely isolated from patients attending the Special Treatment Centre in Aberdeen.

Material and methods

A total of 160 strains of N. gonorrhoeae isolated from 87 male and 73 female patients was available for study. From each male patient, discharge from the urethra was collected on a charcoal-coated swab which was placed immediately in a bottle of Stuart’s transport medium and dispatched to the Laboratory, City Hospital. From each female patient, charcoal-coated swabs from the urethra and cervical os were collected and dispatched similarly. The interval between collection and investigation in the Laboratory was 18 to 24 hours.

The swabs were plated on chocolate agar and chocolate agar containing antibiotics (polymixin B 100 units/ml. and vancomycin 10 µg./ml.), to reduce contamination. Incubated plates were incubated at 37°C. in an atmosphere of 10 per cent. carbon dioxide in an air-tight metal box for 4 days. The plates were inspected daily. Suspicious colonies were examined by Gram-staining and the oxidase reaction. Confirmation of N. gonorrhoeae was based on sugar fermentation tests.

SPECTINOMYCIN SENSITIVITY TESTS

Plates employed for testing the sensitivity of strains of N. gonorrhoeae were prepared as follows. A stock solution of spectinomycin was made by adding 10 ml. sterile distilled water to a vial of spectinomycin dihydrochloride pentahydrate which contained the equivalent of 100 mg. spectinomycin. Appropriate dilutions of this stock solution were prepared and added to chocolate agar at 55°C. to give final concentrations of spectinomycin of 5, 10, and 20 µg./ml. of chocolate agar medium. A chocolate agar plate without spectinomycin was included in each batch of plates for growth control purposes.

Each confirmed culture of N. gonorrhoeae was subcultured on chocolate agar and grown at 37°C. for 48 hrs. Several colonies were transferred to a tube containing 2 ml. Bacto Tryptose broth (Difco). This was diluted further with the same broth to give a degree of turbidity which was known to produce a dense but not confluent growth of gonococci when inoculated on chocolate agar medium with a sterile swab. Each strain under test was seeded on plates containing the different concentrations of spectinomycin and also on a control plate. The plates were incubated at 37°C. in a metal box in 10 per cent. carbon dioxide for 48 hrs.

The minimum inhibitory concentration (MIC) of spectinomycin was taken as the lowest concentration which inhibited the growth of N. gonorrhoeae.

Results

Of the 160 strains of N. gonorrhoeae examined 87 were from male and 73 from female patients. The minimum inhibitory concentrations are shown in the Table.

Thus 153 (95·6 per cent.) of the 160 isolates of
TABLE  MICs of spectinomycin for 160 strains of N. gonorrhoeae

<table>
<thead>
<tr>
<th>MIC (µg./ml.)</th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td>5</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>6 to 10</td>
<td>42</td>
<td>35</td>
</tr>
<tr>
<td>11 to 20</td>
<td>5</td>
<td>2</td>
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N. gonorrhoeae were sensitive to 10 µg. or less per ml. of spectinomycin dihydrochloride.

Discussion
Penicillin is the antibiotic most commonly used in the treatment of gonorrhoea. Although some strains of N. gonorrhoeae have been reported as showing increased resistance to penicillin the use of increased dosage of this antibiotic has usually been effective in the treatment of infections due to such organisms. However, it is advantageous to have available other drugs for the treatment of those patients shown to be infected with strains of N. gonorrhoeae markedly resistant to penicillin. Patients known to be allergic to penicillin also benefit from the availability of other antibiotics such as the tetracylines and kanamycin.

From reports published during the decade up to around 1970 spectinomycin sulphate would appear to have been a suitable alternative to penicillin for gonorrhoeal infections. Spectinomycin dihydrochloride pentahydrate, which has the advantages of greater solubility and less discomfort on injection, replaces the earlier spectinomycin sulphate with equal therapeutic benefit.

The results obtained from the testing of strains of N. gonorrhoeae isolated from patients in N.E. Scotland indicate that the MICs of spectinomycin lie well within the blood level, viz. 105 µg./ml. after 1 hour (Levy, Wicher, and Rose, 1973) achieved after a single intramuscular injection of 2 g.

Summary
The minimum inhibitory concentrations of spectinomycin for 160 isolates of N. gonorrhoeae from 87 males and 73 females attending the Special Treatment Centre in Aberdeen were ascertained; 153 (95.6 per cent.) were sensitive to 10 µg. or less and the remaining seven required up to 20 µg.

We are grateful to Upjohn Limited for supplies of spectinomycin dihydrochloride pentahydrate (Trobicin) used in this investigation.

References

Spectinomycine: concentration inhibitrice minimale pour Neisseria gonorrhoeae

SOMMAIRE
On a établi les concentrations inhibitrices minimales de spectinomycine vis-à-vis de 160 souches de N. gonorrhoeae isolées à partir de 87 hommes et 73 femmes fréquentant le Centre Spécial de Traitement d’Aberdeen; 153 (95,6 pour cent) étaient sensibles à 10 µg. ou moins, et les 7 autres à 20 µg.