A comparison of the in vitro activity of metronidazole, tinidazole, and nimorazole against Gram-negative anaerobic bacilli

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SYNOPSIS The in vitro activities of metronidazole, nimorazole, and tinidazole were compared against 69 strains of obligately anaerobic Gram-negative bacilli. Geometric mean MICs were 0.34, 1.05, and 0.28 μg/ml respectively. Thirty-six strains were also tested by the disk method. Correlation between MIC and diameter of the zones of inhibition was poor.

Metronidazole (1-β-hydroxymethyl-2-methyl-5-nitroimidazole, Flagyl, May & Baker) is at present the drug used most widely for the treatment of Trichomonas vaginalis infection. It is of interest that it has also been effective in the treatment of acute ulcerative gingivitis (Shinn, 1962; Davies et al, 1964; Shinn et al, 1965), an infection in which anaerobic bacteria (Treponema vincentii and Fusobacterium spp.) are believed to have an aetiological rôle. Recent studies have shown that all the clinically important species of anaerobic bacteria are sensitive to therapeutically attainable concentrations of metronidazole (Füzi and Csukas, 1969a, b, c, 1970; Prince et al, 1969; Ueno et al, 1971; Videau, 1971; Nastro and Finegold, 1972; Tally et al, 1972, 1974; Finegold and Sutter, 1972; Whelan and Hale, 1973; Mitchell, 1973; Washington et al, 1974; Dornbusch and Nord, 1974; Phillips, 1974; Staneck and Washington, 1974). In addition, metronidazole is consistently bactericidal in action, which is a property not shown by clindamycin, rifampicin or chloramphenicol (Nastro and Finegold, 1972; Finegold and Sutter, 1972; Whelan and Hale, 1973). Two reports have appeared on the clinical use of metronidazole: Tally et al (1972) successfully treated three patients with infections caused by Gram-negative anaerobic bacteria, and the Study Group (1974) showed that metronidazole was effective both therapeutically and prophylactically in gynaecological patients with pelvic sepsis.

Nimorazole \(^1\) (1-(N-β-ethylmorpholinolo)-5-nitroimidazole, Nagoxin, Carlo Erba) and tinidazole (1(2-ethylsulphonylethyl)-2-methyl-5-nitroimidazole Fasigyn, Pfizer) are also 5-nitroimidazoles whose antitrichomonal activity in vitro is similar to that of metronidazole (de Carneri et al, 1969; Howes et al, 1970). Both have been used successfully to treat trichomoniasis, with reduction in both daily doses and treatment period compared with metronidazole (Rognoni and Sideri, 1969; Györik and Wenner, 1971). Nimorazole was also found to be as effective as metronidazole in the treatment of acute ulcerative gingivitis (Lozdan et al, 1971). Considering the superior pharmacokinetic properties of nimorazole and tinidazole, we thought it worthwhile to carry out a comparison of the in vitro activity of these three 5-nitroimidazoles against Gram-negative anaerobic bacteria.

Methods and Materials

5-NITROIMIDAZOLES

Pure samples of metronidazole, nimorazole, and tinidazole were supplied by May & Baker, Carlo Erba, and Pfizer, respectively.

Bacterial Strains

Sixty-nine strains were studied: all were obligately anaerobic Gram-negative rods. Twenty-four strains (8 Fusobacterium spp. and 16 Bacteroides fragilis) were either reference strains (from the National Collection of Type Cultures, London or Center for Disease Control, Ga, USA) or identified clinical isolates from other hospitals. The majority of the remaining 45 strains had been isolated from clinical

\(^1\)Previously known as nitrimidazone.

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material from the Royal Free Hospital but some were kindly donated by other hospitals. We provisionally identified 43 of these as B. fragilis, since they were resistant to polymyxin B (10 µg/ml), kanamycin (2 mg/ml), and benzylpenicillin (2 µg/ml) (Finegold et al., 1967). The other two strains were sensitive to benzylpenicillin and thus were classified as Bacteroides spp.

**MIC Determination**

Throughout these experiments, media were supplemented with yeast extract (1%), haemin (5 µg/ml), and menadione (0.5 µg/ml).

Serial doubling dilutions of the drugs were made in brain heart infusion agar (Oxoid, CM 375) containing 5% lysed, defibrinated horse blood. Bacteria were grown in 10 ml amounts of thioglycolate broth (Baltimore Biological Laboratories, no. 135C) incubated anaerobically at 37°C for 22 hr, using the GasPak system. Such cultures were diluted 1:500 in sterile 0.06 M sodium phosphate buffer containing 0.3 g/l cysteine hydrochloride, and inoculated on to the agar with a multiple inoculating device (see Reynolds et al., 1974). Plates were incubated at 37°C for 42 hr using the GasPak system. Control plates containing no antibiotic were inoculated and incubated both aerobically and anaerobically. MIC was taken as the lowest drug concentration that completely inhibited growth or allowed the development of no more than two discrete colonies; such a finding is evidence of at least 99.95% inhibition.

**Disk Diffusion Tests**

Broth cultures were grown as above and diluted 1:50 in buffer. Fifteen millilitre amounts of supplemented (see above) brain heart infusion agar + 5% whole horse blood in 9 cm plates were flooded with the diluted cultures and excess fluid was removed. Disks containing 2.5 µg of drug were prepared by dipping 6 mm AA disks (Whatman) into solutions of each individual drug which contained 125 µg/ml. These were then placed on to the surface of the agar. Only one disk was put on to each plate. Plates were incubated anaerobically using the GasPak system for 18 hr at 37°C, and zones of inhibition were measured with calipers.

**Analysis of Data**

Graphs were constructed to compare zone size with MIC for each compound; in addition, results for all three compounds were combined onto a single plot. Analysis was as described by Ericsson and Sherris (1971).

**Results**

As can be seen from table I, there was no clear

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<table>
<thead>
<tr>
<th>MIC (µg/ml)</th>
<th>0.062</th>
<th>0.125</th>
<th>0.25</th>
<th>0.5</th>
<th>1.0</th>
<th>2.0</th>
<th>4.0</th>
<th>8.0</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>4</td>
<td>1</td>
<td></td>
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<tr>
<td>B. fragilis</td>
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<td>24</td>
<td>4</td>
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<tr>
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<tr>
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<td>41</td>
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<td>Tinidazole</td>
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<tr>
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<td>15</td>
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<tr>
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<tr>
<td>Geometric mean MIC</td>
<td>Metronidazole 0.34 µg/ml</td>
<td>Nimorazole 1.05 µg/ml</td>
<td>Tinidazole 0.28 µg/ml</td>
<td></td>
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</table>

**Table I** Activity of metronidazole, nimorazole, and tinidazole against Gram-negative anaerobic bacteria

<table>
<thead>
<tr>
<th>Nitroimidazole</th>
<th>Fitted Line</th>
<th>Correlation Coefficient</th>
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<tr>
<td></td>
<td>y = ax + b</td>
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<tr>
<td>Metronidazole</td>
<td>-2.23</td>
<td>41.45</td>
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<tr>
<td>Nimorazole</td>
<td>-2.84</td>
<td>43.67</td>
<td>0.63</td>
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<td>Tinidazole</td>
<td>-1.62</td>
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<tr>
<td>Composite</td>
<td>-2.90</td>
<td>45.41</td>
<td>0.75</td>
</tr>
</tbody>
</table>

**Table II** Correlation of MIC to zone diameter
Nitroimidazoles between was about Nimorazole strains. geometric both have shown tinidazole, anaerobic bacteria A Discussion of diagram scatter available of trichomoniasis Dornbusch et Edwards not to zole metronidazole Gram-negative organisms. nimorazole, slightly compounds. allowing activity of bacteria, after since stated. this shows that 1052 size (taking the ug/ml). 30
Thirty-six correlation number and seen of metronidazole against anaerobic bacteria, including nimorazole, slightly more active than metronidazole, of the two related compounds allied to their equivalent antimicrobial activity make clinical studies of tinidazole and nitrometazole mandatory.

We should like to thank Dr McFadzean, Dr Rooney, and Dr O'Neill East for the gifts of metronidazole, nimorazole, and tinidazole respectively.

References


