

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.

Nimorazole¹ (1-(N-β-ethylmorpholino)-5-nitro-

imidazole, 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; Györök 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

¹Previously known as nitrimidazine.

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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 u/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

| | MIC (µg/ml) | | | | | | | |
|---------------------------|--|-------|------|-----|-----|-----|-----|-----|
| | 0.062 | 0.125 | 0.25 | 0.5 | 1.0 | 2.0 | 4.0 | 8.0 |
| Metronidazole | | | | | | | | |
| <i>Fusobacterium</i> sp | 1 | 2 | 4 | | 1 | | | |
| <i>B. fragilis</i> | | 3 | 27 | 24 | 4 | | 1 | |
| <i>Bacteroides</i> sp | | | 1 | 1 | | | | |
| Nimorazole | | | | | | | | |
| <i>Fusobacterium</i> sp | | | | | 2 | 5 | 1 | |
| <i>B. fragilis</i> | | | 3 | 7 | 41 | 6 | 1 | 1 |
| <i>Bacteroides</i> sp | | | | | 2 | | | |
| Tinidazole | | | | | | | | |
| <i>Fusobacterium</i> sp | | 2 | 5 | 1 | | | | |
| <i>B. fragilis</i> | 2 | 7 | 31 | 15 | 3 | 1 | | |
| <i>Bacteroides</i> sp | | | 1 | 1 | | | | |
| Geometric mean MIC | Metronidazole 0.34 µg/ml Nimorazole 1.05 µg/ml Tinidazole 0.28 µg/ml | | | | | | | |

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

| Nitroimidazole | Fitted Line | | Correlation Coefficient | SE of Estimate |
|----------------|-------------|-------|-------------------------|----------------|
| | y = ax + b | | | |
| | a | b | | |
| Metromidazole | -2.23 | 41.45 | 0.59 | 3.33 |
| Nimorazole | -2.84 | 43.67 | 0.63 | 3.62 |
| Tinidazole | -1.62 | 36.46 | 0.53 | 2.71 |
| Composite | -2.90 | 45.41 | 0.75 | 3.49 |

Table II Correlation of MIC to zone diameter

difference between the sensitivity patterns of *Fusobacterium* spp and *B. fragilis*; therefore it was considered to be justified to combine data for all strains. Tinidazole was slightly more active than was metronidazole, the two compounds having geometric mean MICs of 0.28 and 0.34 $\mu\text{g/ml}$ respectively. Nimorazole was about threefold less active, having a geometric mean MIC of 1.05 $\mu\text{g/ml}$.

Thirty-six strains were also tested by the disk method, and the results are summarized in table II. To increase the spread of values and thereby make the interpretation of results easier a composite scatter diagram was constructed and a statistical analysis of this is also shown in table II.

Discussion

A large number of reports over the past few years have shown metronidazole to be highly active against both Gram-positive and Gram-negative anaerobic bacteria (for review see Hamilton-Miller, 1975). Two other nitroimidazoles, nimorazole and tinidazole, are currently available for the treatment of trichomoniasis (although tinidazole is not at present available on prescription in the UK.) We have not seen any reports on the antibacterial activity of nimorazole, and only two concerning tinidazole. Edwards *et al* (1973) reported that tinidazole was slightly less active than metronidazole against eight strains of *Clostridium* spp, a conclusion confirmed by Dornbusch and Nord (1974), who also found tinidazole to be more active than metronidazole against Gram-negative organisms. Our results have been extended to include nimorazole but in terms of metronidazole and tinidazole they agree very closely with those of Dornbusch and Nord (1974).

Summation of published data available on the activity of metronidazole against anaerobic bacteria shows that 1052 out of 1071 strains were sensitive (taking the break-point as being either 12.5 or 16 $\mu\text{g/ml}$).

Poor correlation was found in the disk test between zone size and sensitivity. However, since all the strains tested here may be regarded as sensitive, criteria allowing precise interpretation cannot be stated. We suggest an MIC of 16 $\mu\text{g/ml}$ as the break-point, since this value should be obtainable systemically after a 1 g dose of any of these three compounds. If this value is accepted, strains giving a zone size greater than 13 mm for metronidazole, 7 mm for nimorazole, and 15 mm for tinidazole can be considered as sensitive.

Three groups of compounds containing a primary nitro group among other features have now been shown to possess good activity against anaerobic bacteria, chloramphenicol, nitroimidazoles, and

nitrofurans. The possession of such a group may be the determining factor in such activity, and it will be of interest to test this hypothesis with further compounds.

The nitroimidazoles are at present available only as oral preparations, which may be a disadvantage if the treatment of critically ill patients is contemplated. In view of the fact that metronidazole has been used successfully on a small scale for the treatment of systemic bacterial infections, the superior pharmacokinetics (Welling and Monro, 1972; de Carneri, 1969) of the two other related compounds allied to their equivalent antimicrobial activity make clinical studies of tinidazole and nimorazole mandatory.

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