In vitro susceptibility to metronidazole of bacteria from the small intestine of tropical sprue patients

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Anaerobic, microaerophilic and aerobic bacteria isolated from upper jejunum, either from luminal aspirate or mucosal biopsy, of tropical sprue patients were tested in vitro for susceptibility against metronidazole. Hundred percent Bacteroides and over 80 percent of Veillonella and fusobacteria were sensitive with minimum inhibitory concentration (MIC) of the drug up to 8 μg/ml. Most billi-bacteria as also 100 percent of microaerophilic Lactobacilli and aerobic organisms were resistant (MIC exceeded 64 μg/ml). Metronidazole appears to be a useful therapeutic agent for intestinal infections due to anaerobic bacteria.

Material & Methods

All the organisms studied were isolated either from the jejunal luminal fluid or jejunal mucosal biopsies obtained from the first loop of jejunum of patients with tropical sprue. The methods of isolation and characterization of anaerobes, microaerophiles and aerobes were as described earlier.36,21

Pure metronidazole powder was obtained from May and Baker, India. For testing the antimicrobial activity of the drug, 48 h old cultures of anaerobes and microaerophiles in basal glucose phosphate (BGP) broth (peptone 10%, NaCl 5%, Lab-Lemco 3%, yeast extract 5%, cysteine hydrochloride 0-4%, glucose 1%, Na₂HPO₄ 4%, and agar 0-8%, pH 7-4),
6 h old cultures of Gram negative aerobic rods in peptone water and 24 h old cultures of aerobic streptococci in Todd-Hewitt broth were employed. Ten µl of the cultures were applied on to 7 per cent freshly prepared sheep blood agar plates containing metronidazole in doubling dilutions (256 µg/ml to 0.1 µg/ml; Table). Anaerobic cultures were incubated in an atmosphere of 90 per cent hydrogen and 10 per cent carbon dioxide, and microaerophiles in an atmosphere of 10 per cent carbon dioxide and 90 per cent air for 72 h. Aerobes were incubated in the laboratory atmosphere for 24 to 48 h. All cultures were incubated at 37°C. The results were recorded in terms of minimum inhibitory concentration (MIC the lowest concentration of the drug that completely inhibited visible growth). The number of different isolates of each organism that was tested is shown in the Table.

### Results

All isolates of *Bacteroides* were completely inhibited at a concentration of 2 µg/ml. All but two strains of *Veillonella* (88.2%) were also inhibited at this concentration but inhibition of growth of all strains was achieved only at a concentration of 32 µg/ml. 77.8 per cent (7 of 9) strains of *fusobacteria* were inhibited at a concentration of 4 µg/ml but total inhibition was achieved only at a concentration of 128 µg/ml. Only 36.4 per cent (4 of 11 isolates) of bifidobacteria were inhibited by 8 µg/ml of metronidazole but at 32 µg/ml one more strain of the organism was susceptible. Even at 256 µg/ml three strains were resistant to metronidazole (Table).

None of the microaerophilic or aerobic organisms tested (total isolates 93)
were inhibited by 256 μg/ml of metronidazole except 2 strains each of lactobacilli and streptococci which were inhibited by concentration of 128 μg/ml (Table).

Discussion

The peak blood levels achieved after oral metronidazole have varied from 6.1 μg/ml (200 mg of metronidazole) to 35.1 μg/ml (600 mg of metronidazole)22-24. The wide range of peak serum concentrations is not only dependent on the dose administered but also on individual variations which are probably determined by rates of gastric emptying and intestinal absorption. Finegold18 has therefore recommended that a concentration of 8 μg/ml should be the level taken for in vitro testing of metronidazole susceptibility. Organisms are considered susceptible if growth is inhibited by 8 μg/ml or less of metronidazole, moderately susceptible when inhibition occurs with concentrations between 16 to 64 μg/ml and resistant when anaerobes grow at concentrations greater than 64 μg/ml.

The results presented here show that the susceptibility of enteric anaerobic bacteria to metronidazole is similar to that of isolates from other tissues and sites6,7,13,14,17. Bacteroides are the most susceptible to the drug, all isolates tested being inhibited by concentrations of ≤ 2 μg/ml. Isolates of fusobacteria and Veillonella were less susceptible but since nearly all strains are inhibited at 8 μg/ml the drug can be therapeutically efficient against these organisms. However, only less than 50 per cent of the isolates of bifidobacteria were inhibited at a concentration of 64 μg/ml and these findings confirm the reported lack of susceptibility of this organism to metronidazole17,18. Enteric microaerophiles and aerobes were resistant to metronidazole.

In addition to anaerobes being present in large numbers in human colon, it has recently been shown that the autochthonous flora of the human jejunal mucosa is predominantly anaerobic30. The close association of this anaerobic flora with the enterocytes suggests that they are an important part of the microbiology and their metabolites could affect enterocyte function25. From experimental studies metronidazole appears to be a useful agent for altering this flora and to evaluate the effect of the latter on enterocyte function. The clinical significance of enteric anaerobic bacteria is being recognised only recently3,26,27. Metronidazole appears to be a potent therapeutic tool for intestinal infections due to anaerobic bacteria.

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