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Bactericidal activity of metronidazole against *Bacteroides fragilis*

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SYNOPSIS Metronidazole was found to be active against *Bacteroides fragilis* strains isolated from human lesions. The minimal inhibitory concentrations (MIC) were from 0·16 to 2·5 μg/ml and the minimal bactericidal concentrations (MBC) were from 0·16 to 2·5 μg/ml; usually the MIC and MBC figures were equivalent. These levels are easily attainable in the serum following normal therapeutic doses. The drug is not toxic and side effects are rare and it would therefore seem highly suitable for treating Bacteroides infections and also may be considered prophylactically in certain situations that are described.

Organisms of Bacteroides species and particularly *Bact. fragilis* have been incriminated in a large number of infections. They are frequently observed in sepsis following gastrointestinal surgery (Gillespie and Guy, 1956), in puerperal sepsis (Rotheram and Schick, 1969), lung abscess (Tillotson and Lerner, 1968), and brain abscess (Ingham, Selkon, Codd, and Hale, 1970). Lincomycin has been shown to possess bactericidal activity *in vitro* against Bacteroides species (Ingham, Selkon, Codd, and Hale, 1968; Nastro and Finegold, 1972) and Tracey, Gordon, Moran, Love, and McKenzie (1972) described four cases of Bacteroides infection successfully treated with lincomycin.

The drug metronidazole has been shown to be bactericidal *in vitro* against *Bacteroides fragilis* by Nastro and Finegold (1972). We present here the results of testing metronidazole against 23 strains isolated from clinical conditions in Britain.

Materials and Methods

Twenty-three strains of *Bacteroides fragilis* were obtained which had been isolated from a variety of lesions. They were maintained in cooked meat medium and on 10% blood agar under anaerobic conditions.

ANTIBACTERIAL ACTIVITY *in vitro*

The minimal inhibitory concentration (MIC) of metronidazole was determined using two-fold serial dilution in 10% blood agar incorporating the drug in the medium at a starting concentration of 50 μg/ml with the first isolates and at 10 μg/ml in the later estimations. A 48-hour culture grown on 10% blood agar under anaerobic conditions at 37°C was used to inoculate the drug-containing blood agar plates. The latter were incubated overnight at 37°C in an anaerobic jar under 90% hydrogen and 10% carbon dioxide. The MIC values were recorded after 24 hours' incubation and the minimum bactericidal concentrations (MBC) were determined by the replica plate method of Elek and Hilson (1954) using 10% blood agar plates. The recovery plates were incubated overnight under anaerobic conditions. The criterion for bactericidal effect was that there should be no colonies on the corresponding replica plates.

PATHOGENICITY EXPERIMENTS

Attempts to produce lesions in animals were made. Two slopes of horse blood agar were inoculated with each strain, incubated for 48 hours, and the organisms on each slope washed off with 1·0 ml of saline. This suspension was injected in 0·03 ml amounts intracerebrally into 20 g albino mice. The remainder of the saline suspension was diluted with 5% hog mucin and injected intraperitoneally in 0·5 ml volumes into 20 g mice. Similar slopes were inoculated and harvested and 0·5 ml volumes of the suspension injected subcutaneously into 150 g guinea pigs and into 1·5 kg rabbits.

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Results

The minimal inhibitory and minimal bactericidal concentrations of metronidazole against 23 strains of *Bacteroides fragilis* are shown in the table. In all but one case the MIC and MBC figures were equivalent. One strain provided an MIC of 1·2 μg/ml and a MBC of 2·5 μg/ml. The highest MIC or MBC was 2·5 μg/ml.

Experiments designed to demonstrate the activity of metronidazole against Bacteroides *in vivo* failed as all attempts to produce infective lesions by any route in any of the species employed were in vain, although pathogenicity for the guinea pig and the rabbit is described (Wilson and Miles, 1964).

Strain No.	*Clinical Source*	*MIC (μg/ml)*	*MBC (μg/ml)*
17	Blood culture	0·7	0·7
18	Brain abscess	0·7	0·7
21	Peritoneal swab perforated appendix	0·7	0·7
26	High vaginal swab	0·7	0·7
28	Peritonitis	0·16	0·16
37	Blood culture	0·7	0·7
41	Appendix abscess	0·7	0·7
43	Appendix abscess	0·7	0·7
50682	Blood culture postpartum pyrexia	0·6	0·6
51747	Peritoneal swab perforated appendix	0·6	0·6
52227	Urine, after ureteroplasty	0·6	0·6
52419	High vaginal swab, carcinoma uterus c pyometrium	1·2	1·2
54740	High vaginal swab puerperal pyrexia	1·2	1·2
55506	High vaginal swab vaginal discharge	0·3	0·3
55721	Bladder washings	1·2	1·2
56515	Abdominal wound	1·2	1·2
56728	Episiotomy wound	1·2	2·5
57292	Peritoneal dialysis effluent	0·6	0·6
58914	Appendix abscess	1·2	1·2
58966	Abdominal wound	0·6	0·6
2787 (1)	Appendix wound	0·3	0·3
2787 (2)	Appendix wound	2·5	2·5
3060	Appendix wound	1·25	1·25

Table *Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of metronidazole against 23 strains of* Bacteroides fragilis *isolated from clinical material*[1]

[1]Estimations performed on 10% horse blood agar.

Discussion

Metronidazole has been shown to be active against parasites and bacteria which exhibit mainly an anaerobic metabolism. Thus, its activity against *Trichomonas vaginalis* (Durel, Roiron, Siboulet, and Bonel, 1960) and *Entamoeba histolytica* (Powell, Macleod, Wilmot, and Elsdon-Dew, 1966) has made it the most effective therapy in the treatment of infection with these parasites. Its activity against the organisms associated with acute ulcerative gingivitis (Vincent's disease) has secured it a position in the treatment of this condition (Shinn, Squires, and McFadzean, 1965). The effectiveness of metronidazole against Clostridia and its value in the prevention of experimental infections in mice with *Cl. tetani* and *Cl. welchii* were shown by Freeman, McFadzean, and Whelan (1968). Füzi and Csukás (1970) showed activity by metronidazole against the anaerobic bacteria, Fusobacteria, Bacteroides, Leptotrichia, Clostridia, and Veillonella, and suggested that the pronounced efficacy of the drug offered new possibilities in the therapy of infections due to anaerobes. Nastro and Finegold (1972) showed that, of five antimicrobial agents—rifampin, 7-chlorolincomycin, vancomycin, tetracycline, and metronidazole—only metronidazole had complete and consistent bactericidal activity at concentrations easily attainable in serum. It has been shown (Kane, McFadzean, and Squires, 1961) that ingestion of 200 mg of metronidazole produced serum levels of about 5·0 μg/ml at one to two hours later and that the levels fell gradually to 1·0 μg/ml at 24 hours. Further records (Squires, 1973) indicate that patients receiving 400 mg attain a level of 9 μg/ml and that serum concentrations rise progressively with increased dosage of this non-toxic compound. A single dose of 2 g, frequently used for trichomoniasis and amoebiasis, will produce a serum level of 46 μg/ml, which at 24 hours will have receded to 13 μg/ml and at 48 hours to 3·0 μg/ml.

Information on the development of resistance to metronidazole is limited largely to observations with Trichomonads which have shown no development of resistance to the drug in spite of wide use in the past 10 years (McFadzean, Pugh, Squires, and Whelan, 1969; Keighley, 1971; Benazet and Guillaume, 1971). Habituation of Clostridia to metronidazole was attempted over 10 passages by Freeman (1968) with no increase in inhibitory concentration.

Tetracycline has been considered as suitable treatment in Bacteroides infections but Nastro and Finegold (1972), Keusch and O'Connell (1966), and Kislak (1972) have demonstrated resistance to tetracycline in this species. Lincomycin and clindamycin (Ingham *et al*, 1968) are regarded as treatments of choice but Nastro and Finegold (1972), using zero colonies as the endpoint, showed that 7-chlorolincomycin provided bactericidal activity against only one or two strains.

The use of an association of penicillin G and tetracycline has been suggested for the prevention of postoperative infection in certain types of case (Todd, 1968). For appendicectomy complicated by

peritonitis, or surgery involving transection of the gastrointestinal tract, we feel that ampicillin and metronidazole may prove a better combination. The latter is active against Bacteroides, an important cause of infection in such patients; it is virtually non-toxic and does not have the side effects that may be associated with tetracycline, especially in young children. Further we have shown (although not yet published) that metronidazole materially lowers the *Cl. welchii* content of the colon.

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