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In Vitro Activity of the Two Principal Oxidative Metabolites of Metronidazole Against *Bacteroides fragilis* and Related Species

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Metronidazole and its two principal oxidative metabolites were tested in vitro against 20 clinical isolates of the *Bacteroides fragilis* group. Both metabolites were bactericidal, and they exhibited 65 and 5%, respectively, of the inhibitory effect of metronidazole. Additive or weak synergistic effects resulted in combination with the parent compound.

Metronidazole is metabolized in humans by oxidative mechanisms or by conjugation of the parent compound (6). The major metabolic product, 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (hydroxy metabolite), has been shown to occur in considerable amounts in the plasma after oral or intravenous administration of metronidazole (2, 3, 7). A second oxidative metabolite, 1-acetic acid-2-methyl-5-nitroimidazole (acid metabolite), was found predominantly in the urine, but was also identified in the plasma (3, 7). Little is known about the activity of these two metabolites against anaerobic bacteria. In bioassays performed with two strains of *Clostridium perfringens* and *C. sporogenes*, they exhibited 30% (hydroxy metabolite) and 5% (acid metabolite) of the activity of the parent compound (4).

In this study the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrations (MBCs) of metronidazole and its two principal oxidative metabolites were compared, using 20 clinical isolates of *Bacteroides fragilis* (10 strains), *B. fragilis* subsp. *thetaiotaomicron* (5 strains), and *B. fragilis* subsp. *vulgatus* (5 strains) as test organisms. The MICs were measured on Wilkins-Chalgren agar (8) by the reference dilution method proposed by Sutter et al. (5); results are summarized in Table 1. As indicated by the geometric means of the MICs, the hydroxy metabolite and the acid metabolite exhibited about 65 and 5% of the activity of the parent compound, respectively. The concentration required to inhibit 90% of the isolates was 2 μg/ml for metronidazole and for the hydroxy metabolite and was 32 μg/ml for the acid metabolite. All of the test strains except one isolate of *B. fragilis* subsp. *thetaiotaomicron* showed a similar pattern of susceptibility.

Since agar dilution does not permit MBC determination, the bactericidal action of the two metabolites was measured by microdilution technique in Schaedler broth, using an inoculum of about 10^6 bacteria per ml. Viable cell counts were performed at time zero and after 48 h of incubation, and the MBC was read as the lowest concentration to yield a 99.9% kill rate. Inhibition of growth was read from the same experiments; MICs differed by no more than two dilutions from the reference agar dilution meth-

TABLE 1. MICs and MBC/MIC ratios of metronidazole and its principal oxidative metabolites as determined against *B. fragilis* (10 strains), *B. fragilis* subsp. *thetaiotaomicron* (5 strains), and *B. fragilis* subsp. *vulgatus* (5 strains)

Test substance	MICs (μg/ml)		MBC/MIC ratios
	Range	Geometric mean	
Metronidazole	0.25–2	0.84	1 (12 strains)[a] to 2 (8 strains)
Hydroxy metabolite	0.5–2 (−8[b])	1.32	1 (9 strains) to 2 (11 strains)
Acid metabolite	4–32 (−64[b])	18.38	1 (10 strains) to 2 (10 strains)

[a] Twelve strains had an MBC/MIC ratio of one, and eight strains had an MBC/MIC ratio of two.
[b] MIC for one strain of *B. thetaiotaomicron*.

TABLE 2. Combined inhibitory effect of metronidazole and its principal oxidative metabolites as assessed by checkerboard titration (same 20 test strains as in Table 1)

Antibiotic combination	No. of strains having inhibitory effect	
	Additive	Synergistic[a]
Metronidazole + hydroxy metabolite	9	11
Metronidazole + acid metabolite	13	7

[a] In these experiments the optimal FIC indices (1) measured ranged between 0.375 and 0.5, indicating a weak synergistic effect.

od. As indicated by the MBC/MIC ratios (Table 1) both metabolites and the parent compound exhibited bactericidal action, with at least 99.9% of the test organisms being killed at the MIC or at twice this concentration.

To detect potential interferences between metronidazole and its oxidative metabolites, the combined inhibitory effect was examined by checkerboard titration. Test conditions were identical to those of the reference agar dilution method (5). As summarized in Table 2, additive or weak synergistic effects resulted for all test strains. No antagonistic interactions were seen in the range of concentrations which may occur in vivo.

Together with the pharmacokinetic data on the oxidative metabolism of metronidazole our results indicate that the hydroxy metabolite may contribute considerably to the therapeutic effect of metronidazole in the treatment of *B. fragilis* infections. The acid metabolite, on the other hand, does not seem to be of significant clinical importance in this respect. For both metabolites, antagonistic interactions with metronidazole, which could impair the inhibitory effect against *B. fragilis*, are unlikely to occur in vivo.

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