Comparative Evaluation of the 2-Methyl-5-Nitroimidazole Compounds Dimetridazole, Metronidazole, Secnidazole, Ornidazole, Tinidazole, Carnidazole, and Panidazole against *Bacteroides fragilis* and Other Bacteria of the *Bacteroides fragilis* Group

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Tube-dilution MICs of seven 2-methyl-5-nitroimidazole compounds varying at the 1-substitution were determined against *Bacteroides fragilis*. Activities on a molar basis were ranked: tinidazole > panidazole > ornidazole > metronidazole ≥ secnidazole > carnidazole > dimetridazole. Geometric mean MICs varied from 0.5 to 6.6 μM, and MICs against individual strains varied up to 50-fold between compounds. In general, the MIC of each drug correlated with that of each of the other drugs, but with tinidazole the correlation coefficients tended to be small and were not significant versus metronidazole and secnidazole. MICs against 14 other bacteria of the *B. fragilis* group, including *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, and *B. vulgatus*, were within the range determined for *B. fragilis*.

Increased awareness of anaerobic infections and the demonstration of the excellent activity of metronidazole against a wide range of obligately anaerobic bacteria (4–6, 18) have raised new medical interest in nitroimidazole drugs. Other derivatives have subsequently been found to be active against *Bacteroides fragilis* and considered not essentially different from metronidazole (2, 3, 9, 12, 20); there has seemed to be little reason to look for alternatives. However, significant differences exist between closely related 5-nitroimidazole compounds (Fig. 1) with respect to pharmacokinetics (10, 11, 13, 15, 21, 23, 24), therapeutic results (13), and mutagenicity (1, 17, 25). Since such gradually accumulating information may cause reevaluations of the choice of drugs, we extended structure-activity investigations on the susceptibility of *B. fragilis* and report on a comparison of the seven 2-methyl-5-nitroimidazole derivatives which have reached clinical use (primarily in treating protozoal infections).

MATERIALS AND METHODS

Bacteria. We studied *B. fragilis* ATCC 23745 (American Type Culture Collection, Rockville, Md.) and 33 other strains of *B. fragilis* originating from diagnostic clinical material. They had been isolated with enriched nonselective anaerobic blood agar and stored at −70°C in skim milk supplemented with 15% glycerol. Fourteen strains of *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. uniformis*, or *B. vulgatus* were similarly isolated and stored.

Drugs. The chemistry of the compounds used appears in Table 1. Dimetridazole (batch P 0132-1) and metronidazole (batch 8222200) were gifts from Rhône-Poulenc Pharma Norden A/S, Birkerød, Denmark; secnidazole (RP 14539; lot 18) was from Rhône-Poulenc, Paris, France; ornidazole (Ro 7-0207) was from Hoffmann-La Roche Inc., Nutley, N.J.; tinidazole (lot 207 TZ 030) was from Pfizer Inc., New York,

N.Y.; carnidazole (R 25831; batch A 8301) was from Janssen Pharmaceutica N.V., Beerse, Belgium; and panidazole (batch 4 RI/A) was from Ward Blenkinsop & Co. Ltd., Widnes, United Kingdom. Each was dissolved in sterile distilled water to a 10-mM concentration, except carnidazole (4 mM), and stored in 1-ml samples in the dark at −20°C. Each solution was sterilized with a 0.22-μm (pore size) filter (Optex for ophthalmic solutions; Millipore Corp., Bedford, Mass.) before use.

Inoculum. All work with the bacteria took place at room temperature inside an anaerobic chamber (model 800 anaerobic environmental system; Capco, Sunnyvale, Calif.), and the cultures were incubated at 37°C in transfer modules (model 830; Capco). All media were prereduced for 24 h or longer before use and not exposed to air before the end of the experiment. Stock cultures were checked for purity; a few fresh colonies were suspended in 1 ml of Schaedler broth (BBL Microbiology Systems, Cockeysville, Md.) and incubated for 24 h, after which the cultures in preliminary studies contained between 10^8 and 10^9 CFU/ml. For colony counting, cultures were diluted in water in 10-fold steps, and 0.1-ml samples were plated and incubated for 48 h. Similar 24-h cultures were diluted 1:10^4 in Schaedler broth to obtain inoculum suspensions for susceptibility tests, and the number of CFU in each inoculum was counted.

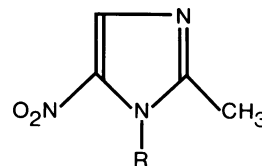**MIC determination.** Drug dilutions with 2- or 2.5-fold steps

FIG. 1. Chemical structure of 2-methyl-5-nitroimidazole R-substituted at position 1.

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TABLE 1. Chemical structures of R substitutions at position 1 of the 2-methyl-5-nitroimidazole compounds studied[a]

-R	Chemical name	Mol wt	Generic name
-CH₃	1,2-dimethyl-5-nitroimidazole	141.1	Dimetridazole
-CH₂CH₂OH	1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole	171.2	Metronidazole
-CH₂CHOHCH₃	1-(2-hydroxypropyl)-2-methyl-5-nitroimidazole	185.2	Secnidazole
-CH₂CHOHCH₂Cl	1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole	219.6	Ornidazole
-CH₂CH₂SO₂CH₂CH₃	1-(2-(ethylsulphonyl)-ethyl)-2-methyl-5-nitroimidazole	247.3	Tinidazole
-CH₂CH₂NHCSOCH₃	1-(2-ethylcarbamothioic acid O-methyl ester)-2-methyl-5-nitroimidazole	244.3	Carnidazole
-CH₂CH₂⟨◯⟩N	1-(2-(4-pyridyl)-ethyl)-2-methyl-5-nitroimidazole	232.2	Panidazole

[a] See the structure of the 2-methyl-5-nitroimidazole nucleus (Fig. 1).

ranging from 10^{-4} to 10^{-7} M were made in 1-ml volumes of Schaedler broth, 1 ml of inoculum suspension was added, and the mixtures were vortexed and incubated for 24 h. MIC was the lowest drug concentration without visible turbidity. The seven nitroimidazoles were always studied in parallel, and the weekly choices of *Bacteroides* strains to be tested were randomized. The median of three MICs was recorded as the result.

Statistical analysis. The logarithms of MICs were used in the calculation of correlation coefficients and in comparisons with the Student t test or the two-tailed paired-sample t test (30).

RESULTS

According to earlier experience, the MICs of metronidazole, ornidazole, and tinidazole were essentially unaffected by increasing the inoculum concentration above the conventional 10^5 CFU/ml (12), and the present 45 preliminary tests with 34 strains covered lower concentrations. The results with metronidazole appear in Table 2, and those with the other six 2-methyl-5-nitroimidazoles (results not shown) agreed, in that below 10^3 CFU, MICs tended to decrease with inoculum size. We decided to accept MICs obtained with inocula between 10^3 and 10^5 CFU per tube, because within this range the MIC was stable.

The MICs of each drug against 17 strains of *B. fragilis* varied within a 10-fold range, except that the two extreme MICs of tinidazole were 20-fold different (Fig. 2). Secnidazole did not differ from metronidazole; dimetridazole ($P < 0.001$ [paired-sample t test]) and carnidazole ($P < 0.001$) were less active; and ornidazole ($P < 0.005$), panidazole ($P < 0.001$), and tinidazole ($P < 0.001$) were more active than metronidazole. The extreme deviations

from metronidazole with individual strains were 10-fold inferior activity (dimetridazole and carnidazole) and 50-fold superior activity (tinidazole) (Fig. 2). Considering all drug pairs against a single bacterium at a time (results not shown), 50-fold different MICs were seen three times, 25-fold differences five times, and 20-fold differences three times. The accepted inocula ranged from 1.4×10^3 to 9.0×10^4 CFU, and the accepted MIC with each bacterium-drug combination was reproducible within 2 adjacent dilutions; i.e., it varied less than the MICs between strains. Despite wide scattering (Fig. 2), the activities of all other drug pairs correlated significantly, but tinidazole-metronidazole and tinidazole-secnidazole behaved as unrelated pairs (Table 3).

The MICs of the seven 2-methyl-5-nitroimidazoles against 14 non-*B. fragilis* members of the *B. fragilis* group (results not shown) fell, in all cases, within the respective range observed with *B. fragilis*.

Table 4 shows the comparison of the seven compounds against *B. fragilis* ATCC 23745. The 16 clinical isolates tended to be more susceptible to each compound (Table 5). Within the present series of 2-methyl-5-nitroimidazoles, with the exception of carnidazole, activity against *B. fragilis* seemed to increase with molecular weight, i.e., the size of the substitution at position 1 (Tables 1, 4, and 5). This was true of the MICs expressed either as molar concentrations or on a weight basis (Tables 4 and 5).

DISCUSSION

It was not surprising to find all the nitroimidazoles very active against *B. fragilis*. There seems to be no disagreement about this general principle, although the actual geometric mean MICs reported by various authors for the same drug against the same organism, *B. fragilis*, differ from each other
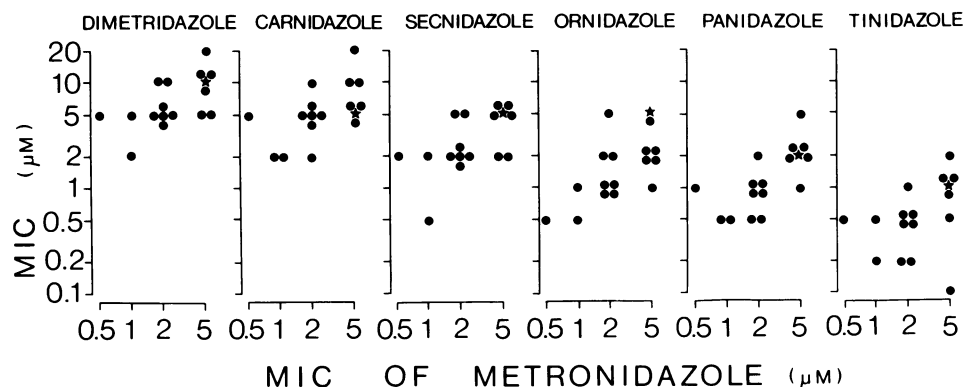

FIG. 2. Comparison of the activities of six 2-methyl-5-nitroimidazoles with that of metronidazole against 17 strains of *B. fragilis*. The vertical axis is the MIC of the drug indicated above the respective panel, and each symbol stands for one strain, the star signifying ATCC 23745.

TABLE 2. Inoculum dependence of the MIC of metronidazole against *Bacteroides* spp.

No. of tests[a]	Inoculum (CFU)	MIC (μM)	
		Geometric mean	Log of the mean ± SEM
6	>10^5	2.7	0.43 ± 0.161
13	10^4–10^5	2.8	0.45 ± 0.072
17	10^3–10^4	2.1	0.33 ± 0.073
9	<10^3	1.5	0.18 ± 0.090[b]

[a] Thirty-four bacteria of the *B. fragilis* group were tested, a few of them with two or three different inoculum concentrations.

[b] MICs with <10^3 CFU were lower than those with 10^4 to 10^5 CFU ($P < 0.05$ [Student t test]); the others were not.

TABLE 4. Activity of seven 2-methyl-5-nitroimidazole derivatives against *B. fragilis* ATCC 23745

Compound	Geometric mean MIC[a]	
	μM	μg/ml
Dimetridazole	10.0	1.41
Metronidazole	3.7	0.63
Secnidazole	3.7	0.68
Ornidazole	3.7	0.81
Tinidazole	0.8	0.20
Carnidazole	6.3	1.54
Panidazole	2.2	0.50

[a] Three experiments each with the seven compounds in parallel.

up to 10-fold or more. Accordingly, comparisons between individual compounds must be based on MICs determined in parallel, and such studies have been infrequent. It has been confirmed that tinidazole is slightly more active than metronidazole against *B. fragilis* (2, 9, 12, 20, 28, 29), and the threefold (fivefold on a molar basis) difference found in the present investigation was somewhat greater than those published previously. Ornidazole has been found to be either marginally more active than (3, 8, 29), equally as active as (12), or marginally less active than metronidazole (7). Our result agreed with the majority, confirming that the MICs of ornidazole are slightly lower than those of metronidazole. Tinidazole has been reported to equal ornidazole (29), but we confirmed our previous finding that tinidazole is more active than ornidazole (12). Secnidazole has been found to be marginally more active than metronidazole (22), although our MICs tended to show the opposite. Since secnidazole was the only compound which did not differ significantly from metronidazole in the present study, similar activity of these two nitroimidazoles is the unifying conclusion.

The above four comparisons could be evaluated against reports from other laboratories, but our remaining 17 comparisons appear to be new and indicate the following order of decreasing activity: tinidazole > panidazole > ornidazole > metronidazole ≥ secnidazole > carnidazole > dimetridazole. This order was based on MICs as molar concentrations, and the last two compounds change places if MICs are expressed in micrograms per milliliter. Molar concentrations may be more relevant in comparative evaluations, since the various biological activities of 5-nitroimidazoles depend on reduction of the nitro group, one per molecule (16, 17, 19). This does not exclude the possibility that other parts of the molecule might also have antibacterial activity. In fact, one explanation for the poor or

undetectable correlation of the MIC of tinidazole and that of other 5-nitroimidazoles is that the activity of this compound may have additional mechanisms. The finding suggests extended studies of the mechanisms of the antimicrobial activity of tinidazole and emphasizes the fact that results with one drug, metronidazole in this case, may not sufficiently describe the whole family.

Among 2-methyl-5-nitroimidazoles varying only at the 1-substitution, over 10-fold differences as to mean MIC and up to 50-fold differences as to the MICs against individual strains of *B. fragilis* were demonstrated. Thus, antibacterial activity is a point to be considered in the choice of nitroimidazole drugs for the treatment of anaerobic infections. Very little is known about other nitroimidazoles or the susceptibilities of bacteria other than *B. fragilis*, and extension in these two directions is likely to reveal additional variability of MIC. Likewise, comparative pharmacological and toxicological studies are few, and their extension may conceivably affect the choice. It seems clear that secnidazole is characterized by a relatively long serum half-life of 17 h (21, 23) and that the order of decreasing mutagenic activity for enterobacteria in vitro is metronidazole > dimetridazole > ornidazole ≃ carnidazole ≃ panidazole > tinidazole (1, 25–27), i.e., very different and almost opposite to the other of antibacterial activity. The clinical significance of these comparisons is unknown. In light of the presently available information, metronidazole may still be sufficient to occupy first place among nitroimidazoles for treating anaerobic infections, but exceptions, like major differences in therapeutic outcome (13) associated with seemingly minor differences in vitro (14), which have been documented in protozoal infections, may conceivably appear in bacterial

TABLE 3. Correlations between the MICs of seven 2-methyl-5-nitroimidazole derivatives against 17 strains of *B. fragilis*

Compound	Correlation coefficient[a]					
	Metronid-azole	Secnid-azole	Ornid-azole	Tinid-azole	Carnid-azole	Panid-azole
Dimetridazole	0.584	0.947	0.719	0.577	0.791	0.670
Metronidazole		0.575	0.655	0.331	0.535	0.647
Secnidazole			0.788	0.475	0.698	0.692
Ornidazole				0.574	0.575	0.755
Tinidazole					0.563	0.621
Carnidazole						0.732

[a] $r > 0.482$ is statistically significant ($P < 0.05$).

TABLE 5. Activity of seven 2-methyl-5-nitroimidazole derivatives against 16 clinical isolates of *B. fragilis*

Compound	MIC			
	μM		μg/ml	
	Geometric mean	Range	Geometric mean	Range
Dimetridazole	6.39	2.0–20	0.90	0.28–2.82
Metronidazole	2.37	0.5–5.0	0.41	0.09–0.86
Secnidazole	2.59	0.5–5.0	0.48	0.09–0.93
Ornidazole	1.45	0.5–5.0	0.32	0.11–1.10
Tinidazole	0.49	0.1–2.0	0.12	0.02–0.49
Carnidazole	5.23	2.0–20	1.28	0.49–4.89
Panidazole	1.15	0.5–5.0	0.27	0.12–1.16

infections as well. Drug development and in vitro evaluation may help to prepare for unexpected as well as expected times of decision.

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