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Antibacterial Activities of Nitrothiazole Against *Campylobacter jejuni* and *Campylobacter coli*

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Niridazole (Ambilhar^R) and three other newly synthesized nitrothiazole derivatives were highly active against 19 microaerophilic campylobacters (minimum concentration required to inhibit 50% of strains [MIC_{50}], 0.0075 to 0.015 mg/liter). There were, however, considerable differences in the susceptibility among strains tested, and one nitrothiazole derivative was rather inactive (MIC_{50}, 2 mg/liter). Nitroimidazole derivatives, such as metronidazole and tinidazole, were less active (MIC_{50}, 2 and 4 mg/liter, respectively). The nitrofuran derivatives, such as nitrofurazone and nitrofurantoin, were also less active (MIC_{50}, 1 mg/liter). Niridazole and another potent nitrothiazole derivative killed the campylobacters rapidly at low concentrations. In contrast, much higher concentrations of metronidazole were required to achieve bactericidal values.

Niridazole (Ambilhar^R), a nitrothiazole derivative, is much more active on anaerobic bacteria than is the related compound metronidazole, which is a nitroimidazole derivative (3, 5). Also, microaerophilic campylobacters are more susceptible to niridazole than they are to metronidazole (1, 4, 5) or any other common antibiotic (4). Whereas anaerobic bacteria are practically all susceptible to niridazole (3, 5), certain *Campylobacter* strains display less susceptibility (4, 5).

Recently, we have described the antibacterial activity of several other nitrothiazole derivatives against anaerobic bacteria (H. Hof, O. Zak, E. H. Schweizer, and A. Denzler, J. Antimicrob. Chemother., in press). Some of these synthetic compounds with a common nitrothiazole ring moiety but different side chains were found to be as active as niridazole itself. There were, however, a few nitrothiazole derivatives with low or absent activity.

This paper describes the antibacterial activity of some nitrothiazole derivatives on microaerophilic campylobacters. In comparison to other nitroarene compounds, such as nitroimidazole and nitrofuran derivatives, this new group of antibacterial agents appears to be more active.

MATERIALS AND METHODS

Bacteria. Two reference strains (*Campylobacter jejuni* NCTC 11168 and *Campylobacter coli* NCTC 11353) and 17 other strains (11 strains of *C. jejuni* and 6 strains of *C. coli*) isolated from stool specimens from patients with diarrhea were tested. They were cultured on brucella agar supplemented with $FeSO_4 \cdot H_2O$, sodium metabisulfite, and sodium pyruvate (FBP) (2) and incubated at 37°C in a microaerophilic milieu (8).

Antimicrobial agents. Besides niridazole (lot no. 4407841, 100% purity and activity), kindly supplied by Ciba-Geigy, Wehr, Federal Republic of Germany, three other newly synthesized nitrothiazole derivatives (9; P. Schmidt, M. Wilhelm, and K. Eichenberger, Swiss patent 455 806, July 1968) were tested.

The chemical structures of these compounds are given in Table 1. These substances were dissolved in *N*,*N*-dimethylformamide and further diluted in distilled water. The nitroimidazole compounds, metronidazole (Clont) and tinidazole (Simplotan), were obtained from Bayer (Leverkusen, Federal Republic of Germany) and Pfizer (Karlsruhe, Federal Republic of Germany), respectively. These substances were dissolved in distilled water. The nitrofuran derivative, nitrofurazone, was kindly supplied by Röhm Pharma (Darmstadt, Federal Republic of Germany), whereas nitrofurantoin was purchased from Sigma (München). Both these drugs were dissolved in *N*,*N*-dimethylformamide and further diluted in distilled water.

Determination of the MIC. Susceptibility was determined by an agar dilution method with Mueller-Hinton agar supplemented with 7% sheep blood. The density of the bacterial suspension was adjusted to one-half of a McFarland no. 1 standard. After a further 1:5 dilution in Mueller-Hinton broth (Oxoid Ltd.), 0.6 μl of each suspension was transferred to an agar plate. The inoculated plates were incubated in a microaerophilic atmosphere for 18 h at 37°C (5).

Bactericidal activity. Killing of bacteria was determined in Mueller-Hinton broth. To 9 ml of the broth containing the antimicrobial agent, 1 ml of bacterial suspension containing 10^6 to 10^7 viable bacteria was added. Both the reference strain of *C. jejuni* (NCTC 11168) and the reference strain of *C. coli* (NCTC 11353) were examined. This culture was incubated in microaerophilic atmosphere. At different intervals thereafter, samples were withdrawn and serially diluted in Mueller-Hinton broth. FBP agar plates were inoculated with an aliquot of each dilution. The bacterial colonies on the surface on the agar were counted after incubation in microaerophilic milieu at 37°C for 24 h.

RESULTS

(i) Comparative inhibitory activities of nitrothiazole derivatives with nitroimidazole and nitrofuran compounds. The nitroimidazole derivatives metronidazole and tinidazole showed similar activities against the 19 strains of *C. jejuni* and *C. coli* tested. Single strains differed considerably in their susceptibilities; species-related differences did not exist. The nitrofuran agents nitrofurantoin and nitrofurazone displayed identical activities against all strains tested.

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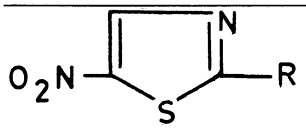
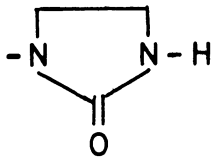
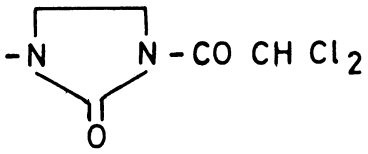
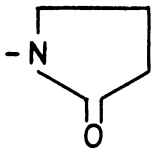
TABLE 1. Chemical structure of nitrothiazole derivatives

Designation	Side chain (−R)
Nitrothiazole ring moiety	
Niridazole (Ambilhar^R)	
Ba 30515	−NH CO NH CH_2 CH_2 Cl
Ba 40922	
Ba 42517	

Most nitrothiazole derivatives were found to be much more active. Most of the strains tested were inhibited at very low concentrations. For example, for reference strain *C. coli* NCTC 11353, nitrothiazole had an MIC of 0.0075 mg/liter. On the other hand, strains such as reference strain *C. jejuni* NCTC 11168 were relatively resistant to nitrothiazole (MIC, 0.5 mg/liter). These same strains were also more resistant to nitroimidazole compounds.

It was found that one nitrothiazole derivative, Ba 42517, was as active as the nitroimidazole derivatives (Table 2). MICs of this compound for the two reference strains of *C. jejuni* and *C. coli* were found to be 16 and 0.5 mg/liter, respectively.

(ii) Bactericidal activities. Niridazole was rapidly bactericidal for the reference strain *C. coli* NCTC 11353 at low concentrations (0.06 mg/liter). The relatively resistant *C. jejuni* NCTC 11168 was also killed by niridazole at a higher concentration (16 mg/liter). Nitrothiazole derivative Ba 30515 was almost as active as niridazole. Nitrothiazole derivative Ba 42517 was definitely less active; however, it was at least as active as metronidazole (Fig. 1).

DISCUSSION

The above results indicate that not only niridazole, which is the single representative of the nitrothiazoles used therapeutically (6), but also some other members of this chemical group (Table 1) were highly active against most campylobacters. The same agents have been found to display pronounced activities in vitro against virtually all anaerobic bacteria tested. Certain aerobic bacteria were also susceptible to these agents (Hof et al., in press). One substance, however, i.e., Ba 42517, which is poorly active against anaerobic and aerobic bacteria (Hof et al., in press), was likewise relatively inactive against microaerophilic campylobacters (Table 2). This compound, however, had antibacterial activity similar to that of metronidazole or tinidazole (Table 2).

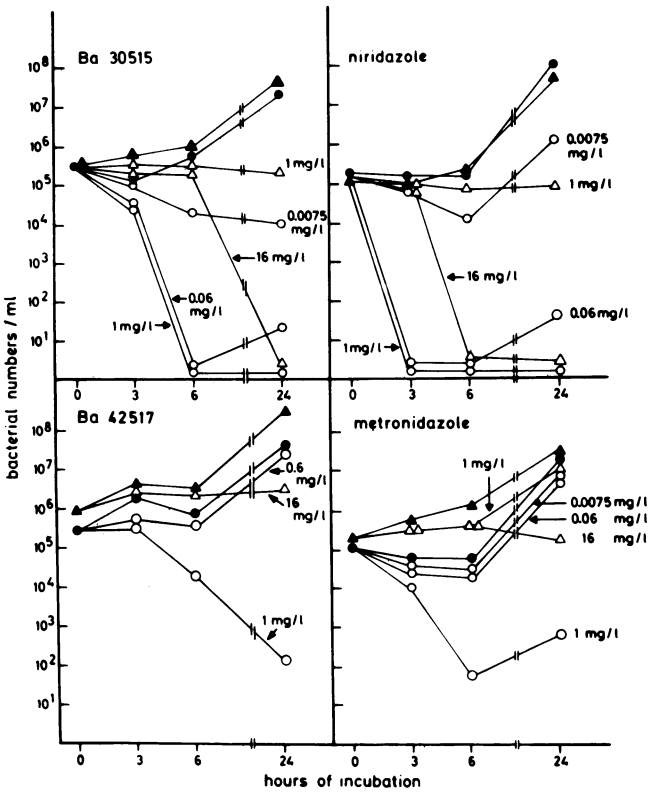


FIG. 1. Bactericidal activities of nitroarene compounds on *C. jejuni* NCTC 11168 (△) and *C. coli* NCTC 11353 (○). The closed symbols (▲, ●) represent the untreated controls.

Nitrofuran derivatives, such as nitrofurazone and nitrofurantoin, were much less active than the potent nitrothiazole derivatives (Table 2). There was, however, a striking difference between the nitrofurans and the other nitroarene compounds. All of the strains tested were uniformly susceptible to nitrofuran derivatives, whereas they differed considerably in their susceptibility to the nitrothiazole as well as to the nitroimidazole derivatives. Thus, the strains with relative resistance to nitrothiazoles were also relatively resistant to

TABLE 2. Comparative activities of some nitrothiazole derivatives and other nitroarene compounds against 19 *C. jejuni* and *C. coli* isolates

Drug	MIC (mg/liter)[a]		
	Range	MIC_{50}	MIC_{90}
Nitrothiazoles			
Niridazole	0.0037–0.5	0.075	0.25
Ba 30515	0.0037–1	0.015	0.5
Ba 40922	0.0037–2	0.015	1
Ba 42517	0.125–16	2	8
Nitroimidazoles			
Metronidazole	0.5–16	2	8
Tinidazole	0.5–16	4	8
Nitrofurans			
Nitrofurantoin	0.5–2	2	2
Nitrofurazone	0.5–2	2	2

[a] MIC_{50}, Minimum concentration required to inhibit 50% of strains; and MIC_{90}, minimum concentration required to inhibit 90% of strains.

nitroimidazoles (Table 2). So, possibly different mechanisms of action exist between nitroimidazoles and nitrothiazoles on the one hand and nitrofurans on the other hand. Although the mode of action of the nitrothiazoles is not yet understood, it might, in a manner analogous to that of the nitroimidazoles, reduce the nitrogroup to several intermediate products such as nitroso- and hydroxylamin- groups which damage the bacterial DNA, resulting ultimately in the death of the target organisms (7). The new nitrothiazole derivatives appear to be bactericidal (Hof et al., in press). Campylobacters were also rapidly killed at low concentrations of each of the nitrothiazoles except Ba 42517. In contrast, the bactericidal activity of metronidazole nearly approximated that of the less effective nitrothiazole derivative Ba 42517 (Fig. 1).

In conclusion, niridazole and several other nitrothiazole derivatives have marked antibacterial activity against *Campylobacter* species.

ACKNOWLEDGMENT

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