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Antibacterial Activities of the Antiparasitic Drugs Nifurtimox and Benznidazole

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Both nifurtimox and benznidazole, which are used for the treatment of Chagas' disease, also display marked antibacterial activities. Characteristically for nitroheterocyclic compounds, they are much more active against anaerobic and microaerophilic bacteria than against aerobic bacteria. Nitroreductase-deficient aerobes are completely resistant, whereas SOS-repair-deficient strains are moderately susceptible. Those strains are rapidly killed.

The antitrichomonadal activity of metronidazole (4) was known long before its antibacterial activity was detected (5). The broad and strong antimicrobial effect of this drug is due to its nitro group coupled onto a heterocyclic ring (6, 12). Several other nitroheterocyclic compounds also show a marked antimicrobial activity (11) that is directed against bacteria, protozoa, and even worms (7, 8).

Nifurtimox (Bay 2502; Lampit; Bayer AG, Leverkusen, Federal Republic of Germany) and benznidazole (Ro 7-1051; Rochagan; Hoffmann-La Roche AG, Basel, Switzerland) are used still now for the therapy of acute infections with *Trypanosoma cruzi* (3, 13). Both of these drugs possess a nitro group at either the 5 or the 2 position of a heterocyclic ring. Therefore, it could be anticipated that these drugs also exert antibacterial activities besides their antiparasitic potency. Their MICs against some aerobic (15) or anaerobic (14) bacteria were determined by an agar dilution method with Mueller-Hinton or Wilkins-Chalgren agar, respectively, and compared with those of metronidazole and niridazole. The microaerophilic campylobacters were tested as described previously (10).

Niridazole was the most active nitro compound. Nifur-

TABLE 1. Comparative activities of nitroheterocyclic compounds

Strain	MIC (μg/ml)			
	Metronidazole	Nifurtimox	Benznidazole	Niridazole
Aerobes				
Pseudomonas aeruginosa ATCC 27853	>128	>128	128	>128
Staphylococcus aureus ATCC 25293	>128	8	64	>128
Escherichia coli ATCC 25922	>128	128	>128	8
W3110 *recA*$^+$	>128	>128	>128	4
W3110 *recA*	128	64	8	0.25
WP2 *polA*$^+$ *uvrA*$^+$	>128	64	64	4
WP67 *polA uvrA*	128	32	16	0.5
Salmonella typhimurium LT2 *uvrB*$^+$	>128	>128	>128	4
LT2 *uvrB*$^+$ NR[a]	>128	>128	>128	>128
TA98 *uvrB*	>128	8	128	0.25
TA98 *uvrB*, NR	>128	64	128	128
TA1538 *uvrB*	128	4	128	0.25
Anaerobes				
Bacteroides fragilis ATCC 25285	0.5	0.5	2	0.0075
Bacteroides variabilis	1		4	0.25
Bacteroides distasonis	1	1	2	0.03
Clostridium perfringens	0.5	1	2	0.015
Clostridium bifermentans	0.5	0.5	4	0.0075
Clostridium septicum	0.5	1	1	0.0075
Clostridium sordelii	1	0.5	2	0.0075
Clostridium tetani	0.5	0.5	2	0.0075
Clostridium sporogenes	0.5	0.5	4	0.0075
Peptostreptococcus sp.	1	1	2	0.03
Actinomyces sp.	8	2	8	4
Propionibacterium acnes	128	128	128	64

[a] NR, Nitroreductase deficient.

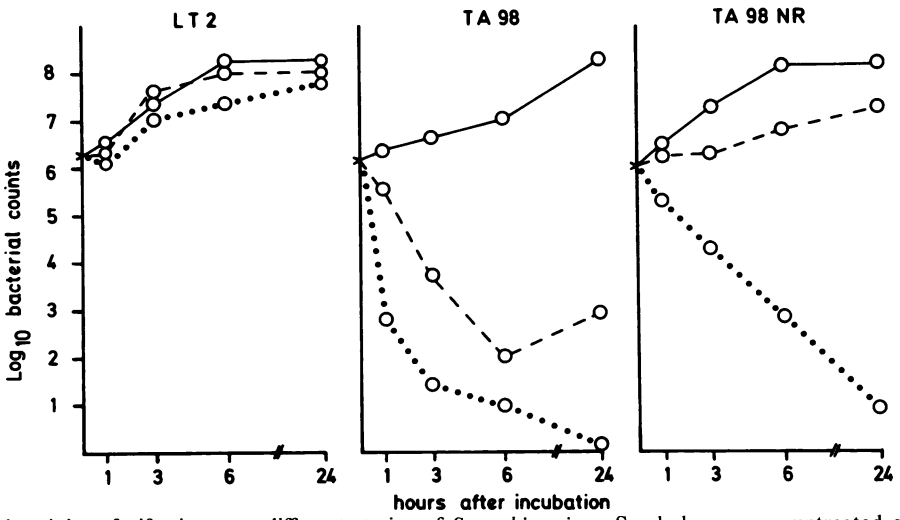

FIG. 1. Bactericidal activity of nifurtimox on different strains of *S. typhimurium*. Symbols: ———, untreated controls; – – –, 8 μg of nifurtimox per ml; . . . . ., 64 μg of nifurtimox per ml.

timox, benznidazole, and metronidazole were rather similar. Few strains were even more susceptible to nifurtimox than to metronidazole, whereas benznidazole was only marginally less active. Anaerobic bacteria other than *Actinomyces* and *Propionibacterium* were much more susceptible to these substances than were aerobic and facultatively anaerobic bacteria (Table 1). The microaerophilic campylobacters were heterogeneous. Of 20 strains of *Campylobacter jejuni* and *Campylobacter coli*, 8 were as susceptible as anaerobic bacteria (MIC, 0.5 to 1 μg/ml), 4 were as resistant as aerobic and facultatively anaerobic bacteria (MIC, 32 to 128 μg/ml), and 8 were intermediate.

Among the aerobic and facultatively anaerobic bacteria tested, strains deficient in their SOS DNA repair system, i.e., with mutations of the *uvr*, *rec*, and *pol* genes (for example, *Escherichia coli* W3110 *recA* and *Salmonella typhimurium* TA98 and TA1538 [9]), were relatively susceptible, indicating that nifurtimox and benznidazole exert their antimicrobial activities by damaging the microbial DNA as metronidazole and niridazole do (1, 6, 9, 12).

Anaerobic bacteria are said to contain highly efficient enzymes that reduce the nitrogroup after penetration of the antimicrobial agent into the cell (2, 12). Aerobic and facultatively anaerobic bacteria and possibly some campylobacters are much less active. A loss of the nitroreductase activity, as in strain TA98NR, leads to a complete resistance (Table 1).

These findings indicate that nifurtimox and benznidazole act like other nitroheterocyclic compounds by damaging the microbial DNA after activation by microbial enzymes. Obviously, this action is bactericidal as shown by a time-kill assay. *Salmonella* strains in the logarithmic phase were diluted to a final concentration of about 10^6/ml in Mueller-Hinton broth containing either 0, 8, or 64 μg of nifurtimox per ml. Subcultures were done at 1, 3, 6, and 24 h after incubation at 37°C. Samples of 0.5 ml of each culture were withdrawn. Serial 10-fold dilutions were plated with 25 ml of liquified Mueller-Hinton agar and incubated for 24 h at 37°C. The susceptible strain *S. typhimurium* TA98, but not the resistant strains such as the innate resistant parent strain LT2 and the mutant TA98NR, was rapidly killed (Fig. 1).

LITERATURE CITED

1. **Andrews, P. J., M. A. Quilliam, B. E. McCarry, D. W. Bryant, and D. R. McCalla.** 1986. Identification of the DNA adduct formed by metabolism of 1,8 dinitropyrene in *Salmonella typhimurium*. Carcinogenesis **7**:105–110.
2. **Angermeier, L., and H. Simon.** 1983. On nitroaryl reductase activities in several *Clostridia*. Hoppe Seyler's Z. Physiol. Chem. **364**:1653–1663.
3. **Brisseau, J. M., J. P. Cebron, T. Petit, M. Majolet, P. Cuilliere, J. Godin, and J. Y. Grolleau.** 1988. Chagas' myocarditis imported into France. Lancet **i**:1046.
4. **Durel, P., V. Roiron, A. Siboulet, and L. J. Borel.** 1960. Systemic treatment of human trichomoniasis with a derivative of nitroimidazole, 8823 R.P. Br. J. Vener. Dis. **36**:21–26.
5. **Füzi, M., and Z. Csukas.** 1970. Das antibakterielle Wirkungsspektrum des Metronidazols. Zentralbl. Bakteriol. Parasitenkd. Infektionskr. Hyg. Abt. 1 Orig. Reihe A **213**:258–262.
6. **Goldmann, P.** 1982. The development of 5-nitroimidazoles for the treatment and prophylaxis of anaerobic bacterial infections. J. Antimicrob. Chemother. **10**(Suppl. A):23–33.
7. **Grunberg, E., and E. H. Titsworth.** 1973. Chemotherapeutic properties of heterocyclic compounds: monocyclic compounds with five-membered rings. Annu. Rev. Microbiol. **27**:317–346.
8. **Hamilton-Miller, J. M. T., and W. Brumfitt.** 1976. The versatility of nitro-compounds. J. Antimicrob. Chemother. **2**:5–8.
9. **Hof, H., T. Chakraborty, R. Royer, and J. P. Buisson.** 1987. Mode of action of nitro-heterocyclic compounds on *Escherichia coli*. Drugs Exp. Clin. Res. **13**:635–639.
10. **Hof, H., V. Sticht-Groh, and K. M. Müller.** 1982. Comparative in vitro activities of niridazole and metronidazole against anaerobic and microaerophilic bacteria. Antimicrob. Agents Chemother. **22**:332–333.
11. **Hof, H., J. Ströder, J.-P. Buisson, and R. Royer.** 1986. Effect of different nitroheterocyclic compounds on aerobic, microaerophilic, and anaerobic bacteria. Antimicrob. Agents Chemother. **30**:679–683.
12. **Müller, M.** 1979. Mode of action of metronidazole on anaerobic microorganisms. R. Soc. Med. Intl. Congr. Symp. Ser. **18**: 223–227.
13. **Ribeiro-dos-Santos, R., A. Rassi, and F. Köberle.** 1981. Chagas' disease. Antibiot. Chemother. **30**:115–134.
14. **Sutter, V. L.** 1985. Susceptibility testing of anaerobes, p. 988–990. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
15. **Washington, J. A.** 1985. Susceptibility tests: agar dilution, p. 967–971. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.