APPLIED MICROBIOLOGY, Nov. 1969, p. 728-730
Copyright © 1969 American Society for Microbiology
Vol. 18, No. 5
Printed in U.S.A.

Effects of 1-(2-Nitro-1-Imidazolyl)-3-Methoxy-2-Propanol and 2-Methyl-5-Nitroimidazole-1-Ethanol Against Anaerobic and Aerobic Bacteria and Protozoa

HERBERT N. PRINCE, E. GRUNBERG, E. TITSWORTH, AND W. F. DELORENZO
Department of Chemotherapy, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110

Received for publication 7 August 1969

1-(2-Nitro-1-imidazolyl)-3-methoxy-2-propanol (RO 7-0582) and 2-methyl-5-nitroimidazole-1-ethanol (Metronidazole), substances known to be potent trichomonacides, were shown to inhibit obligate anaerobic bacteria in vitro but were essentially without effect at the doses tested against bacteria capable of growing aerobically. A similar effect was noted in vivo in that both substances exhibited good chemotherapeutic activity against infections produced by three species of anaerobic protozoa but were essentially inactive at the doses tested against three species of aerobic protozoa.

It has been reported that 1-(5-nitro-2-thiazolyl)-4-acetyl piperazine, a substance exhibiting marked antitrichomonad activity in vitro and in vivo, is also a selective inhibitor of anaerobic microorganisms (5). To the best of our knowledge, this observation of a selective anaerobic effect has not been extended to other heterocyclic nitro-compounds such as the nitroimidazoles, a group which has also been described as having marked antiprotozoan activity. Accordingly, 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (RO 7-0582), a substance recently claimed to be active not only against *Trichomonas vaginalis* but also against *Endamoeba histolytica* (1), and 2-methyl-5-nitroimidazole-1-ethanol (Metronidazole, G. D. Searle and Co., Chicago, Ill.), a substance employed clinically as an antiprotozoan agent, were tested against anaerobic and aerobic bacteria and protozoa employing both in vitro and in vivo methods.

MATERIALS AND METHODS

In vitro tests. All bacteria were grown in thioglycolate broth for the preparation of inocula. For determination of the minimal inhibitory concentration (MIC) of the substances, a standard twofold serial dilution test was employed. All tubes containing the antimicrobial agents plus controls were inoculated with 1 drop of undiluted, 24-hr broth culture, except that the *Escherichia coli* and *Staphylococcus aureus* seed cultures were diluted 1:1000. The tubes were read for turbidity after 24 hr at 37 C. *T. vaginalis* was grown in STS broth, and *T. foetus* was grown in CPLM medium (3). The tubes containing the antimicrobial agents and the controls were inoculated with 0.2 ml of undiluted, 24-hr broth culture and were examined microscopically for the presence of motile trichomonads after 24 hr of incubation at 37 C.

In vivo tests. The methods employed to determine the activity of compounds in mice infected subcutaneously with *T. vaginalis* and intraperitoneally with *T. foetus* have been described in detail elsewhere (3). In brief, the mice infected with *T. vaginalis* were treated either orally four times or by infiltration into the site of infection two times and were sacrificed after 5 days so that the subcutaneous tissue could be examined for the presence or absence of characteristic abscesses. The mice infected with *T. foetus* were treated orally three times and observed for death up to 14 days. The intracecal infection of rats with *E. histolytica* and the evaluation of antiamebic activity were performed as previously reported (2), except that the weanling animals were anesthetized with 30 mg of sodium pentobarbital per kg instead of with ether. The rats were treated once orally and were sacrificed 6 days after infection so that scrapings from the cecal wall could be examined microscopically for the presence or absence of trophozoites. The intraperitoneal infection of mice with *Trypanosoma brucei*, *T. cruzi*, and *T. equiperdum* has been described elsewhere (1). With this prophylactic method, the mice were treated orally five times and the tail blood was examined microscopically for the presence or absence of motile trypanosomes for a period of 42 days.

RESULTS AND DISCUSSION

Table 1 summarizes the antibacterial effects of the two nitroimidazoles when tested against re-

TABLE 1. *Antimicrobial effect of 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (I) and Metronidazole (II) against anaerobic and aerobic organisms in vitro*[a]

Anaerobic	I	II	Aerobic	I	II
Clostridium tetani	62.5	0.01	*Lactobacillus acidophilus*	1,000	1,000
C. septicum	15.6	0.03	*L. arabinosus*	>2,000	>2,000
Fusobacterium polymorphum	7.8	0.03	*Staphylococcus aureus*	1,000	2,000
Veillonella alcalescens	62.5	0.03	*Streptococcus pyogenes*	>1,000	500
Trichomonas vaginalis	31.2	1.0	*Escherichia coli*	250	1,000
T. foetus	62.5	4.0	*Pseudomonas aeruginosa*	>1,000	>1,000
			Proteus vulgaris	1,000	1,000

[a] Results are expressed as MIC (μg/ml).

TABLE 2. *Chemotherapeutic effect of 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (I) and Metronidazole (II) against anaerobic and aerobic protozoa*[a]

Anaerobic	I[b]	II	Aerobic	I[b]	II
Trichomonas vaginalis	8[c]	43[c]	*Trypanosoma brucei*	>500	>1,000
T. vaginalis	12	16	*T. cruzi*	>500[d]	>1,000
T. foetus	5	17	*T. equiperdum*	>500	>1,000[d]
Endamoeba histolytica	61	49			

[a] Treatment schedules for each infection were as follows: *T. vaginalis*, 1 ml subcutaneously 2 and 24 hr after infection, or 1 ml orally 1, 24, 48, and 72 hr after infection; *T. foetus*, 1 ml orally 1, 24, and 48 hr after infection; *E. histolytica*, 1 ml orally 24 hr after infection; trypanosomes, 1 ml orally immediately after infection and then once daily for 4 additional days. Compounds were tested simultaneously under identical conditions. Results are expressed as the CD_{50} (mg/kg orally).

[b] Cited from Grunberg et al. (1).

[c] Amount (μg) infiltrated into site of infection.

[d] Slight delay (4 to 7 days) in appearance of hemoflagellates.

presentative anaerobic and aerobic organisms. It is obvious that the selective inhibition of obligate anaerobes originally described for the nitrothiazole substance (5) is also a feature of members of the nitroimidazole group of compounds. 2-Methyl-5-nitroimidazole-1-ethanol (Metronidazole) was appreciably more active in vitro against anaerobic bacteria than the 2-nitroimidazole compound. Both substances were similar in that they displayed little or no activity at the doses tested against the aerobic and facultative bacteria studied.

It may be thought that the selective activity noted for these compounds is a function of the anaerobic conditions of growth as opposed to the biochemical nature of these obligately anaerobic cells. That this is not true can partially be assumed from the observation that the fluid thioglycolate medium employed would allow the greatest portion of the bacterial population in each tube to grow anaerobically. However, since it would be possible for cells growing aerobically in the oxidized portion of each tube to produce the resultant turbidity, a more rigorous test was made to exclude anaerobiosis per se as a cause of the specific antimicrobial effect. Thus, all of the facultative bacteria were tested for sensitivity to these agents by employing tubes of Trypticase Soy Broth incubated anaerobically in a hydrogen-carbon dioxide anaerobe jar (BBL GasPak). For the lactobacilli, *S. aureus*, *Streptococcus pyogenes*, and *Proteus vulgaris*, the MIC values were the same as those obtained when the tubes were incubated in air. Only in the case of *E. coli* did the anaerobic environment seem to potentiate the slight antimicrobial effect exerted by 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol (MIC = 250 μg/ml in air versus 100 μg/ml anaerobically).

Since the activity of antimicrobial agents can be affected by alterations in *p*H or protein concentration, these variables were studied in the case of species of clostridia. The activity of these substances against *Clostridium septicum* was virtually unaltered over the *p*H range of 5.5 to 8.0 in thioglycolate broth. In addition, calf serum up to a final concentration of 5% did not affect the activity of the two compounds against *C. tetani* and *C. septicum*.

Table 2 summarizes the chemotherapeutic effects of the two nitroimidazoles when tested in standardized experimental infections of mice. The protozoa therein described have been categorized

as anaerobic or aerobic on the basis of the need for a suitably reduced culture environment and high Q_{O_2} values (4).

The infections produced by the anaerobic protozoa responded well to both of the nitroimidazole compounds. The trypanosome infections, except for some slight delays in the appearance of hemoflagellates in the peripheral blood, were essentially unresponsive to these agents at the doses tested. Additional studies with pathogenic anaerobic and aerobic protozoa would have to be undertaken with a number of nitroimidazoles in order to be able to determine categorically whether a bifurcation into responsive and unresponsive protozoan infections can be made on the basis of oxygen tolerance. In addition, studies employing in vitro antimicrobial assays against the trypanosomes would be required. However, from the limited data presented, a general pattern emerges in vivo similar to that seen under in vitro conditions. This effect must be considered to be only qualitative in nature, since, in contrast to the greater activity seen with Metronidazole in the in vitro studies, 1-(2-nitro-1-imidazolyl)-3-methoxy-2-propanol and Metronidazole were quantitatively similar in activity under in vivo conditions.

LITERATURE CITED

1. Grunberg, E., G. Beskid, R. Cleeland, W. F. DeLorenzo, E. Titsworth, H. J. Scholer, R. Richle, and Z. Brener. 1968. Antiprotozoan and antibacterial activity of 2-nitroimidazole derivatives. Antimicrobial Agents and Chemotherapy—1967, p. 513–519.
2. Grunberg, E., H. N. Prince, E. Titsworth, G. Beskid, and M. D. Tendler. 1966. Chemotherapeutic properties of anthramycin. Chemotherapia 11:249–260.
3. Grunberg, E., and E. Titsworth. Toxicity and antitrichomonal activity of 2-nitroimidazole and 2-nitrobenzimidazole derivatives. 1966. Antimicrobial Agents and Chemotherapy—1965, p. 478–480.
4. Lwoff, A., and S. Hutner. 1951. Biochemistry and physiology of protozoa. Academic Press Inc., New York.
5. Prince, H. N. 1960. Specific inhibition of obligate anaerobes. Nature (London) 186:817–818.