5-Nitroimidazole Drugs Effective against Metronidazole-Resistant 
Trichomonas vaginalis and Giardia duodenalis

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Received 1 June 2005/Returned for modification 22 June 2005/Accepted 12 October 2005

Metronidazole (Mz)-resistant Giardia and Trichomonas were inhibited by 1 of 30 new 5-nitroimidazole drugs. Another five drugs were effective against some but not all of the Mz-resistant parasites. This study provides the incentive for the continued design of 5-nitroimidazole drugs to bypass cross-resistance among established 5-nitroimidazole antiparasitic drugs.

Metronidazole (Mz) and a related 5-nitroimidazole, tinidazole, are the only drugs recommended for the treatment of trichomoniasis and are the most-prescribed drugs for the treatment of giardiasis. However, clinical resistance to these drugs has been well documented; and in the event of overt clinical resistance to Mz in trichomonads, there is no alternative for treatment, when one keeps in mind the documented cross-resistance between the currently used 5-nitroimidazole drugs and their worldwide availability (7, 8, 18, 23). On the positive side, a great deal of flexibility is offered by the side chains attached to the imidazole ring structure that bear the all important nitro group (17).

The mechanisms of Mz resistance in Giardia and Trichomonas have been well studied in laboratory-induced resistance (18). It occurs by down-regulation of pathways, especially the enzyme pyruvate:ferredoxin oxidoreductase (PFOR) and ferredoxin (Fd) pathway, that activate Mz to its toxic radical state. The PFOR-Fd couple has an electron potential sufficiently low to activate Mz, while no such electron couple is present in the mammalian host (9). In the laboratory we see a threefold down-regulation of PFOR activity in Mz-resistant (Mz') Giardia duodenalis (14), and in highly Mz' Trichomonas vaginalis the activity of the hydrogenosome organelle is down-regulated such that there is no detectable PFOR or Fd expression (4, 11, 18). Thus, Mz is not activated to its toxic radical state in these cells. On the other hand, it is well documented that clinically Mz' T. vaginalis strains do not have down-regulated hydrogenosomes, and the mechanism of Mz resistance in these cells is not understood (8).

Previously, we showed that some 5-nitroimidazole derivatives were significantly more effective antiprotozoal agents (based on in vitro molar drug concentrations) than Mz against Mz-susceptible (Mzs) parasites but were not as effective against Mz' parasites (17). Given the impetus for the development of 5-nitroimidazole drugs that vary markedly in their efficacies (both positively and negatively), we tested 30 new 5-nitroimidazoles in our anaerobic drug susceptibility screening assay (16) for their efficacies against T. vaginalis and G. duodenalis, with the focus on laboratory-derived Mz' (Mz') lines and clinical isolates derived from patients with treatment failures.

Parasites were cultured axenically in anaerobic TYI-S-33 (6), which was modified as described previously (16). Mzs G. duodenalis isolates WB-1B, BRIS/87/HEPU/713 (713), and BRIS/83/HEPU/106 (106) were tested along with their respective Mz' lines WB-M3, 713-M3, and 106-2ID10 (13, 16). Mzs T. vaginalis isolate BRIS/92/HEPU/F1623 (F1623) and the highly Mz' line derived from it, F1623-M1 (16), were used throughout. BRIS/92/HEPU/F1623 (F1623) and the highly Mz' line derived from it, F1623-M1 (16), were used throughout. BRIS/92/HEPU/F1623 (F1623) and the highly Mz' line derived from it, F1623-M1 (16), were used throughout. BRIS/92/HEPU/F1623 (F1623) and the highly Mz' line derived from it, F1623-M1 (16), were used throughout.

Metronidazole MICs were previously described as an MIC of 3.2 µM (with a maximum of 6.3 µM and minimum of 1.6 µM) for Mzs T. vaginalis and 6.3 µM (with a maximum of 3.2 µM and minimum of 12.5 µM in a few cases) for Mzs G. duodenalis (16). The minimum MIC among all assays for Mz' parasites was 25 µM (16). For the purposes of screening new 5-nitroimidazoles, we have directly compared their MICs with those for Mz.

All new 5-nitroimidazole compounds (Fig. 1) were identified by spectral data, purified by chromatography on silica gel columns, and recrystallized from appropriate solvents. Their purities were checked with appropriate controls by thin-layer chromatography and elemental analysis (C, H, N). The purity was always over 99.6%. The syntheses of compounds 1 to 10, 10 to 16, 17 to 19, 20 to 23, and 24 to 28 were as described in references 2, 1, 20, 21, and 22, respectively. The reaction of compound 28 with 2-nitropropane or nitrocyclohexane anion led to the C-alkylation products 29 and 30, respectively, by the SRN1 mechanism.

Of the 30 compounds tested, compounds 11, 12, 13, 24, 26, and 30 demonstrated MICs against all three Mzs G. duodenalis isolates of ≥100 µM (Mz MIC ≤ 10 µM) (data not shown).
FIG. 1. Structures of the 5-nitroimidazole drugs used in this study compared with that of Mz. MW, molecular weight.
Compounds 6, 10, 15, 16, 19, 20, 21, 22, 23, 28, and 29 had higher MICs than Mz against one or more of the Mz\textsuperscript{r} isolates tested (data not shown). As a result, these drugs were not considered further, except where stated below.

Compounds 1, 2, 3, 4, 5, 7, 8, 9, 14, 17, 18, 25, and 27 demonstrated MICs equal to or less than that of Mz (≤10 μM) for all Mz\textsuperscript{r} Giardia and Trichomonas isolates tested and were subsequently assessed against all the Mz\textsuperscript{r} and Mz\textsuperscript{e} parasites described above. Of these compounds, compounds 1, 2, 4, 5, 9, 16, and 7, or a dioxole nucleus (compound 18) were noted, but the deprotection of the remote dioxole ring in compound 18 resulted in the formation of the diphenol compound 19, which is not effective against Mz against 31 T. vaginalis, which supposedly does not have the native 2 oxoacid oxidoreductase (OR) activity in Mz\textsuperscript{r} T. vaginalis (4), and it is possible that these alternative ORs are responsible for the activation of the diphenol compound 19, which was detrimental to protozoocidal activity.

These data suggest that the 5-nitro group is highly active in compound 14, but it does not explain why this drug is so active against Mz\textsuperscript{r} T. vaginalis, which supposedly does not have the ability to reduce Mz (4). We have previously identified alternative 2 oxoacid oxidoreductase (OR) activity in Mz\textsuperscript{d} T. vaginalis (4), and it is possible that these alternative ORs are responsible for the activation of compound 14 to its toxic radical state.

There are examples in the literature of patients who have failed to respond to all antiangiarial or antitrichomonal treatments (7, 23). Here we have shown that at least three different 5-nitroimidazole compounds, including one that is readily synthesized, inhibited highly Mz\textsuperscript{d} G. duodenalis and Mz\textsuperscript{e} T. vaginalis isolates. The clinical application of such drugs may well be the single most cost-effective step in human immunodeficiency virus infection incidence reduction (5, 10, 12), and for this reason the development of drugs for the treatment of cases of Mz\textsuperscript{e} T. vaginalis has been a focus of our work (8, 16).

Previously, we reported that among a 5-nitroimidazole series of compounds, the most antimicrobial and antiparasitic compounds showed a greater resonance conjugation in the molecular structure (17, 19). For this study, we have synthesized under mild conditions and in good yields highly conjugated 5-nitroimidazole derivatives using electron transfer reactions (S\textsubscript{RN1}, LD-S\textsubscript{RN1}, bis-S\textsubscript{RN1}, and E\textsubscript{RC1} [1, 2, 20, 21, 22]). By increasing the conjugated system, we have developed some highly active compounds, especially the C-alkylation products obtained by LD-S\textsubscript{RN1}, compounds 8 and 14. The present study again demonstrates the importance of the 5-nitroimidazole side chain in antiparasitic activity (e.g., compounds 3 and 7 are E and Z isomers, and the differences between the antiprotozoal activities of compounds 14 and 16 and between compounds 18 and 19). These dramatic differences may relate to solubility and membrane permeability. The positive influence of bromine (compound 17), a sulfonyl group (compounds 3 and 7), or a dioxole nucleus (compound 18) were noted, but deprotection of the remote dioxole ring in compound 18 resulted in the formation of the diphenol compound 19, which was detrimental to protozoocidal activity.
We gratefully acknowledge Wim Sturm, Sarita Naidoo, and staff at the Medical Faculty, University of KwaZulu Natal, Durban, South Africa.

This work was supported by ACITHN: a grant to support a field visit to Durban in 1999 and continued support of Jacqueline Upcroft. We gratefully acknowledge the Winston Churchill Memorial Trust for a fellowship to Jacqueline Upcroft in 2003 for work carried out in South Africa and the NHMRC, which has supported some of this work.

REFERENCES
15. Reference deleted.