In Vitro Metronidazole and Tinidazole Activities against Metronidazole-Resistant Strains of *Trichomonas vaginalis*

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The in vitro activities of tinidazole and metronidazole against *Trichomonas vaginalis* isolates clinically resistant to metronidazole were compared. Minimal lethal concentrations (MLCs) of tinidazole were significantly lower than MLCs of metronidazole. Increased metronidazole resistance correlated with increased tinidazole resistance. These data support a role for tinidazole in the treatment of trichomoniasis.

*Trichomonas vaginalis* is a common sexually transmitted infection that is increasingly recognized as an important infection in women and men (9, 15). Recent estimates have suggested that *T. vaginalis* infections account for nearly one-third of the 15.4 million cases of sexually transmitted diseases in the United States (2). In 1995, the World Health Organization estimated the number of adults with trichomoniasis at 170 million worldwide, more than the numbers for gonorrhea, syphilis, and chlamydia combined (23).

*T. vaginalis* is recognized as a common cause of vaginitis (16) and as a factor contributing to preterm birth and low birth weight (4). *T. vaginalis* infections have also been linked with increased human immunodeficiency virus (HIV) transmission (1, 8, 21, 22). Surwillo and colleagues have suggested that *T. vaginalis* could facilitate HIV acquisition in women by causing lesions and an influx of leukocytes in the genital area (21, 22). Studies have also shown a sixfold increase in viral concentration in the semen of HIV-infected men with symptomatic trichomoniasis, compared to HIV-positive men not infected with *T. vaginalis* (7).

Metronidazole has been the drug of choice for *T. vaginalis* infections since 1960 (5) and remains effective today, with a cure rate of approximately 95% (3). Clinical resistance to metronidazole, though, has been reported since 1962 (18). Recently, there has been an increase in the recognition of metronidazole-resistant trichomoniasis, but whether this is due to an increase in resistance among trichomonas strains or simply to better surveillance is unknown. Most patients with *T. vaginalis* infections that are refractory to initial metronidazole treatment will eventually respond to increased doses of the drug (11). Indeed, the current protocol for treating metronidazole-refractory trichomoniasis infections is to increase the dose of metronidazole and/or prescribe multiple doses. However, side effects from metronidazole treatment are common, and nausea has been reported for up to 12% of patients (19). Hypersensitivity reactions, usually manifesting as dermatological symptoms, can also result, though the frequency of these reactions is unknown (10).

Unfortunately for those patients for whom metronidazole treatment has proven ineffective or contraindicated, there is no other drug licensed in the United States for the treatment of trichomoniasis. Tinidazole, another nitroimidazole, has emerged as an effective antitrichomonas medication in other countries (6). It has also been shown to be effective in clinical trials conducted in the United States (20), though it is not yet Food and Drug Administration approved.

Despite the international use of tinidazole and its suggested use in the United States, a direct comparison of the activities of these nitroimidazoles on a large number of metronidazole-resistant isolates has never been published. The objective of this study was to compare the relative in vitro efficacies of metronidazole and tinidazole against a set of clinically resistant isolates.

A total of 104 clinical isolates collected during the years 1995 to 2001 from health care practices throughout the United States were tested at the Centers for Disease Control and Prevention (CDC) by a modified Meingasser method (12, 13). These isolates were sent to the CDC for confirmation of metronidazole resistance. Vaginal swabs were collected by a physician, placed in Diamond’s (Trypticase-yeast-maltose medium with agar) culture medium, and sent to the CDC for resistance testing. Isolates were maintained in Diamond’s Trypticase-yeast-maltose medium without agar at 37°C.

Metronidazole and tinidazole (Sigma, St. Louis, Mo.) were dissolved in 100% dimethyl sulfoxide (Sigma). Parasites (2 × 10⁶) were added to serial twofold dilutions (400 to 0.1 µg/ml) of each 5-nitroimidazole in round-bottomed 96-well microtiter plates. Each plate contained three replicates of each drug concentration and duplicate medium controls (dimethyl sulfoxide) at the same final dilution. Each isolate was tested at least twice under both aerobic and anaerobic conditions. Anaerobic conditions were generated by using a GasPak jar and CO₂-generating GasPak Plus anaerobic system envelopes (Becton Dickinson, Sparks, Md.) and monitored with GasPak disposable anaerobic indicator strips (Becton Dickinson). Standard metronidazole-resistant (CDC 085) and standard metronidazole-sensitive (CDC 520) isolates were included as controls in each clinically resistant isolate assay.
After 48 h of incubation at 37°C, plates were examined by using an inverted phase-contrast microscope. The lowest drug concentration at which no motile trichomonads were observed was recorded as the minimum lethal concentration (MLC). When different MLC scores were observed on duplicate assays, the average score was used. Statistical comparisons of drug effect under aerobic and anaerobic conditions were made with the Wilcoxon signed rank test.

Under aerobic conditions, the isolates evaluated in this study were much more sensitive to tinidazole than to metronidazole (Fig. 1). The mean MLC for metronidazole was 2,618 ± 1,922.4 μM, while the mean MLC for tinidazole was 1,014.9 ± 1,314.4 μM. The medians were 197.7 and 102.6 μM, respectively. For 60% of the isolates, the MLC for tinidazole was lower than that for metronidazole (P < 0.0001, Wilcoxon signed rank test).

Correlation analysis revealed a strong relationship between the MLC for metronidazole and the MLC for tinidazole (r = 0.8709, P < 0.0001), indicating that those isolates with increased resistance to metronidazole have decreased sensitivity to tinidazole (Fig. 2). This finding is not surprising, as both compounds are 5-nitroimidazoles. However, as drug resistance in trichomoniasis is relative rather than absolute, the increased sensitivity of freshly isolated trichomonads to tinidazole suggests clinical applicability for this compound.

These results are consistent with a previous study comparing in vitro efficacies of furazolidone, metronidazole, and tinidazole (14). In that study, for a limited number of recent isolates (four) clinically resistant to metronidazole, the MLCs of tinidazole were lower than those of metronidazole. However, long-term standard isolates characterized as having very high resistance to metronidazole were similarly insensitive to tinidazole (14). The previously evaluated recent or standardized laboratory isolates (other than the assay controls CDC 085 and CDC 520) were not included in the present study.

Whether the increased recognition of metronidazole-resistant T. vaginalis infections reflects an increased incidence or simply increased recognition of the problem, a therapeutic alternate to metronidazole is needed. While tinidazole may not be effective for every patient with metronidazole-resistant trichomoniasis, the increased sensitivity (lower MLC) of metronidazole-resistant trichomonads to tinidazole suggests that patients refractory to metronidazole treatment may respond to tinidazole, as demonstrated by Sobel et al. in a study of patients who failed to respond to metronidazole (20). Differences in the pharmacokinetics of the two drugs may also play a significant role in their relative in vivo efficacies. In addition, tinidazole appears to have less severe side effects than those of metronidazole (6). Thus, broader testing of tinidazole as an alternative to metronidazole could be beneficial.

FIG. 1. Comparison of metronidazole and tinidazole activities against 104 T. vaginalis isolates with clinical metronidazole resistance. The MLC was determined for each drug as described in the text. Each line represents a separate isolate and is generated by connecting the two points representing the MLCs for metronidazole and tinidazole. The MLCs, measured in micromolar concentrations, for metronidazole were consistently higher than those for tinidazole (P < 0.0001, Wilcoxon signed rank test).

FIG. 2. Regression analysis of MLCs for metronidazole and tinidazole. The diagonal represents the line of identity, indicating equal concentrations of the two drugs. Each point represents an individual isolate. MLCs of metronidazole strongly correlated with MLCs of tinidazole (r = 0.8709, P < 0.0001).
adjunct or alternative to metronidazole, especially for patients with metronidazole-resistant *T. vaginalis* infections, is clearly indicated.

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REFERENCES