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In vitro Testing of *Trichomonas vaginalis* Drug Susceptibility: Evaluation of Minimal Lethal Concentration for Secnidazole that Correlates with Treatment Success

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Abstract

We determined the in vitro minimum lethal concentration (MLC) of secnidazole (SEC) and assessed correlation with clinical susceptibility among *T. vaginalis* isolates obtained from 71 women, of whom 66 were successfully treated with this medication. An MLC ≤12.5 μg/ml correlated with clinical susceptibility in this study.

Brief Summary:

This study found that an in vitro minimal lethal concentration of ≤12.5 μg/ml for secnidazole (SEC) correlated with clinical susceptibility in patient isolates of *Trichomonas vaginalis*.

Keywords

Trichomonas vaginalis ; secnidazole; minimum lethal concentration; 5-nitroimidazoles; drug resistance

Trichomonas vaginalis is a parasitic protozoan and the causative agent of the sexually transmitted infection (STI), trichomoniasis. *T. vaginalis* is the most common non-viral STI worldwide (1). It is more common in women than men (2). Signs and symptoms in women may include malodorous/discolored vaginal discharge, genital pruritus, dysuria, and dyspareunia (1). Symptomatic men may experience penile discharge, urethritis,

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epididymitis, and prostatitis (3). Asymptomatic infection may also occur. *T. vaginalis* is associated with multiple adverse health outcomes including adverse birth outcomes, increased risk of HIV/STI acquisition, infertility, and cervical cancer (1, 4, 5).

Drugs in the 5-nitroimidazole class are the only FDA-approved treatments for *T. vaginalis*; these include oral metronidazole (MTZ), tinidazole (TDZ), and secnidazole (SEC) (1). MTZ has been used to treat *T. vaginalis* since the 1960s and can be used during pregnancy (6). However, MTZ resistance in *T. vaginalis* was observed within a few years of its introduction (7). TDZ was FDA-approved as an additional treatment option in 2004; however, it is more costly than MTZ and should not be used in pregnancy or while breastfeeding (8); resistance has also emerged (9). SEC is a next-generation oral 5-nitroimidazole with a longer half-life (17–19 hours) than MTZ (7–8 hours) and TDZ (11–12 hours) (10). It has been used internationally since the 1960s for the treatment of parasitic infections (i.e., giardiasis, amoebiasis, trichomoniasis) (11). More recently, SEC has been FDA-approved for the treatment of bacterial vaginosis and trichomoniasis in non-pregnant women. There are minimal treatment options beyond MTZ for MTZ-resistant *T. vaginalis* infection in pregnancy (1). Longer courses of treatment with higher doses of MTZ can be considered in this situation (i.e., MTZ 2 g/d orally, for 7–14 days) (1, 8).

Resistance rates in *T. vaginalis* for MTZ and TDZ can range from 4.3–10% (12). In vitro breakpoints for susceptibility of MTZ and TDZ to *T. vaginalis* have previously been determined (13, 14). Additionally, a prior in vitro study of the susceptibility of 100 *T. vaginalis* isolates to MTZ and SEC found that 96% of isolates demonstrated a lower minimum lethal concentration (MLC) to SEC than MTZ, suggesting SEC could have better *in vivo* activity than MTZ (15). However, the cut-off for SEC susceptibility in *T. vaginalis* was not determined in this study as isolates from participants who had failed SEC treatment were not available. Given its longer half-life and prior in vitro susceptibility testing results, SEC may be a useful treatment option, particularly for MTZ- or TDZ-resistant *T. vaginalis* infections, although this requires further evaluation (16).

The primary aim of this study was to determine the in vitro MLC of SEC that correlates with clinical susceptibility of *T. vaginalis* among isolates obtained from *T. vaginalis*-infected women in a randomized controlled, delayed-treatment trial (RCT) of oral SEC vs. placebo (17). A secondary aim was to compare in vitro MLC values of MTZ and TDZ for these *T. vaginalis* isolates to results from prior studies (13, 14, 18).

METHODS

This research was designated as not human subjects' research by the UAB Institutional Review Board (IRB), Protocol #IRB-300008770. A subset of stored, frozen *T. vaginalis* isolates (N=71) from women who had been evaluated for cure after treatment with 2-g oral SEC were used in this study (17).

Each *T. vaginalis* isolate was initially grown anaerobically in Diamond's Trypticase-Yeast-Maltose media supplemented with heat-inactivated horse serum at 37°C for a minimum of three days. The anaerobic environment was accomplished using a 7-L AnaeroPack™-

Anaero rectangular jar and AnaeroPouch™-Anaero oxygen absorber-CO_2 generator sachets (Mitsubishi Gas Chemical, Inc., Tokyo, Japan).

A modified CDC protocol (14, 19) was used to perform 5-nitroimidazole susceptibility assays for MTZ, TDZ, and SEC under aerobic conditions. Stock solutions of each 5-nitroimidazole were prepared in dimethyl sulfoxide (DMSO) and diluted further in Diamond's media. DMSO diluted in Diamond's media without any drugs served as a vehicle-control solution.

The 5-nitroimidazole susceptibility assays were performed in 96-well plates using Diamond's media. The final concentration of DMSO in the plate was the same as the amount of DMSO in the corresponding drug dilution. The concentrations of tested compounds ranged from 0.2 μg/mL to 400 μg/mL. The plates were incubated at 37°C for 46–50 hours under aerobic conditions and then examined using an inverted microscope at 100X magnification to evaluate cell motility. Parasite viability was confirmed in the wells with the equivalent concentration of DMSO only. The lowest concentration at which no viable parasite(s) were observed was recorded as the MLC.

Data from *T. vaginalis* isolates of women successfully treated with SEC were analyzed to determine the median and 95th percentile MLC for each 5-nitroimidazole. Using the 95th percentile MLC of susceptible isolates as the cutoff for potential in vitro resistance, we compared the agreement of the in vitro SEC MLC for each isolate with the corresponding treatment outcome. Similar analyses were performed for MTZ and TDZ MLCs. A related-samples Friedman's two-way analysis of variance by ranks and a post-hoc Wilcoxon signed-rank test were performed using IBM SPSS Statistics 27 software (IBM, SPSS Inc., Armonk, New York) to assess any significant differences in the MLCs of the tested 5-nitroimidazoles.

RESULTS

T. vaginalis isolates (n=71) obtained from women in the RCT were analyzed for their susceptibility to 5-nitroimidazoles MTZ, SEC, and TDZ (Figure 1). Of the 71 isolates tested, 5 (7%) were from women who failed SEC treatment in the parent RCT while 66 (93%) were from women successfully treated with SEC. The median SEC MLC of the isolates obtained from women successfully treated with SEC was 3.1 μg/ml and the 95th percentile was 12.5 μg/ml (Table 1). Of the isolates from women successfully treated with SEC, 55/66 (83.3%) had MLCs <12.5 μg/ml, while 11/66 (16.7%) had MLCs ≥12.5 μg/ml. Among the 5 women failing SEC treatment, 2/5 (40%) had isolates with MLCs ≥12.5 μg/ml while 3/5 (60%) had isolates with MLCs <12.5 μg/ml. By comparison, 64/71 (90.1%) isolates were sensitive to TDZ (MLCs <6.3 μg/ml) while 7/71 (9.9%) isolates had TDZ MLCs consistent with TDZ resistance (≥6.3 μg/ml). Of the 7 TDZ-resistant isolates, 1 was from a woman who failed SEC treatment while 6 were from women successfully treated with SEC. Almost all the isolates (70/71; 98.6%) were susceptible to MTZ in vitro, including 4 isolates from women who had failed SEC (Supplementary Table 1). There was a statistically significant difference in the MLCs measured for the 70 MTZ-sensitive isolates used in the 5-nitroimidazole susceptibility assays, χ^2 (2)=15.43, p<0.001 (Supplementary Table 2). Forty-four MTZ-sensitive isolates had a lower TDZ MLC than their MTZ MLC. Likewise,

36 had a lower TDZ MLC than their SEC MLC (Supplementary Tables 3 and 4). This is consistent with findings from previous studies where *T. vaginalis* isolates had lower in vitro TDZ MLCs compared to MTZ MLCs (13). A post-hoc Wilcoxon signed-rank test showed there was no significant difference between the MTZ and SEC MLCs (Z=−1.559, p=0.119) for MTZ-sensitive isolates (Supplementary Table 4). By contrast, there was a statistically significant difference between both the MTZ and TDZ MLCs (Z=−5.166, p< 0.001) and the TDZ and SEC MLCs (Z=−3.435, p<0.001) for the MTZ-sensitive isolates.

The median MLCs for MTZ and TDZ were 3.1 and 0.8 μg/ml, respectively, which were similar to values previously obtained in a Centers for Disease Control and Prevention (CDC) study (14). The 95th percentile of MLCs was 25 μg/ml for MTZ and 6.3 μg/ml for TDZ (Table 1). Only one isolate from a woman failing SEC treatment had MTZ and TDZ MLCs that were in the range of resistance, at 100 μg/ml and 50 μg/ml, respectively. This resistant isolate was compared to 14 MTZ-resistant control *T. vaginalis* isolates (Supplementary Table 5) obtained from the CDC and UAB biorepositories. There was a statistically significant difference in the MLCs measured for MTZ-resistant isolates, χ^2 (2)=11.66, p=0.003 (Supplementary Table 6). A majority of the SEC MLCs (n=11) for MTZ-resistant isolates were lower than the MTZ MLCs, while none of the SEC MLCs were higher than the MTZ MLCs (Supplementary Tables 7 and 8). These results suggest that SEC has a greater in vitro activity against MTZ-resistant *T. vaginalis* isolates compared to MTZ (15). The Wilcoxon signed-rank test showed a significant difference between the MTZ and TDZ MLCs (Z=−1.995, p=0.046), but the TDZ and SEC MLC difference was not significant (Z=−0.879, p=0.379). However, there was a statistically significant difference between the MTZ and SEC MLCs (Z=−2.937, p=0.003) (Supplementary Table 8).

CONCLUSION

T. vaginalis isolates with SEC MLCs <12.5 μg/ml correlated with successful treatment outcomes in this study. SEC appears to be as effective as MTZ at successfully killing MTZ-sensitive *T. vaginalis* based on similar MLCs for MTZ and SEC determined in this study. However, TDZ had greater in vitro activity against MTZ-sensitive *T. vaginalis* compared to SEC and MTZ. Conversely, SEC had a greater in vitro activity against MTZ-resistant *T. vaginalis* isolates. These results, combined with the longer half-life of SEC compared to MTZ, suggest that SEC may be particularly useful for the treatment of MTZ-resistant *T. vaginalis* infections. Future clinical trials should be conducted to directly compare the effectiveness of these 5-nitroimidazole medications in *T. vaginalis*-infected individuals, including those with MTZ-resistant *T. vaginalis* infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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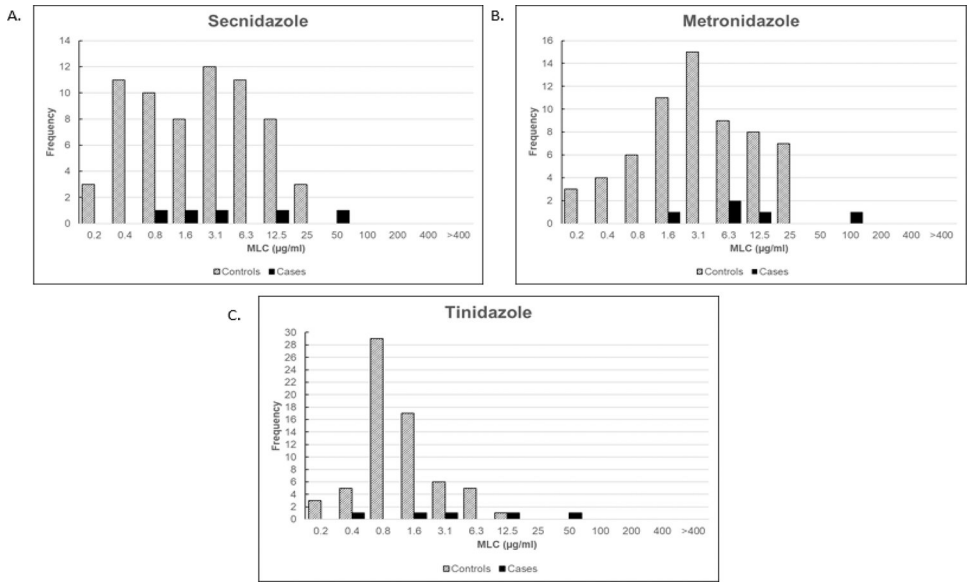

Figure 1.

5-Nitroimidazole minimum lethal concentration (MLC) distributions. **A)** Secnidazole MLC distribution; **B)** Metronidazole MLC distribution; **C)** Tinidazole MLC distribution. **Controls:** women successfully treated with secnidazole; **Cases:** women who failed secnidazole treatment.

Table 1.

Sensitivity of *T. vaginalis* isolates obtained from women successfully treated with SEC to 5-nitroimidazole drugs used to treat trichomoniasis.

	SEC (N=66)	MTZ (N=66)	TDZ (N=66)
Mean (μg/ml)	4.66	6.14	1.94
Median (μg/ml)	3.1	3.1	0.8
95% Percentile (μg/ml)	12.5	25	6.3

Abbreviations: SEC=secnidazole; MTZ=metronidazole; TDZ=tinidazole

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