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Pharmacodynamics of Metronidazole Determined by a Time-Kill Assay for *Trichomonas vaginalis*

DAVID E. NIX,[1]* ROBERT TYRRELL,[1] AND MIKLÓS MÜLLER[2]

Clinical Pharmacokinetics Laboratory, Millard Fillmore Health System, Buffalo, New York,[1] *and The Rockefeller University, New York, New York*[2]

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The pharmacodynamic effects of metronidazole on *Trichomonas vaginalis* have been poorly characterized. The present in vitro study was performed to characterize the relationship between killing of trichomonads and metronidazole exposure (metronidazole concentration and time of exposure). Five laboratory strains and five recent clinical isolates of *T. vaginalis* were studied. The minimum lethal concentrations (MLCs) of metronidazole for the strains ranged from 0.8 to 25 μg/ml under anaerobic conditions. Metronidazole exhibited concentration-dependent killing against *T. vaginalis* at concentrations ranging from 0.1 to >10 times the MLC. The endpoint measurement, the kill rate constant, which was derived from the reduction in the logarithm of the colony count divided by exposure time, compared with the kill rate constant for the growth control was not affected by the time of assessment between 2 and 24 h. The kill rate constant-versus-metronidazole exposure curves were similar when concentration was expressed as a multiple of the MLC. There were no apparent differences between the clinical isolates and laboratory strains. These data suggest that peak metronidazole concentration and/or area under the plasma concentration-versus-time curve are the important pharmacodynamic parameters to be optimized.

Metronidazole is the treatment of choice for genitourinary infections caused by *Trichomonas vaginalis*, but it is also used in the treatment of amebiasis, giardiasis, and anaerobic bacterial infections (7). Overall, a large number of different dosing regimens have been used in clinical trials resulting in cure rates of between 55 and 100% (4). Dosing regimens vary from the 1- to 2-g single-dose regimens to the regimens that use 600 to 1,000 mg as divided doses daily for 3 to 10 days. Yet, all regimens appear to have similar efficacies. Metronidazole appears to be equally effective against *T. vaginalis* when it is administered orally as a single 2-g dose or as a 7-day regimen of 250 mg three times daily (5). The single oral dose provides advantages in terms of compliance; however, the 7-day regimens minimize the occurrences of reinfection while sexual contacts are being treated (6, 11). A 2-g total dose given once is equal in efficacy to 5.25 g (250 mg three times a day for 7 days). However, a 1-g single dose is less effective for the treatment of *Trichomonas* vaginitis. This suggests that either a high peak concentration or area under the plasma concentration-time curve is the important pharmacodynamic property for optimizing the activity of metronidazole. The study described here was performed to investigate the relationship between killing of *T. vaginalis* and the concentration of metronidazole.

No official methods have been adopted by the National Committee for Clinical Laboratory Standards for testing the susceptibility of *T. vaginalis*; however, an assay introduced by Meingassner et al. (8) for *Tritrichomonas foetus* that was subsequently applied to *T. vaginalis* (9, 10) appears to differentiate clinically resistant strains from those that readily respond to treatment. Although the resultant minimum lethal concentration (MLC) is useful for categorizing organisms by their relative susceptibilities, this parameter is not useful for characterizing the pharmacodynamics of metronidazole. Optimization of antimicrobial therapy requires consideration of the relationship between concentration and killing and potential postantibiotic effects. The in vitro antitrichomonal activity of metronidazole has been studied extensively, but with the endpoint used the effect was usually determined at only one time point.

In one recent study (3), kill curves were established for *T. vaginalis* at concentrations ranging from 0.025 to 5 μg/ml by using an initial inoculum of 500 trichomonads per ml. The influence of drug carryover was not addressed in that study, and the minimum quantifiable colony count was not stated. Assuming a minimal detectable colony count of 10 trichomonads per ml, only a 1.5-log decrease in the colony count could have been assessed. Complete killing of all isolates studied was observed at low concentrations of metronidazole (≤1.56 μg/ml) within 4 to 8 h of exposure. The possibility of drug carryover cannot be excluded since the culture was not diluted and the cells were not washed prior to plating. It is possible that the rapid and complete killing represented an artifact because metronidazole remained present at concentrations sufficient to inhibit growth.

We studied the concentration-dependent killing of *T. vaginalis* by metronidazole. Two major methodological differences from the previous study were used, including a higher initial inoculum (approximately 10^4 trichomonads per ml) and the avoidance of metronidazole carryover in assessing colony counts.

MATERIALS AND METHODS

Medium. Diamond's modified TYM medium described by Müller et al. (10) was used for all studies. This medium consisted of 900 ml of distilled-deionized water, 20 g of tryptose (Difco, Ann Arbor, Mich.), 10 g of yeast extract (Difco), 5.0 g of D-maltose monohydrate (Aldrich, Milwaukee, Wis.), 1.0 g of L-cysteine hydrochloride (Sigma, St. Louis, Mo.), 0.20 g of L-ascorbic acid (Gibco, Grand Island, N.Y.), 0.80 g of potassium dihydrogen phosphate (Aldrich), 0.80 g of dipotassium hydrogen phosphate (Aldrich), and 1.6 ml of concentrated hydrochloric acid (final pH at 42°C, approximately 6.0). This solution was stirred and heated to a slow boil. Liquid and semisolid forms of the medium were prepared. For the liquid medium, 0.5 g of agar (Sigma) was added to 900 ml of medium, and for the semisolid medium, 3.6 g of agar was added to 900 ml of medium. Both types of medium were dispensed while hot into glass culture tubes, and the

* Corresponding author. Mailing address: The Clinical Pharmacokinetics Laboratory, Millard Fillmore Hospital, 3 Gates Circle, Buffalo, NY 14209. Phone: (716) 887-4688. Fax: (716) 887-4566.

tubes were then autoclaved at 121°C for 15 min. The liquid medium was stored at room temperature but was brought to 37°C prior to use. The semisolid medium was kept at 42°C. Both media were used within 3 days. Immediately prior to use, heat-inactivated horse serum (Sigma) containing 400 μg of gentamicin per ml was prewarmed, and 0.5 ml was added to all tubes.

Organisms. Ten *T. vaginalis* isolates were obtained from three sources. Five strains were obtained from the American Type Culture Collection (ATCC) including ATCC 30001 (C-1:NIH), ATCC 50143 (CDC-0H5), ATCC 50144 (CDC-337), ATCC 50145 (CDC-409), and ATCC 50148 (NYH-286). These strains were used as reference control strains. Four clinical isolates (PH001, PH002, PH004, and PH006) were obtained from females at the Erie County Public Health Department in New York State between March and April 1994. An additional isolate (CPL001) was obtained from the urine of a male visiting the outpatient clinic at the Veterans Administration Hospital in Buffalo, N.Y. Vaginal samples were collected by swab and were transported by using an anaerobic swab transport device. The swabs were inoculated into liquid medium within 8 h of collection. The following antibiotics (final concentrations) were added to reduce the contamination by other microorganisms in the medium; penicillin (1,000 U/ml), gentamicin (40 μg/ml), and amphotericin B (5 μg/ml). All strains were passaged serially for at least 3 days in prewarmed (37°C) medium and were incubated at 37°C in closed glass culture tubes. The clinical isolates were passaged between four and six times prior to susceptibility testing or kill curve studies. Passage was accomplished by removing a 100-μl sample from the tube containing the growing trichomonads and adding this inoculum to 9 ml of fresh medium.

Inocula of *T. vaginalis* were prepared by culturing the isolates for 24 to 48 h at 37°C. The colony count plating method was validated with an inoculum of 10^5 cells per ml prepared by using a hemocytometer to determine cell density. This inoculum was diluted 1:1,000, and then plate counts were performed. The mean colony count was determined to be 6.7×10^4 colonies per ml (coefficient of variation, 22%). This indicates that the colony counts determined by plate counting were lower than the cell counts determined by using a hemocytometer, presumably because of the presence of nonviable or clumped organisms. Consequently, hemocytometer counts of 20% greater than the desired colony counts were prepared. All dilutions were made with prewarmed medium.

Metronidazole solutions. Stock solutions of metronidazole (lot B90045; Searle, Caguas, Puerto Rico) in water were prepared as solutions of 200 and 5,000 μg/ml and were subjected to no more than one freeze-thaw cycle. The 200-μg/ml solution was used in the susceptibility assay, while both solutions were used in the time-kill curve assay. Working solutions were diluted with the liquid medium.

Metronidazole susceptibility assay. The microdilution methods introduced by Meingassner and Thurner (9) and subsequently used by Müller et al. (10) were used with minor modifications. Cultures of 24 to 48 h that had not entered the stationary phase ($<10^7$ organisms per ml) were used for susceptibility tests. The cell density of the cultures was determined by using a hemocytometer and, if necessary, was adjusted with prewarmed (37°C) medium to a concentration of approximately 670,000 cells per ml and was then diluted 10-fold. To the first column of 96-well plates containing eight rows of 12 columns, 50 μl of the metronidazole (200 μg/ml) working solution was added. Fifty microliters of medium was then added to this column and to all other wells. By mixing and taking 50 μl from the first column and working sequentially, 1:2 serial dilutions were made in wells of columns 2 to 11. To each well, 150 μl of a cell suspension containing approximately 10^4 cells was added with a pipeter with 1-ml pipet tips. The final concentration of metronidazole in the first column was therefore 25 μg/ml. The plates were covered with sterile plastic covers and were incubated at 37°C in a GasPak (BBL, Cockeysville, Md.) chamber. The anaerobic atmosphere was established with the use of a commercial generator (CO_2, H_2, N_2; BBL) and was monitored with a methylene blue indicator strip. After 48 h, samples from each well were placed on a slide, covered with a cover slip, and examined with the high-power (×40) objective of a conventional light microscope. The plates were kept at 37°C during the examination. For wells in which cell movement could be detected, between 10 and 50 cells were typically examined. The usual time for complete examination of a plate was approximately 2 to 3 h. Each well was studied to determine the approximate proportion of cells showing motility, defined as any movement of the flagella or undulating membrane. Scoring was as follows: 0, no motile cells; 1, <10% of cells were motile; 2, 10 to 50% of cells were motile; 3, 51 to 100% of cells were motile; and 4, increase in the number of cells.

The endpoint of the assay was the minimal concentration of drug at which no motile cells were observed. This value was designated the approximate MLC, because a lack of cell motility has been correlated with the death of the organism (10). All microscopic examinations were performed by a single investigator.

Time-kill assay. Twenty-four- to 48-h cultures that had not entered the stationary phase were used in time-kill experiments. The cell densities of these cultures were determined by using a hemocytometer, and the concentration of trichomonads was adjusted, if necessary, to 10^6 cells per ml with prewarmed (37°C) liquid medium. One hundred microliters of this standardized cell suspension was added to tubes containing 9.9 ml of prewarmed liquid medium and 80 μl of water or metronidazole working solution. The final concentration of the inoculum was approximately 10^4 trichomonads per ml; however, plate counting was used to estimate the actual inoculum. For each isolate a series of seven glass culture tubes containing the following concentrations of metronidazole was used: 0, 0.16, 0.4, 1.6, 8.0, 16.0, and 40.0 μg/ml. For one isolate (ATCC 50143), the concentrations used were 10-fold higher in each of the seven tubes. The tubes with tightened screw-top caps were gently inverted three times following inoculation or immediately before sample withdrawal. They were incubated at 37°C during the experiment. Sample volumes of 100 or 1,000 μl were withdrawn from the growth control tube and the metronidazole-containing tubes at intervals of 2, 4, 6, 24, and 48 h. The sample withdrawn was added to 10 ml of prewarmed Hanks' balanced salt solution in polypropylene tubes and was immediately centrifuged at $1{,}000 \times g$ for 10 min in a heated (37°C) centrifuge. After centrifugation, while keeping the tubes at 37°C, the supernatant was removed until 1 ml remained in each of the plastic tubes. Nine milliliters of prewarmed Hanks' balanced salt solution was again added to the tubes, which were inverted once and centrifuged as described earlier. After this second centrifugation, the supernatant was removed until 0.5 ml remained in each tube. Depending on the possible number of trichomonads in the sample, the 0.5-ml volume was placed directly or after dilution with Hanks' balanced salt solution into sterile petri dishes (100 by 15 mm). We attempted to keep the number of trichomonads to between 10 and 200 per plate. Semisolid medium kept at 42°C was poured into the petri dishes containing the samples and was mixed by gentle agitation, and the agar was allowed to solidify for about 5 min at room temperature. The plates were placed upright into the GasPak (BBL) chamber. After anaerobic incubation for 4 to 5 days, the numbers of colonies on each plate were counted and recorded. Thus, for each isolate, there were seven plates for each of the five time periods plus at least one plate to confirm the initial inoculum.

Data analysis. Kill curve data were summarized by using the average first-order kill rate constant for each time interval relative to the kill rate constant for the control growth. The control growth rate constant was determined as the natural logarithm of the change in the density of organisms (colonies per milliliter at time t compared with that at time zero (initial inoculum) by the following equation: growth rate = $[\ln CC(t) - \ln CC(0)]/(t - 0)$, where $CC(t)$ and $CC(0)$ are the colony counts per milliliter at time t and time zero, respectively, and t is the time of incubation. The same equation was used for cases in which metronidazole was added. The growth rate constant is positive when colony counts increased compared with the baseline counts and negative when the colony counts decreased. The apparent kill rate constant is determined as the difference in the growth rate of the control and the growth rate of the metronidazole-supplemented cultures, as follows: kill rate constant (in $hours^{-1}$) = growth rate constant (control) − growth rate constant (test). The data were presented as the actual colony counts per milliliter versus time of exposure to metronidazole and as the average kill rate constant assessed at 2, 4, 6, and 24 h at each metronidazole concentration.

A separate analysis was performed by using an inhibitory E_{max} model for colony counts taken at the 24-h time point. The data were expressed as the colony count at each test concentration divided by the control (no metronidazole) colony count at 24 h versus the metronidazole concentration. Weighted least-squares regression [weight $1/(f \cdot 0.1 + 0.25)^2$] was performed with the program PCNONLIN, version 3.0 (Statistical Consultants Inc., Lexington, Ky.), and the model $1 - (100 - C^h)/(EC_{50} + C^h)$, where C is the concentration of metronidazole, h is Hill's constant for slope, and EC_{50}^h is the concentration of metronidazole required to cause a 50% reduction in the colony count relative to the colony count of the control.

RESULTS

The MLCs of metronidazole for the 10 strains of *T. vaginalis* ranged from 0.4 to 25 μg/ml under anaerobic conditions. The MLCs for the five control strains were 0.4 μg/ml for ATCC 30001, 1.6 μg/ml for ATCC 50145, 3.1 μg/ml for ATCC 50144 and ATCC 50148, and 25 μg/ml for ATCC 50143. Duplicate MLC testing was performed for four of the ATCC strains with the following results; 0.8 μg/ml for ATCC 30001, 3.1 μg/ml for ATCC 50144, 1.6 μg/ml for ATCC 50148, and 25 μg/ml for ATCC 50143. Two repeat MLCs were therefore identical; one was a single dilution above and one was a single dilution below the initial MLCs. These MLCs are similar to those reported previously (10). For the five clinical isolates, the MLCs were 0.8 μg/ml for isolate PH001, 1.6 μg/ml for isolates PH002 and PH006, 3.1 μg/ml for isolate PH004, and 6.3 μg/ml for isolate CPL001. In previous studies, MLC testing has been described with incubation for 48 h in both aerobic and anaerobic environments (9, 10). Under aerobic incubation by a microdilution procedure, the number of viable organisms was reduced at 48 h in the control experiment without any added metronidazole. Since growth was not sustained in the aerobic environment in

TABLE 1. Viable colony counts of *T. vaginalis* control strains at times of 0 and 24 h following exposure to metronidazole at various concentrations[a]

Metronidazole concn (μg/ml)[b]	Colony counts of the following isolates at the indicated times (h):									
	ATCC 50143		ATCC 50144		ATCC 50145		ATCC 50148		ATCC 30001	
	0	24	0	24	0	24	0	24	0	24
0	9,800	281,000	12,000	194,000	10,200	240,000	17,900	291,000	10,400	340,000
0.16	9,800	21,000	12,000	9,400	10,200	110,000	17,900	142,000	10,400	14,700
0.40	9,800	6,300	12,000	2,600	10,200	9,300	17,900	38,900	10,400	840
1.6	9,800	320	12,000	1,410	10,200	110	17,900	330	10,400	11
8.0	9,800	<10	12,000	<10	10,200	<10	17,900	<10	10,400	<10
16	9,800	<10	12,000	<10	10,200	<10	17,900	<10	10,400	<10
40	9,800	<10	12,000	<10	10,200	<10	17,900	<10	10,400	<10

[a] The concentrations of metronidazole which caused a 50% decrease in the colony count associated with the control at 24 h were 0.291, 0.008, 0.152, 0.170, and 0.060 μg/ml for isolates ATCC 50143, ATCC 50144, ATCC 50145, ATCC 50148, and ATCC 30001, respectively. Hill's constants (the slope parameter for the inhibitory E_{max} curve) were 1.47, 1.04, 3.32, 2.3, and 3.17 for ATCC 50143, ATCC 50144, ATCC 50145, ATCC 50148, and ATCC 30001, respectively.

[b] Concentrations for isolate number ATCC 50143 were 10-fold greater than stated because of the higher MLC for this organism.

the present study, MLC testing was performed only under relative anaerobic conditions.

Metronidazole exhibited a concentration-dependent killing of *T. vaginalis*. Changes in the log colony counts between 2 and 48 h compared with the baseline counts were assessed. Tables 1 and 2 provide the initial and 24-h colony counts for the 10 isolates at various concentrations of metronidazole. At concentrations of ≥8 μg/ml, all organisms were killed to levels of ≤10 colonies per ml following 24 h of exposure. Typical kill curves for isolates ATCC 50148 and PH001 are given in Fig. 1A and B, respectively. Figure 1A and B highlights the dependence of killing on time and metronidazole concentration.

Kill rate constants could not be determined at the later endpoints or for the higher metronidazole concentrations because killing was complete (≤10 colonies per ml). Consequently, 6-h kill rate constants were used to show concentration dependence in Fig. 2A. The curve for each strain, characterized by a different kill rate constant-versus-concentration curve, exhibited a similar shape but varied in the position on the concentration axis. Curves were shifted to the right for strains for which MLCs were higher, suggesting that concentration/MLC is a more relevant measure of drug exposure. Figure 2B shows the relationship between killing rate constants and concentration/MLC.

Killing or growth inhibition was first apparent at concentrations of approximately 1/10 of the MLC. Moreover, there was no evidence of a plateau of the killing rate for concentrations between 1 and 10 times the MLC. Kill rate constant-versus-concentration relationships were similar for the fresh clinical isolates compared with those for the control strains obtained from ATCC when concentration was expressed as a multiple of the MLCs. Figure 3A and B shows the kill rate as a function of metronidazole concentration divided by the MLC when the values were assessed at 2, 4, 6, 24 and 48 h for isolates ATCC 50148 and PH001, respectively. The kill rate constant, when it was assessed at the lower concentrations of metronidazole (<8 μg/ml) or within 6 h of exposure, was independent of the time of assessment, as shown in Fig. 3A and B.

DISCUSSION

The present study demonstrated a clear concentration-dependent kill rate for *T. vaginalis* exposed to metronidazole under anaerobic conditions. Maximal kill rates were achieved at concentrations of at least 10 to 25 times the MLC of metronidazole. Since the kill rate never reached a plateau, higher concentrations may provide kill rates even faster than those observed in the present study. Our results differed from those of a previous study that demonstrated very rapid killing at metronidazole concentrations of ≤1.56 μg/ml (3). The more pronounced kill rates estimated in the previous study could have resulted from the low initial inoculum, the limited range for detecting viable colony counts (0 to 1.5 log), or the presence of metronidazole carryover in the plating procedure. None of these three issues was addressed in the previous study (3).

TABLE 2. Viable colony counts of *T. vaginalis* clinical strains at times 0 and 24 h following exposure to metronidazole at various concentrations[a]

Metronidazole concn (μg/ml)	Colony counts of the following isolates at the indicated times:									
	PH001		PH002		PH004		PH006		CPL001	
	0	24	0	24	0	24	0	24	0	24
0	12,100	121,000	14,200	97,000	10,100	450,000	16,000	121,000	9,900	310,000
0.16	12,100	11,600	14,200	52,000	10,100	94,000	16,000	95,000	9,900	159,000
0.40	12,100	1,150	14,200	9,300	10,100	16,100	16,000	12,000	9,900	10,100
1.6	12,100	14	14,200	140	10,100	173	16,000	101	9,900	180
8.0	12,100	<10	14,200	14	10,100	<10	16,000	<10	9,900	<10
16	12,100	<10	14,200	<10	10,100	<10	16,000	<10	9,900	<10
40	12,100	<10	14,200	<10	10,100	<10	16,000	<10	9,900	<10

[a] The concentrations of metronidazole which caused a 50% decrease in the colony count associated with the control at 24 h were 0.195, 0.171, 0.087, 0.224, and 0.125 μg/ml for PH001, PH002, PH004, PH006, and CPL001, respectively. Hill's constants (the slope parameter for the inhibitory E_{max} curve) were 6.48, 2.66, 2.18, 3.81, and 2.00 for PH001, PH002, PH004, PH006, and CPL001, respectively.

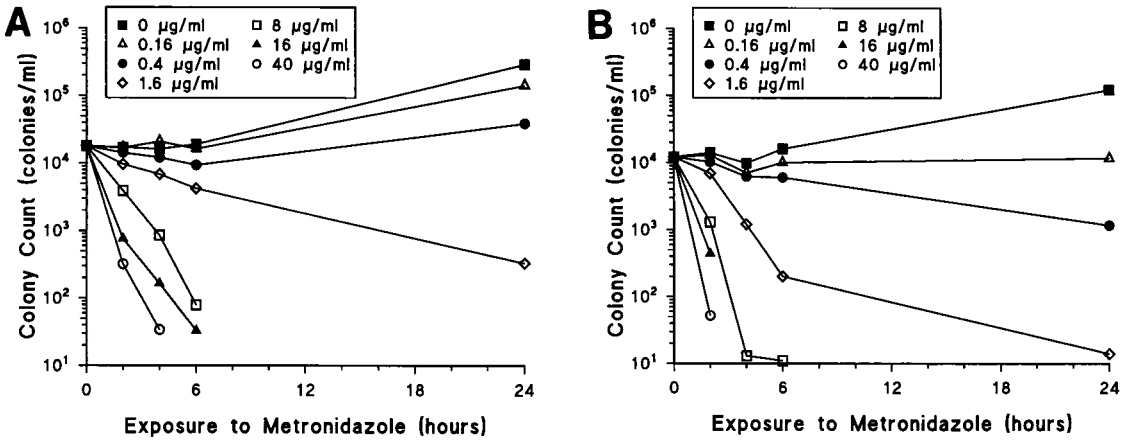

FIG. 1. (A) Colony counts of isolate ATCC 50448 following exposure to various concentrations of metronidazole for 0, 2, 4, 6, and 24 h in an anaerobic atmosphere at 37°C. (B) Colony counts of isolate PH001 following exposure to various concentrations of metronidazole for 0, 2, 4, 6, and 24 hours in an anaerobic atmosphere at 37°C.

In our preliminary studies, we found several variables which adversely affected the MLC determination and kill curve study results. Growth of trichomonads was optimized when the growth medium was adjusted to pH 6.0. In contrast, growth was much slower when the medium was adjusted to pH 7.0. Growth was also optimized when anaerobic conditions were maintained; thus, we determined MLCs and incubated colony count plates under anaerobic conditions. For kill studies, a 10-ml volume of liquid medium in tightly capped tubes was used, which provided growth indistinguishable from growth under anaerobic incubation conditions. One-milliliter volumes of medium, however, did not support growth unless incubation was performed under anaerobic conditions. Thus, the organism was present in an anaerobic or oxygen-reduced environment when tubes containing 10 ml of medium were incubated in an aerobic atmosphere. Despite all attempts to minimize medium changes or to avoid trauma to the organisms, there was a lag period before logarithmic growth occurred in the control cultures. Slight reductions in colony counts were often observed in the control cultures at the 2- and 4-h time points.

Müller et al. (10) suggested that MLCs for resistant strains of *T. vaginalis* are ≥3.1 μg/ml, as determined under anaerobic conditions. By using this criterion, two of the control strains and one of the clinical strains exhibited borderline resistance. In addition, one of the control strains was highly resistant (MLC, 25 μg/ml). In our studies killing rates for these isolates were similar to those for the susceptible strains as long as the same concentration/MLC ratio was used.

The administration of metronidazole at doses of 250 mg three times a day provides mean maximal concentrations in plasma of approximately 8.7 μg/ml and trough concentrations of approximately 4.1 μg/ml. These values were predicted on the basis of pharmacokinetic parameters reported previously (2). Thus, concentrations in plasma should exceed the MLCs for most strains of *T. vaginalis* for most of the dosing interval. On the basis of the concentration-dependent killing observed in the present study, the achievement of higher peak plasma concentration-to-MLC ratios may be desirable. However, relationships between concentrations in plasma and concentrations at the site of effect have not been well characterized for *Trichomonas* vaginitis. *T. vaginalis* has a doubling time of between 4 and 6 h in vitro under optimal growth conditions. The remaining questions revolve around the length of a dosing interval and whether any clinical consequences result if the concentration in plasma is allowed to fall below the MLC for some portion of the dosing interval. In a preliminary study, we found a postantibiotic effect with *T. vaginalis* of approximately 6 h after exposure to 4 μg of metronidazole per ml (one to five

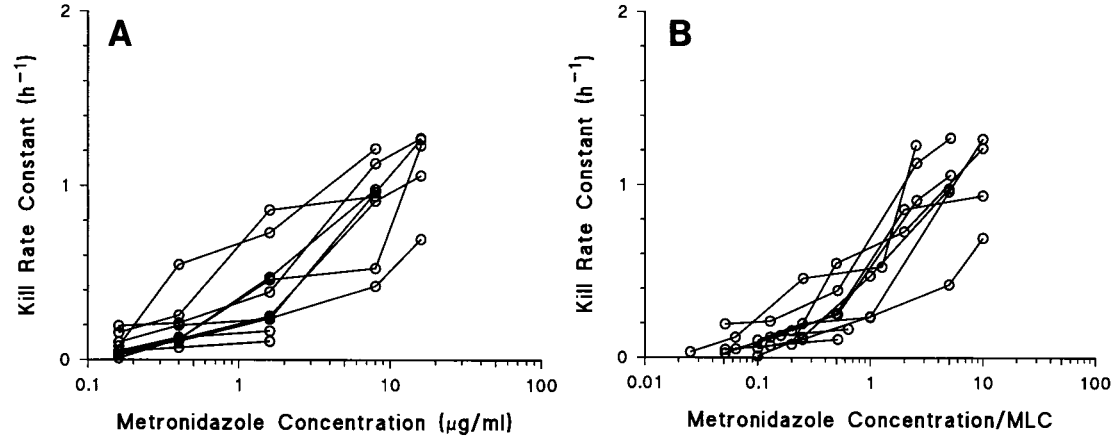

FIG. 2. (A) Kill rate constant versus metronidazole concentration for all 10 isolates after 6 h of exposure. (B) Kill rate constant versus metronidazole concentration/MLC for all 10 isolates after 6 h of exposure.

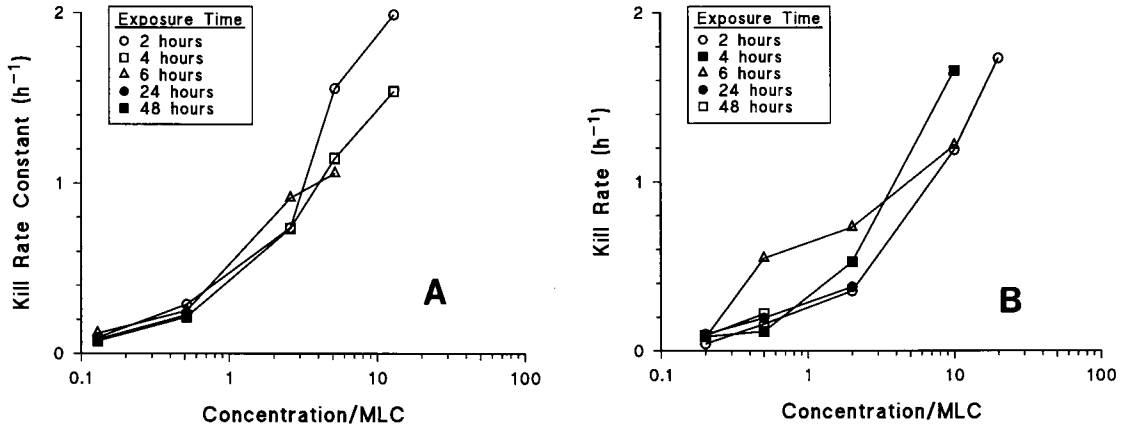

FIG. 3. (A) Kill rate constant of isolate ATCC 50148 as a function of metronidazole concentration divided by MLC determined at the endpoint times 2, 4, 6, 24, and 48 h. (B) Kill rate constant of isolate PH001 as a function of metronidazole concentration divided by MLC determined at the endpoint times 2, 4, 6, 24, and 48 h.

times the MIC) for 4 h. This finding in combination with the relative slow growth of the organism should permit a longer dosage interval than that which is currently recommended. However, further study to confirm the postantibiotic effect is required.

Following administration of a single 2-g oral dose, the mean maximum concentration in serum was 40.6 μg/ml and the apparent half-life was 8.8 h. After 24 h, the mean metronidazole concentrations remained greater than 5 μg/ml (1). Thus, a typical *T. vaginalis* isolate would be exposed to concentrations of metronidazole greater than the MLC for 24 to 48 h. Although treatment with a single 2-g oral dose of metronidazole is often recommended for the treatment of *Trichomonas* vaginitis, gastrointestinal tolerance of this regimen is poor in many patients, and this may be the reason why a 7-day regimen of 250 mg three times daily is more often prescribed. On the basis of our studies, it would seem logical to divide this dosage into two or even one daily dose, as opposed to three, in order to take advantage of the pronounced concentration-dependent killing. Fewer daily doses also improves compliance. Consequently, development of a new metronidazole regimen which maintains treatment for 7 days and allows once- or twice-daily dosing is highly desirable and quite consistent with the in vitro killing behavior of this anti-infective agent.

Metronidazole exhibits a strong concentration-dependent killing of *T. vaginalis* up to concentrations greater than 10 to 25 μg/ml. This supports the use of individual doses greater than 250 mg. Clearly, a 2,000-mg single dose is effective for treatment, because it allows complete killing in one dosage and a smaller total dosage than the 7-day regimen (250 mg three times daily or 5.25 g total) for similar efficacy. The single 2,000-mg dose, however, is undesirable because of poor gastrointestinal tolerance. The optimal dose regimen must be chosen to maximize peak concentrations and/or area under the concentration-time curve relative to the MLC for *T. vaginalis* and to minimize gastrointestinal intolerance. The present study was not designed to separate whether the peak concentration/MLC ratio or area under the concentration-time curve/MLC ratio is more important. However, these data taken with the clinical data suggest that more convenient regimens, twice daily or once daily, with doses of between 250 and 2,000 mg may be useful.

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