

Efficacy of tinidazole for treatment of cats experimentally infected with *Tritrichomonas foetus*

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Objective—To determine the efficacy of tinidazole for treatment of cats with experimentally induced *Tritrichomonas foetus* infection.

Animals—8 specific-pathogen-free kittens.

Procedures—Tinidazole was tested for activity against a feline isolate of *T foetus* in vitro. Kittens were infected orogastrically with the same isolate and treated or not with tinidazole (30 mg/kg, PO, q 24 h for 14 days). Amoxicillin was administered 28 weeks after completion of tinidazole administration to induce diarrhea. Feces were repeatedly tested for *T foetus* by use of PCR assay and microbial culture for 33 weeks.

Results—Tinidazole killed *T foetus* at concentrations $\geq 10 \mu\text{g/mL}$ in vitro. In experimentally induced infection, tinidazole administered at 30 mg/kg decreased *T foetus* below the limit of molecular detection in 2 of 4 cats. Recrudescence of *T foetus*, as elicited by amoxicillin-induced diarrhea, was diminished in cats that received prior treatment with tinidazole.

Conclusions and Clinical Relevance—Although tinidazole decreased the detection of *T foetus* and treated cats were resistant to later efforts to incite the infection, inability of tinidazole to eradicate infection in many cats poses a serious impediment to the drug's effectiveness in practice. (*Am J Vet Res* 2007;68:1085–1088)

Tritrichomonas foetus is a flagellated protozoan parasite of domestic cats that resides in the lumen of the colon and causes colitis and chronic, foul-smelling diarrhea.^{1,2} The infection is prevalent among cattery cats,³ in which transmission via the fecal-oral route is suspected. Infected cats may have persistent diarrhea for up to 2 years and can remain infected for life.⁴ The susceptibility of trichomonads to 5-nitroimidazole antiprotozoan drugs is caused by reductive pathways that convert these drugs into autotoxic free-radical metabolites.^{5,6} Treatment of feline *T foetus* infection with metronidazole, a 5-nitroimidazole drug, has not been successful. Cats treated with metronidazole have transient improvement in diarrhea but remain infected with the organism.^{4,7} A related nitroimidazole, ronidazole, is effective in eradicating infection from experimentally infected cats on the basis of PCR assay results.⁸ Unfortunately, ronidazole is not approved by the FDA or commercially available in the United States. The pharmacokinetics of ronidazole are not known for cats, and in our experience, cats may be at more risk for ronidazole neurotoxicosis than metronidazole neurotoxicosis.

Tinidazole is a second-generation 5-nitroimidazole that is FDA approved for use in treating metronidazole-resistant *Trichomonas vaginalis*, *Giardia* spp, and *Entamoeba histolytica* infections in humans.⁹ Susceptibility of feline *T foetus* to tinidazole has been detected in vitro,⁸ and pharmacokinetic studies in cats reveal a long elimination half-life that would be consistent with once-per-day administration.¹⁰ The purpose of the study reported here was to determine the ability of tinidazole to eradicate *T foetus* on the basis of PCR assay results when given orally once per day to cats with experimentally induced infection.

Materials and Methods

Tritrichomonas foetus—*Tritrichomonas foetus* was isolated from feces of a cat with diarrhea and established in antimicrobial-free culture medium as described.¹ The isolate was identified as *T foetus* on the basis of single-tube nested PCR assay of rRNA genes by use of species-specific primers. The isolate was used for both in vitro susceptibility testing and experimental infections.

In vitro susceptibility assay—*Tritrichomonas foetus* in log-phase culture was inoculated at a concentration of 10^3 organisms/mL into 10-mL culture tubes containing antimicrobial-free culture medium^a into which tinidazole^b was directly dissolved at concentrations of 0, 0.1, 1, 10, and 100 $\mu\text{g/mL}$ (3 replicates each) and incubated at 37°C (primary cultures). After 24, 48, and 72 hours of incubation, organisms were suspended by light vortexing and a 0.1-mL aliquot of the suspended culture was removed for purposes of cell counting.

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The aliquot was diluted and fixed in 0.9 mL of 10% formaldehyde because live organisms with rapid motility are difficult to accurately count. Cell counts were performed with a hemocytometer and reported as the mean \pm SD of each triplicate dilution. At 72 hours, 1 primary culture tube at each dilution was centrifuged and 0.1 mL of pelleted organisms was subcultured into 10 mL of antimicrobial-free medium. After 24 hours, a 10- μ L aliquot of each subculture was examined via light microscopy for motile trichomonads. Susceptibility of *T foetus* to a given drug concentration was defined by the absence of replication in primary culture over a 72-hour period and failure of the organisms to replicate after transfer to antimicrobial-free medium.

Experimentally induced infection—Specific-pathogen-free 7-week-old sexually intact female (n = 4) and male (4) domestic shorthair cats were purchased from a commercial vendor^c and fed dry food^d ad libitum throughout the study. Cage liners were scraped clean, and a new litter box was provided daily. Cats were transferred to newly disinfected cages every 1 to 2 weeks. Cats were housed under conditions of controlled lighting and temperature and were maintained in compliance with biosafety level 2 guidelines. The North Carolina State University Institutional Animal Care and Use Committee approved this protocol.

During an initial 7-week acclimatization period, each cat was determined to be healthy on the basis of weekly physical examination and results of a CBC. Fecal examination for each cat included antigen testing for *Giardia* spp^e and single-tube nested PCR assay for detection of the rRNA gene unit of *T foetus*¹¹ (performed on each of 2 fecal samples/cat).

Inoculation with *T foetus*—Each cat was sedated with ketamine (11 mg/kg, IV) and midazolam (0.15 mg/kg, IV) and received 3 mL of media containing approximately 2×10^6 live *T foetus* via orogastric tube.

Tinidazole—Treatment with tinidazole was initiated 4 weeks after induced infection. At that time, cats were paired on the basis of body weight into 2 groups containing equal numbers of males and females. Groups were housed at opposite ends of the same room and in individual cages. Tinidazole tablets^f (250 mg) were quartered, and group A cats received 62.5 mg (1/4 tablet) orally once per day for 14 days. Actual doses ranged from 26 to 30 mg/kg. Group B cats were not treated with tinidazole or placebo. Administration of tinidazole and identity of which group received treatment were restricted to 1 investigator (JLG). All diagnostic samples were coded by personnel not involved in analyses or interpretation.

Detection of *T foetus* infection—For the purpose of PCR assay or culture, feces were collected from the colon of each cat by use of a rectal loop weekly during the 3-week period between induction of infection and initiation of treatment, twice during treatment, and on 28 occasions during a 33-week period following completion of treatment. During this 33-week period, for each cat, 21 fecal samples were tested via PCR assay and 10 fecal samples were tested via culture for *T foetus*. On week 28 after treatment, all cats in group

A and group B were subjected to induction of antimicrobial-induced diarrhea by administration of 250 mg of amoxicillin orally twice per day for 2 days. Actual doses ranged from 54 to 88 mg/kg. Feces were collected 24 and 72 hours later for microscopic examination of a fecal smear, microbial culture of feces in commercially available medium,^g and single-tube nested PCR assay for detection of the *T foetus* rRNA gene unit by use of species-specific primers.¹¹

PCR assay—The DNA was extracted from each fecal sample and subjected to PCR amplification of an 876-bp gene sequence of bacterial 16S rRNA and a 180-bp gene sequence of the partial *T foetus* rRNA gene unit, as described.⁸ By performing PCR assay for bacterial 16S rRNA gene, the possibility that a negative PCR assay result for *T foetus* could be attributed to the presence of endogenous PCR inhibitors in the extracted DNA was ruled out for each sample.

On week 29 after treatment of group A cats, the study was unmasked and group B cats received 125 mg of tinidazole orally once per day for 14 days. Actual doses ranged from 27 to 44 mg/kg. The PCR assay was performed weekly for 3 weeks following treatment. After completing treatment, feces were collected from the colon of group B and group A cats by use of a rectal loop weekly for an additional 3 weeks. All cats were again subject to induction of antimicrobial-induced diarrhea by administration of 250 mg of amoxicillin orally twice per day for 2 days. Feces were collected 24 and 72 hours later for microscopic examination of a fecal smear, microbial culture of feces in commercially available medium,^g and single-tube nested PCR assay for detection of the *T foetus* rRNA gene unit by use of species-specific primers.¹¹

Results

In vitro susceptibility—Tinidazole arrested growth of *T foetus* in vitro at concentrations ≥ 10 μ g/mL (Figure 1). Trichomonads did not replicate after transfer from tubes containing ≥ 1 μ g of tinidazole/mL to drug-free medium.

***Tritrichomonas foetus* infection**—No cat had positive results for *T foetus* or *Giardia* sp before experimentally induced infection. Feces from each cat repeatedly were positive for *T foetus* via PCR assay during the 3 weeks following infection. No cat developed diarrhea as a result of infection. In feces from each untreated cat (group B), positive PCR assay results for *T foetus* were observed intermittently throughout the study (mean \pm SD percentage positive results per cat, $43 \pm 2.1\%$; range, 27% to 60%). In 3 cats from group B, fecal cultures also intermittently were positive for *T foetus*. However, culture was less effective than PCR assay for detection of infection (mean \pm SD percentage positive fecal results per cat, $21 \pm 1\%$; range, 0% to 33%).

Effect of tinidazole—All 4 cats that received tinidazole (group A) had negative results for *T foetus* infection based on PCR assay results within 3 days of initiation of treatment. In 1 cat, an amplification product consistent with *T foetus* was observed after PCR assay of DNA extracted from the feces 6 and 8 weeks after treat-

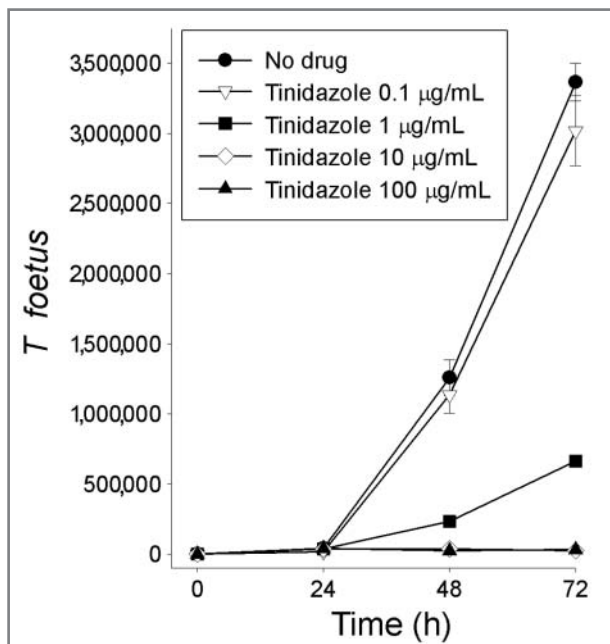


Figure 1—Mean \pm SD concentrations (No. of organisms/mL [3 replicates/datum point]) of a feline *Trichomonas foetus* isolate in culture medium at various times after inoculation. Organisms were cultured with various concentrations of tinidazole.

ment, but not thereafter. The PCR assay did not reveal *T foetus* in the feces of any of the remaining group A cats. Positive culture results for *T foetus* were not observed in any group A cats at any time.

Administration of amoxicillin to each cat in groups A and B resulted in fecal consistencies ranging from soft to liquid. When examined within 24 hours of completion of administration of amoxicillin, all cats in group B had numerous *T foetus* in wet-mount preparations of feces. In group A, *T foetus* was not observed via light microscopy or microbial culture of feces of any cat. However, in 1 cat with negative results for *T foetus* in all prior samples obtained after treatment, PCR assay results were positive. The DNA amplified from the feces of the cat was confirmed to have 100% sequence identity to *T foetus*.

When group B cats were subsequently treated with tinidazole, fecal samples were negative for *T foetus* via PCR assay for the next 3 weeks, at which time amoxicillin treatment of all cats was repeated. Administration of amoxicillin resulted in fecal consistencies ranging from soft to cow pat. When examined 24 hours later, *T foetus* was not observed in the feces of any cats from group A or B via light microscopy, culture, or PCR assay. Adverse drug effects such as lethargy, inappetence, or signs of neurotoxicity were not observed in any cats in the study at any time.

Discussion

In this study, tinidazole suppressed *T foetus* infection below the limit of molecular detection in only 2 of 4 cats during a 33-week period after completion of treatment. Failure to eradicate *T foetus* was indicated by PCR assay results of 1 and 2 fecal samples from each cat, respectively. False-positive test results were con-

sidered unlikely because all control PCR reactions and DNA extractions were negative for *T foetus* throughout the study and because the identity of the reaction products was confirmed via DNA sequencing.

Results of PCR assay or culture identified *T foetus* in feces from all untreated (group B) cats, but only intermittently. Lower numbers of *T foetus* in feces of group A cats could potentially have resulted in false-negative PCR assay and culture results. Because trichomonads depend on host bacterial flora for acquisition of essential nutrients¹² and orally administered antimicrobials cause cats with subclinical infection to shed increased numbers of *T foetus*,^{4,7} all cats were subjected to amoxicillin-induced diarrhea. Amoxicillin-induced diarrhea resulted in the appearance of copious numbers of *T foetus* in the feces of each cat in group B. Notably, in cats in which treatment with tinidazole was completed 28 weeks earlier (group A), *T foetus* was not detected after amoxicillin-induced diarrhea developed, although PCR assay results were positive in 1 cat. When group B cats were subsequently treated with tinidazole and resubjected to amoxicillin-induced diarrhea, *T foetus* could not be detected by any means. These observations suggest that tinidazole suppressed the numbers of *T foetus* below the level of molecular detection in most of the fecal samples and impaired shedding after the intestinal insult. Whether this effect diminishes the potential for contagion cannot be answered by this study. Failure to clear *T foetus* infection from 2 of 4 cats in this study indicated that tinidazole is unlikely to be clinically useful in eradicating the infection.

It is not clear why cats in the present study did not develop diarrhea in response to *T foetus* infection, as has been reported in prior experimental infections.^{8,13} Strain differences in pathogenicity have been reported for *T vaginalis*¹⁴ and may also be characteristic of *T foetus*. However, the isolate used in the present study was low passage and obtained from a cat with diarrhea. Whether the absence of diarrhea contributed positively or negatively to the efficacy of tinidazole in these cats was unknown. Importantly, prolonged periods of subclinical infection are common in *T foetus*-infected cats⁴ and failure to eradicate the organisms from cats without diarrhea would be a serious impediment to the drugs' effectiveness in practice.

The reason for failure of tinidazole to eliminate *T foetus* from at least 2 cats reported in this study was unclear. The minimum inhibitory concentration of tinidazole against the *T foetus* isolate used for experimental infection (10 µg/mL) was 2 log-fold higher than that of the *T foetus* isolate of an earlier report (0.1 µg/mL).⁸ Plasma tinidazole concentration in cats 24 hours after a single dose of 30 mg/kg, as was used in the present study, is approximately 8 µg/mL.¹⁰ Tinidazole is completely absorbed after oral administration in cats,¹⁰ and in humans, tinidazole penetrates well into various tissues where drug concentrations are similar to those in plasma. Thus, it is possible that tinidazole did not achieve sufficient concentration in the colon of cats reported here to be entirely effective against this strain of *T foetus*. In contrast to metronidazole, for which drug withdrawal is often associated with heavy shedding of *T foetus*,⁷ administration of tinidazole resulted in nega-

tive test results for *T foetus* for a prolonged period, and treated cats were resistant to later efforts to cause shedding of the organism. These observations were consistent with prior in vitro observations that *T foetus* are resistant to direct effects of metronidazole, but susceptible to tinidazole.⁸

In humans, tinidazole has greater tissue distribution, fewer and milder adverse effects, and more efficacy at lower doses than metronidazole. In humans infected with *E histolytica*, delivery of tinidazole can be targeted to the colon by controlled-release formulations that release the active drug in the colon, rather than the small intestine. Controlled release can be accomplished by pH-sensitive coatings or coating tablets with carriers such as guar gum that are degraded exclusively by colonic bacteria.^{15,16} Whether this approach could be used to enhance colonic concentration and activity of tinidazole in cats with *T foetus* infection is worthy of further study, as is the potential efficacy of tinidazole for treatment of *Giardia* spp infections.

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- a. Modified Diamond medium, Remel, Lenexa, Kan.
 - b. Tinidazole [1-(2(ethylsulfonyl)ethyl)-2-methyl-5 nitroimidazole] T3021, Sigma Chemical Co, St Louis, Mo.
 - c. Liberty Research Inc, Waverly, NY.
 - d. Hills' Science Diet Feline Growth, Hills' Nutrition Inc, Topeka, Kan.
 - e. IDEXX *Giardia* antigen test kit, IDEXX Laboratories, Westbrook, Me.
 - f. Tindamax, Mission Pharmacal, San Antonio, Tex.
 - g. In Pouch TF, Biomed Diagnostics Inc, White City, Ore.
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