

## Standard Article

J Vet Intern Med 2017;31:1163–1169

## Pharmacokinetics and Relative Bioavailability of Orally Administered Innovator-Formulated Itraconazole Capsules and Solution in Healthy Dogs

A.E. Hasbach, D.K. Langlois , E.J. Rosser Jr, and M.G. Papich 

**Background:** Itraconazole is commonly used for treatment of systemic and cutaneous mycoses in veterinary medicine. Two formulations, capsule and solution, are used interchangeably in dogs. However, marked differences in bioavailability have been reported in other species. Similar investigations have not been performed in dogs.

**Objective:** To determine and compare pharmacokinetics of itraconazole in dogs after oral administration of commercially available capsule and solution formulations intended for use in humans.

**Animals:** Eight healthy, adult, purpose-bred dogs.

**Methods:** Dogs received approximately 10 mg/kg of innovator-formulated itraconazole solution and capsule PO in randomized, crossover design with a 10-day washout period. To ensure maximal absorption, solution was administered to fasted dogs, whereas capsules were co-administered with food. Blood samples were collected at predetermined time points, and plasma drug concentrations were measured using high-pressure liquid chromatography. Pharmacokinetic parameters were determined with compartmental analysis.

**Results:** The mean relative bioavailability of the capsule was 85% that of the solution, but drug absorption was variable, and overall drug concentrations were similar between formulations. Mean elimination half-lives of both formulations were nearly identical at approximately 33 hours. Regardless of formulation, simulations suggest that a loading dose of 20 mg/kg, followed by 10 mg/kg once every 24 hours, will result in plasma concentrations considered to be adequate in most dogs.

**Conclusions and Clinical Importance:** Contrary to findings reported in other species, overall drug exposures after capsule and solution administration are not substantially different in dogs. Despite some pharmacokinetic differences between itraconazole capsule and solution, formulation-specific dosages do not appear to be necessary.

**Key words:** Blastomycosis; Histoplasmosis; Mycology; Sporanox<sup>®</sup>.

Successful treatment of many fungal infections requires systemic antifungal therapy. Azole antifungals, which block ergosterol biosynthesis by inhibition of cytochrome P-450, are routinely used in veterinary medicine for the treatment of fungal infections.<sup>1,2</sup> Itraconazole (ITZ) is a triazole antifungal drug that can effectively treat infections caused by many species of yeasts, molds, dermatophytes, and dimorphic fungi.<sup>2–5</sup> It possesses greater activity than fluconazole, is less

From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, MI (Hasbach, Langlois, Rosser); Molecular Biomedical Sciences Department, College of Veterinary Medicine, North Carolina State University, Raleigh, NC (Papich).

Work: The study was conducted at the Michigan State University College of Veterinary Medicine. Sample analysis was performed at the North Carolina State University Veterinary Clinical Pharmacology Laboratory.

Prior presentation: An oral abstract was presented April 27, 2017 at the 2017 North American Veterinary Dermatology Forum in Orlando, FL.

Corresponding author: Dr. Daniel K. Langlois, Department Small Animal Clinical Sciences, Michigan State University College of Veterinary Medicine, 736 Wilson Road, East Lansing, MI 48824; e-mail: langlo21@cvm.msu.edu

Submitted January 14, 2017; Revised April 17, 2017; Accepted May 9, 2017.

Copyright © 2017 The Authors. Journal of Veterinary Internal Medicine published by Wiley Periodicals, Inc. on behalf of the American College of Veterinary Internal Medicine.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

DOI: 10.1111/jvim.14779

## Abbreviations:

AUC	area under the plasma drug concentration vs. time curve
$C_{max}$	maximum plasma drug concentration
CV%	coefficient of variation
HPLC	high-pressure liquid chromatography
ITZ	itraconazole
$k_{01}$ $T_{1/2}$	oral absorption rate constant and accompanying half-life
QC	quality control
Relative $F$	relative bioavailability
SD	standard deviation
$T_{1/2}$	elimination half-life
$T_{lag}$	lag-time
$T_{max}$	time to maximum plasma drug concentration

expensive than newer triazoles, and unlike its predecessor ketoconazole, has minimal effects on mammalian enzymes.<sup>4–7</sup> For these reasons among others, ITZ frequently is used by veterinary clinicians for treatment of fungal infections.

Currently, 2 oral formulations of ITZ are commercially available for people, 100 mg capsules and 10 mg/mL solution. These formulations are used extralabel and often administered interchangeably in veterinary medicine. A Food and Drug Administration (FDA) approved ITZ solution in a hydroxypropyl- $\beta$ -cyclodextrin vehicle also is available for dermatophyte treatment in cats,<sup>a</sup> but this formulation has not been tested in dogs. The commercially available capsules are prepared using solid dispersion of ITZ in a hydroxypropyl methylcellulose matrix (ITZ layered on glucose spheres)

to facilitate absorption,<sup>8</sup> and compounded preparations lacking this formulation are poorly absorbed.<sup>9</sup> Despite the microencapsulated sugar sphere technology, absorption of the ITZ capsule is associated with more variability as compared to the solution in humans.<sup>10</sup> Oral absorption is dependent on the granules inside the capsules dissolving in the stomach and intestine. This process can be altered by the pH of the stomach, stomach emptying time, and the presence of a meal, particularly the fat content of a meal. Oral absorption is facilitated by gastric acid production, optimized by co-administration with food, and impaired by co-administration of antacids.<sup>5,11,12</sup> Itraconazole solution, on the other hand, is better absorbed with less variability because the drug is already in solution and does not depend on other factors such as food or acid production for dissolution.<sup>13–15</sup> In studies conducted in humans, overall drug exposures are markedly higher after administration of solution as compared to capsule, and this difference can approach 60%.<sup>10,13</sup> Consequently, FDA guidelines explicitly state that the 2 formulations should not be used interchangeably.<sup>16</sup> Increased bioavailability of the oral solution also has been reported in cats. One report documented ITZ solution bioavailability to be 78%, whereas capsule bioavailability was speculated to be as low as 14%.<sup>17</sup> Another report described the oral absorption of ITZ oral solution in cats to be 52%, but the vehicle used in the oral formulation was not described.<sup>18</sup> Furthermore, a more recent study in cats has established that drug exposures after solution administration are 420% higher than after capsule administration, and maximum serum drug concentrations are 3.4 times higher.<sup>b</sup> Given these observations in other species, a similar difference could exist in dogs. The consequences for failing to recognize a potential difference could be substantial and include unnecessary cost, increased risk of toxicity, inadequate tissue drug concentrations, and treatment failures.

Although the pharmacokinetics of orally administered ITZ capsules has been reported in dogs, little is known about the pharmacokinetics of ITZ solution, and no direct comparisons have been made between these 2 formulations in dogs.<sup>8,9</sup> The purpose of our study was to establish and compare the pharmacokinetic parameters of orally administered innovator-formulated ITZ capsules and solution in dogs in a crossover study. We hypothesized that drug exposures and maximum plasma drug concentrations would be higher after solution administration.

## Material and Methods

### Sample Size Calculation

A study of a continuous response variable (area under the plasma concentration versus time curve [AUC]) from matched pairs of study subjects receiving capsule in 1 study phase and solution in another phase was planned. The study was designed to detect an approximate 30% difference in drug exposure between formulations. This potential difference is less than that observed in cats and humans, but a difference that we deemed to be clinically relevant. Prior data on the pharmacokinetics of ITZ solution in

dogs are unavailable, but pharmacokinetic data after ITZ capsule administration were used to aid in sample size determination. If the true difference in the mean response (AUC) of matched pairs is 30%, 8 dogs would be required to reject the null hypothesis that this response difference is 0 with probability (power) of 0.8. The Type I error probability associated with this test of the null hypothesis is 0.05.

### Animals

Eight purpose-bred dogs, 4 intact males and 4 intact females, were used in the study. Breeds included Beagle and Beagle-mixes. The median age and weight of the dogs were 1.25 years (range, 1–3 years) and 11.3 kg (range, 8.0–19.2 kg), respectively. All dogs were considered healthy on the basis of normal physical examination findings and the absence of clinical abnormalities. The study was approved by the Institutional Animal Care and Use Committee at Michigan State University.

### Study Design

Ours was a single-dose, randomized, crossover study with 2 phases. Dogs were randomly assigned to either the 10 mg/kg capsule<sup>c</sup> or 10 mg/kg solution<sup>d</sup> group. Study phases were separated by 10 days to allow sufficient time for drug elimination and blood volume recovery.<sup>19</sup> After an overnight fast a 16 gauge IV catheter was placed in each dog. Itraconazole capsules were administered concurrently with a small meal<sup>e</sup> comprising 40–50% of the dog's resting energy requirements<sup>f</sup>, whereas the oral solution was administered after the dogs were fasted. This approach was used to maximize absorption of both formulations.<sup>11,14</sup> Dogs were reintroduced to their regular feeding schedule 8 hours after ITZ administration. Seven of 8 dogs received 100 mg ITZ, whereas the 1 larger dog (body weight, 19.2 kg) received 200 mg. Overall, the median dosage was 9.5 mg/kg (range, 8.0–12.5 mg/kg). The dogs were monitored for any adverse events from the time of drug administration through the end of sample collection.

### Sample Collection and Processing

Venous blood samples (4 mL) were collected before and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 48 hours after ITZ administration. Upon collection, all samples were immediately transferred into plastic EDTA collection tubes, placed on ice, and centrifuged within 2 minutes at 4°C for 10 minutes × 1,200 g. Plasma was transferred into plastic cryovials for storage at –80°C. Samples were shipped frozen on dry ice to the North Carolina State University Clinical Pharmacology Laboratory for ITZ concentration determinations. All analyzes were performed within 2 months of sample collection.

### Itraconazole Analysis

Quantitative determination of ITZ in canine plasma samples was performed by high-performance liquid chromatography (HPLC) as previously described and validated.<sup>9,20</sup> Briefly, all experimental plasma samples, quality control (QC) samples, calibration samples, and blank (control) plasma samples were prepared identically. The analytical reference standard of ITZ was obtained as a pure substance.<sup>g</sup> Itraconazole was dissolved in HPLC-grade acetonitrile to make a 1 mg/mL stock solution. From this stock solution, further dilutions were made to use as fortifying solutions for plasma to generate calibration curves and QC standards in canine plasma. The stock solution was kept at 4°C in a tightly sealed, dark vial, and was determined to be

stable throughout the duration of the study. Itraconazole spiking solutions were added to blank canine (control) plasma, to prepare 9 calibration standards (range, 0.01–10 µg/mL). Blank plasma samples also were analyzed with each day's run to check for interfering peaks and estimate background noise. All calibration curves were linear with an  $R^2$  value  $\geq 0.99$ . Limit of quantification (LOQ) for ITZ in canine plasma was 0.01 µg/mL, which was determined from the lowest point on a linear calibration curve that produced an acceptable signal-to-noise ratio with a range of 0.01–20 µg/mL. Quality control samples were analyzed each day and compared against the calibration curve. The laboratory used guidelines published by the US Pharmacopeial Convention.<sup>21</sup>

### Pharmacokinetics Analysis

A computer program was used to determine the pharmacokinetics in each animal.<sup>h</sup> Plasma drug concentrations were plotted on linear and semilogarithmic graphs for visual inspection and initial selection of appropriate models for pharmacokinetic analysis. Plasma drug concentrations, weighted by a factor of  $1/(\text{predicted } Y)^2$ , were used for pharmacokinetic analysis, where  $Y$  is the plasma concentration. The specific model (e.g. 1, 2, etc. compartments) was determined for best fit on the basis of a smaller value for the Akaike's Information Criterion (AIC).<sup>22</sup> The model that was best fit to the data was a 2-compartment model with first-order absorption and elimination.

For both oral doses, pharmacokinetic parameters were calculated using the following formula:

$$C(T) = A \times e^{-\alpha T} + B \times e^{-\beta T} + C \times e^{-k_{01} T}$$

Where  $C$  is the plasma concentration,  $T$  is time,  $k_{01}$  is the non-IV absorption rate, assuming first-order absorption,  $\alpha$  is the distribution rate,  $\beta$  is the elimination rate,  $A$  is the distribution  $y$ -axis intercept,  $B$  is the elimination rate  $y$ -axis intercept, and  $C$  is the intercept for the oral absorption rate. In this model, it is assumed that the absorption rate constant is greater than the elimination rate constant ( $k_a \gg k$ ), or that there is no "flip-flop" effect caused by slow absorption from the gastrointestinal tract. A lag-time ( $T_{\text{lag}}$ ) was added to the model to account for dissolution of oral formulation capsule and stomach emptying time. Secondary parameters from the model included the peak concentration ( $C_{\text{max}}$ ), time to peak concentration ( $T_{\text{max}}$ ), area under the plasma concentration versus time curve (AUC), and the respective absorption and terminal half-lives ( $T_{1/2}$ ). In addition, pharmacokinetic parameters generated here were used to simulate plasma drug concentration profiles to investigate dosing strategies necessary for achieving target plasma drug concentrations.<sup>23,24</sup>

### Statistical Analysis

Pharmacokinetic parameters are reported as mean  $\pm$  standard deviation (SD). Data were assessed for normality with Kolmogorov-Smirnov testing. Although most pharmacokinetic parameters were normally distributed,  $k_{01}$  and  $T_{\text{lag}}$  were not. Normally distributed response variables (pharmacokinetic parameters) were evaluated by means of split plot analysis of variance with the grouping factor of sex and the repeat factors of period and formulation. A Wilcoxon signed rank test was used to evaluate  $k_{01}$  and  $T_{\text{lag}}$  given the distribution of data. Statistical analysis was performed using commercially available software.<sup>i</sup> For all analyzes,  $P \leq 0.05$  was considered significant.

## Results

The oral ITZ solution and capsules both were administered successfully to all 8 dogs, and no dogs vomited or experienced adverse effects during the sampling period. Sex and study period had no effect on pharmacokinetic parameters. Pharmacokinetics for each formulation is summarized in Table 1, and plasma drug concentration versus time curves are displayed in Figure 1. As demonstrated by plasma drug concentration versus time curves (Fig 1), a rapid rate of oral absorption was observed for both formulations, and although absorption ( $k_{01} T_{1/2}$ ) appeared slower graphically for the capsule, differences were not significant ( $P = 0.13$ ). A substantial lag-time ( $T_{\text{lag}}$ ) also was observed for the oral capsule ( $1.6 \pm 1.3$  hours) compared to the oral solution ( $0.54 \pm 0.24$  hours;  $P = 0.023$ ). After oral absorption, peak drug concentrations occurred at approximately 2.9 hours for the oral capsule and 1.6 hours for the oral solution ( $P = 0.034$ ). After this peak, in both study groups, an initial steep phase in drug decrease (considered the distribution phase in this model,  $\alpha$ ) was observed, followed by a longer elimination phase ( $\beta$ ). The 2 components of the decrease in plasma drug concentration are apparent in the drug concentration versus time curve plotted on a semilogarithmic axis (Fig 1). Although differences were observed in the absorption rate constant and oral lag time, once absorbed, drug elimination was nearly identical for both formulations with an elimination half-life ( $T_{1/2}$ ) of  $33.2 \pm 11.7$  hours for the oral capsule and  $32.9 \pm 7.6$  hours for the oral solution. The relative bioavailability (Relative  $F = \text{AUC}_{\text{CAPSULE}}/\text{AUC}_{\text{SOLUTION}}$ ) was  $0.85 \pm 0.26$ , indicating that, on average, the oral capsule was absorbed 15% less than the oral solution. However, it is important to note that although 5 of 7 dogs had higher drug exposures after solution administration as compared to capsule administration, results were variable ( $P = 0.16$ ). Plasma drug concentration profiles were simulated for repeated once daily dosing of both orally administered ITZ solution and capsule (Fig. 2). Simulations were based on the mean pharmacokinetic values produced from the dogs in our study.

Other pharmacokinetic parameters were calculated such as clearance and volume of distribution. These parameters are not relevant without knowing the absolute oral absorption, which cannot be calculated without an accompanying IV dose; therefore, they are not reported here. One dog in our study was considered an extreme outlier for the oral capsule and was not included in the modeling, statistical comparisons, or the mean values presented in Table 1 and Figure 1. This dog had a  $T_{1/2}$  of 317 hours and an AUC of 101 h\*µg/mL, which were well outside the range of other dogs in the group.

## Discussion

The pharmacokinetic parameters established here are similar to those of a previous report of ITZ capsule pharmacokinetics in healthy dogs.<sup>9</sup> Although some

**Table 1.** Pharmacokinetic values in dogs after oral administration of itraconazole capsules and solution at an approximate dosage of 10 mg/kg.

Parameter	Units	Oral Capsule (n = 7) <sup>b</sup>			Oral Solution (n = 8)		
		Mean	SD	CV%	Mean	SD	CV%
<i>A</i>	µg/mL	8.90	11.23	126.25	7.98	11.08	138.92
$\alpha$	1/h	0.65	0.37	56.55	0.57	0.19	34.05
$\alpha T_{1/2}$	h	1.29	0.53	40.82	1.41	0.72	50.92
AUC	h*µg/mL	22.95	9.49	41.36	28.03	11.24	40.11
<i>B</i>	µg/mL	0.42	0.13	29.98	0.54	0.21	39.53
$\beta$	1/h	0.02	0.01	37.73	0.02	0.01	25.46
$\beta T_{1/2}$	h	33.24	11.69	35.16	32.85	7.59	23.09
<i>C</i> <sub>max</sub>	µg/mL	1.26	0.43	34.20	1.54	0.53	34.41
<i>k</i> <sub>01</sub>	1/h	1.76 <sup>a</sup>	1.07	60.82	2.57 <sup>a</sup>	1.67	65.20
<i>k</i> <sub>01</sub> <i>T</i> <sub>1/2</sub>	h	0.57	0.41	70.75	0.41	0.28	69.14
<i>k</i> <sub>10</sub>	1/h	0.10	0.02	23.66	0.09	0.04	46.89
<i>k</i> <sub>12</sub>	1/h	0.43	0.30	69.16	0.36	0.15	40.61
<i>k</i> <sub>21</sub>	1/h	0.15	0.07	50.41	0.14	0.05	33.17
<i>T</i> <sub>max</sub>	h	2.89 <sup>a</sup>	1.20	41.55	1.61 <sup>a</sup>	0.59	36.98
<i>T</i> <sub>lag</sub>	hr	1.60 <sup>a</sup>	1.29	80.55	0.54 <sup>a</sup>	0.24	44.31
Relative <i>F</i>	Cap/Sol	0.85	0.26	30.74	–	–	–

Values represent the mean, SD, and CV% after administration of itraconazole capsules (n = 7) and solution (n = 8) to healthy dogs. SD, standard deviation; CV, coefficient of variation; *A*, intercept for the distribution phase;  $\alpha$ , rate constant for distribution phase and accompanying half-life (*T*<sub>1/2</sub>); *B*, intercept for the elimination phase;  $\beta$ , rate constant for elimination phase and accompanying half-life (*T*<sub>1/2</sub>); AUC, area under the curve; *C*<sub>max</sub>, maximum (peak) plasma drug concentration; *k*<sub>01</sub>, oral absorption rate constant and accompanying half-life (*T*<sub>1/2</sub>); *k*<sub>10</sub>, *k*<sub>12</sub>, *k*<sub>21</sub>, microdistribution rate constants; *T*<sub>max</sub>, time to peak drug concentration; *T*<sub>lag</sub>, lag time; Relative *F*, the relative extent of oral absorption for oral capsule compared to oral solution (calculated from AUC<sub>CAPSULE</sub>/AUC<sub>SOLUTION</sub> ratio).

<sup>a</sup>*P* < 0.05.

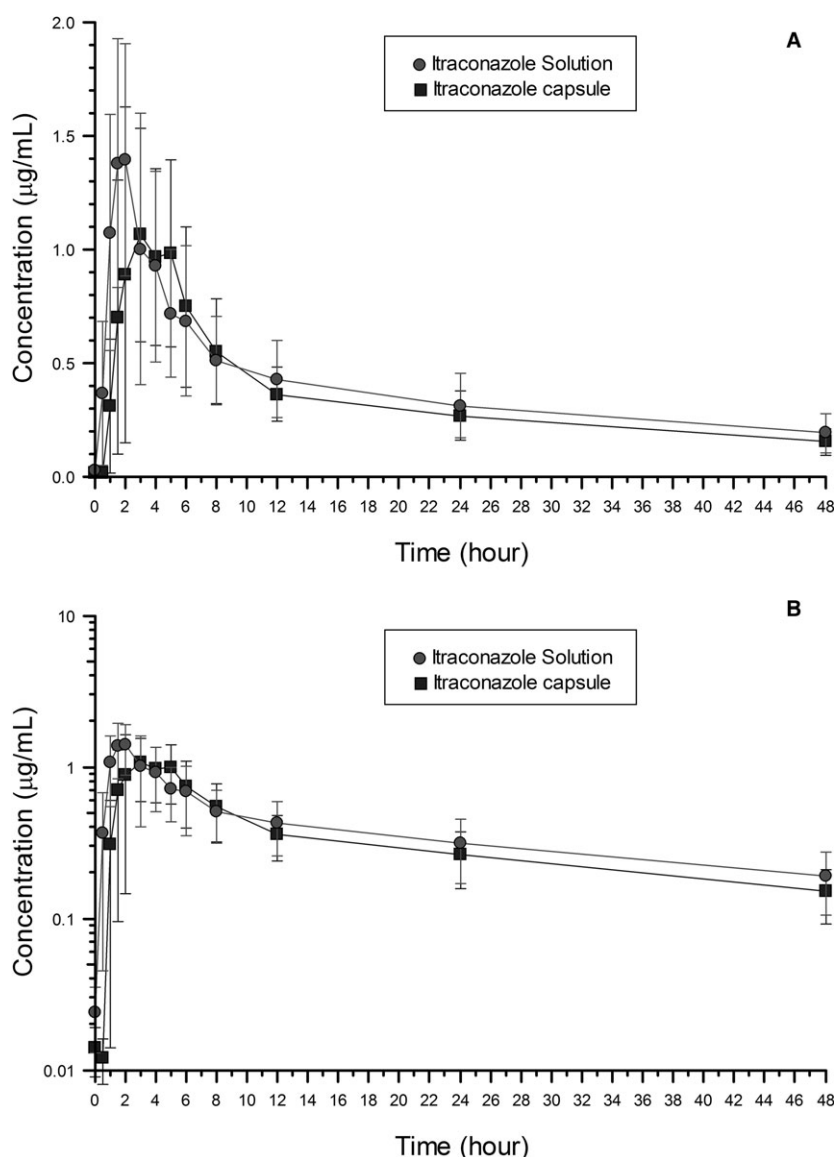
<sup>b</sup>One dog was an extreme outlier and was excluded from this table.

differences in pharmacokinetics were detected between formulations, these results are in stark contrast to what has been reported in humans and cats in which maximum drug concentrations and overall drug exposures are markedly higher after ITZ solution administration as compared to ITZ capsule administration.<sup>10,17,b</sup> In these other species, failure to modify dosage based on formulation could lead to unwanted consequences ranging from unnecessary client cost to treatment failures, and FDA dosing guidelines for humans clearly state that the formulations should not be used interchangeably.<sup>16</sup> Our results suggest that similar dosage modifications are not necessary for dogs and underscore the importance of utilizing species specific pharmacokinetic data when making treatment recommendations. It is possible that differences in gastric acidity or gastrointestinal motility could contribute to these differences between species, but actual mechanisms remain undetermined until additional investigations occur.<sup>25</sup>

In our study, relative bioavailability of the capsule to solution was 85 ± 26%, and maximum drug concentrations after solution administration were 22% higher than after capsule administration. These findings suggest that ITZ solution may be absorbed to a higher extent than ITZ capsules, but the differences in AUC and *C*<sub>max</sub> between formulations were not significant. We believe this finding is likely due to a type 2 statistical error, because our study was not designed to detect such small differences in drug absorption. A delay in oral absorption was observed for the capsule compared to the solution, shown by the longer lag time (*T*<sub>lag</sub>),

greater oral absorption rate constant (*k*<sub>01</sub>), and longer absorption *T*<sub>1/2</sub>. These differences and potential differences in AUC and *C*<sub>max</sub> can be explained by the higher solubility of the oral solution. Itraconazole is a weak base and is classified as “practically insoluble” by the United States Pharmacopeia.<sup>21</sup> Because of its insolubility, ITZ is classified by the Biopharmaceutical Classification System (BCS)<sup>26</sup> as Class 2, which means that oral absorption is highly dependent on the ability of the drug to dissolve in the gastrointestinal tract. For the capsule formulation, gastric acidity is required for dissolution and absorption (pKa 3.7).<sup>5,12</sup> Because of high variability in the canine stomach pH, as well as stomach emptying profile, there is inherent high variability in dissolution and oral absorption of the oral capsules. The proprietary oral solution is solubilized by hydroxypropyl- $\beta$ -cyclodextrin (400 mg/mL) as a molecular inclusion complex to maintain solubility.<sup>27</sup> Cyclodextrin is an oligosaccharide in the form of a cylindrical structure, which is hydrophilic on the outside and hydrophobic on the inside, and therefore, the ITZ molecule is placed in the hydrophobic tunnel. As a result, absorption does not require acidity, resulting in increased bioavailability of the solution in comparison to the capsule formulation.<sup>5,13,15</sup>

Plasma concentrations of ITZ necessary for therapeutic success in dogs are undetermined. Therefore, veterinarians have extrapolated concentrations based on drug concentration data obtained in humans<sup>23,24</sup> in which trough serum ITZ concentrations of at least 0.5–1.0 µg/mL, as measured by HPLC, have been associated with

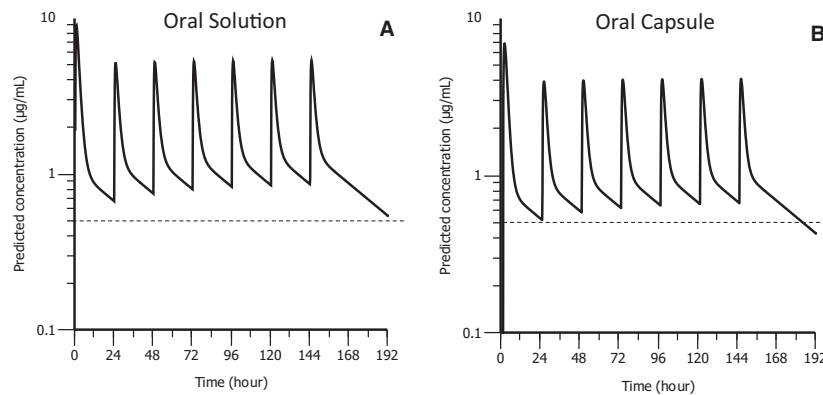


**Fig 1.** Plasma drug concentration profiles for dogs after administration of 10 mg/kg itraconazole solution and capsule. Results are given as mean ( $\pm$ SD) from 7 (capsule) and 8 (solution) dogs. Panel (A) contains a linear axis, whereas panel (B) contains a semilogarithmic axis.

therapeutic success for various systemic fungal infections.<sup>23,24</sup> Based on our results that showed a long terminal  $T_{1/2}$  of approximately 33 hours, we simulated plasma drug concentrations to demonstrate that with repeated dosing to steady-state, accumulation occurs and a once daily dose is reasonable in clinical patients. This proposed dosing strategy is demonstrated with simulations in Figure 2. These simulations were derived from the average pharmacokinetic values generated from the research dogs in our study, which do not necessarily represent an “average dog” in a population. Nonetheless, they suggest that with an initial loading dose of 20 mg/kg administered to dogs for the first dose, followed by 10 mg/kg once every 24 hours, plasma drug concentrations may achieve the target of 0.5 µg/mL identified in studies conducted in humans.<sup>23,24</sup> However, ideal ITZ dosages could be

influenced by many factors such as organ system involvement, individual differences in drug absorption, and variations in minimum inhibitory concentrations among fungal species. For example, the minimum inhibitory concentration for many *Blastomyces dermatitidis* isolates is much lower than 0.5 µg/mL.<sup>28</sup> As such, ITZ dosages of 5 mg/kg can be used to treat systemic blastomycosis,<sup>29,30</sup> whereas dosages of 10 mg/kg may increase the risk of adverse drug effects without improving cure rates.<sup>30</sup> Because our study was not intended to determine efficacy, or predict efficacy from the doses studied, clinical studies are encouraged to determine the optimal dose and plasma drug concentrations needed for routine treatment of fungal infections in veterinary patients.

One limitation of our investigation was the small number of dogs used in the study, which may not



**Fig 2.** Simulated plasma drug concentration profiles for orally administered itraconazole solution (A) and capsule (B) in dogs using the mean pharmacokinetic values generated from this study. The simulated dosages were an initial loading dose of 20 mg/kg as the first dose, followed by 10 mg/kg once every 24 hours. The dashed line at 0.5 µg/mL represents desired trough concentration associated with efficacy in humans.

represent a clinical population of dogs with fungal disease. Sample size was further limited because 1 dog's pharmacokinetic parameters after capsule administration were deemed to be outliers, and were not included in analyses. Without further study, we cannot determine the cause of this discrepancy from the other animals. Regardless, our study was powered to detect an approximate 30–40% difference in drug exposures between formulations, a difference that is less than what has been reported in cats but similar to what has been reported in some studies of humans.<sup>10,13,b</sup> Also, this difference is what the authors deemed to be clinically relevant and a difference that could impact treatment recommendations. Given the intrinsic properties of ITZ, it would be reasonable to assume that some differences in drug absorption and maximum drug concentrations exist.<sup>5</sup> Based on our data, approximately 24–26 dogs would be required in a similarly designed crossover study to detect 15 and 22% differences in AUC and  $C_{max}$ , respectively. Likewise, a bioequivalence study meeting FDA guidelines for comparing oral formulations would require a similar number of dogs. However, as mentioned above, the clinical consequence of a potential 15% difference in drug exposure is likely marginal. The simulations (Fig 2) further support this speculation as both formulations are able to maintain adequate plasma drug concentrations with identical dosing strategies.

It is worth mentioning that the ITZ solution and capsular formulation were administered under different conditions, fasted and fed, respectively. The oral capsule should be administered with food to stimulate drug dissolution, whereas the oral solution does not require a meal because the product is already in a dissolved form.<sup>5,11,13,14,31</sup> In fact, co-administering the solution with food has been shown to decrease drug absorption and overall drug exposures.<sup>16,31</sup> Although formulation differences can affect stomach emptying time in dogs, and subsequently influence parameters such as  $k_{01}$ ,  $T_{max}$ , and  $T_{lag}$ , it is also possible that the different administration protocols resulted in variability in gastric

emptying time and small intestinal transit time and thus contributed to some of these differences.<sup>32,33</sup> However, our protocol was designed to optimize gastrointestinal absorption of each formulation and allow comparisons of the presumed maximal drug concentrations and exposures achievable with either formulation. Also, this methodology mimics veterinary clinical drug administration practices and is in accordance with FDA drug administration guidelines for humans.<sup>16</sup>

In conclusion, ITZ concentrations and exposures after capsule and solution administration are similar in dogs under conditions tested in our study, which is contrary to what has been reported in others species. As such, formulation-specific dosage modifications do not appear to be necessary. Based on simulations, plasma drug concentrations that reach extrapolated therapeutic targets<sup>27</sup> can be obtained with either formulation using a once daily dosing regimen of 10 mg/kg. Additional clinical studies are needed to determine optimal dosing strategies and effective drug concentrations for treating fungal infections in dogs, and the results reported here should provide a framework for such investigations.

## Funding

This work was supported by an American College of Veterinary Dermatology Research Award.

## Footnotes

<sup>a</sup> Itrafungol® (10 mg/mL), Elanco US Inc, Greenfield, IN

<sup>b</sup> Mawby D, Fowler L, Papich M, Whittemore J. Itraconazole absorption from proprietary and compounded formulations in healthy cats. *J Vet Intern Med* 2016;30:1494-1495

<sup>c</sup> Sporanox® capsules (100 mg), Janssen Pharmaceutica, N.V., Olen, Belgium

<sup>d</sup> Sporanox® oral solution (10 mg/mL), Janssen Pharmaceutica, N.V., Beerse, Belgium

<sup>e</sup> Purina Pro Plan® Savor® Classic Adult Chicken and Rice Entrée. Nestle Purina, Wilkes-Barre, PA

<sup>f</sup> Resting energy requirements in kcal/day = {30 × (ideal BW [kg])} + 70

<sup>g</sup> Itraconazole analytical reference standard, Research Diagnostics Inc, Flanders, NJ

<sup>h</sup> Phoenix<sup>®</sup> WinNonlin<sup>®</sup> software; Certara, St. Louis, MO

<sup>i</sup> SAS version 9.1; SAS Institute Inc, Cary, NC

## Acknowledgments

The authors acknowledge Janice Querubin for her technical assistance, and Delta R. Dise for her expertise in measuring itraconazole concentrations.

*Conflict of Interest Declaration:* Authors declare no conflict of interest.

*Off-label Antimicrobial Declaration:* Itraconazole is not approved for use in dogs in the United States.

## References

- VandenBossche H, Engelen M, Rochette F. Antifungal agents of use in animal health – chemical, biochemical and pharmacological aspects. *J Vet Pharmacol Therap* 2003;26:5–29.
- Foy DS, Trepanier LA. Antifungal treatment of small animal veterinary patients. *Vet Clin Small Anim* 2010;40:1171–1188.
- Brömel C, Sykes JE. Epidemiology, diagnosis, and treatment of blastomycosis in dogs and cats. *Clin Tech Small Anim Pract* 2005;20:233–239.
- Greene CE. Antifungal chemotherapy. In: Green CE, ed. *Infectious Disease of the Dog and Cat*, 4th ed. St. Louis: Elsevier Saunders; 2012:579–587.
- Lester J, Hope WW. Itraconazole: An update on pharmacology and clinical use for treatment of invasive and allergic fungal infections. *Expert Opin Drug Metab Toxicol* 2013;9:911–926.
- Boothe DM. Treatment of fungal infections. In: Booth DM, ed. *Small Animal Clinical Pharmacology and Therapeutics*, 2nd ed. Philadelphia, PA: W.B. Saunders Company; 2001:222–236.
- Willems L, van der Geest R, de Beule K. Itraconazole oral solution and intravenous formulations: A review of pharmacokinetics and pharmacodynamics. *J Clin Pharm Ther* 2001;26:159–169.
- De Smet L, Saerens L, De Beer T, et al. Formulation of itraconazole nanocrystals and evaluation of their bioavailability in dogs. *Eur J Pharm Biopharm* 2014;87:107–113.
- Mawby DI, Whittemore JC, Genger S, Papich MG. Bioequivalence of orally administered generic, compounded, and innovator-formulated itraconazole in healthy dogs. *J Vet Intern Med* 2014;28:72–77.
- De Beule K, Van Gestel J. Pharmacology of itraconazole. *Drugs* 2001;61(Suppl 1):27–37.
- Barone JA, Koh JG, Bierman RH, et al. Food interaction and steady-state pharmacokinetics of itraconazole capsules in healthy male volunteers. *Antimicrob Agents Chemother* 1993;37:778–784.
- Jaruratanasirikul S, Sriwiriyan S. Effect of omeprazole on the pharmacokinetics of itraconazole. *Eur J Clin Pharmacol* 1998;54:159–161.
- Barone JA, Moskovitz BL, Guarnieri J, et al. Enhanced bioavailability of itraconazole in hydroxypropyl-β-cyclodextrin solution versus capsules in healthy volunteers. *Antimicrob Agents Chemother* 1998;42:1862–1865.
- Van de Velde VJ, Van Peer AP, Heykants JJ, et al. Effect of food on the pharmacokinetics of a new hydroxypropyl-beta-cyclodextrin formulation of itraconazole. *Pharmacotherapy* 1996;16:424–428.
- Johnson MD, Hamilton CD, Drew RH, et al. A randomized comparative study to determine the effect of omeprazole on the peak serum concentrations of itraconazole oral solution. *J Antimicrob Chemother* 2003;51:453–457.
- U.S. Food and Drug Administration. Sporanox<sup>™</sup> (itraconazole) oral solution: Safety Labeling Changes Approved by FDA Center for Drug Evaluation and Research (CDER). 14 Nov 2015. [www.fda.gov/Safety/MedWatch/SafetyInformation/ucm403762.htm](http://www.fda.gov/Safety/MedWatch/SafetyInformation/ucm403762.htm).
- Boothe DM, Herring I, Calvin J, et al. Itraconazole disposition after single oral and intravenous and multiple oral dosing in healthy cats. *Am J Vet Res* 1997;58:872–877.
- Liang C, Shan Q, Zhong J, et al. Pharmacokinetics and bioavailability of itraconazole oral solution in cats. *J Feline Med Surg* 2016;18:310–314.
- Diehl KH, Hull R, Morton D, et al. A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 2001;21:15–23.
- Middleton SM, Kubier A, Dirikolu L, et al. Alternate-day dosing of itraconazole in healthy adult cats. *J Vet Pharmacol Therap* 2015;39:27–31.
- United States Pharmacopeia (USP-NF). USP 38-NF 33. General chapter 1225. Validation of Compendial Procedures. Rockville, Md: United States Pharmacopeial Convention, 2015.
- Yamaoka K, Nakagawa T, Uno T. Application of Akaike's information criterion (AIC) in the evaluation of linear pharmacokinetic equations. *J Pharmacokinetic Biopharm* 1978;6:165–175.
- Ashbee HR, Barnes RA, Johnson EM, et al. Therapeutic drug monitoring (TDM) of antifungal agents: Guidelines from the British Society for Medical Mycology. *J Antimicrob Chemother* 2014;69:1162–1176.
- Goodwin ML, Drew RH. Antifungal serum concentration monitoring: An update. *J Antimicrob Chemother* 2008;61:17–25.
- Beasley DE, Koltz AM, Lambert JE, et al. The evolution of stomach acidity and its relevance to the human microbiome. *PLoS ONE* 2015;10:e0134116.
- Papich MG, Martinez MN. Applying biopharmaceutical classification system (BCS) criteria to predict oral absorption of drugs in dogs: Challenges and pitfalls. *AAPS J* 2015;17:948–964.
- Sykes JE, Papich MG. Antifungal drugs. In: Sykes JE, ed. *Canine and Feline Infectious Diseases*. St. Louis: Elsevier Saunders; 2014:87–96.
- Sabatelli F, Patel R, Mann PA, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother* 2006;50:2009–2015.
- Arceneaux KA, Taboda J, Hosgood G. Blastomycosis in dogs: 115 cases (1980–1995). *J Am Vet Med Assoc* 1998;213:658–664.
- Legendre AM, Rohrbach BW, Toal RL, et al. Treatment of blastomycosis with itraconazole in 112 dogs. *J Vet Intern Med* 1996;10:365–371.
- Barone JA, Moskovitz BL, Guarnieri J, et al. Food interactions and steady-state pharmacokinetics of itraconazole oral solution in healthy volunteers. *Pharmacotherapy* 1998;18:295–301.
- Martinez MN, Papich MG. Factors influencing the gastric residence of dosage forms in dogs. *J Pharm Sci* 2009;98:844–860.
- Fleisher D, Li C, Zhou Y, et al. Drug, meal and formulation interactions influencing drug absorption after oral administration. *Clin Pharmacokinetic* 1999;36:233–254.