Pharmacokinetics and tissue distribution of itraconazole after oral and intravenous administration to horses

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Objective—To determine the pharmacokinetics of itraconazole after IV or oral administration of a solution or capsules to horses and to examine disposition of itraconazole in the interstitial fluid (ISF), aqueous humor, and polymorphonuclear leukocytes after oral administration of the solution.

Animals—6 healthy horses.

Procedure—Horses were administered itraconazole solution (5 mg/kg) by nasogastric tube, and samples of plasma, ISF, aqueous humor, and leukocytes were obtained. Horses were then administered itraconazole capsules (5 mg/kg), and plasma was obtained. Three horses were administered itraconazole (1.5 mg/kg, IV), and plasma samples were obtained. All samples were analyzed by use of high-performance liquid chromatography. Plasma protein binding was determined. Data were analyzed by compartmental and noncompartmental pharmacokinetic methods.

Results—Itraconazole reached higher mean ± SD plasma concentrations after administration of the solution (0.41 ± 0.13 µg/mL) versus the capsules (0.15 ± 0.12 µg/mL). Bioavailability after administration of capsules relative to solution was 33.83 ± 33.08%. Similar to other species, itraconazole has a high volume of distribution (6.3 ± 0.94 L/kg) and a long half-life (11.3 ± 2.84 hours). Itraconazole was not detected in the ISF, aqueous humor, or leukocytes. Plasma protein binding was 98.81 ± 0.17%.

Conclusions and Clinical Relevance—Itraconazole administered orally as a solution had higher, more consistent absorption than orally administered capsules and attained plasma concentrations that are inhibitory against fungi that infect horses. Administration of itraconazole solution (5 mg/kg, PO, q 24 h) is suggested for use in clinical trials to test the efficacy of itraconazole in horses. (*Am J Vet Res* 2005;66:1694–1701)

Itraconazole is a broad-spectrum, orally administered, triazole antifungal drug used for the treatment of humans and other animals with infections caused by *Aspergillus* spp, *Histoplasma* spp, and *Blastomyces* spp.[1]

Received January 3, 2005.
Accepted March 2, 2005.
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Presented in part at the American College of Veterinary Internal Medicine Forum, Minneapolis, June 2004.
The authors thank Tammy Stewart for assistance with the horses and Dr. Butch Kukanich and Delta Dise for technical assistance.
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It acts by blocking the action of the cytochrome P450 enzyme 14α-demethylase, which converts lanosterol to ergosterol in the cell membrane of fungi.[2] This leads to alterations in the cell membrane of fungi, inhibition of cell growth, and, ultimately, cell death. Itraconazole has a higher affinity for the fungal cytochrome P450 enzymes than do other azole antifungal drugs; however, it may also inhibit mammalian enzymes, and drug-drug interactions are possible.[3] In people and laboratory animals, itraconazole is highly protein bound; extremely lipophilic; and capable of attaining high concentrations in tissues, including the lungs, kidneys, brain, skin, and esophagus, with concentrations often higher in the tissues than in plasma.[4,5] Its major metabolite, hydroxyitraconazole, has equal antifungal activity in vitro, although conversion to this metabolite is not necessary for efficacy.[3]

Itraconazole reportedly[6-8] is effective in the treatment of horses with mycotic rhinitis, osteomyelitis, and mycosis of the diverticulum of the auditory tube (ie, guttural pouch). It has been administered for up to 6 months without adverse effects as determined during physical examination or serum biochemical analysis.[6-8] One of the most frequent clinical manifestations of fungal infections in horses is keratomycosis. *Aspergillus* spp are the most commonly isolated organisms from horses with keratomycosis, and 1 report[9] revealed 100% susceptibility of these isolates to itraconazole. Fungal invasion of the cornea can be devastating, requiring surgical correction or resulting in blindness or loss of the globe.[9] Topically applied antifungal compounds are the mainstay of treatment for keratomycoses; however, absorption of topically applied drugs is usually < 5% because of poor penetration of the drug through the cornea and tear washout.[10] Therefore, in animals that do not respond to topical medications or that have involvement of the posterior segment of the eye, adjunctive systemic administration of antifungal compounds may be beneficial.

Our laboratory group has conducted pharmacokinetic studies[11,a] in horses to determine appropriate doses of fluconazole. Although fluconazole has excellent absorption and tissue distribution after oral administration as well as favorable pharmacokinetic properties in horses, it has weak activity against *Aspergillus* spp. This may be overcome in clinical situations as a result of high plasma and intraocular concentrations of the drug. Ketoconazole also does not have substantial activity against *Aspergillus* spp and is not absorbed in horses after oral administration because of insolubility of the drug in the equine stomach.[12] Of the azole antifungals, only itraconazole and voriconazole have substantial in vitro activity against

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filamentous fungi, such as *Aspergillus* spp.[13] Activity against *Fusarium* spp, another group of fungi commonly isolated from horses with keratomycosis, is limited, and many strains are resistant.[9,13] Pharmacokinetics and dosing recommendations have not been determined for horses; therefore, the dosages used in horses have been extrapolated from dosages used in dogs and range from 2.6 to 5.0 mg/kg, PO, every 12 hours.[6,12,13] Studies in which investigators examined the in vitro susceptibilities of itraconazole revealed a **minimum inhibitory concentration (MIC)** of 0.125 to 32 µg/mL against *Fusarium* spp[14] and an **MIC at which growth of 90% of the organisms is inhibited (MIC_{90})** of 0.03 to 0.12 µg/mL against *Aspergillus* spp.[15]

Three formulations of itraconazole are currently marketed for use in humans: an orally administered solution,[b] orally administered capsules,[c] and a product formulated for IV injection.[d] A solution is also marketed in Europe for oral administration to cats.[e] Human pharmacokinetic studies[3,16] have revealed that oral administration of the solution resulted in improved absorption and **bioavailability (F)** with no substantial increase in the incidence of adverse effects. Intermittent-pulse dosing has been used in humans and cats for the treatment of dermatophytosis, which resulted in similar or better outcomes than for once-daily dosing.[17,18]

The purpose of the study reported here was to describe pharmacokinetics after oral administration of the solution and capsules and IV administration of itraconazole in horses and to establish the comparative F of the 2 oral formulations. Protein-bound and -unbound concentrations of itraconazole are considered important for efficacy as determined on the basis of an in vitro study[19]; therefore, plasma protein binding was determined in the horses.

We also intended to further classify the tissue distribution of itraconazole in horses and evaluate whether the activity of the drug at the tissue site was potentially related to unbound concentrations within aqueous fluids, concentrations delivered to the site of infection via polymorphonuclear leukocytes, or intracellular concentrations and total tissue concentrations. To do this, we collected interstitial fluid, aqueous humor, and leukocytes from a representative sample of the horses after oral administration of the itraconazole solution and determined the drug concentrations within those matrices. Information from this part of the study was used to establish whether efficacy may be related to total drug concentrations in plasma or unbound drug concentrations within each of those compartments.

Materials and Methods

Animals—Six healthy adult horses (3 males and 3 females) that weighed between 424 and 615 kg were used in the study. Horses included 2 Quarter Horses, 1 Hanoverian, 1 Thoroughbred, 1 Arabian, and 1 Standardbred. Three of the 6 horses were used for collection of samples of interstitial fluid, aqueous humor, and leukocytes. On the basis of preliminary results obtained from those horses, these samples were not collected from the remaining 3 horses. Horses were housed in stables beginning the day prior to drug administration and for the duration of the study. Water was available ad libitum throughout the study. This study was approved by the North Carolina State University Institutional Animal Care and Use Committee.

Drug administration—By use of a 2-way crossover design, itraconazole in the form of a commercially available solution or capsules was administered orally. Three horses received the solution first, followed by the capsule formulation. The other 3 horses received the capsules first, followed by the solution. A minimum 2-week washout period was allowed between administrations. Food was withheld for 12 hours before and 4 hours after drug administration. The solution was administered at a dosage of 5 mg/kg via a nasogastric tube. For administration of the capsules, the capsules were opened and the contents mixed with corn syrup in a dosing syringe (administration volume, 60 mL). Oral administration was achieved within 15 minutes after capsule contents were mixed with corn syrup. The amount of drug administered was rounded to the nearest 100 mg, and the actual dosage range for the capsules was 5.05 to 5.14 mg/kg.

In addition, 3 horses were randomly chosen to receive IV injections of itraconazole at a dosage of 1.5 mg/kg to allow for the calculation of absolute F and total systemic clearance. Only 3 horses were administered the drug in this manner because of the high cost of the IV preparation (approx $586/dose). For IV administration, itraconazole injection was mixed in accordance with the manufacturer's directions to achieve a final concentration of 3.33 mg/mL. It was slowly administered IV; infusion times ranged from 14 to 30 minutes, depending on the volume injected.

Collection of blood samples—Blood samples were collected via a catheter inserted in the jugular vein. Samples were collected into heparinized tubes 0 (before treatment), 15, and 30 minutes and 1, 2, 4, 8, 12, 24, 48, and 72 hours after oral administration. For the IV administration, 2 catheters were inserted (1 in each jugular vein) in each horse. Itraconazole was administered via one of these catheters, and blood samples were collected into heparinized tubes via the other catheter. Samples were collected 0, 10, 20, and 40 minutes and 1, 2, 4, 8, 12, 24, and 48 hours after the end of the IV infusion.

After collection, all tubes were immediately centrifuged at approximately 1,000 × *g* for 10 minutes. Plasma was harvested and then stored at –70°C until assayed.

Collection of samples of tissue fluid—Collection of tissue fluid from the subcutaneous tissues was performed by use of an in vivo ultrafiltration sampling kit[f] that has been tested by our laboratory group.[20] The probe was inserted into the subcutaneous tissues of 3 of 6 horses 12 hours before drug administration to allow a state of equilibrium to develop. Interstitial fluid was collected immediately before (time 0) and 2, 4, 8, 12, and 24 hours after oral administration of the solution at a dosage of 5 mg/kg. Immediately after collection, the fluid was stored at –70°C until analyzed.

Collection of samples of aqueous humor—Samples of aqueous humor were collected 2.5 hours (1 sample) or 4.5 hours (2 samples) after oral administration of the itraconazole solution. These time points were chosen to coincide with peak plasma concentrations predicted on the basis of results of preliminary studies and to allow for a 30-minute equilibrium period between plasma and tissues. For sample collection, the horses were sedated by IV administration of detomidine[g] (0.01 to 0.015 mg/kg) and manually restrained by application of a nose twitch. Auriculopalpebral, supraorbital, and retrobulbar nerve blocks were performed, and a topical anesthetic was applied to the eye. The eye was cleansed with dilute (10%) aqueous iodine solution.

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Aqueous humor was aspirated by inserting a sterile 27-gauge, 0.5-inch needle through the conjunctiva and sclera in the dorsolateral limbus of the eye until the needle penetrated the anterior chamber. Once in the anterior chamber, gentle suction by use of a 1-mL tuberculin syringe was used to aspirate approximately 200 to 300 µL of fluid, and then the needle was withdrawn. Samples were frozen at –70°C until analyzed.

Isolation of polymorphonuclear leukocytes— Leukocytes were harvested from blood samples collected from 3 horses immediately before (time 0) and 1, 4, 8, and 24 hours after itraconazole administration. Leukocytes were harvested by a method published elsewhere.[21,22] The remaining cell pellets were frozen at –70°C until analyzed.

Plasma protein binding—Protein binding was determined by use of a microcentrifugation system.[h] Pooled plasma obtained from 6 healthy horses that did not receive any itraconazole was used in the analysis. Aliquots of equine plasma were fortified (ie, spiked) with itraconazole to make solutions containing 2, 1, and 0.5 µg/mL. Three replicates of spiked plasma samples/concentration were prepared and incubated in a water bath at 37°C for 30 minutes. Each spiked plasma sample was added to a microcentrifugation system and centrifuged at 1,000 X g for 10 minutes. A protein-free ultrafiltrate was obtained in the reservoir of the system, extracted, and analyzed as described for the drug analysis. The percentage of nonspecific binding of the drug to the microcentrifugation device and filter was determined in vitro. There was no measured adherence of the drug to the device, and adherence to the filter was < 5%. Protein binding was determined by use of the following equation:

$$\text{Protein binding percentage} = ([\text{Total concentration} - \text{unbound concentration}]/\text{total concentration}) \times 100.$$

Drug analysis—Concentrations of itraconazole and its active metabolite, hydroxyitraconazole, were analyzed by use of reverse-phase **high-performance liquid chromatography (HPLC)** with UV detection after solid-phase extraction by use of an assay developed and validated in our laboratory. The HPLC apparatus consisted of a pump,[i] autosampler,[j] UV detector,[k] and computer for data collection and analysis.[l] Solid-phase extraction of itraconazole in plasma was performed by use of cyano-bonded extraction cartridges.[m] Cartridges were initially conditioned with 1 mL of methanol and 1 mL of 0.05M dibasic potassium phosphate buffer (pH, 7.5). One milliliter of plasma was then extracted and washed with a solution of 0.05M dibasic potassium phosphate buffer:methanol (95:5). Samples were eluted with 1 mL of methanol, evaporated under a stream of nitrogen gas for 25 minutes at 40°C, and reconstituted with 200 µL of mobile-phase solution prior to injection into the HPLC apparatus. A reverse-phase column[n] was used for separation. The mobile-phase solution consisted of distilled water and HPLC-grade acetonitrile in a 50:50 mixture with 0.02% trifluoroacetic acid. Ultraviolet detection was performed at 263 nm, and injection volume was 50 µL/sample.

Tissue fluid samples were analyzed directly by use of HPLC without extraction because they were derived from a cell-free, protein-free matrix. A liquid-liquid extraction technique that used heptane:isoamyl alcohol (9:1) was modified from another published report[23] for use on the samples of leukocytes and aqueous humor. Briefly, for leukocyte samples, the cell pellet was diluted with 200 µL of 0.05M dibasic potassium phosphate buffer (pH, 7.5). The heptane–isoamyl alcohol mixture (800 µL) was added to the cell-buffer mixture. Samples were then sonicated for 15 seconds and vortexed for 15 seconds. Then, the samples were centrifuged for 15 minutes at 13,500 X g. An aliquot (500 µL) of the top organic layer was pipetted into a glass test tube and evaporated under a stream of nitrogen gas for 20 minutes. The resulting samples were then reconstituted with 200 µL of mobile-phase solution and injected directly into the HPLC apparatus. For samples of aqueous humor, the extraction process was identical, with the exception that 800 µL of heptane–isoamyl alcohol was added directly to 200 µL of aqueous humor.

Standard curves were prepared by use of pure itraconazole and hydroxyitraconazole reference standards[o] dissolved in 100% acetonitrile. When frozen at –70°C in a plastic container, the standards were stable for > 2 months. Standard curves were prepared fresh daily in pooled plasma obtained from untreated horses (for plasma and protein binding analyses), mobile-phase solution (for samples of tissue fluid), aqueous humor from untreated horses (for samples of aqueous humor), and 0.05M dibasic potassium phosphate buffer (for leukocyte samples). Calibration curves were linear between the concentrations of 2 and 0.0156 µg/mL, with a coefficient of determination of > 0.99 and all values within 20% of the expected range. The lower limit of quantification was the lowest concentration that was determined to be on a linear regression line on the calibration curve. This value was 0.0156 µg/mL for plasma, 0.05 µg/mL for interstitial fluid, and 0.025 µg/mL for aqueous humor and leukocytes. At concentrations of 1.0, 0.125, and 0.0625 µg/mL, the accuracy of the HPLC assay was within (mean ± SD) 3.66 ± 3.03% of the true value and intra-assay precision was within 4.35 ± 1.86% of the mean.

Pharmacokinetic analysis—Drug concentrations were analyzed by use of standard pharmacokinetic methods to determine the drug disposition for each drug in each horse. A computer program[p] was used to determine pharmacokinetic variables in accordance with methods described elsewhere.[24] Pharmacokinetic variables were calculated separately for each horse, and the results for all horses were reported as mean ± SD. Noncompartmental and compartmental methods were used.

Noncompartmental analysis was used to calculate the **area under the curve (AUC)** for the plasma concentration versus time data for each drug formulation. Each AUC was calculated by use of the trapezoidal rule. Absolute F for oral administration of the solution and capsules was then calculated for the 3 horses that also received IV infusions of itraconazole. Values for F were calculated by use of the following equation:

$$F = (AUC_{oral} \times Dose_{IV})/(Dose_{oral} \times AUC_{IV}),$$

where AUC_{oral} is the AUC after oral administration, $Dose_{IV}$ is the dose administered IV, $Dose_{oral}$ is the dose administered orally, and AUC_{IV} is the AUC after IV administration. Relative F after oral administration of the capsules was determined by dividing the AUC after oral administration of the capsules by the AUC after oral administration of the solution (ie, the ratio between the 2 drug formulations).

Results

Oral administration of the solution resulted in more consistent absorption and a higher maximum concentration, compared with results after oral administration of the capsule formulation **(Figure 1)**. Mean ± SD elimination half-life after oral administration of the solution (11.3 ± 2.84 hours) was almost twice that after oral administration of the capsules (7.97 ± 3.11 hours) or IV administration (6.52 ± 0.20 hours). Concentrations of itraconazole were initially

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detected 15 minutes after oral administration of the solution in 2 of 6 horses and 30 minutes after oral administration of the solution in the remaining 4 horses and were still detectable 48 hours after administration in 4 of 6 horses. Drug was not detected until 1 hour after oral administration of the capsules in 4 of 6 horses and 2 hours after administration in the remaining 2 horses. At time points at which the drug was not detected or was below the limit of quantification, the concentration was considered 0 for the calculations. Relevant noncompartmental pharmacokinetic variables for each formulation were summarized (**Table 1**).

For compartmental analysis of the data obtained after IV administration of the drug, a 2-compartment model was determined to provide the best fit on the basis of evaluation of the plasma concentration versus time data plotted on a logarithmic scale as well as the Akaike inclusion criteria (**Figure 2**). An infusion time was added to the calculations by use of the following equation:

$$C = A(e^{-\alpha t} - e^{-\alpha[t - t^*]}) + B(e^{-\beta t} - e^{-\beta[t - t^*]}),$$

where C is the plasma concentration of itraconazole, A is the intercept of the distribution phase, e is the base of the natural logarithm, α is the slope of the distribution phase, t is time after the end of the itraconazole infusion, t* is t minus the duration of the infusion (ie, ti) for t > ti and 0 for t < ti, B is the intercept of the elimination phase, and β is the slope of the elimination phase.

Pharmacokinetic variables for a 2-compartment analysis after IV administration of itraconazole were summarized (**Table 2**). Following IV administration of the drug, mean ± SD apparent volume of distribution was 6.3 ± 0.94 L/kg. Mean absolute F after oral administration of the solution and capsules was 64.96 ± 26.34% and 12.18 ± 5.6%, respectively. Relative F of the capsule formulation, compared with F of the solution, was highly variable at 33.83 ± 33.08%.

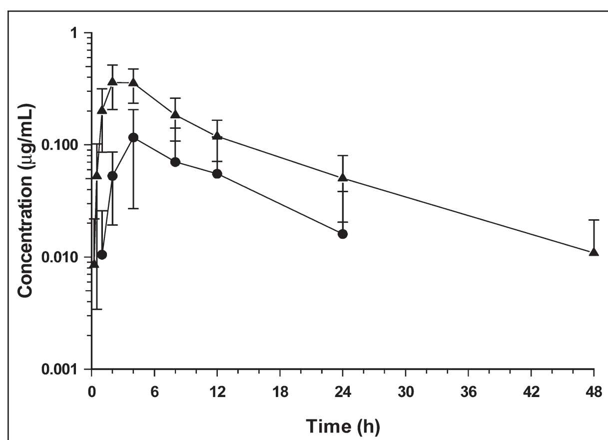

Figure 1—Mean ± SD values for the concentration versus time data after oral administration of itraconazole (5 mg/kg) in the form of a solution (solid triangles) or in capsules (solid circle) to 6 horses.

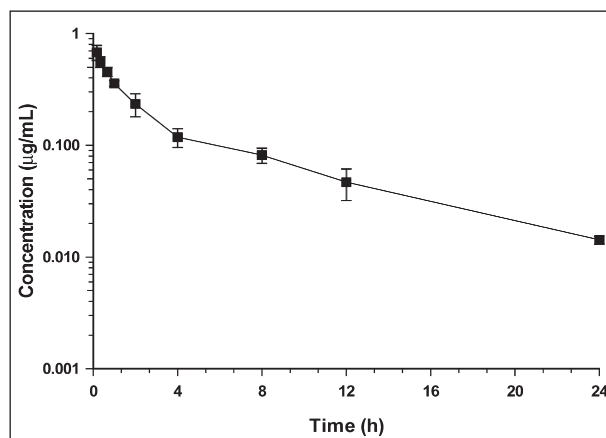

Figure 2—Mean ± SD values for plasma concentration versus time data after IV administration of itraconazole at a dosage of 1.5 mg/kg to 3 horses. Infusion of itraconazole required 14 to 30 minutes. Time 0 = Completion of IV administration.

Table 1—Mean ± SD values for noncompartmental pharmacokinetic variables after IV administration of a single dose of itraconazole (1.5 mg/kg) or after oral administration of a single dose of itraconazole (5 mg/kg) in the form of a solution or in capsules to horses.*

Pharmacokinetic variable	IV	Oral	
		Solution	Capsules
Cmax (µg/mL)	NA	0.41 ± 0.13	0.15 ± 0.12
Tmax (h)	NA	3.33 ± 1.03	3.33 ± 1.15
Vd_{area} (L/kg)	6.30 ± 0.94	NA	NA
MRT (h)	6.94 ± 0.05	13.83 ± 2.73	12.59 ± 4.21
AUC ([h × µg]/mL)	2.27 ± 0.28	4.73 ± 1.85	1.60 ± 1.66
AUMC(h × {h × µg}/mL])	16.13 ± 2.08	67.44 ± 36.32	23.79 ± 28.95
λ (/h)	0.11 ± 0.00	0.06 ± 0.02	0.10 ± 0.05
$t_{1/2\lambda}$ (h)	6.52 ± 0.20	11.30 ± 2.84	7.97 ± 3.11
F (%)	NA	64.96 ± 26.34	12.18 ± 5.6

*Itraconazole was administered IV to 3 horses and orally (solution and capsules) to 6 horses.

Cmax = Maximum concentration. Tmax = Time to Cmax. Vd_{area} = Apparent volume of distribution (area method). MRT = Mean residence time. AUC = Area under the concentration versus time curve. AUMC = Area under the first moment versus time curve. λ = Rate constant of the terminal phase. $t_{1/2\lambda}$ = Half-life of the terminal phase. F = Systemic availability. NA = Not applicable.

Table 2—Mean ± SD values* for pharmacokinetic variables after IV administration of itraconazole (1.5 mg/kg) to 3 horses.

Pharmacokinetic variable	Mean ± SD
A (µg/mL)	0.69 ± 0.17
α (/h)	1.02 ± 0.36
B (µg/mL)	0.17 ± 0.01
β (/h)	0.11 ± 0.01
$t_{1/2\alpha}$ (h)	0.75 ± 0.32
$t_{1/2\beta}$(h)	6.54 ± 0.51
k_{10} (/h)	0.38 ± 0.11
k_{12} (/h)	0.47 ± 0.22
k_{21} (/h)	0.28 ± 0.04
Vd_{ss} (L/kg)	4.47 ± 0.23
Cl (mL/min/kg)	11.14 ± 1.33

*Values were determined by use of a 2-compartment model. A = Coefficient of the distribution phase. α = Rate constant of the distribution phase. B = Coefficient of the elimination phase. β = Rate constant of the elimination phase. $t_{1/2\alpha}$ = Half-life of distribution. $t_{1/2\beta}$ = Half-life of elimination. k_{10} = Elimination rate from compartment 1. k_{12} = Rate of movement from compartment 1 to compartment 2. k_{21} = Rate of movement from compartment 2 to compartment 1. Vd_{ss} = Apparent volume of distribution at steady state. Cl = Systemic clearance.

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No adverse effects were observed during the study following oral or IV administration of itraconazole. The metabolite, hydroxyitraconazole, was not detected in any samples.

Itraconazole concentrations were less than the detection limit of the assay in all of the samples of interstitial fluid, aqueous humor, and leukocytes analyzed; therefore, these samples were obtained from only 3 of the 6 horses in the study. Subcutaneous insertion of the ultrafiltration probe was easily accomplished in awake, sedated horses. Fluid collection rates ranged from 74.2 to 113.3 µL/h. Probes were tolerated well for up to 5 days with no adverse reactions evident at the insertion site. Results of in vitro plasma-protein binding analyses revealed that itraconazole was highly protein bound in these horses (98.81 ± 0.17%).

Discussion

Analysis of results of the study reported here indicated that itraconazole solution was absorbed following oral administration at a dosage of 5 mg/kg in horses and maintained plasma concentrations above the targeted MIC of 0.06 µg/mL against *Aspergillus* spp for > 12 hours in all horses in the study and > 24 hours in 2 horses in the study. Plasma concentrations did not consistently reach values sufficiently high to treat horses with *Fusarium* infections; therefore, this drug is not recommended for the treatment of horses infected with this pathogen unless adequate susceptibility can be documented. The capsule formulation was variably absorbed with a lower peak concentration and smaller AUC, compared with results for the solution. Overall F after oral administration of the solution was high (64.96 ± 26.34%), whereas F after oral administration of the capsules was much lower (12.18 ± 5.6%). Relative F of the capsules, when compared with F for the solution, was 33.83 ± 33.08%. The high degree of variability was attributable to 1 horse that had almost 100% bioequivalence between capsules and solution.

These findings in horses are similar to data obtained from human pharmacokinetic trials in which oral administration of the solution resulted in improved absorption and F, without a substantial increase in the incidence of adverse effects.[3] The physicochemical properties of itraconazole can be used to explain this phenomenon. Itraconazole is a highly lipophilic (logarithm of the partition coefficient, 5.66) weak base; as such, it will become minimally soluble in aqueous solutions only at a low pH. The capsule formulation relies on the natural acid environment of the stomach to dissolve the drug into a solution prior to absorption; therefore, its absorption is subject to variability.[3] The pKa for this weak base is < 2 for the base and 3.7 for the piperazine portion of the molecule.[25] At these pKa values, it will only be protonated (and therefore solubilized in aqueous solutions) at an extremely low pH. The pH in the stomach of horses may not be sufficiently acidic and may be too variable to ensure consistent absorption.[26]

A newer oral formulation is a combination of itraconazole with cyclodextrins. Cyclodextrins are commonly used permeability enhancers consisting of oligosaccharides with a hydrophilic outer surface and a lipophilic inner surface.[27] Cyclodextrins are not absorbed to any major degree after oral administration and are rapidly cleared from the system by renal excretion after IV administration; therefore, they do not add substantially to the toxic effects of most drugs, although gastrointestinal tract disturbances have been reported[28,29] after long-term administration. The main function of cyclodextrins is to form complexes with extremely lipophilic drugs, such as itraconazole, and allow them to remain soluble in solution. Because dissolution is the rate-limiting step in the absorption of most orally administered lipophilic drugs, this formulation would be expected to be absorbed better than would the capsule formulation in which factors such as gastric pH may be limiting. However, because of the low concentration of drug in the solution (10 mg/mL), a large volume must be administered orally (250 mL for a 500-kg horse treated at a dosage of 5 mg/kg), which makes administration inconvenient for horse owners. The IV formulation is not considered practical for administration to horses because of the high cost per dose, large volume administered, instability of the compound, and need for a slow infusion.

In humans, itraconazole has patterns that trend toward nonlinear pharmacokinetics as determined on the basis of comparisons of the AUC for various doses.[30] Steady-state conditions in plasma are not reached until up to 10 days after oral administration in humans and 21 days after oral administration in cats,[31,32] and the elimination half-life after multiple doses is often prolonged, indicating a saturable mechanism for excretion at clinical doses or inhibition of metabolism through inhibition of cytochrome P450 enzymes.[16,33] The longer half-life observed for oral administration of the solution, compared with that for the IV formulation in the horses of the study reported here, was most likely attributable to a prolonged absorption phase and a potential flip-flop phenomenon, as evidenced by discrepancies in the terminal elimination phase between IV administration and oral administration of the solution.

Elimination in humans is mainly through metabolism by the liver and biliary excretion of itraconazole and its metabolites.[13] More than 30 metabolites of itraconazole have been identified in humans, and one of these (hydroxyitraconazole) has comparable activity in vitro to that of the parent compound.[34] In humans, this metabolite often reaches concentrations that are 2 to 3 times higher than itraconazole in plasma.[34] Hydroxyitraconazole was not detected in any of the samples analyzed in our study, indicating that metabolizing pathways must differ between horses and humans.

Another favorable feature of itraconazole in other species is that it reaches concentrations in tissues that are often several fold higher than plasma concentrations and may last up to 4 weeks after cessation of treatment in some organs, particularly the skin and esophagus.[5,35] In those studies, investigators determined concentrations of the drug in whole tissue biopsy specimens that measured bound and unbound concentrations of the drug. Itraconazole is highly bound to plasma protein (> 99%) and tissues in humans,[19] and

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analysis of results of the study reported here indicated that plasma-protein binding was similar in horses (98.81%). Because protein binding may influence the distribution of drugs to tissue fluid, we therefore used an in vivo ultrafiltration technique to determine the free, unbound concentrations of itraconazole in the interstitial fluid of horses. These probes have been used by our laboratory group to measure drug concentrations in tissue fluid in dogs and have revealed that those concentrations correlate well with unbound drug concentrations in plasma.[20] The correlation of unbound drug concentrations in plasma and unbound drug concentrations in interstitial fluid was also confirmed in the study reported here because itraconazole was not detected in any of the interstitial fluid samples, indicating that free-drug concentrations in the interstitial tissue fluid were negligible and free drug in plasma represented < 2% of total drug. However, the lack of unbound drug in the interstitial fluid does not imply that drug concentrations of itraconazole in horses are not therapeutic. The large volume of distribution observed in our study is consistent with, but not absolute proof of, high intracellular concentrations. It may also have been attributable to high tissue binding.

Although protein-unbound drug is considered to be the only active component for many antibacterial drugs, this may not be true for some antifungal drugs, including itraconazole and other triazole antifungals (eg, fluconazole and voriconazole). By use of a skin blister technique, investigators documented in 1 study[19] that at doses commonly considered to be effective in clinical cases, the free-drug concentration in the skin is far below reported MIC values for *Candida* spp and dermatophytes. In vitro techniques for examining antifungal activity revealed that itraconazole had comparable efficacy against *Candida albicans* when incubated with or without 4% human serum albumin.[36] Similar results were detected when a microdilution technique was used.[19] On the basis of results for those techniques, the antifungal activity of itraconazole is not related entirely to free-drug concentrations; therefore, the lack of unbound drug detected in our interstitial fluid samples would not necessarily predict therapeutic failure. In fact, a significant effect was documented[37] between total plasma concentrations (bound plus unbound) and antifungal efficacy as a function of *Aspergillus fumigatus* burden in the lungs of immunosuppressed rabbits. Significant correlations have also been found between efficacy and maximum and minimum total plasma concentrations as well as the AUC in neutropenic rabbits with invasive pulmonary aspergillosis, supporting time- and concentration-dependent pharmacodynamic relationships.[30] In the study reported here, orally administered itraconazole solution yielded a plasma concentration of total drug that was typically between 3.4 and 13.6 times the reported MIC_{90} for *Aspergillus* spp.

Although protein-bound itraconazole may be active in some tissues, the high amount of protein binding will influence distribution to fluid compartments that have a relative lack of protein, such as the aqueous humor. Itraconazole was not detected in the aqueous humor samples obtained from noninflamed eyes in the horses of the study reported here. This is consistent with a report[38] of topical application of 1% itraconazole ointment with or without dimethyl sulfoxide in horses. The topical treatment resulted in high corneal concentrations and was effective for clearing infection from 8 of 10 eyes with fungal keratitis.[38,39] However, topical application (with or without dimethyl sulfoxide) did not yield measurable itraconazole concentrations in the aqueous humor. This is most likely a consequence of the high tissue-binding affinity and low aqueous solubility of the drug. Investigators also did not detect itraconazole in the aqueous or vitreous humor of noninflamed eyes of rabbits after systemic administration; however, the drug was detectable at low concentrations in the cornea following administration of a single dose.[40] When endophthalmitis was induced with *C albicans*, drug was detectable in all compartments of the eye examined, although the concentrations were significantly lower than for ketoconazole or fluconazole.[40] Therefore, we can conclude that despite the high lipophilicity of itraconazole, it does not penetrate an intact blood-aqueous barrier. This is in contrast to fluconazole, which readily penetrates into the aqueous humor of noninflamed eyes of horses.[11,a]

Itraconazole was also not detectable in the samples of leukocytes analyzed in our study. In an in vitro study[41] of alveolar macrophages isolated by use of bronchoalveolar lavage from New Zealand White rabbits, investigators revealed a rapid, passive uptake of tritium-labeled itraconazole when incubated in serum-free media. This uptake was drastically reduced when incubated with 5% to 100% serum, indicating an inhibitory effect of serum protein binding on cellular uptake of the drug.[41] The lack of detectable drug in our study may have been attributable to the relatively low plasma concentrations, high plasma protein binding in vivo, or instability of the drug in the cells as a result of metabolism by intracellular enzymes (eg, myeloperoxidase). Because most fungal infections are not intracellular, the lack of penetration of itraconazole into WBCs would not be expected to have a negative effect on clinical efficacy; however, it does potentially eliminate this as a route of drug delivery to the site of infection.

We conclude that concentrations of itraconazole in aqueous fluids (eg, aqueous humor and interstitial tissue fluid) and leukocytes are negligible. We also conclude that itraconazole solution administered orally at a dosage of 5 mg/kg every 24 hours will yield total (bound and unbound) plasma concentrations that are inhibitory against fungi known to infect horses. This regimen is suggested for use in clinical trials conducted with itraconazole for the treatment of horses with susceptible fungal infections. However, oral administration of the solution may be impractical because of the cost and large volume of drug required. Itraconazole capsules are more variable in the extent of absorption and cannot be recommended for use in horses at a dosage of 5 mg/kg. Higher or more frequent dosing with the capsules would be necessary to attain pharmacokinetic patterns similar to those for oral administration of the solution. Use of the IV formulation at a dosage of 1.5 mg/kg once daily would achieve

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concentrations considered to be therapeutic for the treatment of most infections with *Aspergillus* spp, but the expense may prohibit its use in most horses. Multiple-dosing studies are necessary to determine the time needed to reach steady-state conditions and whether the half-life of itraconazole would increase or itraconazole would accumulate in tissues, which would allow for intermittent dosing in horses, similar to its use in other species. The safety of long-term oral administration of a cyclodextrin solution also needs to be assessed.

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c. Sporanox (itraconazole) capsules (100 mg), Janssen Pharmaceuticals, Titusville, NJ.
d. Sporanox (itraconazole) injection (10 mg/mL), Janssen Pharmaceuticals, Titusville, NJ.
e. Itrafungol (itraconazole) oral solution (10 mg/mL), Janssen-Cilag Ltd, High Wycombe, UK.
f. Canine ultrafiltration probe (RUF-3-12), BAS Bioanalytical Systems, West Lafayette, Ind.
g. Dormosedan, Pfizer Animal Health, Exton, Pa.
h. Centrifree micropartition system, Amicon, Beverly, Mass.
i. Waters pump, Millipore Corp, Milford, Mass.
j. Agilent series 1100, Agilent Technologies, Wilmington, Del.
k. Agilent series 1050 variable wavelength detector, Agilent Technologies, Wilmington, Del.
l. Agilent series 1100 Chemstation software, Agilent Technologies, Wilmington, Del.
m. Bond-Elut CN-E extraction cartridges (1 mL), Varian Inc, Harbor City, Calif.
n. Zorbax RX-C8, 4.6 × 150-mm reverse-phase column, Agilent Technologies, Wilmington, Del.
o. Research Diagnostics Inc, Flanders, NJ.
p. WinNonlin, version 4.0, Pharsight Corp, Mountain View, Calif.

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