Pharmacokinetics of fluconazole following intravenous and oral administration and body fluid concentrations of fluconazole following repeated oral dosing in horses

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Objective—To determine the pharmacokinetics of fluconazole in horses.

Animals—6 clinically normal adult horses.

Procedure—Fluconazole (10 mg/kg of body weight) was administered intravenously or orally with 2 weeks between treatments. Plasma fluconazole concentrations were determined prior to and 10, 20, 30, 40, and 60 minutes and 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72 hours after administration. A long-term oral dosing regimen was designed in which all horses received a loading dose of fluconazole (14 mg/kg) followed by 5 mg/kg every 24 hours for 10 days. Fluconazole concentrations were determined in aqueous humor, plasma, CSF, synovial fluid, and urine after administration of the final dose.

Results—Mean (± SD) apparent volume of distribution of fluconazole at steady state was 1.21 ± 0.01 L/kg. Systemic availability and time to maximum plasma concentration following oral administration were 101.24 ± 27.50% and 1.97 ± 1.68 hours, respectively. Maximum plasma concentrations and terminal half-lives after IV and oral administration were similar. Plasma, CSF, synovial fluid, aqueous humor, and urine concentrations of fluconazole after long-term oral administration of fluconazole were 30.50 ± 23.88, 14.99 ± 1.86, 14.19 ± 5.07, 11.39 ± 2.83, and 56.99 ± 32.87 µg/ml, respectively.

Conclusions and Clinical Relevance—Bioavailability of fluconazole was high after oral administration to horses. Long-term oral administration maintained plasma and body fluid concentrations of fluconazole above the mean inhibitory concentration (8.0 µg/ml) reported for fungal pathogens in horses. Fluconazole may be an appropriate agent for treatment of fungal infections in horses. (Am J Vet Res 2001;62:1606–1611).
azole in adult horses. To verify our predictions we measured plasma, CSF, aqueous humor, synovial fluid, and urine concentrations of fluconazole at the end of the 10-day multiple-dose regimen.

Materials and Methods

**Horses**—Six (4 geldings, 2 mares) clinically normal adult horses with a body weight between 390 and 585 kg were used. Results of CBC and serum biochemical analyses performed immediately before the study were within reference values for our laboratory. All horses were vaccinated for tetanus, influenza, and eastern and western encephalitis and were given an anthelmintic paste orally 2 weeks before the pharmacokinetic study. Horses were maintained on pasture except on days when drugs were administered and blood samples collected; on those days, horses were kept in individual stalls. Water and grass hay were available ad libitum, and a commercial grain ration was fed at a rate of 2.2 kg/50 kg of body weight twice daily.

**Experimental design**—Horses were weighed and randomly assigned to an IV or oral dosing group (3 horses/group). All horses were fed approximately 2 hours prior to drug administration, but no effort was made to withhold feed or water in either group prior to drug administration. A 14-gauge 13.3-cm catheter was placed in the right jugular vein for collection of blood samples. A 10-gauge 8.98-cm catheter was placed in the left jugular vein of horses in the IV dosing group for drug administration. A 2 mg/ml solution of fluconazole was administered as a bolus by gravity flow through a nasogastric tube to yield a final dose of 10 mg of fluconazole/kg of body weight. Immediately after drug administration, the left jugular catheter was removed. In the oral dosing group, fluconazole tablets (200 mg/tablet) were crushed and suspended in 2 L of water and administered as a bolus by gravity flow through a nasogastric tube to yield a final dose of 10 mg of fluconazole/kg.

Blood samples (10 ml) were obtained from the right jugular catheter of each horse immediately before and 10, 20, 30, 60 minutes and 2, 4, 6, 8, 10, 12, 24, 36, 48, 60, and 72 hours after fluconazole administration. Catheters were flushed with sterile heparinized saline (0.9% NaCl) solution immediately before and after blood collection. The first 6 ml of blood obtained at each sampling period was discarded prior to obtaining the 10 ml sample for pharmacokinetic determinations to ensure that blood samples were not diluted with saline solution. Blood samples were placed in tubes containing lithium heparin and centrifuged in a refrigerated centrifuge immediately after collection. Plasma was stored at –70 C for determination of fluconazole concentrations.

At the conclusion of the initial sampling period, horses were returned to pasture for 2 weeks. After this washout period, fluconazole was administered to all horses in the alternate-crossover design so that each horse received fluconazole intravenously and orally. Drugs were administered and plasma samples obtained as described for the first part of the crossover. At the conclusion of the second sampling period, horses were returned to pasture for an additional 2-week period. Pharmacokinetic data obtained from the crossover study were used to calculate a long-term oral dosing regimen that would maintain plasma concentrations of fluconazole > 8.0 µg/ml throughout the treatment period.

After the second washout period and approximately 2 hours after the morning feeding, fluconazole tablets (200 mg/tablet) were dissolved in water, mixed with molasses, and administered to each horse with a dosing syringe to yield a final loading dose of 14 mg of fluconazole/kg. Each day thereafter for 10 days, fluconazole was prepared and administered in the same manner to yield a daily dose of 5 mg of flucona-
and transferred to an HPLC vial for analysis. Twenty plasma samples were also sent to an independent laboratory to validate the assay. Results from both laboratories were within 15 to 20% agreement for all samples.

**Pharmacokinetic analyses**—Pharmacokinetic analyses were performed, using standard methods and equations and computer software for kinetic modeling by use of nonlinear least square methods. Because the LOQ of the HPLC assay was 0.5 µg/ml, fluconazole concentrations < 0.5 µg/ml were not used for analyses.

Plasma concentration versus time data obtained after IV administration of fluconazole were first analyzed by use of compartmental pharmacokinetic methods. A 2-compartment model with first order output and bolus input was the most appropriate for analysis. The decision to fit this model versus other models was made on the basis of the sum of squares of the weighted residuals and measurement of the Akaike information criteria (AIC) after data were fit to other models. The model with the lowest AIC was selected as the most appropriate. Model-independent methods were used to calculate the area under the concentration versus time curve (AUC) and mean residence time (MRT). Total area under the curve from zero to infinity (AUC∞) was calculated, using the log-trapezoid method, and included the residual (terminal) area determined as Cn/β, where Cn is the concentration at the last measured time point, and β is the terminal rate constant. Plasma concentrations of fluconazole (C T) after IV administration were determined for the 2-compartment open model with elimination from the central compartment, using the equation:

\[ C_T = A e^{-\alpha T} + B e^{-\beta T} \]

where \( e \) is the base of the natural logarithm, \( T \) is time after drug administration, and \( A \) and \( B \) are the y-axis intercepts for the distribution and elimination phases of the curve, respectively, and \( \alpha \) and \( \beta \) are the rate constants for the distribution and elimination phases of the curve, respectively. A weighting factor of 1/\( T^2 \) was used, where \( Y \) is the plasma concentration of fluconazole.

Half-life of distribution (\( T_{1/2D} \)), half-life of elimination (\( T_{1/2E} \)), total systemic clearance (\( CL_S \)), apparent volume of distribution to the central compartment (\( V_C \)), apparent volume of distribution at steady-state (\( V_{AREA} \)), apparent volume of distribution determined by use of the area method (\( V_{AIC} \)), and microdistribution rate constants (\( K_{G1}, K_{12}, \) and \( K_{21} \)) were calculated with computer software, using standard pharmacokinetic methods.

Data obtained after oral administration of a single dose of fluconazole were described by use of a 1-compartment model with first order input for 2 horses and a 2-compartment model with first order input for the other 4 horses. The decision to fit data to one model or the other was made on the basis of the sum of squares of the weighted residuals and measurement of AIC after data were fit to each model. The model with the lowest AIC was used to describe data obtained from each individual horse. The absorption rate constant (\( K_G \)), terminal elimination rate constant (\( K_{O1} \)), and the corresponding half-lives of these variables were calculated, using standard pharmacokinetic methods. As with data obtained after IV administration, a weighting factor of 1/\( T^2 \) was used, where \( Y \) is the plasma concentration of fluconazole.

From data obtained after oral administration of fluconazole, AUC was calculated in the same manner as described after IV administration. Systemic availability after oral administration (%F) was calculated according to the formula:

\[ %F = \frac{AUC_O}{AUC_IV} \]

where \( AUC_O \) is the AUC determined after oral administration, and \( AUC_IV \) is the AUC after IV administration. Time to maximum plasma concentration (\( T_{MAX} \)) and maximum plasma concentration (\( C_{MAX} \)) of fluconazole after oral administration were determined from the plasma concentration versus time curve. Values for MRT were obtained, using the same methods as described for IV administration. From half-life data after oral administration, a once daily dosing regimen was calculated, using computer software for kinetic modeling, to maintain plasma concentration of fluconazole > 8.0 µg/ml. This value was selected on the basis of the reported MIC of fluconazole against susceptible organisms in horses.

**Results**

Horses did not develop swelling, heat, or signs of pain at the injection site after IV administration of fluconazole. In addition, signs of discomfort or colic were not detected after oral administration of either a single dose or repeated doses of fluconazole. Appetite, water consumption, and fecal consistency remained normal in all horses throughout the study.

The HPLC assay for determination of fluconazole concentrations was specific and reliable; approximately 50 samples could be analyzed each day. Interfering peaks that coincided with the retention time for fluconazole were not identified in blank body fluid samples. Concentrations of fluconazole in plasma samples obtained after IV or oral administration of a single dose of fluconazole remained above the LOQ of the assay. Concentrations of fluconazole in all quality control and calibration standards were within 15% of the true concentration. Plasma fluconazole concentration versus time curves were created and pharmacokinetic variables calculated after IV and oral administration (Fig 1; Table 1). After IV administration, mean \( V_{AREA} \) and \( V_C \) were 1.22 and 1.21 L/kg, respectively, and mean CL S was 0.02 L/kg/h. Mean \( C_{MAX} \) and %F of fluconazole after oral administration of a single dose were 10.73 µg/ml and 101.24%, respectively. The harmonic mean elimination \( T_{1/2} \) after IV administration was 41.6 hours and after oral administration was 37.8 hours.

Mean plasma concentrations of fluconazole after IV or oral administration of a single dose of 10 mg/kg remained ≥ 2.5 µg/ml throughout the 72-hour sample period.
plasma concentration in all horses was 30.50 µg/ml after oral administration. Concentrations of fluconazole in horses after prolonged administration of IV or oral administration were 41.6 and 37.8 µg/ml, respectively. For orally administered fluconazole that we detected indicate that absorption of fluconazole is rapid and nearly complete after oral administration to horses. Oral absorption of fluconazole was excellent in relation to absorption after IV administration.

Mean CMAX of fluconazole after oral administration was 10.73 µg/ml. Again, this value is similar to the reported CMAX in humans treated with 3.3 or 6.6 mg of fluconazole/kg (10.1 and 18.9 mg/L, respectively). Owing to the apparently rapid and complete absorption of orally administered fluconazole in horses, administration of larger doses should result in proportionately higher CMAX and AUC values for treatment of fungi less susceptible to fluconazole.

Mean bioavailability of orally administered fluconazole indicated that absorption was complete in most horses. In comparison, oral administration of 30 mg of ketoconazole/kg to horses did not result in detectable drug concentrations in serum. However, after the same dose was admixed with hydrochloric acid and administered via a nasogastric tube, serum concentrations of ketoconazole averaged 3.76 µg/ml and bioavailability 23%.

Fluconazole is unique among theazole antifungals in that it is minimally protein bound (approx 11%). In humans and other animals, the high VSS and VAREA and low protein binding result in concentrations of fluconazole for most equine fungal pathogens. In other species, fluconazole is excreted unchanged in the urine. Urinary excretion of the unchanged drug approximates 70% of the IV or oral dose in mice, rats, dogs, and humans. In people, the unchanged fraction of the drug excreted in the urine ranges from 64 to 90% of the administered dose. In the present study, urinary excretion of unchanged fluconazole also appeared to be high, because urine concentrations were much higher than concentrations in other body fluids.

### Discussion

To our knowledge, results of the present study represent the first data that describe pharmacokinetics of fluconazole after IV or oral administration to horses. In addition, our results described plasma and body fluid concentrations of fluconazole in horses after prolonged oral administration.

Harmonic mean elimination T1/2 of fluconazole after IV and oral administration were 41.6 and 37.8 hours, respectively. In humans with normal renal function receiving daily doses of fluconazole ranging from 1 to 6.6 mg of fluconazole/kg, these values are 22 and 42 hours, respectively. Because fluconazole has such a long T1/2, plasma steady-state concentrations are not achieved for 6 to 10 days after repeated dosing unless a loading dose is administered. From data obtained after oral administration of a single dose of fluconazole, we calculated a treatment regimen that incorporated a loading dose so that steady-state concentrations could be achieved in less time. Administration of a single loading dose of 14 mg of mg of fluconazole/kg followed by 5 mg/kg orally once daily for 10 days maintained fluconazole concentrations at > 8.0 µg/ml in plasma and other body fluids. Fluconazole appears to have similar pharmacokinetics after oral administration in other species. The Cls of fluconazole after IV administration in the present study was 0.020 L/kg/h, which is similar to the value reported in humans. In addition, we found that mean TMAX after oral administration was 1.97 hours. This value is in close agreement to values reported for humans after oral administration of 0.83 to 6.6 mg of fluconazole/kg (1.4 to 4.3 hours). The short TMAX and high %F for orally administered fluconazole that we detected indicate that absorption of fluconazole is rapid and nearly complete after oral administration to horses. Oral absorption of fluconazole was excellent in relation to absorption after IV administration.

### Table 1—Pharmacokinetics of fluconazole after IV and oral administration of a single dose of fluconazole (10 mg/kg of body weight) to 6 healthy adult horses

<table>
<thead>
<tr>
<th>Variable</th>
<th>IV</th>
<th>Oral</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (µg/ml)</td>
<td>4.87 ± 1.03</td>
<td>NA</td>
</tr>
<tr>
<td>B (µg/ml)</td>
<td>8.09 ± 0.33</td>
<td>NA</td>
</tr>
<tr>
<td>α</td>
<td>0.97 ± 0.00</td>
<td>NA</td>
</tr>
<tr>
<td>β</td>
<td>0.02 ± 0.00</td>
<td>NA</td>
</tr>
<tr>
<td>T1/2α (h)</td>
<td>0.80*</td>
<td>NA</td>
</tr>
<tr>
<td>T1/2β (h)</td>
<td>41.6*</td>
<td>NA</td>
</tr>
<tr>
<td>T1/2β (h)</td>
<td>NA</td>
<td>37.8*</td>
</tr>
<tr>
<td>T1/2β (h)</td>
<td>NA</td>
<td>1.97 ± 1.68</td>
</tr>
<tr>
<td>C0 (µg/ml)</td>
<td>12.97 ± 1.47</td>
<td>NA</td>
</tr>
<tr>
<td>CMAX (µg/ml)</td>
<td>10.73 ± 3.48</td>
<td>NA</td>
</tr>
<tr>
<td>AUC (µg·h/ml)</td>
<td>507.40 ± 78.20</td>
<td>508.22 ± 131.23</td>
</tr>
<tr>
<td>AUMC (µg·h2/ml)</td>
<td>31,822.45 ± 10,278.07</td>
<td>28,197.13 ± 12,072.52</td>
</tr>
<tr>
<td>VAREA (L)</td>
<td>1.22 ± 0.09</td>
<td>NA</td>
</tr>
<tr>
<td>VC (L/kg)</td>
<td>0.78 ± 0.09</td>
<td>NA</td>
</tr>
<tr>
<td>VS (L/kg)</td>
<td>1.21 ± 0.09</td>
<td>NA</td>
</tr>
<tr>
<td>ClS (L/h/kg)</td>
<td>0.02 ± 0.00</td>
<td>NA</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>61.38 ± 11.49</td>
<td>54.16 ± 14.94</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>NA</td>
<td>101.24 ± 27.50</td>
</tr>
</tbody>
</table>

*Reported as harmonic mean. A = Intercept of distribution phase of curve. NA = Not applicable. B = Intercept of elimination phase of curve. α = Rate constant of distribution phase of curve. β = Rate constant of elimination phase of curve. T1/2α = Half-life of distribution. T1/2β = Half-life of elimination. T1/2 = Half-life of terminal portion of the curve. T1/2 = Time to maximum plasma concentration. K01 = Absorption rate constant. K10 = Microdistribution rate constant (IV) or terminal rate constant (oral). K12 = Michaelis elimination rate constants. C0 = Plasma concentration at time zero. CMAX = Maximum plasma concentration. AUC = Area under the concentration versus time curve from zero to infinity. AUMC = Area under the moment curve from time zero to infinity. VAREA = Volume of distribution determined by use of the area method. VC = Volume of distribution to the central compartment. VSS = Apparent volume of distribution at steady state. ClS = Systemic clearance. MRT = Mean residence time.
Adverse effects of fluconazole are infrequently reported in humans and include nausea, headache, skin rash, vomiting, abdominal pain, diarrhea,16 and dose-dependent hepatotoxicity.12 We did not detect adverse effects of fluconazole in either phase of the present study. On the basis of our results, fluconazole appears to be a highly bioavailable azole antifungal compound with a high VSS and VAREA and a long mean elimination T1/2 when administered intravenously or orally to horses. There are no other antifungal drugs currently available with this pharmacokinetic profile for use in horses.

Fluconazole has a limited spectrum of activity against filamentous fungi and Candida spp. In addition, with the exception of itraconazole, theazole group of antifungals has limited activity against Aspergillus spp.14 Indeed, most Aspergillus species isolated from horses with keratomycoses may be resistant to fluconazole.14 Because some filamentous fungi, Candida spp and Aspergillus spp, are important ocular pathogens in horses, the clinical efficacy of fluconazole may be limited despite the favorable pharmacokinetics and high aqueous humor concentrations of this drug following oral administration. However, clinical response to treatment with fluconazole in horses with ocular Fusarium infections should be more favorable.15 Fluconazole appears to be especially useful for the treatment of systemic fungal infections such as histoplasmosis, blastomycosis, and coccidioidomycosis,14,15 even though results of in vitro susceptibility tests for most fungi do not accurately reflect the therapeutic efficacy of this agent.15 The MIC of fluconazole against some fungi may be greater than that of otherazole antifungals. However, fluconazole is often more effective when used experimentally or clinically than results of in vitro susceptibility tests would predict.13,15

Fluconazole (4 or 5 mg/kg, PO, q 24 h) was administered to 2 foals with systemic candidiasis; treatment resulted in clinical resolution of the disease.18 Peak serum concentrations in these foals ranged from 4.12 to 8.08 µg/ml. However, oral administration of fluconazole according to the dosing regimen designed in the present study resulted in a mean plasma fluconazole concentration of 30.50 ± 23.88 µg/ml. The daily dose administered (5 mg/kg) was calculated to maintain plasma concentrations of fluconazole above the MIC of this agent for susceptible fungi (ie, 8.0 µg/ml)19 throughout a 24-hour interval. Predicted plasma concentrations 16 to 22 hours after administration of the last dose of fluconazole, made on the basis of results of computer simulation, ranged from 8.26 to 9.24 µg/ml. Actual plasma concentrations and concentrations in fluids of critical tissues exceeded these predictions. Therefore, long-term oral administration of fluconazole did not result in rapid elimination of the drug, which could compromise efficacy. The precise explanation for the higher than predicted concentrations of fluconazole at the end of the 10-day treatment regimen was not determined, but, clearly, there are factors that result in accumulation of the drug, which, in turn, may enhance its antifungal effects. Our results indicate that administration of a loading dose of 14 mg of fluconazole/kg PO to horses results in rapid achievement of plasma steady state concentrations. Thereafter, once daily oral dosing at 5 mg/kg will maintain plasma concentrations > 8.0 µg/ml and yield measurable concentrations of fluconazole in CSF, synovial fluid, aqueous humor, and urine of healthy adult horses.

References


Correction: Assessment of lithium dilution cardiac output as a technique for measurement of cardiac output in dogs

In the article “Assessment of lithium dilution cardiac output as a technique for measurement of cardiac output in dogs” (AJVR, Aug 2001, pp 1255–1261), The legend for Figure 4 on page 1258 is incorrect. The correct version of the figure is as follows:

![Figure 4](image-url)