Pharmacokinetics of terbinafine after oral administration of a single dose to Hispaniolan Amazon parrots (*Amazona ventralis*)

Erika E. Evans, DVM; Lee C. Emery, BS; Sherry K. Cox, PhD; Marcy J. Souza, DVM, MPH

Objective—To determine pharmacokinetics after oral administration of a single dose of terbinafine hydrochloride to Hispaniolan Amazon parrots (*Amazona ventralis*).

Animals—6 healthy adult Hispaniolan Amazon parrots.

Procedures—A single dose of terbinafine hydrochloride (60 mg/kg) was administered orally to each bird, which was followed immediately by administration of a commercially available gavage feeding formula. Blood samples were collected at the time of drug administration (time 0) and 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after drug administration. Plasma concentrations of terbinafine were determined via high-performance liquid chromatography.

Results—Data from 1 bird were discarded because of a possible error in the dose of drug administered. After oral administration of terbinafine, the maximum concentration for the remaining 5 fed birds ranged from 109 to 671 ng/mL, half-life ranged from 6 to 13.5 hours, and time to the maximum concentration ranged from 2 to 8 hours. No adverse effects were observed.

Conclusions and Clinical Relevance—Analysis of the results indicated that oral administration of terbinafine at a dose of 60 mg/kg to Amazon parrots did not result in adverse effects and may be potentially of use in the treatment of aspergillosis. Additional studies are needed to determine treatment efficacy and safety. (*Am J Vet Res* 2013;74:835–838)

Aspergillosis is a commonly reported fungal disease that results in illness and death in avian species.[1–6] Species reportedly at increased risk of infection include raptors, waterfowl, poultry, and psittacine birds, specifically Amazon parrots and African grey parrots (*Psittacus erithacus*).[1,4] Successful treatment of aspergillosis often requires long-term, systemic treatment with antifungals delivered IV, orally, topically, or via nebulization.[3,4] Drugs commonly used include itraconazole, clotrimazole, fluconazole, voriconazole, and amphotericin B, but adverse effects seen with these drugs, including liver and kidney damage, can sometimes preclude their use in some species and animals. In addition, treatment failures have been reported with the use of itraconazole and voriconazole for infections caused by certain strains of *Aspergillus fumigatus*, the most commonly isolated *Aspergillus* organism.[7]

Terbinafine hydrochloride, an allylamine antifungal commercially available in topical and oral formulations, provides primarily fungicidal action with a broad range of in vitro activity.[8] Terbinafine prevents fungal biosynthesis of ergosterol, necessary for cell membrane synthesis, via the inhibition of squalene expoxidase.[5,6,9] Historically, terbinafine has been used for the treatment of dermatophytosis, but it has been found that terbinafine is useful in the treatment of refractory and systemic fungal infections, particularly aspergillosis.[10,11] An increase in Cmax has been detected in humans when terbinafine is administered with food.[12] Oral administration of terbinafine is generally tolerated better than is other available antifungal drugs, but adverse effects, including mild to moderate gastrointestinal tract discomfort, rash or urticaria, and malaise, can develop.[10]

Currently, terbinafine is not licensed for use in veterinary medicine but is routinely used in an extralabel manner to treat fungal infections, especially dermatophytosis in small animal medicine.[13] Pharmacokinetics and dose recommendations for terbinafine have been reported for cats,[13] dogs,[14,15] horses,[15] red-tailed hawks (*Buteo jamaicensis*),[5] and penguins (*Spheniscus demersus*).[6] The recommended dose of terbinafine is 22 mg/

Abbreviations

$AUC_{0-\infty}$	Area under the plasma concentration–time curve from time 0 to infinity
Cmax	Maximum plasma concentration
MIC	Minimum inhibitory concentration
Tmax	Time to maximum plasma concentration

Received September 20, 2012.
Accepted January 17, 2013.
From the Departments of Small Animal Clinical Sciences (Evans) and Biomedical and Diagnostic Sciences (Emery, Cox, Souza), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996.
The authors thank Dr. Agricola Odoi for technical assistance and Jason Yarbrough for assistance with sample analysis.
Address correspondence to Dr. Souza (msouza@utk.edu).

kg/d and 15 mg/kg/d for red-tailed hawks[5] and penguins,[6] respectively. We are not aware of any published doses derived from pharmacokinetic or pharmacodynamic studies for psittacine species such as Amazon parrots. Oral administration of doses of 15 mg/kg reportedly have been used clinically for the treatment of aspergillosis in psittacine birds with favorable results,[16] but preliminary pharmacokinetic trials with that dose failed to yield measurable plasma concentrations in African grey parrots.[17]

Comparative in vitro testing has revealed that terbinafine is more effective against *Aspergillus* spp than are other drugs (including azole antifungal drugs and amphotericin B) commonly used to treat diseases attributable to those organisms.[4,16,18] The elimination of terbinafine in humans requires < 5% of the total cytochrome P450 capacity of the liver, which may minimize adverse effects when terbinafine is used alone or in combination with other antifungal drugs. Terbinafine also has a better safety profile than the azole antifungals and amphotericin B, with few adverse effects reported in human and veterinary medicine.[5,6,8,9]

The purpose of the study reported here was to determine the pharmacokinetics of terbinafine after oral administration to Hispaniolan Amazon parrots (*Amazona ventralis*). These parrots were selected for use in the study because of their popularity as companion animals and the high frequency of aspergillosis in Amazon parrots, compared with the frequency of the disease in other species of psittacine birds.[4] The size of the parrots also enabled us to safely collect an adequate volume of blood from each bird at all time points.

Materials and Methods

Animals—The study involved 6 apparently healthy adult Hispaniolan Amazon parrots; each parrot weighed between 274 and 329 g. All birds had a healthy appearance and unremarkable results for a physical examination, CBC, and plasma biochemical analysis during the 6 months preceding the study. No drugs had been administered to the birds during the 12 months preceding the study.

All birds were housed separately or in pairs in wire cages. The room was maintained at a constant temperature of 22°C with a photoperiod of 12 hours of light and 12 hours of darkness. The birds were fed a commercially available pelleted diet[a] and were provided water ad libitum; food was changed in the morning and offered continuously. The diet was periodically supplemented with fresh fruit and vegetables. The experimental protocol was approved by the University of Tennessee Institutional Animal Care and Use Committee.

Experimental design—Birds were orally administered a single dose of terbinafine (60 mg/kg) into the crop with a stainless steel feeding tube (time 0). To ensure a fed condition, approximately 6 mL of a commercially available gavage feeding formula[b] was placed directly into the crop immediately after drug administration. The feeding formula was administered via a 12-mL syringe attached to the same stainless steel feeding tube used to administer the drug (the tube was not removed between drug and food administration). Food was also offered continuously during the period following administration when blood samples were collected.

The terbinafine hydrochloride suspension (25 mg/mL) was created as previously described[19] from terbinafine hydrochloride tablets (250 mg/tablet). Briefly, the tablets were ground with a mortar and pestle, and the powder was mixed with 2 suspension vehicles.[c,d] The suspension was mixed until uniform, labeled, and then stored in the refrigerator prior to administration. The terbinafine suspension was administered within 24 to 48 hours after it was created, which is well within the time that the suspension would remain stable.[19]

Venous blood samples were collected from each bird at 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after drug administration. At each time point, each bird was manually restrained and a blood sample (0.25 to 0.3 mL) was collected from the right or left jugular vein by use of a 26-gauge needle attached to a 1-mL plastic insulin syringe. Each blood sample was immediately transferred to a lithium heparin blood tube and placed in a refrigerator for a maximum of 2 hours, after which samples were centrifuged (16,000 × *g* for 10 minutes) and the plasma decanted. Plasma was stored at –80°C until subsequent analysis. All samples were analyzed within 3 months after collection.

Measurement of plasma concentrations of terbinafine—Plasma samples were analyzed by personnel at the Clinical Pharmacology Laboratory at the University of Tennessee via a reverse-phase high-performance liquid chromatography method. The system consisted of a separation module,[e] absorbance detector,[f] and computer equipped with chromatography software.[g] Terbinafine was extracted from plasma samples via liquid extraction. Briefly, frozen plasma samples were thawed and mixed in a vortexer, and 100 µL of plasma was transferred to a clean screw-top test tube. Then, 75 µL of an internal standard (butenafine [1.0 µg/mL]) was added to each tube. Hexane (3 mL) also was added to each tube, and the tubes were mixed in a rocker for 20 minutes and then centrifuged for 20 minutes at 1,000 × *g*. The organic layer was transferred to a clean tube and evaporated to dryness with nitrogen gas. Samples were reconstituted in 250 µL of mobile phase, which was a mixture of 20mM phosphoric acid with 0.1% triethylamine (pH, 3.0) and acetonitrile (65:35), and 100 µL of each reconstituted sample was analyzed.

Compounds were separated on a C18 column[g] (4.6 × 100 mm, 5 µm) with a C18 guard column[h]; the column was maintained at ambient temperature (approx 22°C). A mixture of 20mM phosphoric acid with 0.1% triethylamine (pH, 3.0) and acetonitrile (65:35) was used as the eluent. Flow rate was 1.1 mL/min. Absorbance was measured at 224 nm.

Standard curves for analysis of plasma concentration were created with calibration samples made by fortifying untreated, pooled Hispaniolan Amazon parrot plasma with terbinafine to achieve concentrations (range, 5 to 1,500 ng/mL) that yielded a linear result. Calibration samples were prepared in exactly the same manner as plasma samples obtained from the parrots after drug administration. Mean recovery for terbinafine was 95%, and intra-assay and inter-assay variability ranged from 4.5% to 8.5% and 0.93%

to 8.5%, respectively. The lower limit of quantification was 5 ng/mL.

Pharmacokinetic analyses—Pharmacokinetics for terbinafine were calculated with software.[i] Mean plasma concentrations of terbinafine over time were plotted with software.[i] Values for plasma half-life, Cmax, Tmax, and $AUC_{0-\infty}$ were calculated from noncompartmental analysis. The area under the curve was calculated on the basis of the log-linear trapezoidal rule. Mean residence time was calculated as the total area under the first moment curve from time 0 to infinity/$AUC_{0-\infty}$.

Results

No adverse effects were detected during the study. Data for 1 bird were excluded because the results suggested a possible error in the dose of drug administered. Pharmacokinetic data for the remaining 5 birds were summarized (**Table 1**). Mean plasma concentrations of terbinafine over time were plotted (**Figure 1**). The Cmax ranged from 109 to 671 ng/mL, plasma half-life ranged from 6 to 13.5 hours, and Tmax ranged from 2 to 8 hours.

Discussion

The objective of the present study was to determine whether oral administration of terbinafine to Hispanolian Amazon parrots at a dose consistent with doses used in other species would provide potentially therapeutic plasma concentrations. The in vitro MICs for *Aspergillus flavus* and *A fumigatus* have been reported as 0.01 to 0.5 µg/mL and 0.02 to 5.0 µg/mL, respectively.[8] Target peak therapeutic plasma concentrations used for determining appropriate drug administration in red-tailed hawks have been extrapolated from the MIC for *A fumigatus* and were 1 to 4 µg/mL,[5] but to our knowledge, in vivo therapeutic concentrations have not been established for the treatment of aspergillosis in any species of bird. The peak concentrations achieved by administration at a dose of 60 mg/kg in the present study were within the range for the in vitro MIC, but the maximum concentrations of all parrots failed to reach the therapeutic range of 1 to 4 µg/mL suggested for red-tailed hawks.

In a preliminary study, 3 Hispaniolan Amazon parrots from which food had been withheld were orally administered terbinafine at a dose of 30 mg/kg; the dose was selected on the basis of the fact that potentially therapeutic plasma concentrations were achieved in red-tailed hawks administered that dose of terbinafine[5]; however, the plasma concentrations thought to be therapeutic in red-tailed hawks were not reached in those 3 Hispanolian Amazon parrots. A 60 mg/kg dose in fed birds was chosen for the present study in an attempt to increase plasma concentrations. Absorption characteristics of terbinafine were not affected when the drug was administered with food to red-tailed hawks,[5] but in humans, consumption of food resulted in an increase in plasma concentrations.[12] In addition, ill birds infected with *Aspergillus* organisms often fail to ingest a sufficient number of calories to meet metabolic needs and are routinely fed via gavage to provide nutritional support at the time of medication administration. For this reason, in the opinion of one of the investigators (MJS), terbinafine pharmacokinetics in fed birds are likely more clinically relevant than are pharmacokinetics for unfed birds.

For the 60 mg/kg dose, Tmax ranged from 2 to 8 hours, which is similar to the Tmax of terbinafine when administered to other avian species.[5,6] Substantial variation among the parrots was evident for Cmax (range, 109 to 671 ng/mL) and terminal half-life (5.99 to 13.51 hours), but these values were similar to values reported in other avian species.[5,6] Unfortunately, data for 1 bird were excluded because of possible problems with the dose of drug administered.

Regurgitation is a reported adverse effect after terbinafine administration in avian species, but it has been observed only after frequent administration or administration of high doses.[5] No adverse effects were detected for the Amazon parrots in the present study. Additional studies that include use of the dose of 60 mg/kg as well as higher doses and repeated administration of doses need to be performed to more fully assess the safety of terbinafine in this avian species.

Analysis of results of the present study suggests that higher doses of ter-

Table 1—Pharmacokinetic values after oral administration of terbinafine hydrochloride (60 mg/kg) to fed Hispaniolan Amazon parrots (*Amazona ventralis*).

Variable	Mean	Range
Terminal half-life (h)	8.71	8.56–13.51
Elimination rate constant (1/h)	0.09	0.05–0.12
Cmax (ng/mL)	353	109–671
Tmax (h)	6.4	2.0–8.0
$AUC_{0-\infty}$ (h•ng/mL)	3,345	1,902–4,436
$MRT_{0-\infty}$ (h)	13.03	8.56–21.92

Results represent data for 5 birds; data for 1 other bird were discarded because of a possible error in the dose of drug administered.
$MRT_{0-\infty}$ = Mean residence time from time 0 to infinity.

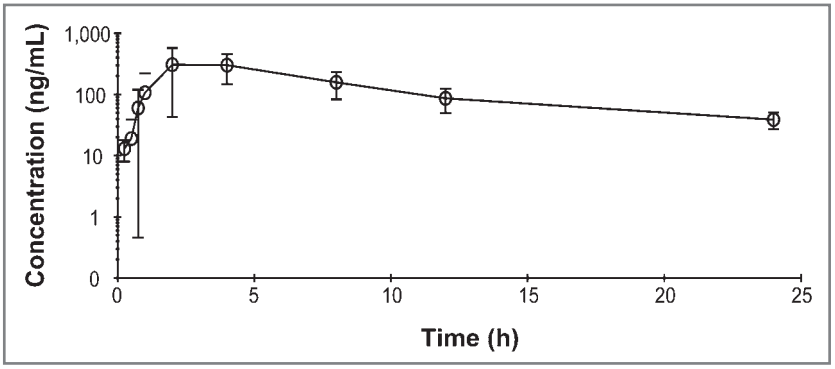

Figure 1—Mean ± SD plasma concentrations after oral administration of a single dose of terbinafine (60 mg/kg) to 5 Hispaniolan Amazon parrots (*Amazona ventralis*). Time of drug administration was designated as time 0. Birds were administered terbinafine, which was followed immediately by administration of a commercially available gavage feeding formula to ensure a fed state. Data for 1 other bird were excluded from analysis because of a possible error in the dose of drug administered.

binafine (≥ 60 mg/kg) may be needed to achieve potentially therapeutic plasma concentrations in Amazon parrots. This is in contrast to the suggested doses of terbinafine for red-tailed hawks (22 mg/kg)[5] or penguins (15 mg/kg).[6] Pharmacokinetic studies of terbinafine in avian species have relied on in vitro–based determinations of MICs, and determining the in vivo concentrations of terbinafine necessary to treat aspergillosis in avian species would be extremely beneficial in future pharmacological studies. No adverse effects were observed in this study; however, additional trials with a larger number of birds that have been fed and, possibly, from which food has been withheld and additional pharmacokinetic and pharmacodynamic studies at higher and varied doses are warranted to evaluate safety and effects of multiple doses.

a. Lafeber's Premium Daily Diet, Lafeber Co, Cornell, Ill.
b. Exact hand feeding formula, Kaytee Products Inc, Chilton, Wis.
c. Ora-Plus oral suspending vehicle (50 mL), Paddock, Minneapolis, Minn.
d. Ora-Sweet syrup (100 mL), Paddock Laboratories Inc, Minneapolis, Minn.
e. 2695 separations module, Waters Corp, Milford, Mass.
f. 2487 absorbance detector, Waters Corp, Milford, Mass.
g. Empower software, Waters Corp, Milford, Mass.
h. Symmetry Shield, Waters Corp, Milford, Mass.
i. WinNonlin, version 5.2, Pharsight Corp, Mountain View, Calif.

References

1. O'Meara D, Witter J. Aspergillosis. In: Davis JW, Anderson RC, Karstad L, et al. *Infectious and parasitic diseases of wild birds*. Ames, Iowa: Iowa State University Press, 1971;153–162.
2. McMillan MC, Petrak ML. Retrospective study of aspergillosis in pet birds. *J Assoc Avian Vet* 1989;3:211–215.
3. Tsai SS, Park JH, Hirai K, et al. Aspergillosis and candidiasis in psittacine and passeriforme birds with particular reference to nasal lesions. *Avian Pathol* 1992;21:699–709.
4. Aguilar R, Redig P. Diagnosis and treatment of avian aspergillosis. In: Bonagura JD, ed. *Kirk's current veterinary therapy XII: small animal practice*. Philadelphia: WB Saunders Co, 1995;1294–1299.
5. Bechert U, Christensen J, Poppenga R, et al. Pharmacokinetics of terbinafine after single oral dose administration in red-tailed hawks (*Buteo jamaicensis*). *J Avian Med Surg* 2010;24:122–130.
6. Bechert U, Christensen JM, Poppenga R, et al. Pharmacokinetics of orally administered terbinafine in African penguins (*Spheniscus demersus*) for potential treatment of aspergillosis. *J Zoo Wildl Med* 2010;41:263–274.
7. Beernaert LA, Pasmans F, Waeyenberghe L, et al. Avian *Aspergillus fumigatus* strains resistant to both itraconazole and voriconazole. *Antimicrob Agents Chemother* 2009;53:2199–2201.
8. Balfour JA, Faulds D. Terbinafine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. *Drugs* 1992;43:259–284.
9. Orosz S, Frazier D, Schroeder E, et al. Pharmacokinetic properties of itraconazole in blue-fronted Amazon parrots (*Amazona aestiva aestiva*). *J Avian Med Surg* 1996;10:168–173.
10. Villars VV, Jones TC. Special features of the clinical use of oral terbinafine in the treatment of fungal diseases. *Br J Dermatol* 1992;126(suppl 39):61–69.
11. Revankar SG, Nailor MD, Sobel JD. Use of terbinafine in rare and refractory mycoses. *Future Microbiol* 2008;3:9–17.
12. Nedelman J, Cramer JA, Robbins B, et al. The effect of food on the pharmacokinetics of multiple-dose terbinafine in young and elderly healthy subjects. *Biopharm Drug Dispos* 1997;18:127–138.
13. Wang A, Ding H, Liu Y, et al. Single dose pharmacokinetics of terbinafine in cats. *J Feline Med Surg* 2012;14:540–544.
14. Sakai MR, May ER, Imerman PM, et al. Terbinafine pharmacokinetics after single dose oral administration in the dog. *Vet Dermatol* 2011;22:528–534.
15. Williams MM, Davis EG. KuKanich B. Pharmacokinetics of oral terbinafine in horses and Greyhound dogs. *J Vet Pharmacol Ther* 2011;34:232–237.
16. Dahlhausen B, Lindstrom J, Radabaugh C. The use of terbinafine hydrochloride in the treatment of avian fungal disease, in *Proceedings*. Annu Conf Assoc Avian Vet 2000;35–39.
17. Orosz S, Flammer K. Avian mycoses: managing these difficult diseases, in *Proceedings*. Annu Conf Assoc Avian Vet 2008;153–163.
18. Orosz SE. Antifungal drug therapy in avian species. *Vet Clin North Am Exot Anim Pract* 2003;6:337–350.
19. Abdel-Rahman SM, Nahata MC. Stability of terbinafine hydrochloride in an extemporaneously prepared oral suspension at 25 and 4 degrees C. *Am J Health Syst Pharm* 1999;56:243–245.