Pharmacokinetics of voriconazole after oral administration of single and multiple doses in Hispaniolan Amazon parrots (Amazona ventralis)

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Objective—To determine the pharmacokinetics and safety of voriconazole administered orally in single and multiple doses in Hispaniolan Amazon parrots (Amazona ventralis).

Animals—15 clinically normal adult Hispaniolan Amazon parrots.

Procedures—Single doses of voriconazole (12 or 24 mg/kg) were administered orally to 15 and 12 birds, respectively; plasma voriconazole concentrations were determined at intervals via high-pressure liquid chromatography. In a multiple-dose trial, voriconazole (18 mg/kg) or water was administered orally to 6 and 4 birds, respectively, every 8 hours for 11 days (beginning day 0); trough plasma voriconazole concentrations were evaluated on 3 days. Birds were monitored daily, and clinicopathologic variables were evaluated before and after the trial.

Results—Voriconazole elimination half-life was short (0.70 to 1.25 hours). In the single-dose experiments, higher drug doses yielded proportional increases in the maximum plasma voriconazole concentration (C_max) and area under the curve (AUC). In the multiple-dose trial, C_max, AUC, and plasma concentrations at 2 and 4 hours were decreased on day 10, compared with day 0 values; however, there was relatively little change in terminal half-life. With the exception of 1 voriconazole-treated parrot that developed polyuria, adverse effects were not evident.

Conclusions and Clinical Relevance—In Hispaniolan Amazon parrots, oral administration of voriconazole was associated with proportional kinetics following administration of single doses and a decrease in plasma concentration following administration of multiple doses. Oral administration of 18 mg of voriconazole/kg every 8 hours would require adjustment to maintain therapeutic concentrations during long-term treatment. Safety and efficacy of voriconazole treatment in this species require further investigation. (Am J Vet Res 2010;71:460–467)

Voriconazole, a second-generation triazole that is available in IV and oral formulations, provides a new and improved treatment option for fungal infections.1 Voriconazole is modified from fluconazole by substitution of a fluoropyrimidine ring for one of theazole groups and addition of an α-methyl group. These modifications result in activity against a greater variety of fungal organisms. Voriconazole interacts with fungal cytochrome P-450, thereby inhibiting C-14 demethylation of lanosterol and preventing the conversion of lanosterol to ergosterol; ergosterol is necessary for cell membrane synthesis.2 This antifungal agent has good in vivo and in vitro activities against a variety of yeast and filamentous and dimorphic fungi (including Candida spp, Cryptococcus neoformans, and Aspergillus spp) but has little or no activity against zygomycetes.3-10 Voriconazole has been shown to be more effective than

ABBREVIATIONS

<table>
<thead>
<tr>
<th>ABBREVIATIONS</th>
<th>DESCRIPTION</th>
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<tr>
<td>AUC</td>
<td>Area under the plasma concentration-versus-time curve</td>
</tr>
<tr>
<td>CI/F</td>
<td>Clearance per fraction absorbed</td>
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<tr>
<td>C_max</td>
<td>Maximum plasma concentration</td>
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<tr>
<td>HPLC</td>
<td>High-pressure liquid chromatography</td>
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<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>NLMEM</td>
<td>Nonlinear mixed-effects model</td>
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<tr>
<td>Vd/F</td>
<td>Volume of distribution per fraction absorbed</td>
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Voriconazole has been used in birds for the treatment of aspergillosis, but the number of pharmacokinetic studies is limited. In chickens, the oral bioavailability of voriconazole was <20% and the half-life was short (1.25 hours). Plasma concentrations achieved via oral administration of 10 mg of voriconazole/kg every 24 hours were low (<0.5 µg/mL), but concentrations in some tissues were considerably higher, which suggested that such treatment would result in concentrations that were effective against Candida spp and Aspergillus spp in most tissues. In that study, chickens, nonlinear pharmacokinetics in single-dose experiments and autoinduction of metabolism enzymes in multiple-dose trials were not demonstrated. In a study involving falcons, the influence of food intake on voriconazole bioavailability and the safety of drug treatment were evaluated. Six falcons were administered voriconazole (12.5 mg/kg, PO [via crop gavage], q12 h) for 14 days. Resultant peak plasma concentrations were high (>1 µg/mL), but trough concentrations were low and sometimes undetectable. Compared with findings in that group, plasma concentrations in another falcon that received doses of voriconazole incorporated into meat were 21% to 26% lower. In another study in African grey parrots, the half-life of voriconazole was short (1.1 to 1.6 hours) and higher doses resulted in disproportional increases in plasma concentrations, suggesting nonlinear pharmacokinetics. Trough plasma concentrations achieved in the multiple-dose trial in that study were lower than those achieved after administration of single doses, suggesting that the drug induces its own metabolism. Dosages of 12 to 18 mg/kg administered orally every 12 hours were recommended as a starting point to target plasma concentrations >0.4 µg/mL, but higher dosages could be needed for long-term treatment. In pigeons, oral bioavailability of voriconazole was determined to be 43.7%; in those birds, nonlinear voriconazole pharmacokinetics were determined in single-dose experiments and autoinduction of metabolism enzymes was identified in a multiple-dose trial. On the basis of those data, oral administration of 10 mg/kg every 12 hours or 20 mg/kg every 24 hours was recommended for pigeons. The reported differences in enteral absorption, saturable nonlinear pharmacokinetics, and induction of metabolism enzymes indicate the necessity of specific studies to determine dosage recommendations for different species.

The purpose of the study reported here was to determine the pharmacokinetics and safety of voriconazole administered orally in single and multiple doses in Hispaniolan Amazon parrots (Amazona ventralis). These parrots were selected because of their availability and the popularity of Amazon parrots as companion animals. The small body size of these birds precluded collection of blood samples at all time points from any single bird, so naive pooling of drug concentrations from multiple birds was used to plot concentration-versus-time curves and calculate pharmacokinetic values for the single-dose experiments. This method has been used successfully in previous studies.

Materials and Methods

Animals—Fifteen adult Hispaniolan Amazon parrots that weighed 260 to 320 g were used. All birds were considered clinically normal on the basis of physical examination findings and results of a CBC. The birds had not received any drug within the last 12 months. Birds were housed individually in suspended wire cages in rooms maintained at 22°C with a 12-hour photoperiod. The birds were fed a pelleted diet and had unlimited access to water. The Louisiana State University Institutional Animal Care and Use Committee approved the experimental protocol.

Experimental design for single-dose experiments—In the single-dose experiments, 2 doses of voriconazole (12 and 24 mg/kg) were evaluated. All 15 birds were used in the 12 mg/kg single-dose experiment, and 12 of the 15 birds were used in the 24 mg/kg single-dose experiment. For the birds that were used in both single-dose experiments, an interval of 6 months was allowed to elapse between treatments. For each single-dose experiment, food was withheld from the birds for 4 hours and they were weighed prior to being randomly assigned to 3 groups (groups A, B, and C). For the 12 mg/kg single-dose experiment, 5 birds were assigned to each group; for the 24 mg/kg single-dose experiment, 4 birds were assigned to each group. Each bird was manually restrained, and voriconazole suspension was administered directly into the crop by use of a 3-ml syringe and a stainless-steel feeding tube. The voriconazole suspension (2.5 mg/ml) was compounded from voriconazole tablets (50 mg); the tablets were ground by use of a mortar and pestle, and the powder was mixed with a suspending vehicle and distilled water (3:1 [vol/vol]).

Venous blood samples were collected from each bird at intervals after administration of the single dose of voriconazole. After dose administration, samples
were collected at 0.5, 4, 12, and 48 hours from birds in group A; at 1, 6, and 24 hours from birds in group B; and at 2, 8, and 32 hours from birds in group C. At each designated time point, each bird was manually restrained and a blood sample (0.7 to 1 mL) was collected from either the right or left jugular vein by use of a 23-gauge needle attached to a 3-mL plastic syringe that was precoated with heparin. Each blood sample was transferred to a 3-mL tube that was not precoated with heparin, centrifuged (10,000 × g for 5 minutes), and decanted within 2 hours of the collection. The plasma was stored at −70°C for later analysis.

Experimental design for the multiple-dose trial—For the multiple-dose trial, 10 of the birds used in both single-dose experiments were used. A 6-month wash-out period was allowed to elapse before the multiple-dose trial was performed. Six birds were allocated to the treatment group, and 4 birds were allocated to the control group. The treatment group received voriconazole (18 mg/kg, PO, q 8 h) for 11 days. The drug was administered directly into the crop by use of a 3-mL syringe and a stainless-steel feeding tube. The voriconazole suspension (2.5 mg/mL) was compounded as described for the single-dose experiments. The mixed suspension was stored at 5°C and protected from light until administered. This formulation is stable under refrigeration for at least 14 days. The control group received an equivalent volume of tap water administered in the same manner every 8 hours for 11 days. The day on which the first dose of the drug or water was administered was designated as day 0.

Venous blood samples were collected after the first drug administration of the day from each bird at 2, 4, and 5 hours on day 0; at 2 and 5 hours on day 7; and at 2, 4, and 5 hours on day 10. At each designated time point, each bird was manually restrained and a blood sample (0.7 to 1 mL) was collected from either the right or left jugular vein by use of a 23-gauge needle attached to a 3-mL plastic syringe that was precoated with heparin. Each blood sample was transferred to a 3-mL tube that was not precoated with heparin, centrifuged (10,000 × g for 5 minutes), and decanted within 2 hours of the collection. The plasma was stored at −70°C for later analysis.

All birds were monitored during the trial for signs of adverse effects. At the times of drug or water administration each day, birds were observed for signs of regurgitation (present or absent) or polyuria (assessed subjectively as none, mild, moderate, or severe) and their activity level was evaluated. They also were weighed and visually examined each morning before administration of the first dose of voriconazole or water. Analysis of PCV, totals solids concentration, and plasma biochemical variables (activities of aspartate aminotransferase and creatine kinase and concentrations of bile acids, uric acid, glucose, calcium, phosphorus, total protein, albumin, globulin, potassium, and sodium) was performed during the week before day 0 and on days 10 and 14. Plasma biochemical analyses were performed by use of a point-of-care analyzer.

Measurement of plasma voriconazole concentration—For both the single-dose experiments and the multiple-dose trial, voriconazole concentrations in plasma samples were determined by use of a method that was previously validated at our institution. Drug concentrations were determined by use of HPLC with UV detection at 263 nm. All plasma samples underwent solid-phase extraction by use of cyano-bonded cartridges prior to injection into the HPLC system, as previously described. Sample volume was 150 to 200 µL. Following extraction, the samples were eluted with 1 mL of methanol and the eluent was evaporated under compressed air at 40°C for 30 minutes. Samples were reconstituted with 200 µL of mobile phase, which consisted of double-deionized water and HPLC-grade acetonitrile (50:50 [vol/vol]). A reverse-phase C8 column was used for separation. The flow rate was 1.0 mL/min, and the sample injection volume was 25 µL.

Prior to each days HPLC run, calibration curves were prepared by fortifying a pooled sample of plasma obtained from 4 untreated Hispaniolan Amazon parrots with known concentrations of voriconazole reference standard (99% pure). A blank sample was processed and analyzed at the beginning of each assay to check for interfering peaks. Calibration curves were linear between the concentrations of 0.05 and 10 µg/mL (r² > 0.99), and all values were within 15% of the expected range. The lower limit of quantification was defined as the lowest concentration on the linear portion of the calibration curve, as determined on the basis of regression analysis of the calibration curve. For these conditions, the value for voriconazole in plasma was 0.05 µg/mL. The analysis of the samples was performed by the Clinical Pharmacology Laboratory at North Carolina State University.

Pharmacokinetic analyses—Naïve pooling of data points was used to generate a mean plasma voriconazole concentration at each time point. A computer program was used to determine pharmacokinetic values by use of standard equations for both noncompartmental and compartmental analysis. Data were uniformly weighted for noncompartmental analysis. On the basis of visual inspection of the plasma concentration-versus-time curve plotted on semilogarithmic paper and by use of the Akaike information criterion, data for both single-dose experiments best fit a 1-compartment open model with first-order input. Fit of the predicted curve was improved by adding a lag phase to the 24 mg/kg single-dose experiment and by weighting the data from both single-dose experiments. The weighting factors were 1/|yhat| for the 12 mg/kg experiment and 1/|y²| for the 24 mg/kg trial, where y is the plasma concentration of voriconazole and what is the predicted voriconazole concentration. The model was described by an equation as follows:

\[
C = (|k_{i0}\times F\times D|)/Vd \times (k_{i0} - k_{i10}) \times (e^{-k_{10}\times t-[dag]} - e^{-k_{i10}\times t-[dag]})
\]

where C is the plasma concentration, k₁₀ is the absorption rate constant, F is the fraction of drug absorbed, D is the orally administered dose, Vd is the apparent volume of distribution, k₁₀ is the elimination rate constant, t is time, and [dag] is the estimated lag time.

The bioavailability of voriconazole was not calculated because the study design did not include evalu-
ation of a dose administered IV. Therefore, results for volume of distribution and clearance are reported on the basis of the fraction absorbed (ie, Vd/F and Cl/F, respectively). The dose proportionality of the kinetics was determined by comparing the AUC-to-dose ratio for each dose. By use of a computer simulation program, distribution and elimination rate constants were calculated for the 24 mg/kg single-dose experiment and used to simulate predicted concentrations after multiple doses of voriconazole.

Data for the single-dose experiments were also evaluated by use of a computer program† and an NLME model. Because of the sparse sampling structure used in the study, it was not possible to estimate nonlinear regression parameters for each individual. Therefore, population parameters were estimated by use of first-order linearization of a hierarchical nonlinear model. Several models were considered with this method in which parameters were a function of both fixed effects and random effects. The fixed effects considered for the study were the values of the primary parameters (eg, Vd, K_{01}, and K_{10}) that described the typical value for the population. The random effect varied randomly among individuals. For the study, an identity model was used in which constant within-subject error variance that had a normal distribution with a constant variance was assumed. The pharmacokinetic model selected was a 1-compartment model with first-order input. The formula for the model was as follows:

\[ C_t = \frac{D \times K_{01}}{V_d/F \times (K_{10} - K_{01})} \times (\exp[-K_{01} \times T] - \exp[-K_{10} \times T]) \]

where D is the dose (mg/kg), K_{01} is the absorption rate, K_{10} is the elimination rate, and T is time (hours). In this model, no covariates were assumed because the study was performed in a uniform population of birds, in which there were no covariates or subpopulations to be examined. The mixed effects for this model were as follows:

\[ \frac{V_d/F}{\theta} = \theta_1 \times \eta_1, \quad K_{01} = \theta_2 \times \eta_2, \quad K_{10} = \theta_3 \times \eta_3, \]

where \( \theta \) represents the fixed effect for the parameter and \( \eta \) represents the corresponding random effect. Various models were tested, including an additive and multiplicative model, but there was no improvement in the fit with more complicated models. Models were compared by observation of plots and examination of the Akaike information criterion.

Statistical analysis—Values are expressed as the mean ± SEM. Plasma concentrations from the 2-, 4-, and 5-hour samples on days 0 and 10 of the multiple-dose trial were compared by use of the Mann-Whitney rank sums test. A value of \( P < 0.05 \) was considered significant.

Results

**Single-dose experiments**—Plasma concentration-versus-time curves were derived from the single-dose experiment data (Figure 1). Pharmacokinetic parameters were calculated by use of noncompartmental and compartmental and nonlinear mixed-effect methods (Tables 1 and 2). The noncompartmental results indicated that an increase in the voriconazole dose from 12 to 24 mg/kg increased the \( \text{C}_{\text{max}} \) and AUC in an approximately dose-proportional manner and that the AUC-to-dose ratios for the 2 experiments were similar. No

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**Figure 1—Mean ± SEM plasma concentrations of voriconazole in Hispaniolan Amazon parrots after oral administration of a single dose of voriconazole (12 mg/kg [n = 15; black circles] or 24 mg/kg [12; white circles]). Fifteen parrots were used in the study, and birds were included in 1 or both single-dose experiments. For each single-dose experiment, birds were assigned to 1 of 3 groups (designated as A, B, and C); each group included 4 (24 mg/kg dose experiment) or 5 (12 mg/kg dose experiment) birds. After dose administration, venous blood samples were collected at 0.5, 4, 12, and 48 hours from birds in group A; at 1, 6, and 24 hours from birds in group B; and at 2, 8, and 32 hours from birds in group C. Naïve pooling of datum points was used to generate a mean plasma voriconazole concentration at each time point. Plasma concentrations were less than the lower limit of quantification after 6 and 8 hours in the 12 and 24 mg/kg experiments, respectively, and are not shown.**

Table 1—Pharmacokinetic parameters derived via noncompartmental analysis after oral administration of voriconazole (single-dose experiments) in Hispaniolan Amazon parrots.*

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<th>Drug dose</th>
<th>12 mg/kg</th>
<th>24 mg/kg</th>
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<tr>
<td>C_{\text{max}} (µg/mL)</td>
<td>2.49</td>
<td>5.08</td>
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<tr>
<td>t_{\text{max}} (h)</td>
<td>0.90</td>
<td>1.25</td>
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<tr>
<td>MRT (h)</td>
<td>1.97</td>
<td>2.61</td>
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<tr>
<td>AUC_{\text{0–72}} (h•µg/mL)</td>
<td>7.61</td>
<td>18.38</td>
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<tr>
<td>Vz/F (mL/kg)</td>
<td>2.094 ± 0.15</td>
<td>2.349 ± 0.18</td>
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<tr>
<td>CI/F (mL/hr/kg)</td>
<td>1,575 ± 56</td>
<td>1,306 ± 01</td>
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<tr>
<td>AUC-to-dose ratio</td>
<td>0.83</td>
<td>0.77</td>
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* Fifteen birds were used in the 12 mg/kg single-dose experiment, and 12 of those 15 birds were used in the 24 mg/kg single-dose experiment. For each single-dose experiment, birds were assigned to 1 of 3 groups (designated as A, B, and C); each group included 4 (24 mg/kg single-dose experiment) or 5 (12 mg/kg single-dose experiment) birds. After dose administration, venous blood samples were collected at 0.5, 4, 12, and 48 hours from birds in group A; at 1, 6, and 24 hours from birds in group B; and at 2, 8, and 32 hours from birds in group C. Naïve pooling of datum points was used to generate a mean plasma voriconazole concentration at each time point, and those mean values were used for pharmacokinetic analysis.

\( \text{AUC}_{\text{0–72}} = \) Area under the plasma concentration-versus-time curve extrapolated to infinity. \( \text{MRT} = \) Mean residence time. \( t_{\text{max}} = \) Half-life of elimination phase. \( t_{\text{max}} = \) Time to achieve maximal concentration. \( \text{Vz/F} = \) Apparent volume of distribution during terminal elimination phase per fraction absorbed.
adverse effects of treatment were observed during the experimental period (48 hours).

Pharmacokinetic parameters were derived from compartmental modeling and used to create plots of actual and predicted plasma concentration versus time for each single-dose experiment (Figure 2; Table 2). There was wide intra-individual variation. Simulated plasma concentrations predicted for an 18 mg/kg dose administered orally every 8 hours varied from a peak concentration of 3.9 µg/mL to a trough concentration of 0.11 µg/mL. At this dose, there was no accumulation, and predicted plasma concentrations were >0.4 µg/mL for approximately 60% to 70% of the dosing interval. Pharmacokinetic parameters estimated by use of the NLMEM were similar to those estimated via naïve pooling of datum points.

Multiple-dose trial—A dose of 18 mg/kg administered orally every 8 hours was selected for the multiple-dose trial. The dose represented a practical compromise of balancing the difficulty of frequent

<table>
<thead>
<tr>
<th>Drug dose</th>
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<th>24 mg/kg</th>
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<tbody>
<tr>
<td>PKM</td>
<td>NLMEM</td>
<td>PKM</td>
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<tr>
<td>T_{max} (h)</td>
<td>1.01</td>
<td>0.96 ± 0.33</td>
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<tr>
<td>C_{max} (µg/mL)</td>
<td>2.58</td>
<td>2.81 ± 0.31</td>
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<tr>
<td>K_{a} (1/h)</td>
<td>0.99</td>
<td>0.98 ± 0.11</td>
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<tr>
<td>K_{e} (1/h)</td>
<td>0.99</td>
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<td>K_{a1} (h)</td>
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<td>0.68 ± 0.07</td>
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<td>K_{e1} (h)</td>
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<tr>
<td>AUC_{0-∞} (h•µg/mL)</td>
<td>7.10</td>
<td>7.62 ± 1.15</td>
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<tr>
<td>Vd/F (mL/kg)</td>
<td>1,703.41</td>
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<td>Cl/F (mL/h/kg)</td>
<td>1,691.32</td>
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<td>AUC-to-dose ratio</td>
<td>0.59</td>
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<td>Lag time (h)</td>
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Values derived by use of the NLMEM are reported as mean ± SEM, where applicable.

*K_{a} = Absorption rate constant. K_{e} = Elimination rate constant. K_{a1}t_{½} = Half-life of absorption phase. K_{e1}t_{½} = Half-life of elimination phase.

See Table 1 for remainder of key.

Pharmacokinetic parameters were derived from compartmental modeling and used to create plots of actual and predicted plasma concentration versus time for each single-dose experiment (Figure 2; Table 2). There was wide intra-individual variation. Simulated plasma concentrations predicted for an 18 mg/kg dose administered orally every 8 hours varied from a peak concentration of 3.9 µg/mL to a trough concentration of 0.11 µg/mL. At this dose, there was no accumulation, and predicted plasma concentrations were >0.4 µg/mL for approximately 60% to 70% of the dosing interval. Pharmacokinetic parameters estimated by use of the NLMEM were similar to those estimated via naïve pooling of datum points.

Multiple-dose trial—A dose of 18 mg/kg administered orally every 8 hours was selected for the multiple-dose trial. The dose represented a practical compromise of balancing the difficulty of frequent
drug administration in birds with maintaining effective plasma concentrations. Compared with day 0 findings, plasma concentrations measured at 2 and 4 hours after the first drug administration of the day were significantly (P = 0.009 on both days) lower on day 10 (Figure 3). The AUC for the 2- to 5-hour interval was also markedly decreased (Table 3); however, there was little change in elimination half-life.

No adverse effects were detected via observation or examination in the control group; 1 parrot in the treatment group had mild polyuria throughout the 11 days of the trial. Clinicopathologic analyses performed before commencement of the multiple-dose trial and at days 10 and 14 revealed that all variables of interest were within reference ranges for both the treatment and control groups. In both groups, there was no clinically relevant change in body weight or activity level; abnormal behavior was not observed.

Discussion

As in most other species, there was a high level of interindividual variability in the plasma concentrations of voriconazole in the treated Hispaniolan Amazon parrots in the present study. The elimination half-life of a single 12 or 24 mg/kg dose of voriconazole following oral administration in Hispaniolan Amazon parrots was short (0.7 to 1.25 hours). This finding was similar to the elimination half-life of voriconazole determined in African grey parrots (Eriithacus crithicus timneh) receiving doses of 6 to 18 mg/kg (1.1 to 1.6 hours) and chickens (Gallus domesticus domesticus) receiving doses of 3 to 15 mg (approx 2 hours), but much shorter than that determined in pigeons (Columbia livia forma domestica) receiving a dose of 10 mg/kg (10.3 hours).10-21 In Hispaniolan Amazon parrots, doubling the orally administered dose from 12 to 24 mg/kg resulted in approximately proportional increases in Cmax and AUC, suggesting that voriconazole kinetics are proportional at these doses. This is in contrast to the nonlinear pharmacokinetics associated with similar dose escalations in African grey parrots and pigeons10-21 and the lack of difference in AUC between doses of 5 and 15 mg/kg in chickens.22 Nonlinear pharmacokinetics for voriconazole in mice, rats, rabbits, guinea pigs, dogs, and adult humans have also been reported13 and were presumed to be associated with saturation of metabolic clearance. It is possible that nonlinear kinetics might be identifi-
voriconazole concentrations after administration of multiple doses. This finding has several possible explanations including decreased absorption or increased clearance. Variable effects have been observed following administration of multiple doses of voriconazole in other avian species. Plasma concentrations and AUC were lower after multiple doses in pigeons and African grey parrots but not chickens.\textsuperscript{19-21} In contrast to findings in Hispaniolan Amazon parrots, pigeons administered 10 mg of voriconazole/kg orally twice daily also had a marked decrease in the elimination half-life at day 4 of treatment (1.6 hours), compared with the elimination half-life determined after administration of a single dose of voriconazole (10.3 hours); this suggested that, in pigeons, there was increased systemic metabolism of the drug via autoinduction of hepatic enzymes,\textsuperscript{21} perhaps in association with presystemic metabolism. In our study, plasma voriconazole concentrations at 4 hours after dose administration were > 0.4 µg/mL on day 10 in only 1 of 6 Hispaniolan Amazon parrots. This decrease in plasma concentrations following administration of multiple doses indicates that adjustments in the voriconazole dose will likely be necessary to maintain therapeutic concentrations during longer-term treatment in Hispaniolan Amazon parrots.

Adverse reactions to voriconazole in humans have been reported\textsuperscript{4,15,18} and include transient visual disturbances, liver function abnormalities, and dermatologic reactions. In a study\textsuperscript{22} of African grey parrots, birds in both the treatment (18 mg of voriconazole/kg, PO, q 12 h) and control groups developed polyuria, which resolved 5 days after treatment ended. In pigeons (n = 12) administered 20 mg of voriconazole/kg orally every 12 hours for 4 days, biochemical and histologic changes consistent with hepatic toxicosis were reported.\textsuperscript{23} Among those pigeons, adverse effects were detected in 8 and biochemical variables were abnormally high in 5; 1 bird died.\textsuperscript{24} Mild hepatic abnormalities without changes in biochemical variables developed in pigeons given 10 mg of voriconazole/kg orally every 12 hours for 4 days.\textsuperscript{25} In 20 falcons with aspergillosis that were treated orally with 12.5 mg of voriconazole/kg for 18 to 100 days, adverse effects included flicker (rapid back and forth movement) of the meat while eating (n = 2), vomiting (2), and anorexia (2).\textsuperscript{26} One of the falcons developed hepatomegaly and high liver enzyme activities after 1 week of treatment.\textsuperscript{20} Finally, in chickens treated orally with 10 mg of voriconazole/kg every 24 hours for 30 days, no adverse effects or abnormalities in biochemical variables were detected.\textsuperscript{27} During the present study, no abnormalities in hematologic or plasma biochemical variables or other adverse effects developed in the Hispaniolan Amazon parrots, except for 1 parrot in the treatment group of the multiple-dose trial that developed polyuria. The polyuria resolved after completion of the study. It is likely that the toxic effects of voriconazole are species specific and dose dependent. Monitoring plasma biochemical variables (e.g., aspartate aminotransferase activity, total plasma bile acids concentrations, and plasma uric acid concentration) is recommended during long-term treatment.

The small body size and number of birds available for the present study precluded frequent collection of the samples, which is needed for a standard 2-phase pharmacokinetic study. This is a common problem when working with small nondomestic species. However, the naive pooling of samples undertaken in our study allowed estimation of pharmacokinetic parameters; such pooling of samples may assist clinical dosing and guide future studies. A more sophisticated pharmacokinetic analysis, NLMEM, was also used to provide estimates of the population and variability. The NLMEM approach provided more information about the variability of the population estimates; however, the values for most pharmacokinetic parameters were similar and it was difficult to fit an NLMEM model to the sparse data sets in the present study. The NLMEM approach could be more useful when there is a large population to be analyzed and there is need for comparison of subpopulations (covariates).

The present study revealed that voriconazole could be safely administered to Hispaniolan Amazon parrots at a dose of 18 mg/kg every 8 hours for 11 days. Treatment of aspergillosis in birds may require a much longer treatment period, and adverse effects and long-term changes in voriconazole pharmacokinetics may occur, although these were not evident during the short time period and small population used in our study. Further studies are needed to determine the safety and efficacy of long-term voriconazole treatment in this species.

References