Single-dose pharmacokinetics of orally administered terbinafine in bearded dragons (*Pogona vitticeps*) and the antifungal susceptibility patterns of *Nannizziopsis guarroi*

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OBJECTIVE
To identify the antifungal susceptibility of *Nannizziopsis guarroi* isolates and to evaluate the single-dose pharmacokinetics of orally administered terbinafine in bearded dragons.

ANIMALS
8 healthy adult bearded dragons.

PROCEDURES
4 isolates of *N guarroi* were tested for antifungal susceptibility. A compounded oral solution of terbinafine (25 mg/mL [20 mg/kg]) was given before blood (0.2 mL) was drawn from the ventral tail vein at 0, 4, 8, 12, 24, 48, 72, and 96 hours after administration. Plasma terbinafine concentrations were measured with high-performance liquid chromatography.

RESULTS
The antifungal minimum inhibitory concentrations against *N guarroi* isolates ranged from 4,000 to >64,000 ng/mL for fluconazole, 125 to 2,000 ng/mL for itraconazole, 125 to 2,000 ng/mL for ketoconazole, 125 to 1,000 ng/mL for posaconazole, 60 to 250 ng/mL for voriconazole, and 15 to 30 ng/mL for terbinafine. The mean ± SD peak plasma terbinafine concentration in bearded dragons was 435 ± 338 ng/mL at 13 ± 4.66 hours after administration. Plasma concentrations remained >30 ng/mL for >24 hours in all bearded dragons and for >48 hours in 6 of 8 bearded dragons. Mean ± SD terminal half-life following oral administration was 21.2 ± 12.40 hours.

CLINICAL RELEVANCE
Antifungal susceptibility data are available for use in clinical decision making. Results indicated that administration of terbinafine (20 mg/kg, PO, q 24 to 48 h) in bearded dragons may be appropriate for the treatment of dermatomycoses caused by *N guarroi*. Clinical studies are needed to determine the efficacy of such treatment.

Bearded dragons (*Pogona vitticeps*) are common companion lizards in the United States and are commonly infected with *Nannizziopsis guarroi*, the causative agent of yellow-fungus disease.1–9 *Nannizziopsis* spp are a genus of keratinophilic fungi that cause cutaneous lesions in lizards ranging from mild scale discoloration and dysecdysis to severe, disfiguring, and ulcerative lesions leading to invasive mycosis and death.1–9 Recent molecular characterization has led to several nomenclature changes; infection with this pathogen has been previously reported under the names *Chrysosporium guarroi* and *Chrysosporium* anamorph of *Nannizziopsis vriesii* (CANV).1,7,8 At this time, genetic sequencing suggests that most cases of CANV in companion reptiles are caused by *N guarroi*.3 However, many fungal pathogens within the genera *Nannizziopsis*, *Ophidiomyces*, *Paranannizziopsis*, and *Emydomyces* were previously assigned to the CANV group.3,7,9,10 Despite its recognition as an emerging disease in reptilian patients, there is limited understanding of the best treatment for *N guarroi*.3,5,8 Antifungal susceptibility information exists for 32 CANV isolates11; however, given the recent nomenclature changes, interpretation of these data is challenging because isolates previously assigned to the CANV group may have been members of various other genera.3,7–10 Few antifungal agents have been investigated for treatment of *N guarroi* in bearded dragons. The azole class of antifungals is the most commonly used, although they have been associated with hepatotoxicity.12,11 Voriconazole has been shown to be
the most efficacious and the least toxic of this group (14% mortality) in the treatment of what was at the time termed CANV in bearded dragons, compared with itraconazole (70% mortality).\textsuperscript{11} Despite clinical and microbiological evidence of resolved infections, the literature also supports a high rate of recurrence of latent infections following treatment with drugs in theazole class.\textsuperscript{2,6,12} Clients and clinicians are understandably dissatisfied with current treatment options for \textit{N} \textit{guarro}i given the expensive nature of azoles, their variable toxicity rates, and the high likelihood of infection recrudescence. There is a need to establish the susceptibility patterns of molecularly confirmed isolates of \textit{N} \textit{guarro}i and to investigate safer, more efficacious antifungals for treatment of this disease.

Terbinafine hydrochloride is a synthetic allylamine antifungal agent, originally developed for use in the treatment of human mycotic nail infections.\textsuperscript{13–16} Terbinafine is fungicidal by inhibiting squalene epoxidase, an enzyme required for ergosterol synthesis. Accumulation of squalene within fungal cells leads to cell death.\textsuperscript{13–16} Terbinafine is an attractive option for antifungal therapy because there are rare reports of toxicity in other species owing to its keratinophilic nature.\textsuperscript{16} The accumulation of terbinafine within keratinized tissues makes it a particularly attractive treatment option for dermatomycoses.\textsuperscript{13–16} Pharmacokinetic studies for terbinafine have been reported for several zoological mammal and avian species and a single reptile species, the cottonmouth snake (\textit{Agkistrodon piscivorus}).\textsuperscript{17–23} Nebulization of terbinafine has become an effective treatment of another important cutaneous reptile fungal pathogen, \textit{Ophidiomyces ophidicolae}, in venomous snakes, for which oral administration is both dangerous and unreliable.\textsuperscript{17} Thus, terbinafine may represent an alternative antifungal treatment for \textit{N} \textit{guarro}i infection in bearded dragons, but the susceptibility of the fungus to terbinafine and the pharmacokinetics of the drug must be established first.

The objectives of the study reported here were to establish the antifungal susceptibility patterns of molecularly confirmed \textit{N} \textit{guarro}i from clinical isolates and to determine the pharmacokinetics of a single dose of terbinafine following oral administration in bearded dragons. We hypothesized that oral administration of terbinafine would provide plasma drug concentrations that exceeded the minimum inhibitory concentration (MIC) against \textit{N} \textit{guarro}i and be safe in bearded dragons.

Materials and Methods

Animals

This study was carried out with the approval of the University of Illinois Institutional Animal Care and Use Committee (protocol No. 17243). Eight bearded dragons (5 males and 3 females) with ages ranging from 2 to 3 years and body weights ranging from 256 to 384 g that were maintained as a teaching and research colony were used for the study. Standard bearded dragon housing and husbandry was provided by the University of Illinois in accordance with Institutional Animal Care and Use Committee protocol No. 18056.\textsuperscript{24} In brief, each bearded dragon was individually housed in a front-opening 91.45 X 71.12 X 45.72-cm enclosure (V332 Vision Cage; LLL Reptile and Supply Co) located within a temperature-controlled room (24 to 25.5 °C). The daily diet included a variety of dark leafy greens (collard greens, kale, turnip greens, and mustard greens) with additional shredded vegetables (carrots, sweet potato, and berries) supplemented with calcium carbonate powder and twice weekly offering of gut loaded king worm (\textit{Zophobas morio}) larvae (High-calcium Cricket Diet; Fluker’s Farms and NOW Foods). Each enclosure had a dedicated heat lamp and a dedicated UV emitting bulb (both UVA and UVB) situated above the habitat. Twelve hours of darkness were provided daily with a basking temperature (Zoo Med Laboratories Inc) reaching 35 to 37 °C. The animals were determined to be free of significant clinical disease on the basis of normal activity, appetite, and urinary and fecal outputs.

Culture and morphologic identification of \textit{N} \textit{guarro}i isolates

Fungal isolates were derived from clinical samples of 2 bearded dragons and 2 green iguanas (\textit{Iguana iguana}). Three isolates were obtained from patients evaluated at the William R. Pritchard Veterinary Medical Teaching Hospital at University of California-Davis, and 1 isolate was obtained as a submission from an outside private practice to the Veterinary Microbiology Laboratory at the University of California-Davis. All isolates were derived from clinical patients with skin lesions considered consistent with \textit{N} \textit{guarro}i infection (crusting and ulceration) as either skin biopsies (2 isolates) or samples obtained during necropsy (1 isolate). The isolate submitted by a private practice was derived from skin and tissue, but it was unknown whether the samples were obtained before or after death.

Samples were inoculated onto inhibitory mold agar (Hardy Diagnostics) and dermatophyte test and rapid sporulation media (Derm Duet; Hardy Diagnostics) then incubated at 30 °C under ambient atmosphere. Plates were examined daily for fungal growth and fungal colonies subcultured to potato flake agar (Biological Media Services, University of California-Davis) and incubated as above. Preliminary identification of fungal isolates as \textit{N} \textit{guarro}i was made by morphologic microscopic examination of a tape preparation stained with lactophenol cotton blue stain (Hardy Diagnostics) from the subculture.\textsuperscript{9}

Molecular identification

Fungal DNA was obtained from each culture by use of a NaOH extraction protocol.\textsuperscript{25} In brief, fresh mycelium was added to 200 μL of 0.5 M NaOH in a 1.5-mL microcentrifuge tube, ground by use of a UV-sterilized pestle, incubated at 4 °C for 20 minutes, centrifuged at 16,873 X g for 2 minutes; 5 μL of the resulting supernatant was added to 495 μL of
100 mM Tris HCl buffered with NaOH to a pH of 8.5 to 8.9 (Tris-HCl-DNA extraction solution). A PCR assay (PTC 200 thermal cycler; Bio-Rad Laboratories Inc) was performed in which the total reaction volume was 25 μL (12.5 μL of a commercially available master mix [GoTaq Green Master Mix; Promega Corp]; 1.2 μL each of 10 μM primer internal transcribed spacer [ITS] 1F and ITS4, 3 μL of the Tris-HCl-DNA extraction solution, and 7.1 μL of DNA-free water) and the thermal cycle parameters were initial denaturation at 94 °C for 2 minutes, 30 cycles of 94 °C for 30 seconds, 55 °C for 45 seconds, and 72 °C for 1 minute with a final extension step of 72 °C for 10 minutes. Gel electrophoresis (1% Tris-borate-EDTA agarose gel stained with ethidium bromide) was used to verify the presence of a PCR product before purification with a clean-up system (Wizard SV Gel and PCR Clean-Up System; Promega Corp). A cycle sequencing kit (BigDye Terminator version 3.1; Applied Biosystems Inc) was used to sequence the ITS in 1 direction with the ITS5 primer on a high-throughput capillary sequencer (3730XL; Applied Biosystems) at the Roy J. Carver Biotechnology Center at the University of Illinois. Sequences were examined and manually corrected with sequence analysis software (Sequencher version 5.1, Gene Codes Corp). Culture identity was confirmed through nBLAST analysis of the ITS1-5.8-ITS2 region using the National Center for Biotechnology Information database.

Antifungal susceptibility
All plated isolates were sent on Sabouraud dextrose agar (Remel) to an external laboratory (Fungus Testing Laboratory, Department of Pathology and Laboratory Medicine, University of Texas San Antonio, San Antonio, Texas) for antifungal susceptibility testing. Each isolate was broth dilution tested against fluconazole, ketoconazole, itraconazole, nystatin, posaconazole, terbinafine, and voriconazole as described.26

Comounded terbinafine
An oral solution (concentration, 25 mg/mL) of terbinafine was compounded from commercially available tablets (Sigma-Aldrich; 250 mg of terbinafine/tablet) crushed and dissolved in commercially available suspending vehicles (Ora-Plus and Ora-Sweet; Paddock Laboratories) using a previously published27 compounding recipe that ensures a stable suspension for 28 days. The time from compounding to administration was < 48 hours.

Pharmacokinetic sampling and analysis
Pilot studies—To determine an appropriate dose and sampling protocol, 2 pilot studies were performed. Three lizards were randomly assigned by pulling numbers from a hat to received 5 mg of terbinafine/kg by gavage, and blood (0.2 mL) was collected from the ventral tail vein at 0 (immediately after), 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after terbinafine administration. A second group of 3 different lizards randomly assigned by pulling numbers from a hat received 20 mg of terbinafine/kg by gavage, and blood (0.2 mL) was collected from the ventral tail vein at 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after drug administration. To determine the duration of sampling needed to evaluate clearance, a fourth lizard received 20 mg of terbinafine/kg by gavage, and blood (0.2 mL) was collected from the ventral tail vein at 0, 0.5, 1, 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 hours. The total amount of blood drawn was less than 0.8% of the body weight for each individual animal. Blood was collected into lithium heparin microtainers (Becton Dickinson), kept on ice until centrifuged (Eppendorf AG22331 Centrifuge; Brinkmann Instruments Inc) within 1 hour after collection, after which plasma was harvested and stored at −20 °C until analysis.

Single-dose pharmacokinetics—Four months following the pilot study, 8 lizards, including the 7 lizards used in the pilot study, received a single oral dose of terbinafine (20 mg/kg), administered by gavage directly into the stomach (total volume administered, 0.22 to 0.31 mL). Immediately after terbinafine gavage, a slurry of commercially available intensive care diet (Intensive Care Herbivore; Emerald LLC; fed at 1.5% of body weight; total volume administered, 4.1 to 5.9 mL) was administered via gavage needle. Blood (0.2 mL) was collected from the ventral tail vein at 0, 4, 8, 12, 24, 48, 72, and 96 hours after drug administration. The total amount of blood drawn was less than 0.8% of the body weight for each subject. Blood was collected into lithium heparin microtainers, kept on ice until centrifuged within 1 hour after collection, after which plasma was harvested and stored at −20 °C until analysis.

Terbinafine analysis
Plasma samples were analyzed with a reverse-phase high-performance liquid chromatography method.28 The system consisted of a 2695 separations module, 2487 absorbance detector, and computer equipped with software (Empower 3 Chromatography Software; Waters Corp). Terbinafine was extracted from plasma samples using liquid extraction. Briefly, previously frozen plasma samples were thawed and vortexed, and 100 μL was transferred to a clean screw-top test tube followed by 75 μL internal standard (butenafine, 1.0 μg/mL). Hexane (3 mL) was added, and the tubes were rocked for 20 minutes and then centrifuged for 20 minutes at 1,000 X 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 and 100 μL was analyzed.

The compounds were separated on a C18 column (Symmetry Shield C18 Chromatography Kit; Waters Corp; 4.6 X 100 mm; 5 μm). The mobile phase was a mixture of 20 mM phosphoric acid with 0.1% triethylamine adjusted to pH 3.0 (A) and acetonitrile (65:35; B). The flow rate was 1.1 mL/min with the column maintained at ambient temperature (22 °C). Absorbance was measured at wavelength of 224 nm.

Standard curves for plasma analysis were prepared by fortifying untreated, pooled plasma with
terbinafine to produce a linear concentration range of 5 to 1,500 ng/mL. Calibration samples were prepared in the same manner as plasma samples. Average recovery for terbinafine was 95%, the intra-assay variability ranged from 4.5% to 8.5%, and the interassay variability ranged from 0.93% to 8.5%. The lower limit of quantification was 5 ng/mL.

**Statistical analysis**
Continuous parameters were summarized as the mean ± SD. For the main study, noncompartmental pharmacokinetic analysis was performed with commercial software (Phoenix WinNonLin; Certara LP). Reported parameters included the terminal rate constant, terminal half-life (t1/2), maximum plasma concentration (Cmax), time to maximum plasma concentration, area under the concentration-versus-time curve (AUC) from time 0 to the last measured concentration, AUC from time 0 to infinity, AUC from the last measured time extrapolated to infinity and expressed as a percentage of the total AUC, and mean residence time (MRT). Accumulation ratios (ARs) were calculated at dosing intervals of 24 and 48 hours using the following formula:

\[
AR = \frac{1}{1 - e^{-\lambda T}}
\]

where \( \tau \) is the proposed dosing interval. The maximum concentration at steady state and concentration just prior to the next dose at steady state were calculated from the AR and single-dose data.

**Results**

**Bearded dragons**
One bearded dragon died following completion of the main study. Postmortem analysis of that animal revealed a focal gastric perforation with localized peritonitis, consistent with iatrogenic trauma from gavage; no other gross or histologic lesions were observed in any of the tissues. No adverse effects were observed in any of the other bearded dragons during any of the experiments, and none of the 3 females laid eggs during the month after study completion. There were no perceived changes in appetite or attitude despite the frequent handling and venipuncture.

**Antifungal susceptibility test results**
Susceptibility test results for the 4 \( N \) guarroi isolates against the antifungals fluconazole, itraconazole, ketoconazole, nystatin, posaconazole, terbinafine, and voriconazole were summarized (Table 1). On the basis of the susceptibility test results of the 4 \( N \) guarroi isolates to terbinafine, the target terbinafine concentration threshold for the pharmacokinetic study was set at > 30 ng/mL.

**Pharmacokinetics**
**Pilot studies**—In the initial pilot study (terbinafine dose, 5 mg/kg), only 1 of the 3 bearded dragons achieved plasma terbinafine concentrations > 30 ng/mL starting at approximately 4 hours and lasting for < 8 hours after drug administration. In the second pilot study (terbinafine dose, 20 mg/kg), all 4 bearded dragons achieved plasma terbinafine concentrations > 30 ng/mL by 0.5 to 8 hours after drug administration and maintained concentrations > 30 ng/mL for 48 hours.

**Single-dose pharmacokinetics**—The mean ± SD plasma terbinafine concentration was plotted over time (Figure 1) and the pharmacokinetic parameters were summarized (Table 2) for all 8 bearded dragons. The median (range) plasma terbinafine concentration at each sample time were also summarized for male and female bearded dragons separately (Table 3). For all 8 bearded dragons, the plasma terbinafine concentration exceeded the target MIC (30 ng/mL) by 4 hours after drug administration and remained about the target MIC for > 24 hours. For 6 of the 8 bearded dragons (3 males and 3 females), the plasma terbinafine concentration remained above the target MIC for

**Table 1**—Antifungal minimum inhibitory concentrations (MICs; ng/mL) for each of 4 \( N \)annizziopsis guarroi isolates obtained from 2 bearded dragons (\( Pogona vitticeps \); BD) and 2 green iguanas (\( Iguana iguana \); GI) with clinical skin infections.

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>BD 1</th>
<th>BD 2</th>
<th>GI 1</th>
<th>GI 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluconazole</td>
<td>4,000</td>
<td>64,000</td>
<td>32,000</td>
<td>&gt; 64,000</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>125</td>
<td>1000</td>
<td>2,000</td>
<td>1,000</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>125</td>
<td>200</td>
<td>2,000</td>
<td>2000</td>
</tr>
<tr>
<td>Posaconazole</td>
<td>125</td>
<td>250</td>
<td>500</td>
<td>1,000</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>60</td>
<td>125</td>
<td>125</td>
<td>250</td>
</tr>
<tr>
<td>Nystatin</td>
<td>4,000</td>
<td>4,000</td>
<td>2,000</td>
<td>2,000</td>
</tr>
<tr>
<td>Terbinafine</td>
<td>30</td>
<td>30</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

**Figure 1**—Mean ± SD plasma terbinafine concentration over time following gavage administration of a single dose of terbinafine (20 mg/kg) to 8 bearded dragons (\( Pogona vitticeps \). The target plasma concentration for terbinafine (30 ng/mL) selected on the basis of culture and susceptibility results for 4 \( N \)annizziopsis guarroi isolates obtained from 2 bearded dragons and 2 green iguanas (\( Iguana iguana \)) with clinical skin infections is also indicated (dotted line).
> 48 hours. Accumulation ratios and predicted steady state concentrations are were summarized (Table 4).

**Discussion**

The data presented in this study support that terbinafine orally administered at a dose of 20 mg/kg was sufficient to reach the target MIC (30 ng/mL) for *N. guarroi* and may be useful for treatment of infections caused by *N. guarroi*. Additionally, the antifungal susceptibilities of the *N. guarroi* isolates assessed in this study differed from those obtained for other Onygenalean fungi and provided clinical-

**Table 2**—Values for noncompartmental pharmacokinetic parameters after gavage administration of terbinafine (20 mg/kg) to 8 bearded dragons.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</td>
<td>434.85 ± 338.27</td>
<td>153–1,158</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h)</td>
<td>13 ± 4.66</td>
<td>8–24</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2,h&lt;/sub&gt; (h)</td>
<td>21.24 ± 12.40</td>
<td>5.21–39.50</td>
</tr>
<tr>
<td>λ&lt;sub&gt;1&lt;/sub&gt; (h&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.049 ± 0.039</td>
<td>0.018–0.13</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-last&lt;/sub&gt; (h•ng/mL)</td>
<td>10,855.97 ± 8,355.24</td>
<td>3,682–29,896</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;cr&lt;/sub&gt; (h•ng/mL)</td>
<td>11,364.19 ± 9,812.74</td>
<td>3,874–34,853.5</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;extrap&lt;/sub&gt; (%)</td>
<td>10.05 ± 5.90</td>
<td>3.59–17.99</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>25.78 ± 9.53</td>
<td>11.84–40.40</td>
</tr>
</tbody>
</table>

AUC<sub>0-last</sub> = Area under the concentration-versus-time curve from time 0 to the last measured concentration. AUC<sub>cr</sub> = Area under the concentration-versus-time curve from time 0 to infinity. AUC<sub>extrap</sub> = Area under the concentration-versus-time curve from the last measured time extrapolated to infinity and expressed as a percentage of the total area under the concentration-time curve. C<sub>max</sub> = Maximum plasma concentration. MRT = Mean residence time. t<sub>max</sub> = Time to maximum plasma concentration. t<sub>1/2,h</sub> = Terminal half-life. λ<sub>1</sub> = Terminal rate constant.

**Table 3**—Median (range) of plasma terbinafine concentration (ng/mL) in male (n = 5) and female (3) bearded dragons following gavage administration of a single dose of terbinafine (20 mg/kg).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>4</td>
<td>51 (29–116)</td>
<td>309 (94–397)</td>
</tr>
<tr>
<td>8</td>
<td>96 (84–158)</td>
<td>511 (253–670)</td>
</tr>
<tr>
<td>12</td>
<td>178 (36–378)</td>
<td>634 (265–1,158)</td>
</tr>
<tr>
<td>24</td>
<td>77 (57–271)</td>
<td>234 (79–424)</td>
</tr>
<tr>
<td>48</td>
<td>49 (0–95)</td>
<td>61 (40–202)</td>
</tr>
<tr>
<td>72</td>
<td>27 (0–51)</td>
<td>16 (0–133)</td>
</tr>
<tr>
<td>96</td>
<td>16 (0–33)</td>
<td>12 (0–87)</td>
</tr>
</tbody>
</table>

**Table 4**—Accumulation ratios (ARs) and the predicted maximum concentration at steady state (C<sub>max,ss</sub>) and concentration just prior to the next dose at steady state (C<sub>t,ss</sub>) following gavage administration of terbinafine (20 mg/kg) to 8 healthy bearded dragons assuming 24- and 48-dosing intervals.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>24-hour dosing interval</th>
<th>48-hour dosing interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR</td>
<td>1.87 ± 0.69 (1.04–2.91)</td>
<td>1.30 ± 0.29 (1.00–1.76)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max,ss&lt;/sub&gt; (ng/mL)</td>
<td>871.51 ± 1,030.67 (281.75–3,368.94)</td>
<td>593.26 ± 605.77 (205.92–2,034.05)</td>
</tr>
<tr>
<td>C&lt;sub&gt;t,ss&lt;/sub&gt; (ng/mL)</td>
<td>348.86 ± 382.50 (80.30–1,233.53)</td>
<td>100.14 ± 111.70 (0–354.82)</td>
</tr>
</tbody>
</table>

Values represent the mean ± SD (range).
rate of bearded dragons. For the bearded dragons of the present study, terbinafine was administered with a meal because administration of terbinafine with food enhances the absorption and increases the $C_{\text{max}}$ for the drug in human patients. The inclusion of a gavage-fed meal with the terbinafine was added to the methods of the full study to facilitate peak absorption. The $C_{\text{max}}$ (434 ± 338 ng/mL) for the bearded dragons of the present study following oral administration of terbinafine at a dose of 20 mg/kg was similar to the $C_{\text{max}}$ for African penguins receiving terbinafine a dose of 15 mg/kg, PO (200 ng/mL);14 horses receiving terbinafine at a dose of 20 mg/kg, PO (310 ng/mL);14 and Hispaniolan Amazon parrots receiving terbinafine at a dose of 60 mg/kg, PO (353 ng/mL).19 but much lower than the $C_{\text{max}}$ measured in red-tailed hawks (1,200 ng/mL),23 cats (3,220 ng/mL),30 and Greyhound dogs (4,010 ng/mL) that received terbinafine at a dose of 30 mg/kg, PO. The low $C_{\text{max}}$ may be the result of slower absorption or differences in terbinafine dosages or formulations administered among studies. It is also possible that the oral bioavailability of terbinafine in bearded dragons is lower than that for species with a higher $C_{\text{max}}$.

The plasma $t_{1/2}$ of terbinafine (21.2 ± 12.4 hours) for the bearded dragons of the present study was longer than that observed in cats (8.01 hours), Greyhound dogs (8.6 hours), horses (8.1 hours), and Hispaniolan Amazon parrots (8.7 hours) and similar to that observed in African penguins (17.0 hours) and red-tailed hawks (17.5 hours). This may represent slow elimination of terbinafine because of slower metabolism of the drug in bearded dragons, although it is unknown whether terbinafine undergoes hepatic biotransformation in this species. The $t_{1/2}$ of terbinafine is also likely influenced by its lipophilic and keratophilic nature, which results in a large volume of distribution as the drug accumulates in peripheral tissues and slowly redistributes back into the blood. The accumulation of terbinafine in keratinized tissues (dermis, epidermis, nail, and hair) is observed in multiple species and makes terbinafine an excellent choice for the treatment of fungal dermatomycoses.

In mammals, terbinafine is highly protein bound, with >99% plasma protein binding occurring in dogs, rabbits, and humans. There are no published data on the protein affinity of terbinafine in any reptile species. However, it must be assumed that a degree of protein binding does occur in bearded dragons and a state of hypoproteinemia or hyperproteinemia will affect terbinafine pharmacokinetics. Vitellogenesis results in elevated protein concentrations in reproductively active females preparing for egg laying. Given the time of year that the present study was performed (March), the female bearded dragons enrolled in the study may have been in an active state of vitellogenesis, despite a lack of gross lipemia in all blood samples and egg laying in the month before and month after study completion. Although the small number of bearded dragons enrolled in this study did not provide enough statistical power to compare the findings between males and females, the higher plasma terbinafine concentrations noted in the females may have represented sex differences associated with seasonal plasma protein levels. The quantification of plasma proteins was not pursued in this study but may be worthwhile in future studies. However, assessment of tissue or cellular drug concentration is likely the most appropriate method for determination of the required kinetic parameters for drugs that are highly protein bound, with a free fraction of <20% in plasma. Clinicians prescribing terbinafine should strongly consider performing plasma protein quantification prior to and during terbinafine treatment.

The presence of hepatic disease may alter terbinafine pharmacokinetics owing to alteration of hepatic biotransformation or as a consequence of hypoproteinemia. Although not evaluated in reptilian species, results of mammalian studies support that terbinafine undergoes extensive hepatic biotransformation through multiple enzymatic pathways. The bearded dragons of the present study did not undergo diagnostic testing to ascertain hepatic health but were deemed healthy on the basis of normal behavior, appetite, and outputs. Additionally, the bearded dragon that died and underwent necropsy following completion of the study did not have any gross or histologic evidence of hepatopathy. Two other bearded dragons not enrolled in the study but maintained in the same research colony as the study animals were euthanized. One of those bearded dragons was euthanized 1 year before study initiation and was determined to have biliary lithiasis. The other bearded dragon was euthanized 1 month after study completion and was found to have an intestinal intussusception. At the time this manuscript was written, the 7 remaining bearded dragons of the present study were alive and appeared healthy without any signs of illness suggestive of hepatic disease and were not being treated for any illness over 1 year later. The findings of this study should be presumed to represent terbinafine pharmacokinetics in healthy bearded dragons, and clinicians utilizing terbinafine should consider pursuing diagnostic testing to assess hepatic health in clinically ill patients.

In the present study, pharmacokinetic analysis confirmed that gavage administration of a single dose (20 mg/kg) of terbinafine to bearded dragons exceeded the MIC of terbinafine (30 ng/mL) necessary to inhibit growth of N. guarroi for at least 24 hours. The pharmacodynamic parameter most predictive of antibiotic efficacy ($T > MIC$, AUC/MIC, or $C_{\text{max}}$/MIC) has not been established for terbinafine and makes terbinafine an excellent choice for the treatment of fungal dermatomycoses.
All 8 bearded dragons of the present study tolerated terbinafine well and no overt clinical signs suggestive of toxicosis were observed. The administration of terbinafine via gavage was performed to improve dosing accuracy but may have resulted in the acute death of 1 study subject. Oral, rather than gavage, administration of terbinafine is recommended for clinical patients. In human patients receiving terbinafine, the most commonly reported adverse effects are gastrointestinal disturbances, headache, skin disorders, and taste abnormalities. Reported adverse effects of terbinafine in veterinary patients include transient periorcular swelling and erythema in dogs,11,19 regurgitation in avian species,16 transient lassitude in cats,30 and transient head shaking and lip curling in horses.31 Careful monitoring of clinical patients is warranted until multidose pharmacokinetic and controlled efficacy and safety trials can be pursued.

The present study had several important limitations, including a small study population, lack of multidose data, and lack of preliminary testing to ascertain the hepatic health and plasma protein concentration of the study subjects, all of which could be addressed in future larger-scale studies. Although the stability of terbinafine in frozen plasma has been demonstrated in other species,23 it was not investigated in plasma samples obtained from the bearded dragons of the present study. Given the accumulation of terbinafine within keratinized tissues, tissue or cellular concentrations may be more appropriate for determining the kinetic and dynamic parameters needed to establish successful dosing regimens. Terbinafine is primarily metabolized in the liver14,16,34; however, the present study did not evaluate terbinafine metabolites (carboxyterbinafine, desmethylhydroxyterbinafine, hydroxyterbinafine or n-desmethylterbinafine) in either the pharmacokinetic or susceptibility experiments. It is possible reptiles may metabolize terbinafine differently than avian and mammalian species or that N guarroí may have differing susceptibility patterns to terbinafine metabolites. Lastly, this study presented preliminary data supporting sex differences in terbinafine pharmacokinetics that should be explored in future studies.

In conclusion, administration of terbinafine (20 mg/kg, PO) every 24 to 48 hours may be useful in treating infections caused by N guarroí in bearded dragons by producing plasma concentrations greater than the target MIC (30 ng/mL) established for the drug in the present study. However, multiple-dose pharmacokinetic and controlled treatment trials are needed to confirm this recommendation. Given the predicted concentrations at steady state, terbinafine doses of 20 mg/kg, PO, administered at 24- or 48-hour intervals are recommended for bearded dragons; however, a lower dose may be sufficient for 24-hour dosing. Further research is needed to determine the details of reptilian terbinafine metabolism, protein binding, and to evaluate its accumulation within keratinized tissues in both infected and healthy reptiles.

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