Pharmacokinetics of terbinafine in little brown myotis (*Myotis lucifugus*) infected with *Pseudogymnoascus destructans*

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OBJECTIVE
To determine the pharmacokinetics of terbinafine in little brown myotis (*Myotis lucifugus*) infected with *Pseudogymnoascus destructans*.

ANIMALS
123 bats from a *P destructans*–infected hibernation site in Virginia.

PROCEDURES
3 bats were euthanized and necropsied to confirm the presence of *P destructans* within the population. The remaining 120 bats were systematically assigned to 6 groups (20 bats/group). Bats in each of 3 groups received 6, 20, or 60 mg of terbinafine/kg, SC, once daily for 10 days. Bats in another group received 200 mg of terbinafine/kg, SC, once daily for 5 days. Bats in 1 group received the terbinafine vehicle solution (0.1 mL/kg, SC, once daily for 10 days). Bats in the remaining group did not receive any treatment. Following the treatment period (days 1 through 10), bats were housed in a hibernation chamber and monitored daily until euthanasia on day 42, 75, or 109. Tissue specimens were collected from all bats as soon as possible after death or euthanasia to determine terbinafine concentration. Within each group and tissue type, terbinafine concentration data were pooled, and pharmacokinetic parameters were calculated by noncompartmental methods.

RESULTS
Adverse neurologic effects and a high mortality rate before day 10 were observed in bats that received the highest terbinafine dose (200 mg/kg) but not those that received lower doses. Presumed therapeutic terbinafine concentrations (≥ 2 μg/g) were maintained in skin and wing for at least 30 and 6 days in bats that received the 60 and 20 mg/kg doses, respectively, but were not achieved in most bats that received the 6 mg/kg dose. Tissue terminal half-life ranged from 14 to 22 days. Terbinafine concentration in hair was positively correlated with that in skin and wing.

CONCLUSIONS AND CLINICAL RELEVANCE
Results indicated terbinafine doses > 6 but < 200 mg/kg should be further evaluated for the treatment of *P destructans*–infected bats. Collection of serial hair specimens may represent a noninvasive method for monitoring terbinafine concentration in treated bats. (*Am J Vet Res* 2017;78:90–99)

White-nose syndrome is a newly emergent fungal disease caused by *Pseudogymnoascus destructans* that is currently decimating bat populations in North America.[1–3] The syndrome is named for the distinctive appearance of fungal growth on the muzzle of affected bats.[4] The fungus colonizes the skin and is psychrophilic (cold loving) in that it prefers to grow at temperatures ranging from 4° to 15°C and will not grow at temperatures > 20°C.[4] The mechanism by which the fungus causes death is the subject of ongoing investigation, but appears to be associated with an increase in energy expenditure during winter hibernation, loss of water and electrolytes, and an inadequate immune response.[5,6] Because certain bat species are at risk of extinction, various intervention strategies are currently being explored, including treatment of infected bats with antifungal agents.

Terbinafine is an allylamine antifungal drug that has both fungistatic and fungicidal properties.[7] The fungistatic effects result from inhibition of the synthesis of ergosterol, the principal sterol in fungi, from squalene by squalene epoxidase, whereas the fungicidal effects are associated with the toxic accumulation of intracellular squalene.[7] In human and veterinary medicine, oral and topical preparations of terbinafine are used to treat cutaneous fungal infections (dermatophytoses) such as ringworm in children,

ABBREVIATIONS

AUC	Area under the concentration-time curve
HPLC	High-performance liquid chromatography
MIC	Minimum inhibitory concentration
$t_{1/2}$	Terminal half-life

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dogs, cats, and other veterinary species and tinea pedis and cruris and nail infections in adult humans.[7]

The pharmacokinetic parameters of terbinafine in human patients suggest the drug has a wide tissue distribution that includes skin and hair and a prolonged tissue $t_{1/2}$, properties that are ideal for the treatment of dermatophyte infections.[8] However, to our knowledge, the pharmacokinetic parameters of terbinafine (or any other pharmaceutical agent) have not been reported for any bat species. Results of preliminary in vitro efficacy studies suggest that terbinafine may be effective against *P destructans*.[a] Compared with other available antifungals (including the azole antifungals), terbinafine has been proposed as an ideal choice for the treatment of dermatophytes because of its better safety and efficacy profiles.[9] In human patients, adverse effects associated with terbinafine are generally mild and include gastrointestinal tract upset and headache.[10,11] Additionally, terbinafine has a lower potential for adverse drug-drug interactions, compared with other antifungals (particularly the azoles).[7]

The purpose of the study here was to determine the pharmacokinetics for terbinafine following SC administration of each of 4 doses (6, 20, 60, and 200 mg/kg) for 5 (200 mg/kg dose) or 10 (6, 20, and 60 mg/kg doses) days to *P destructans*–infected little brown myotis (*Myotis lucifugus*), a species of bat highly susceptible to *P destructans* infection and death.[2]

Materials and Methods

Animals

The study protocol was approved by the Tufts University Institutional Animal Care and Use Committee. In November 2010, little brown myotis were collected under a permit from the Virginia Department of Game and Inland Fisheries (No. 040804) from a hibernation site in northern Virginia that was confirmed to have bats positive for *P destructans* infection the previous winter (2009 to 2010). One-hundred twenty bats were collected. Each bat was weighed, wing banded, and systematically assigned to 1 of 6 treatment groups (an untreated control group, a terbinafine vehicle control group, and 4 terbinafine-treated groups; 20 bats/group). A systematic group assignment was used to minimize the possible effects of the order of collection of the bats from the cave. Three additional bats were collected and designated for euthanasia on the first day of treatment (day 1) for necropsy and histologic examination to confirm the presence of *P destructans* infection. With a permit from the Massachusetts Department of Fish and Game, the bats were transported in coolers to a biosecurity level 2 animal care facility at Tufts University, North Grafton, Mass. At arrival to the animal care facility, the untreated control group (n = 20 bats) was immediately placed into an environmental (hibernation) chamber in which the temperature and humidity were maintained at 8°C and 95%, respectively. The other 5 treatment groups were housed in separate screened cages (83-L reptariums; n = 20 bats/cage) that were maintained at room temperature (approx 22°C) under red-filtered lights set on a 12-hour on-off cycle. The cages were also supplied with heat lamps that enabled roosting at temperatures between 20° and 35°C. All bats were provided water ad libitum and fed meal worms and a nutritional supplement.[b] The bats were allowed to acclimate for 7 days prior to treatment initiation.

Preparation of treatment solutions

Terbinafine hydrochloride powder[c] was dissolved in a 50% (v/v) polyethylene glycol 400[d] and water solution to achieve solutions with 4 different terbinafine concentrations (60, 200, 600, and 2,000 mg/mL). The resulting terbinafine solutions were placed in 20-mL amber-colored glass bottles with crimped multidose injection stoppers and sterilized by autoclaving the bottles for 30 minutes at 121°C. Bottles containing only the vehicle solution (50% polyethylene glycol 400 in water) were sterilized in the same manner.

Study protocol

The day that the assigned treatment was initiated was designated day 1. Each bat in the terbinafine-treated groups received the assigned dose (6, 20, 60, or 200 mg/kg) by SC injection once daily for 5 (200 mg/kg dose) or 10 (6, 20, and 60 mg/kg doses) days. Each bat in the terbinafine-vehicle control (vehicle control) group received the vehicle solution (0.1 mL/kg, SC) once daily for 10 days. Bats in the untreated control group did not receive any treatment and were not handled.

On day 11, all bats in the terbinafine-treated and vehicle control groups were transferred to the hibernation chamber where the untreated control bats were housed. All bats were monitored daily thereafter for adverse effects. Bats that were considered moribund on the basis of the opinion of one of the wildlife veterinarian investigators (AHR) in consultation with the Tufts University laboratory animal care veterinarian were euthanized. Bats designated for euthanasia were anesthetized with isoflurane and then euthanized by cervical dislocation.

Prior to treatment initiation (day 1), 4 bats were randomly selected by means of pulling numbers from a hat from each of the 6 treatment groups to be euthanized for tissue collection on days 42 and 75 (ie, 24 randomly selected bats were scheduled to be euthanized on day 42 and day 75). The remaining 72 bats were scheduled for euthanasia and tissue collection at the end of the observation period (day 109). All bats were euthanized as previously described.

Each bat was necropsied as soon as possible after death or euthanasia. Tissues collected for determination of terbinafine concentration included an entire wing, portion of the skin (including the hair) from the back, hair plucked from the torso, and liver. All collected tissues were immediately frozen and stored

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at –80°C until analysis, which was within 6 months after collection.

Determination of tissue terbinafine concentration

An assay to measure terbinafine concentration in various bat tissues that incorporated sodium hydroxide digestion, liquid-liquid extraction, and HPLC-mass spectrometry quantitation was developed as described[12,13] with some modifications. Pure solutions of terbinafine[e] and terbinafine-D7[e] were used as standards. Briefly, 10 to 100 mg of tissue was placed in a 2-mL polypropylene tube[f] with 400 µL of sodium hydroxide and 1 µg of terbinafine-D7 (internal standard) in 200 µL of methanol. The samples were placed in a hot block and heated to 90°C for 30 (liver and hair) or 90 (skin and wing) minutes. The samples were allowed to cool to room temperature (approx 22°C), and a 4.8-mm stainless steel bead was added to each tube. The samples were placed in a bead mill homogenizer[g] for 3 minutes at a 5.5 speed setting, then 1.2 mL of hexane was added to each tube and the samples were homogenized for an additional 3 minutes. The resulting mixture was centrifuged at 13,000 X *g* for 5 minutes. The top organic layer was transferred to a new 2-mL polypropylene tube, then 400-µL of a solution containing 15% 2-propanol and 10% formic acid in water was added to each tube. The samples were homogenized without a bead for 3 minutes and centrifuged at 13,000 X *g* for 5 minutes. Four hundred microliters of the bottom aqueous layer was transferred to an HPLC vial for analysis (injection volume, 10 µL).

The HPLC–mass spectrometry system[h] consisted of a gradient-capable high-performance liquid chromatograph connected to an ion trap detector and electrospray source that was operated in the positive-ion mode with collision-induced dissociation fragmentation and daughter ion monitoring. A gradient mobile phase (acetonitrile in water with 0.1% formic acid) was pumped through a 50 X 2.1–mm HPLC column[i] at 0.4 mL/min. The mobile phase was begun at 5% acetonitrile and increased linearly to 100% acetonitrile from 0.1 to 2 minutes of the run and then returned to starting conditions (5% acetonitrile) from 3.9 to 4 minutes of the run. The total run time was 5 minutes. Terbinafine (m/z, 292 to 141) and terbinafine-D7 (m/z, 299 to 141) peaks coeluted at 2.9 minutes.

For each run, a standard curve was generated by the addition of known amounts of terbinafine (10 ng to 10 µg) to blank tissue specimens (10 to 100 mg) obtained from untreated bats that were then processed as described for the unknown samples. Unknown concentrations were then calculated from the plots of known concentrations versus peak area ratios for terbinafine to terbinafine-D7 adjusted for tissue weight. The standard curves were linear ($R^2 > 0.99$) over the measured concentration range (0.1 to 1,000 µg of terbinafine/g of tissue). Quality control samples, which consisted of blank tissue samples spiked with 100 ng and 10 µg of terbinafine, were also processed with each run to evaluate assay performance. Preliminary results for assay validation indicated high terbinafine recovery (90% to 100%), good assay precision (coefficient of variation, < 15%), and good accuracy (measured concentrations had < 15% variation from nominal concentrations). The limit of detection for the assay was 0.02 µg/g, and the limit of quantitation for the assay was 0.1 µg/g, which resulted in a terbinafine peak signal height that was 5 times that of the background signal. The terbinafine concentration was determined in duplicate for all tissue samples, and the mean was calculated and used for analysis.

Estimation of pharmacokinetic parameters

Because tissue specimens were collected at only 1 point in time from each bat (ie, after death or euthanasia), it was not possible to derive accurate pharmacokinetic parameters for individual animals. Therefore, for each treatment group, the terbinafine concentration data for each tissue were pooled and pharmacokinetic parameters were determined by noncompartmental methods. The estimated logarithmic mean ± SE $t_{1/2}$ for each tissue was calculated from the slope of the linear regression line[j] for the logarithmically transformed terbinafine concentrations in the tissue of interest over time after treatment initiation. Only terbinafine concentrations in tissues collected after day 10 (ie, the end of the treatment administration) were included in that calculation so that the analysis was restricted to the elimination phase of the drug. For each tissue and terbinafine dose combination, the AUC values from the start of treatment to the last measurement were estimated by use of the trapezoidal technique.[j]

Statistical analysis

Kaplan-Meier log-rank survival analysis was performed to evaluate differences between treatment groups in the duration from treatment initiation (day 1) to death or euthanasia because of moribundity. For that analysis, bats that survived until scheduled euthanasia (day 42, 75, or 109) were designated as removed (ie, censored) from the study at the time of euthanasia and values of $P < 0.05$ were considered significant. Spearman correlation analysis was used to compare terbinafine concentrations between hair and skin and between hair and wing within each treatment group; values of $r_s > 0.5$ and $P < 0.05$ were considered significant. All analyses were performed with commercially available statistical software.[j]

Results

Bats

All 3 bats that were euthanized on day 1 had histologic lesions consistent with *P destructans* infection. Therefore, we were confident that the rest

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Table 1—Descriptive survival data for *Pseudogymnoascus destructans*–infected little brown myotis (*Myotis lucifugus*) that were treated with 1 of 4 dosages of terbinafine (6 [6 mg/kg group; n = 20], 20 [20 mg/kg group; 20], or 60 [60 mg/kg group; 20] mg/kg, SC, once daily for 10 days, or 200 mg/kg, SC, once daily for 5 days [200 mg/kg group; 20]), received the terbinafine vehicle solution only (50% polyethylene glycol 400 in water; 0.1 mL/kg, SC, once daily for 10 days; vehicle control group; 20), or did not receive any treatment (untreated control group; 20).

Treatment group	Median (25th–75th percentile) survival time (d)	No. of bats that died or were euthanized because of moribundity	No. of bats euthanized as scheduled for tissue collection		
			Day 42	Day 75	Day 109
Untreated control	48 (40–57)	16	2	0	0
Vehicle control	47 (36–101)	15	3	1	1
6 mg/kg	55 (34–88)	15	2	3	0
20 mg/kg	47 (15–73)	18	1	1	0
60 mg/kg	62 (31–75)	16	2	0	1
200 mg/kg	14 (5–73)	16	2	1	0

Treatment initiation was designated day 1. Prior to day 1, 4 bats were randomly selected from each of the 6 treatment groups to be euthanized for tissue collection on days 42 and 75 (ie, 24 randomly selected bats were scheduled to be euthanized on day 42 and day 75). The remaining 72 bats were scheduled for euthanasia and tissue collection at the end of the observation period (day 109). Two bats in the untreated control group, 1 bat in the 60 mg/kg group, and 1 bat in the 200 mg/kg group died before treatment initiation and were not included in the above statistics.

of the study bats were likewise infected. Most bats in the terbinafine-treated (6, 20, 60, and 200 mg/kg groups) and vehicle control groups could be hand-fed mealworms and reached or exceeded their original cave weights (ie, body weight at time of collection from the hibernation site in Virginia) before the start of the treatment period (day 1). One bat in the 20 mg/kg group refused to eat during the treatment period (days 1 through 10) and died. None of the bats developed evidence of an inflammatory reaction or infection at the injection site during the treatment period. Some bats in the 200 mg/kg group developed neurologic abnormalities such as ataxia, tremors, and conscious proprioception deficits (inability to correct body position) that were not observed in bats in the other terbinafine-treated groups. The mortality rate for the bats in the 200 mg/kg group was also substantially greater than that for the other terbinafine-treated groups during the early treatment period, and terbinafine administration to that group was discontinued on day 5. No gross or histologic abnormalities associated with treatment administration were identified in any of the major organs evaluated including the liver, kidney, heart, lung, spleen, gastrointestinal tract, brain, and thyroid gland for any of the bats.

Effect of treatment on survival

Two bats in the untreated control group, 1 bat in the 60 mg/kg group, and 1 bat in the 200 mg/kg group died before treatment initiation (day 1) and were not included in the survival analysis. Of the remaining 116 bats in the study population, 88 died and 8 were euthanized because of moribundity during the observation period. The median (25th to 75th percentile) survival times, number of bats that died or were euthanized because of moribundity, and number of bats that were euthanized as scheduled on days 42, 75, and 109 for each treatment group were summarized **(Table 1)**. The Kaplan-Meier survival curves for each of the 6 treatment groups were plotted **(Figure 1)**. Although the overall survival curves did not differ significantly ($P = 0.13$) among the treatment groups,

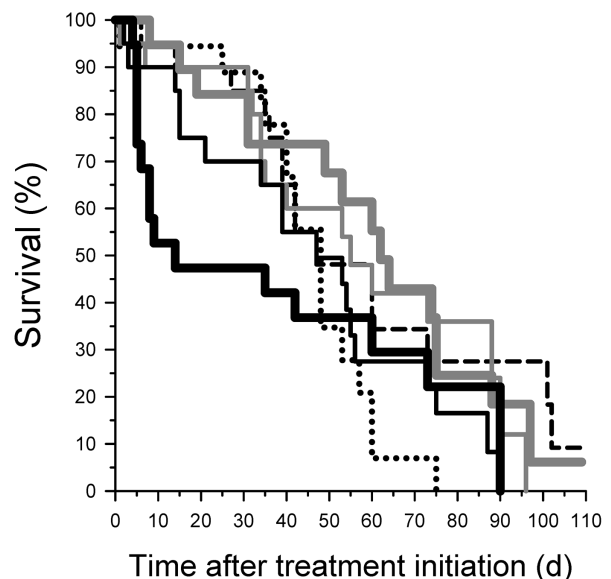

Figure 1—Kaplan-Meier survival curves for *Pseudogymnoascus destructans*–infected little brown myotis (*Myotis lucifugus*) that were treated with 1 of 4 dosages of terbinafine (6 [6 mg/kg group; n = 20; thin gray line], 20 [20 mg/kg group; 20; thin black line], or 60 [60 mg/kg group; 20; thick gray line] mg/kg, SC, once daily for 10 days, or 200 mg/kg, SC, once daily for 5 days [200 mg/kg group; 20; thick black line]), received the terbinafine vehicle solution only (50% polyethylene glycol 400 in water; 0.1 mL/kg, SC, once daily for 10 days; vehicle control group; 20; dashed line), or did not receive any treatment (untreated control group; 20; dotted line). Administration of the assigned treatment was initiated on day 1.

the median survival time for the 200 mg/kg group (14 days) was substantially lower than that (47 to 62 days) for the other 5 treatment groups. Only 1 bat in the vehicle control group and 1 bat in the 60 mg/kg group survived to the end of the observation period (day 109). All bats had either died or were euthanized (either because of moribundity or as scheduled) by day 75 for the untreated control group, by day 90 for the 20 and 200 mg/kg groups, and by day 96 for the 6 mg/kg group.

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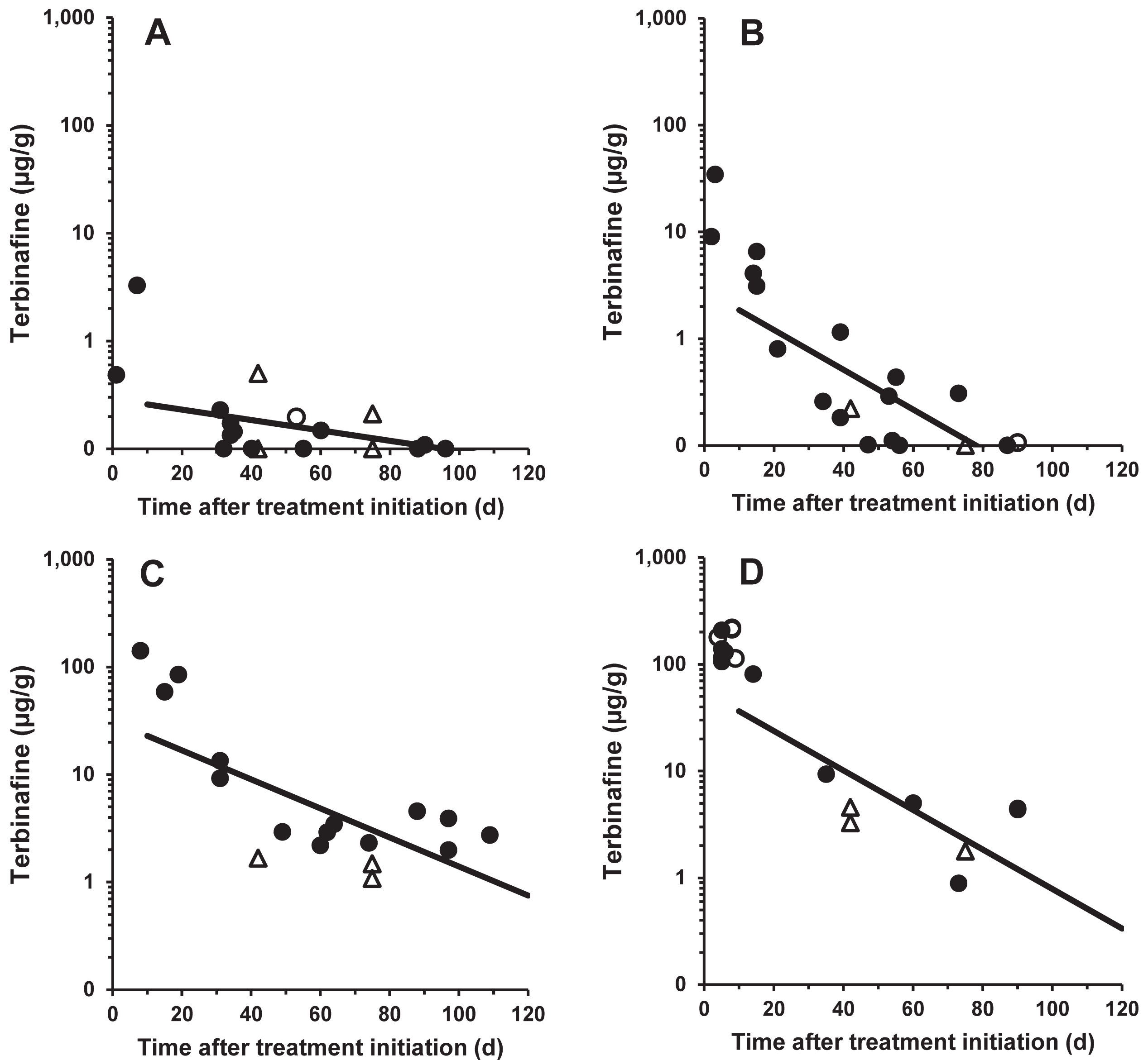

Figure 2—Logarithmic mean wing terbinafine concentration over time for bats assigned to the 6 (A), 20 (B), 60 (C), and 200 (D) mg/kg groups described in Figure 1. Terbinafine concentration was determined in duplicate for all tissue samples, and the mean concentration was calculated and used for analysis. A logarithmic transformation was applied to the terbinafine concentration data for each group to normalize it for regression analysis. Each symbol represents the logarithmically transformed mean wing terbinafine concentration for a bat that died (black circle), was euthanized because of moribundity (white circle), or was euthanized for tissue collection as scheduled on days 42, 75, or 109 (white triangle). The line represents the linear regression line for the data, the slope of which was used to calculate the mean tissue $t_{1/2}$ of terbinafine. ***See*** Figure 1 for remainder of key.

Pharmacokinetic parameters for terbinafine

None of the tissues obtained from bats in the untreated and vehicle control groups contained detectable concentrations of terbinafine (0.02 µg/g). Of the 80 bats that were assigned to the terbinafine-treated groups, tissue terbinafine concentrations were determined for only 77. One bat in the 60 mg/kg group and 1 bat in the 200 mg/kg were found dead on day 1 prior to initiation of terbinafine administration, and the tissues for 1 bat in the 6 mg/kg group that died on day 40 were lost. The terbinafine concentration was less than the assay limit of quantitation (0.1 µg/g) for all liver specimens obtained from bats in the 6 mg/kg group and over half of the liver specimens obtained from the bats in the other terbinafine-treated groups. Therefore, the $t_{1/2}$ and AUC were not calculated for liver.

All terbinafine concentrations were logarithmically transformed for linear regression. The logarithmic mean wing terbinafine concentrations over time for each of the terbinafine-treated groups were plotted **(Figure 2)** as were the logarithmic mean terbinafine concentrations in the skin, wing, hair, and liver for bats in the 60 mg/kg group **(Figure 3)**. All plots, except that for the mean liver terbinafine concentration over time

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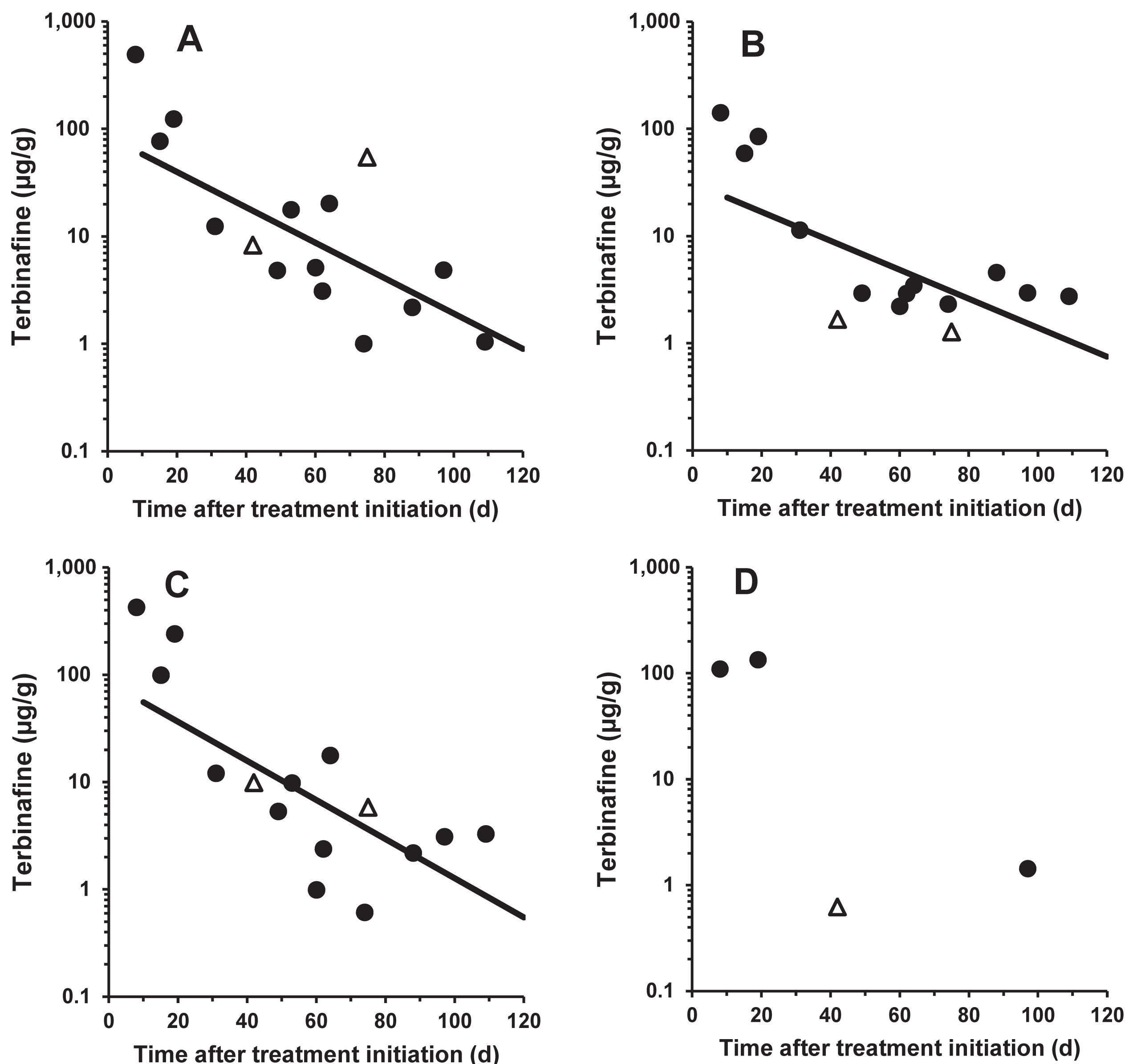

Figure 3—Logarithmic mean terbinafine concentration in the skin (A), wing (B), hair (C), and liver (D) for the bats in the 60 mg/kg group described in Figure 1. The terbinafine concentration in the liver was less than the assay limit of quantitation (0.1 µg/g) for 16 of the 20 bats; therefore, the linear regression line was not calculated for the liver. ***See*** Figures 1 and 2 for remainder of key.

for the 60 mg/kg group, indicated a logarithmic decline in tissue terbinafine concentrations after the end of the treatment period (ie, after day 10) that was consistent with a first-order concentration-dependent elimination process. For the 200 mg/kg group, there was a steep initial decline in terbinafine concentration in all tissues after the treatment period, presumably because over half of the bats in that group died during the treatment period. Conversely, the decline in tissue terbinafine concentrations during and after the treatment period for the other terbinafine-treated groups was less abrupt than that observed for the 200 mg/kg group, presumably because few bats in those groups died during the treatment period.

The respective estimated logarithmic mean $t_{1/2}$s for terbinafine in wing, skin, and hair were similar for bats in the 20, 60, and 200 mg/kg groups **(Table 2)** and substantially shorter than those for the 6 mg/kg group. However, unlike tissue specimens collected from bats in the 20, 60, and 200 mg/kg groups, some of the tissue specimens collected from bats in the 6 mg/kg group after day 60 had terbinafine concentrations that were below the assay limit of quantitation, which might have artificially increased the $t_{1/2}$.

The AUCs for the cumulative dose of terbinafine administered during the treatment period for skin and hair were 2- to 8-fold greater than that for the wing

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Table 2—Estimated logarithmic mean ± SE $t_{1/2}$ for terbinafine in the wing, skin, and hair of bats in the terbinafine-treated groups described in Table 1.

Treatment group	$t_{1/2}$ (d)		
	Wing	Skin	Hair
6 mg/kg	50 ± 32	40 ± 19	25 ± 8
20 mg/kg	16 ± 4	14 ± 2	21 ± 5
60 mg/kg	22 ± 7	18 ± 5	17 ± 4
200 mg/kg	16 ± 3	18 ± 5	19 ± 7

Of the 80 bats that were assigned to the terbinafine-treated groups, tissue terbinafine concentrations were determined for only 77. One bat in the 60 mg/kg group and 1 bat in the 200 mg/kg were found dead on day 1 prior to initiation of terbinafine administration, and the tissues for 1 bat in the 6 mg/kg group that died on day 40 were lost. Because tissue specimens were collected at only 1 point in time from each bat (ie, after death or euthanasia), it was not possible to derive accurate pharmacokinetic parameters for individual animals. Therefore, for each treatment group, the terbinafine concentration data for each tissue were pooled, and the estimated logarithmic mean ± SE $t_{1/2}$ for each tissue was calculated from the slope of the linear regression line for the logarithmically transformed terbinafine concentrations in the tissue of interest over time after treatment initiation. ***See*** Table 1 for remainder of key.

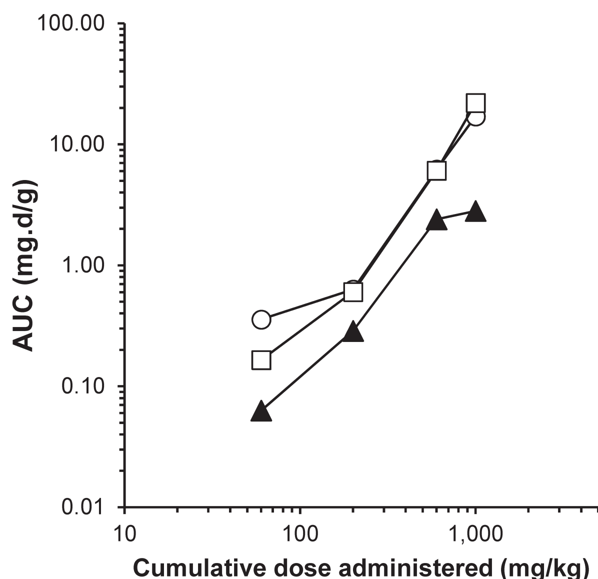

Figure 4—Mean AUC versus the cumulative terbinafine dose administered for hair (white circles), skin (white squares), and wing (black triangles) specimens of the terbinafine-treated bats of Figure 1. The cumulative terbinafine dose administered was 60 mg/kg for the 6 mg/kg group, 200 mg/kg for the 20 mg/kg group, 600 mg/kg for the 60 mg/kg group, and 1,000 mg/kg for the 200 mg/kg group. Notice that both the AUC and cumulative terbinafine dose are reported on a logarithmic scale. ***See*** Figure 1 for remainder of key.

(Figure 4). The AUC values were generally proportional to the cumulative dose of terbinafine administered with 1 exception. The AUC in the wing for the 200 mg/kg group (2.8 mg•d/g) was only slightly greater than that (2.4 mg•d/g) for the 60 mg/kg group.

The terbinafine concentration in hair was significantly ($P < 0.001$ for both comparisons) and positively correlated with the terbinafine concentration in skin ($r_s = 0.92$) and wing ($r_s = 0.82$; **Figure 5**).

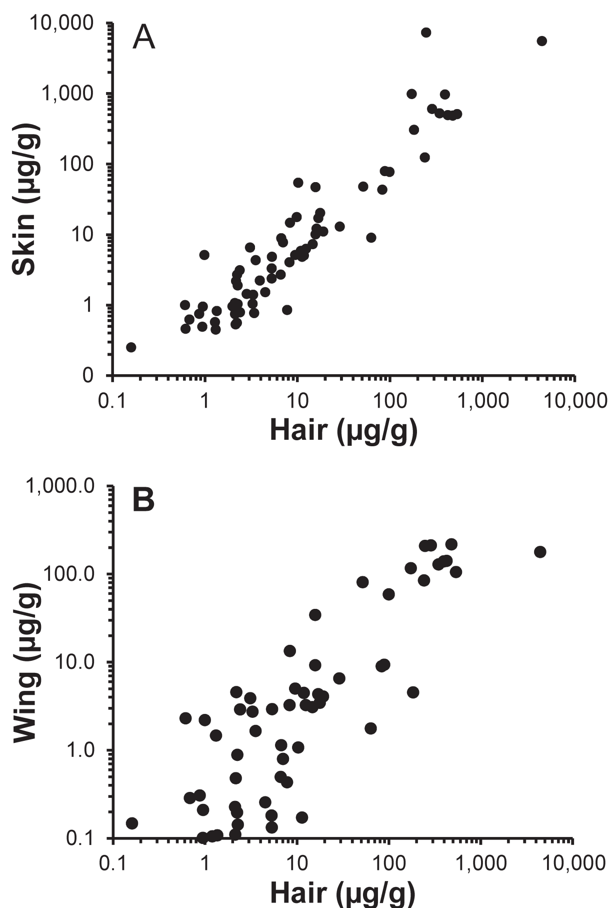

Figure 5—Scatterplots of the logarithmic mean terbinafine concentration in skin versus hair (A) and in wing versus hair (B) for the terbinafine-treated bats of Figure 1. The data for all 80 bats were pooled for this analysis. There was a strong positive correlation between the terbinafine concentration in hair and that in both the skin ($r_s = 0.92$; $P < 0.001$) and wing ($r_s = 0.82$; $P < 0.001$). ***See*** Figures 1 and 2 for remainder of key.

Discussion

To our knowledge, the present study was the first to describe the pharmacokinetics of any therapeutic agent in any bat species. In the present study, the $t_{1/2}$ of terbinafine in bat tissues was fairly long and ranged from 14 to 22 days following administration of 20 and 60 mg of terbinafine/kg, SC, for 10 days and from 16 to 19 days following administration of 200 mg of terbinafine/kg, SC, for 5 days. Those values were similar to the $t_{1/2}$ reported for terbinafine in the plasma (6 to 23 days)[8,11,14] and skin (5 days)[15] of humans and plasma of African penguins (*Spheniscus demersus*; 6 days)[16] and red-tailed hawks (*Buteo jamaicensis*; 7 days)[17] and were much longer than the $t_{1/2}$ reported for terbinafine in the plasma of horses (8 hours),[13] Greyhounds (9 hours),[13] Hispaniolan parrots (*Amazona ventralis*; 6 to 14 hours),[18] and cats (8 hours).[19] The reason for the large discrepancies in the $t_{1/2}$ of terbinafine among species is unclear. The short $t_{1/2}$s might be a reflection of the sampling duration of those studies[13,18,19] or the use of an assay with sensitivity that was insufficient to measure the true

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$t_{1/2}$ and instead measured the redistribution half-life of the drug. Interestingly, the $t_{1/2}$ of terbinafine in the hair (13 days)[20] of cats was much longer than that in plasma (8 hours),[19] although caution should be used when comparing those values because they were derived from different studies. The metabolism and elimination rates of terbinafine may also be affected by species-specific factors. Because the metabolic processes of bats during prolonged torpor (hibernation) are substantially decreased from those during more active periods, it is possible that the prolonged tissue $t_{1/2}$s observed in the present study were a function of the fact that the bats were maintained in a state of torpor. Further investigation to determine the pharmacokinetic parameters of terbinafine in bats that are not in a state of torpor is necessary to confirm whether bat activity level affects the drug's metabolism. Regardless, pharmacokinetic data for drugs in other species should be extrapolated to bats with considerable caution.

Although bats were scheduled to be euthanized for tissue collection at specific times (day 42, 75, or 109) after terbinafine administration was initiated, most died or were euthanized because of moribundity prior to their scheduled euthanasia date. Bats were inspected only once daily; therefore, the bats that died may have been dead for almost 24 hours before tissues were harvested and frozen. That delay between death and tissue harvest could have adversely affected measurement of terbinafine concentrations owing to autolysis. However, following the treatment period (ie, after day 10), all bats were housed in a hibernation chamber where the temperature was maintained at 8°C, which should have minimized temperature-dependent tissue degradation in any bats that died. Our results appeared to support that supposition because the tissue terbinafine concentrations in the bats that died were not consistently lower than those for bats that were euthanized because of moribundity or as scheduled for tissue collection (Figures 2 and 3).

In the present study, the terbinafine concentrations in liver were substantially lower than those in skin, wing, and hair and were frequently unquantifiable. That finding might have been an artifact associated with the propensity for liver to undergo rapid autolysis after death. However, the terbinafine concentrations for liver specimens harvested immediately after euthanasia were not higher than those for liver specimens harvested at various times after death. Consequently, we believe that finding may simply reflect the lower partitioning of terbinafine (a lipophilic drug) in the liver, an organ that generally has low lipid content, compared with other more lipid-rich tissues such as skin and fat, as has been reported for rats.[21]

The AUC for terbinafine in wing tissue was lower than that in hair and skin. This may reflect a propensity for terbinafine to accumulate in hair[15] given that bat wings are essentially hairless, whereas the skin specimens were obtained from the torso and contained a substantial amount of hair. Because hair can be collected noninvasively, we decided to determine whether the terbinafine concentration in the hair of treated bats would accurately reflect the terbinafine concentrations in the skin and wing of those bats such that it could be used to monitor terbinafine concentrations over time. Results indicated that there was a strong positive correlation between the terbinafine concentration in the hair and that in both the skin and wing of treated bats, which suggested that hair terbinafine concentration could be used as a proxy for skin and wing terbinafine concentrations.

Results of the present study indicated that *P destructans*–infected bats could be administered terbinafine at dosages up to 60 mg/kg, SC, once daily for 10 days without any evidence of toxicosis. However, administration of 200 mg of terbinafine/kg, SC, once daily for 5 days resulted in adverse neurologic effects such as ataxia, muscle tremors, and conscious proprioceptive deficits. Additionally, the mortality rate for bats in the 200 mg/kg group was much higher than that for bats in the other terbinafine-treated groups during the treatment period, and over half of the bats in the 200 mg/kg group died or were euthanized because of moribundity by day 9. In human patients treated with oral terbinafine, the most common adverse effects are gastrointestinal tract disturbances, headache, skin disorders, and taste abnormalities that are mild and reversible.[10,11] The product label[22] for terbinafine tablets that are approved for use in human patients lists taste and smell disturbances (sometimes permanent), hepatotoxicosis, depression, neutropenia, and Stevens-Johnson syndrome (immune complex–mediated hypersensitivity) as potential adverse effects. Adverse effects reported by human patients who received up to 20 times the recommended daily treatment dose of terbinafine include nausea, vomiting, abdominal pain, dizziness, rash, frequent urination, and headache.[22] We could not find any specific reports regarding the neurotoxic potential of terbinafine that could provide a possible mechanism for the adverse neurologic effects observed for many of the bats in the 200 mg/kg group. Nevertheless, we found it interesting that many of the adverse effects associated with terbinafine administration in human patients also appear to have neurologic origins (eg, taste and smell disturbances, headache, and dizziness).

Although results of preliminary in vitro efficacy studies suggest that terbinafine may be effective against *P destructans*,[a] the MIC of terbinafine for that organism has not been established. Results of a study[23] in which the susceptibility of *P destructans* to various antifungals was evaluated indicate the MICs of amphotericin B, fluconazole, itraconazole, ketoconazole, and voriconazole against that organism are comparable to the MICs of those agents against other fungal pathogens. The MICs of terbinafine reported for 119 strains of dermatophytes and nondermatophyte filamentous fungi range from

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0.003 to 2 μg/mL.[24] In the present study, a terbinafine concentration ≥ 2 μg/g (ie, equivalent to 2 μg/mL) was consistently observed in wing tissue for at least 30 days after treatment initiation in the 60 mg/kg group (the highest tolerated dose) and 6 days after treatment initiation in the 20 mg/kg group, but was observed for only 1 bat in the 6 mg/kg group (lowest dose evaluated).

The survival rate did not differ significantly between bats that received the highest tolerated terbinafine dosage (60 mg/kg, SC, once daily for 10 days) and bats in the vehicle control group. *Pseudogymnoascus destructans* is a slow-growing fungus. It is possible that the duration of treatment (10 days) and predicted duration of effective tissue terbinafine concentrations (30 days; estimated on the basis of the duration of wing terbinafine concentrations ≥ 2 μg/g for the 60 mg/kg group) were insufficient to completely eliminate this slow-growing fungus from infected tissues. Also, the bats were likely stressed by relocation from their native environment to a hibernation chamber and treatment administration, which could have affected the results. We attempted to minimize stress by hand-feeding the bats in the terbinafine-treated groups and the vehicle control group during the 7-day acclimation period prior to treatment initiation and throughout the treatment period before they were placed in the hibernation chamber. That hand-feeding appeared to have some beneficial effects because the bats in the vehicle control group that were hand-fed had a lower mortality rate between days 50 and 109 than did the bats in the untreated control group that were not hand-fed.

Successful treatment of *P destructans*–infected bats will likely require terbinafine administration for at least 3 months, but additional research is necessary to confirm or refute this. However, repeated dosing of a drug to individual animals by means of injection, oral administration, or topical application is not feasible for large populations of infected bats, which are typically encountered when dealing with outbreaks of naturally occurring white-nose syndrome. Administration of 1 dose of a sustained-release preparation of terbinafine might be a viable alternative. Results of an in vitro study[25] indicate that terbinafine was released for up to 6 months from a terbinafine-impregnated SC implant, even at temperatures as low as 4°C, the typical body temperature for little brown myotis during hibernation.

Results of the present study indicated that terbinafine doses > 6 mg/kg but < 200 mg/kg should be evaluated further for the treatment of *P destructans*–infected bats. Also, because there was a strong positive correlation between the terbinafine concentration in hair and that in both the skin and wings of treated bats, collection of serial hair specimens may represent a noninvasive method for monitoring the terbinafine concentration in treated bats over time.

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Footnotes

a. Ghannoum MA, Department of Dermatology, School of Medicine, Case Western Reserve University, Cleveland, Ohio: Personal communication, 2016.
b. FerretVite, 8 in 1 Pet Products Inc, Islandia, NY.
c. Terbinafine hydrochloride powder, Novartis Pharmaceuticals Corp, Hanover, NJ.
d. Sigma-Aldrich, St Louis, Mo.
e. Toronto Research Chemicals, Toronto, ON, Canada.
f. Safelock, Eppendorf, Hauppauge, NY.
g. Bullet Blender, Next Advance Inc, Averill Park, NY.
h. Surveyor HPLC with Deca XP Plus mass spectrometer, Thermo Fisher Scientific, Waltham, Mass.
i. Zorbax C18 XDB, Agilent Technologies, Wilmington, NC.
j. Sigmaplot, version 12, Systat Software, San Jose, Calif.

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