

Pharmacokinetics of oral terbinafine in adult horses

by

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Abstract

The primary study objective was to compare the pharmacokinetics of p.o. terbinafine alone to p.o. terbinafine administered with p.o. cimetidine in healthy adult horses. The second objective was to assess the pharmacokinetics of terbinafine when administered per rectum in two different suspensions at 30 mg/kg to adult horses. Six healthy adult horses were included in this crossover study. Plasma terbinafine concentrations were quantified with liquid chromatography and mass spectrometry. The half-life (geometric mean) was 8.38 and 10.76 h, for p.o. alone and p.o. with cimetidine, respectively. The mean maximum plasma concentrations were 0.291 lg/mL at 1.54 h and 0.418 lg/mL at 1.28 h for p.o. alone and p.o. with cimetidine, respectively. Terbinafine with cimetidine had an average CMAX 44% higher and the relative F was 153% compared p.o. terbinafine alone but was not statistically different ($P > 0.05$). Terbinafine was infrequently detected when administered per rectum in two different suspensions (water or olive oil). Minor adverse effects included oral irritation, fever, and colic. All resolved spontaneously. More pharmacokinetic studies are indicated assessing drug–drug interactions and using multiple dosing intervals to improve our knowledge of effective oral dosing, the potential for drug accumulation, and systemic adverse effect of terbinafine in horses.

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Chapter 1 - Introduction

Aspergillus sp. cause life-threatening fungal pneumonia, guttural pouch infections, and fungal keratitis in horses (Gerard et al. 2006). Fungal infections in horses are clinically challenging with regard to limited therapeutic treatments and overwhelming expense for the client. The polyene antibiotics and the azoles are the two common classes of antifungal drugs used in equine medicine. Unfortunately, options for the oral treatment of fungal infections are limited by bioavailability and cost. Itraconazole and voriconazole have activity against *Aspergillus sp.* and are absorbed following oral administration (Davis et al. 2005, 2006), but the cost prohibitive nature of most oral antifungal treatments in equine patients limits owner compliance, thus worsening the prognosis for fungal diseases in horses.

Terbinafine (Lamisil ®) is an allylamine antifungal drug approved for use in humans. This allylamine is both fungicidal and fungistatic. Terbinafine has antifungal activity alone or in multidrug combinations against fungal organisms including *Candida albicans*, *Malassezia pachydermatis*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Microsporium canis*, and *Aspergillus species* (Shadomy et al., 1985; Goudard et al., 1986; Petronyi et al., 1987; Saarikoski et al., 2015).

Terbinafine is well absorbed after oral administration in small animal species and humans, but oral bioavailability in horses is incomplete due to first pass metabolism (Williams et al., 2010). Drug–drug interactions with terbinafine have been published (Saarikoski et al., 2015). For instance, CYP450 is a hepatic enzyme system that metabolizes terbinafine; alterations in this enzyme system have direct effects on subsequent plasma drug concentrations. Cimetidine is a CYP450 inhibitor that is occasionally administered in combination with terbinafine in humans.

This drug interaction results in an increased plasma concentration of terbinafine and a 33% decrease in terbinafine clearance in humans (Anonymous, 2015).

Objective

The first objective of this study was to compare the pharmacokinetics of oral terbinafine alone to p.o. terbinafine administered in combination with p.o. cimetidine in healthy adult horses. The second objective of this study was to assess the pharmacokinetics of terbinafine (30 mg/kg) when administered per rectum in two different formulations, an aqueous suspension and separately in an olive oil suspension to adult horses.

Chapter 2 - Literature Review

Aspergillus species

The most ubiquitous and pathogenic *Aspergillus* species documented in equine veterinary literature are *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus versicolor*, *Aspergillus flavus*, and *Aspergillus nidulans* (Cafarchia C. et al 2012). These opportunistic environmental fungi inhabit dead leaves, stored grain, compost piles, hay, and are present in normal equine airways. The spores are inhaled by horses and may cause fungal keratitis, guttural pouch mycosis, or fungal pneumonia (Gerard et al, 2006). Fungal infections occur in immunocompromised animals suffering from endocrinopathies, neoplasia or animals administered prolonged antibiotics and corticosteroids. However, in horses the etiology is unclear as to why some horses do and some horses do not develop an infection with this common upper respiratory fungus (Cafarchia C. et al 2012). Clinical signs of *Aspergillus sp.* infections depend upon the location of the disease. Horses with fungal keratitis may present with ptosis, blepharospasm, miosis, and may have a concurrent deep stromal abscess (Gerard et al, 2006). Horses with guttural pouch mycosis may present for epistaxis, cranial nerve deficits, and/or secondary pneumonia. Also, horses that develop fungal pneumonia infection have a cough and are lethargic. The horses that acquire fungal pneumonia infections are commonly hospitalized patients that have been administered prolonged antibiotics therapies. (Cafarchia C. et al 2012)

Diagnosis

Diagnosis is difficult in the early stages of the disease processes. Diagnosis is based on history, clinical signs, and location of the infection. Horses with fungal keratitis *Aspergillus sp.* can be identified with cytology and/or culture. Horses infected with guttural pouch mycosis endoscopy is a vital diagnostic to evaluate the upper respiratory tract in the horse. Horses that

develop fungal pneumonia radiology is utilized to evaluate the lower respiratory tract. Fungal species can be identified with trans-tracheal wash cytology and/or culture (Cafarchia C. et al 2012; Gerard et al 2006)

Treatment

Treatments of fungal infections include medical and surgical therapies depending on the site of infection and the severity of disease. Fungal infections in horses are clinically challenging with regards to limited therapeutic treatments and an overwhelming expense for the client. The polyene antibiotics and the azoles are the two common classes of antifungal drugs used in equine medicine. Unfortunately, options for the oral medical treatment of fungal infections in horses are limited by the drug's bioavailability and cost. The pharmacokinetics of ketoconazole, itraconazole, fluconazole, and voriconazole in horses have been studied (Latimer, et al, 2001; Davis, et al, 2005; Davis, et al, 2006). Itraconazole and voriconazole have activity against *Aspergillus sp.* and are absorbed following oral administration (Davis et al. 2005, 2006). The cost prohibitive nature of most oral antifungal treatments in equine patients limits owner compliance thus worsening the prognosis for fungal diseases in horses.

Terbinafine antifungal agent

Terbinafine (Lamisil ®) is an allylamine antifungal drug approved for use in humans. This allylamine is both fungicidal and fungistatic. Terbinafine prevents the biosynthesis of ergosterol (necessary for cell membrane synthesis) by the inhibition of squalene epoxidase. The intracellular accumulation of squalene is cytotoxic (Ryder & Favre, 1997). The depletion of ergosterol interferes with cell membrane function and growth. Terbinafine has antifungal activity alone or in multi-drug combinations against fungal organisms including: *Candida albicans*, *Malassezia pachydermatis*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma*

capsulatum, *Microsporium canis*, and *Aspergillus sp.* (Shadomy et al 1985; Goudard et al 1986; Petranyi 73 et al 1987; Saarikoski T et al 2015). Terbinafine is well absorbed after oral administration in small animal species and humans, but oral bioavailability in horses is incomplete due to first pass metabolism (Williams et al 2010). Drug clearance is primarily through hepatic biotransformation into numerous inactive metabolites. Williams and colleagues found that the terminal half-life after oral administration was 8.1 hours, but the mean maximum plasma concentration (C_{MAX}) was only 0.31 $\mu\text{g/ml}$ after a single 20-mg/kg PO dose. The drug was well tolerated but did not achieve adequate plasma concentrations to exceed the MIC (0.05-2 $\mu\text{g/ml}$) for relevant *Aspergillus spp* (Shadomy et al 1985; Goudard et al 1986; Petranyi et al 1987). Pharmacokinetic drug interactions with terbinafine have been published (Saarikoski T et al 2015). For instance, CYP450 is a hepatic enzyme system that metabolizes terbinafine; alterations in this enzyme system have direct affects on subsequent plasma drug concentrations. Cimetidine is a CYP450 inhibitor that is frequently administered in combination with terbinafine in humans. This drug interaction results in an increased plasma concentration of terbinafine and a 33% decrease in terbinafine clearance (Anonymous, 2015). The veterinary literature on terbinafine administered alone or in antifungal drug combinations has been shown to be clinically effective and well tolerated in domesticated and non-domesticated animals. However, investigations testing application of terbinafine in horses is limited. Williams et al 2010 investigated terbinafine absorption in horses when administered a single 20-mg/kg oral dose over 24 hours. A separate report indicated that terbinafine administered to horses at 30-mg/kg PO for up to 14 days 96 resulted in no observed adverse effects. There are no studies that examined the pharmacokinetics of terbinafine in horses when administered alternate routes aimed at achieving MICs effective against *Aspergillus sp.*

Chapter 3 - Material and Methods

Animals

The Institutional Animal Care and Use Committee at Kansas State University approved the study. This crossover systematically randomized study used six adult healthy horses, ranging in age and weight from 8 to 17 years and 490 to 620 kg, respectively. The breeds included three Quarter Horses, one Paint, one Trakehner, and one Thoroughbred. Three of the horses were mares, and three were geldings. The horses were acclimated to the indoor stalls the day prior to drug administration. Horses were offered brome or prairie hay and water ad libitum throughout the study, consistent with their routine roughage diet. Horses were fed their routine ration between 2 and 3 pounds of pelleted feed, twice daily. Prior to study enrollment, a physical examination and complete blood count were conducted and were found to be within species- and age-specific reference intervals. Animals were monitored for 24 h after drug administration.

Drug dosing treatments

The study consisted of four treatments using terbinafine tablets (terbinafine hydrochloride, 250 mg tablets; Liconsa, Guadalajara, Spain). Terbinafine was administered p.o. (30 mg/kg) to all six horses; terbinafine was administered p.o. (30 mg/kg) to all six horses 2 h after 30 mg/kg p.o. cimetidine (cimetidine, 400 mg tablet; Novopharm Limited, Toronto, ON, Canada); three randomly chosen horses received terbinafine tablets (30 mg/kg) crushed and suspended in water per rectum, and three horses received terbinafine tablets (30 mg/kg) crushed and suspended in olive oil per rectum. For the oral administration, both terbinafine and cimetidine tablets were crushed individually with a mortar and pestle and were combined with water and molasses to make a paste-like consistency. Two 35- cc dosing syringes were used to

administer total drug volume. The tablet total administered to each horse was rounded to the nearest whole tablet, regardless of route. At least 7 days was allowed between each treatment as a washout period.

Blood sampling for plasma analysis of terbinafine

Intravenous jugular catheters were aseptically placed for blood collection. Whole blood (9 mL) was collected and discarded prior to sample collection to ensure that samples were not contaminated with heparinized saline. Whole blood (9 mL) was collected at each time point followed by the catheter being flushed with 12 mL of heparinized (4 IU/mL) saline. The whole blood was transferred into tubes containing lithium heparin at times 0 (prior to drug administration), 10 min, 20 min, 30 min, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h after drug administration. Blood was kept on ice until separation by centrifugation. Blood was centrifuged for 15 min at 3000g; plasma was separated and stored frozen at -70 °C until analysis with LC/MS/MS.

Plasma concentrations of terbinafine

Plasma concentrations of terbinafine were measured with ultraperformance liquid chromatography (Acquity UPLC, Waters, Milford MA, USA) with triple quadrupole mass spectrometry (TQD, Waters) UPLC/MS. The qualifying and quantifying ions for terbinafine (Sigma-Aldrich, St Louis, MO, USA) were mass/charge (m/z) 292.27 and 140.994, respectively. The qualifying and quantifying ions for the internal standard tolnaftate (Sigma-Aldrich) were m/z 308.26 and 148.027, respectively. Plasma standards were prepared in untreated equine plasma by adding standard solution in 50% methanol to untreated plasma to not exceed 10% spiking solution per standard. Standard curves achieved a linear range 0.005– 1 $\mu\text{g/mL}$ with 0.005 $\mu\text{g/mL}$ being the lower limit of quantification. Daily runs were accepted if the plasma standards

and quality control samples were within 15% of the actual concentration. Plasma standards, quality control samples, and plasma samples were extracted identically using pass-through sample preparation to remove phospholipids and proteins (Ostro Pass-through Sample plates, Waters). Plasma, 50 μ L, was added to the plates, followed by 150 μ L acetonitrile with 1% formic acid and 500 ng/mL tolinaftate, which was mixed and then the precipitate passed through the sample plates. The injection volume was 0.5 μ L. A C18 column (CSH C18+, 50 \times 9.21 mm, 1.7 μ m pore size, Waters, Milford, MA, USA) maintained at 55 $^{\circ}$ C was used for separation. The mobile phase consisted of A: 0.2% formic acid in deionized water and B: acetonitrile at a flow rate of 0.8 mL/min. The mobile phase gradient started at 70% A with a linear gradient to 100% B at 0.3 min, which was held until 0.89 min with a step to 70% A at 0.9 min with a total run time of 1.5 min. The accuracy of the assay was determined on replicates of five quality control samples in pooled untreated equine plasma fortified with 0.005, 0.05, and 1 μ g/mL terbinafine and were within 1, 1, and 4% of expected concentrations, respectively. The coefficient of variation was determined on replicates of five quality control samples at 0.005, 0.05, and 1 μ g/mL and were 6, 8, and 4%, respectively.

Pharmacokinetics and statistical analysis

Noncompartmental pharmacokinetic analysis was completed using computer software (Phoenix 64, Certara, Princeton, NJ, USA). The pharmacokinetic values for each individual were calculated, and the geometric mean and range (minimum– maximum) are reported (Julious & Debarnot, 2000). The following pharmacokinetic parameters were calculated: AUC₀-INF = area under the curve extrapolated to infinity using the linear trapezoidal method; AUC_{extrap} = percent of the AUC extrapolated to infinity; AUC₀-LAST = area under the curve from time 0 to the last concentration above the lower analytical limit of quantification; Cl/F = clearance per

fraction of the dose absorbed; $T_{1/2}$ = terminal half-life; k_z = terminal rate constant; MRT = mean residence time extrapolated to infinity; V_z/F = volume of distribution (area method) per fraction of the dose absorbed. The CMAX = maximum plasma concentrations and TMAX = time of CMAX were determined directly from the data. The relative F = fraction of the dose of terbinafine with cimetidine absorbed relative to oral terbinafine alone was calculated with the following equation:

$$\frac{\textit{Terbinafine AUC with Cimetidine}}{\textit{Terbinafine AUC administered alone}}$$

Statistical analysis of the pharmacokinetic data was performed with computer software (Sigma Plot 12.5, Systat, Chicago IL, USA) using Mann–Whitney rank-sum analysis (Powers, 1990). The significance was set at $P < 0.05$. The pharmacokinetic parameters of p.o. terbinafine administered alone were compared to the pharmacokinetic data of p.o. terbinafine administered concurrently with p.o. cimetidine. One horse each in the terbinafine (horse #1, 61%) and terbinafine with cimetidine (horse #2, 47%) had excessive extrapolation of the AUC, which could lead to inaccurate estimates of some pharmacokinetic parameters. Therefore, the only PK parameters reported and statistically assessed from those horses were the AUC_{0-LAST}, CMAX, and TMAX. The relative fraction of the dose absorbed was calculated in four horses as horse #1 (terbinafine) and horse #2 (terbinafine with cimetidine) had excessive extrapolation of the AUC_{0-INF}. Per rectum pharmacokinetic analysis was not conducted for either suspension due to the low number of plasma concentrations exceeding the lower limit of analytical quantification.

Chapter 4 - Results

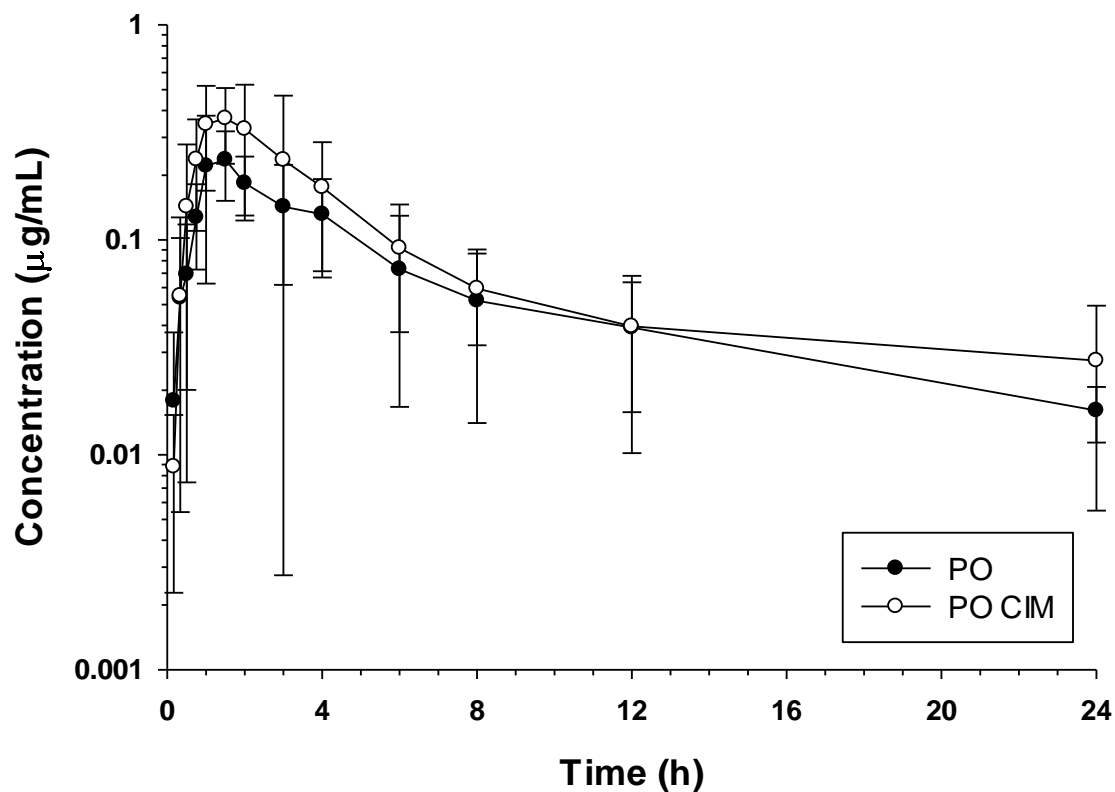


Figure 1.4 The mean and standard deviation plasma concentration of terbinafine following oral terbinafine administered alone (PO) and combination of terbinafine orally with cimetidine (PO-CIM)

The mean maximum plasma concentrations of p.o. terbinafine administered alone and terbinafine with cimetidine were 0.291 µg/mL at 1.54 h and 0.417 µg/mL at 1.28 h, respectively.

Parameter	Units	Oral Terbinafine			Oral Terbinafine with Cimetidine			P-Value
		N	Mean	Range	N	Mean	Range	
AUC _{Extrap}	%	5	10.4	7.3 - 14.5	5	14.1	10.1 - 18.0	0.222
AUC	hr*ug/mL	5	1.66	1.18 - 2.38	5	1.943	0.935 - 3.73	0.548
AUC _{0-LAST}	hr*ug/mL	6	1.39	0.992 - 2.20	6	1.81	0.788 - 3.355	0.310
Cl/F	mL/min/kg	5	0.300	0.211 - 0.420	5	0.257	0.134 - 0.539	0.549
C _{MAX}	ug/mL	6	0.291	0.176 - 0.510	6	0.418	0.156 - 0.701	0.156
T _{1/2}	hr	5	8.38	5.98 - 10.88	5	10.76	8.66 - 13.58	0.151
λ _z	1/hr	5	0.083	0.064 - 0.116	5	0.0644	0.0510 - 0.080	0.095
MRT	hr	5	9.74	8.69 - 11.11	5	10.76	8.68 - 12.50	0.548
T _{MAX}	hr	6	1.54	1.00 - 4.00	6	1.28	1.00 - 2.00	0.699
V _Z /F	L/kg	5	0.218	0.109 - 0.370	5	0.239	0.106 - 0.507	1.000
Relative F	%		N/A	N/A	4	153	108 - 257	N/A

Table 1.4 Pharmacokinetics of oral terbinafine and oral terbinafine administered concurrently with oral cimetidine (geometric mean and ranges).

AUC_{Extrap} = percent of the AUC extrapolated to infinity; AUC_{0-LAST} = area under the curve from time 0 to the last concentration above the lower analytical limit of quantification; AUC = AUC extrapolated to infinity; Cl/F = clearance per fraction of the dose absorbed; C_{MAX} = maximum plasma concentrations; T_{1/2} = terminal half-life; λ_z = terminal rate constant; MRT = mean residence time extrapolated to infinity; T_{MAX} = time of C_{MAX}; V_Z/F = volume of distribution (area method) per fraction of the dose absorbed; relative % F = fraction of the dose absorbed relative to oral terbinafine. The P value comparing oral terbinafine to oral terbinafine concurrently with oral cimetidine was calculated using the Mann-Whitney Rank sum test.

Terbinafine with cimetidine achieved an average 44% higher C_{MAX} and 30% higher AUC_{0-LAST} compared to oral terbinafine alone. The mean relative F of terbinafine with cimetidine was 153% using AUC_{0-INF}. However, these parameters were not statistically different (P > 0.05). No statistical difference in any pharmacokinetic parameter was found between the two treatment protocols. The highest measured concentration of terbinafine after per rectum administration of terbinafine suspended in water to three horses was 0.059 lg/mL. The

highest measured concentration of terbinafine after per rectum administration of terbinafine suspended in olive oil to three horses was 0.013 lg/mL. Most of the plasma concentrations after per rectum administration of terbinafine were below the analytical lower limit of quantification (0.005 lg/mL) precluding pharmacokinetic analysis.

Chapter 5 - Discussion

To the authors' knowledge, this is the first terbinafine pharmacokinetic report of a 30-mg/kg oral dose and terbinafine combined with cimetidine. Additionally, this is the first report of terbinafine administered per rectum in adult horses. The pharmacodynamics of terbinafine suggests clinical efficiency against *Aspergillus* spp, *Fusarium* spp., *Paecilomyces* spp., *Candida albicans*, dematiaceous molds, and *Scedosporium prolificans*, but efficacy studies are needed to confirm clinical efficacy (Shadomy et al 1985; Goudard et al, 1986; Petranyi et al 1987). The reported MIC for *Aspergillus* species are as follows: *Aspergillus fumigatus* MIC range – 0.2-5 ug/ml, *Aspergillus flavus* MIC range 0.02-0.05 ug/ml, and *Aspergillus niger* MIC range 0.02-0.2 ug/ml. *Aspergillus* spp are commonly treated with itraconazole, clotrimazole topically, fluconazole, voriconazole, and amphotericin B, but the *in vitro* activity of terbinafine suggests it could be a reasonable treatment choice.

The single 30 mg/kg oral dose, 30 mg/kg PO in combination with PO cimetidine, and terbinafine 30 mg/kg per rectum all failed to achieve plasma concentrations exceeding the reported MIC levels required for all *Aspergillus* spp. However, plasma concentrations exceeding the MIC ranges for *Aspergillus niger* and *A. flavus* were achieved, but the MIC ranges were not for equine isolates so it is unclear if the MIC for equine isolates are within similar ranges. Multiple dose administration could result in drug accumulation and achieve the MIC requirements of all *Aspergillus* spp. However, further studies are needed to document the multiple dose pharmacokinetics of terbinafine in horses.

A study by Cavalherio et al (2009) observed a synergistic pharmacodynamic interaction of terbinafine when combined with other antifungal agents. That investigation concluded combination of terbinafine with fluconazole achieved the highest terbinafine plasma

concentrations when compared to the other protocols. Therefore, the therapeutic efficacy of terbinafine may be enhanced not only by pharmacokinetic interactions, but pharmacodynamic interactions in the horse. However further studies are needed to assess the efficacy and safety of drug combinations in horses.

Although in the current study the combination of terbinafine with cimetidine resulted in a higher plasma concentration (C_{MAX} and AUC's) than terbinafine administered alone, this difference did not reach statistical significance. The increase in plasma concentration may be due to the inhibitory effects of cimetidine acting on the hepatic metabolizing enzyme system. An interaction of similar magnitude is documented in humans administered terbinafine with cimetidine resulting in a 33% decrease in clearance which should produce a 33% increase in AUC (Anonymous, 2015). The FDA defines a weak inhibitor resulting in a 25-100% increase in the AUC of the substrate. Based on the results of this study, cimetidine can be considered a weak inhibitor of terbinafine in the horse. If moderate (100-500% increase in terbinafine AUC) or strong inhibitors (>500% increase in terbinafine AUC) of terbinafine in horses are found, then the potential clinical utility of terbinafine would be markedly increased.

Per rectum drug administration had low plasma concentrations when compared to the oral route. The rationale of using the per rectal route in horses is aimed at reducing adverse effects observed with oral administration while in addition, potentially improving systemic drug exposure due to reduced first pass metabolism. Low rectal bioavailability may have been due to poor water solubility of terbinafine, pre-systemic metabolism, or interactions with rectal contents. A pilot study using three horses each assessed terbinafine suspended in water and in olive oil at a doses of 30 mg/kg terbinafine. However, data from the pilot study using terbinafine suspended with olive

oil revealed poor rectal absorption, similar to the water suspension. Based on this data, terbinafine administered orally produced superior systemic drug exposure compared to per rectal.

Adverse affects demonstrated by the horses in this study included asymptomatic fever and oral irritation. This elevation in temperature may have been due to environmental causes in the barn, thermometer error, or systemic drug adverse effect. Oral adverse affects observed in this report correlated with the previous study by Williams et al 2010. In our study the primary investigator found mixing crushed terbinafine tablets with water made a paste consistency. This suspension may have caused the minor oral adverse effects observed in three horses.

Limitations of this study include only a single terbinafine dose was administered, so no conclusions can be made on multiple dosing protocols in horses at this time. A healthy population of adult horses was used in this investigation, so conclusions of drug plasma concentrations in sick individuals or foals cannot be concluded. The horse's liver and kidney values were not evaluated after the terbinafine administration so no conclusions could be made on systemic adverse effects, however clinical examination of all horses was normal throughout the study duration. Relative bioavailability was calculated due to the lack IV drug formulation to obtain the true percent bioavailability. The number of horses investigated in this study is a limitation when determining a statistical difference among the drug protocols in question due to the observed variability. In conclusion, orally dosed terbinafine with cimetidine did not produce severe adverse effects when a single dose was administered to the adult horse. Orally dosed terbinafine compared to per rectal is superior with regard to drug plasma concentration. Potential oral irritation, palatability, or fever should be considered adverse effects when administering terbinafine to horses. More pharmacokinetic studies are indicated to determine the safest, highest, and most cost effective terbinafine dose administered orally alone and/or in combination with cimetidine in adult horses.

Indications for PK studies using multiple dosing intervals will improve our knowledge of a potential residual drug accumulation and potential systemic side effects in horses. Further randomized case control studies are indicated to assess the clinical efficacy of terbinafine for the treatment of fungal diseases in adult horses.

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