Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses

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**Objective**—To determine penetration of topically and orally administered voriconazole into ocular tissues and evaluate concentrations of the drug in blood and signs of toxicosis after topical application in horses.

**Animals**—11 healthy adult horses.

**Procedure**—Each eye in 6 horses was treated with a single concentration (0.5%, 1.0%, or 3.0%) of a topically administered voriconazole solution every 4 hours for 7 doses. Anterior chamber paracentesis was performed and plasma samples were collected after application of the final dose. Voriconazole concentrations in aqueous humor (AH) and plasma were measured via high-performance liquid chromatography. Voriconazole concentrations in AH after a single orally administered dose of voriconazole (4 mg/kg); anterior chamber paracentesis was performed, and voriconazole concentrations in AH were measured.

**Results**—Mean ± SD voriconazole concentrations in AH after topical administration of 0.5%, 1.0%, and 3.0% solutions (n = 4 eyes for each concentration) were 1.43 ± 0.37 µg/mL, 2.35 ± 0.78 µg/mL, and 2.40 ± 0.29 µg/mL, respectively. The 1.0% and 3.0% solutions resulted in significantly higher AH concentrations than the 0.5% solution, and only the 3.0% solution induced signs of ocular toxicosis. Voriconazole was detected in the plasma for 1 hour after the final topically administered dose of all solutions. Mean ± SD voriconazole concentration in AH after a single orally administered dose was 0.86 ± 0.22 µg/mL.

**Conclusions and Clinical Relevance**—Results indicated that voriconazole effectively penetrated the cornea in clinically normal eyes and reached detectable concentrations in the AH after topical administration. The drug also penetrated noninflamed equine eyes after oral administration. Low plasma concentrations of voriconazole were detected after topical administration. (Am J Vet Res 2006;67:296–301)

Fungal keratitis is a common ophthalmic disease in horses, accounting for approximately 13% of the corneal problems reported in horses over the past 40 years.1 3 The condition often develops in association with traumatic injury of the cornea, which allows resid-
penetrates the anterior and posterior segments of the eye in humans and rabbits after oral and topical administration. These factors suggest that voriconazole may provide an improved treatment option for keratomycosis in horses. The purpose of this study was to evaluate the ocular penetration and toxicity of topically and orally administered voriconazole in clinically normal horses.

Materials and Methods

Horses—Eleven adult male and female horses of various breeds were used for the study. Use of horses was approved and monitored by the North Carolina State University Institutional Animal Care and Use Committee. Horses used in the study had normal findings of complete ophthalmologic examinations consisting of slit-lamp biomicroscopy and direct ophthalmoscopy. During the study, horses were stabled or kept in a paddock. Horses receiving medication orally were not allowed food for 12 hours prior to and 4 hours after drug administration.

Formulation and stability of ophthalmic preparation of voriconazole—A commercially available preparation of voriconazole for IV injection was used for in vitro determination of the formulation’s stability for extended use and for use in the topical portion of the study. The product is packaged as a lyophilized powder complexed with sulfobutyl ether β-cyclodextrin to increase solubility in water. For stability testing and topical administration, voriconazole was reconstituted with sterile water for injection as 0.5%, 1.0%, and 3.0% solutions. All solutions were refrigerated at 4°C in tightly sealed containers and were protected from light. The color and clarity of each solution were evaluated daily. A 10-µL sample was collected from each solution immediately after reconstitution (day 0); on each day after reconstitution for 7 days; and again on days 10, 14, 21, and 28. Samples were analyzed for concentration of voriconazole by means of HPLC.

Topical voriconazole administration—Six horses were used for this portion of the study. Horses received a 0.5%, 1.0%, or 3.0% solution of voriconazole in each eye so that each concentration of voriconazole was administered to 4 eyes (Table 1). Horses received 7 topically administered doses of a given concentration of voriconazole at 4-hour intervals (times 0, 4, 8, 12, 16, 20, and 24 hours). Each administration consisted of 0.2 mL of solution delivered via a 1-mL syringe through the hub of a 25-gauge needle (0.5% and 1% solutions) or a 22-gauge needle (3% solution). Prior to and immediately after each administration, horses were observed for signs of ocular irritation (eg, blepharospasm, blepharoedema, conjunctival hyperemia, or epiphora). In addition, examination with a slit lamp was performed prior to each administration to evaluate the conjunctiva, cornea, and anterior chamber.

One hour after administration of the final dose (at time 25 hours), samples of AH were collected. This time was selected on the basis of reports of fluconazole in human eyes reaching peak concentrations 15 minutes after topical administration and decreasing to minimal concentrations by 60 minutes and a study in rabbits in which voriconazole was detected in AH 75 minutes after the final topical application. In our study, each horse was sedated with detomidine administered IV (0.1 mg/kg), and auriculopalpebral and ciliary nerve blocks were performed. In addition, retrobulbar anesthesia (injection of lidocaine into the retrobulbar orbital cone through the orbital fossa) was performed according to published protocols. An anesthetic was applied topically to the cornea, and a dilute povidone-iodine solution was used to irrigate the corneal surface. Aqueous humor was obtained by inserting a sterile, 27-gauge needle through the conjunctiva at the limbus into the anterior chamber of the eye. Gentle aspiration was performed with a 1-mL syringe until 0.3 to 0.5 mL of AH was obtained. Samples were analyzed by use of HPLC within 1 hour of collection. Each eye was treated prophylactically with a triple-antimicrobial ophthalmic ointment every 6 hours for 24 hours, and each horse received 1 dose of flunixin meglumine (1.1 mg/kg, IV) immediately after the paracentesis procedure. To determine whether systemic absorption of voriconazole resulted from topical administration, blood samples were collected by venipuncture into tubes containing lithium heparin at time 0 (prior to first treatment) and at 15, 30, and 60 minutes after the final treatment. The plasma was harvested and stored at −70°C until analysis.

Oral voriconazole administration—Five horses were used for this portion of the study. A 4 mg/kg dose of voriconazole powder (99.9% pure) was weighed for accuracy, mixed with corn syrup in a 60-mL dosing syringe, and administered orally. This dose was extrapolated from doses reported in humans and dogs. Aqueous humor was obtained as described 2.5 hours after administration. This sampling time was chosen to allow adequate time for drug absorption and was similar to sampling times used in a human study. The samples were stored at −70°C until analysis. Plasma samples were also collected simultaneously to determine the percentage of voriconazole in AH relative to the plasma.

Analysis of voriconazole concentrations—Concentrations of voriconazole in the formulation, AH, and plasma were determined by use of reverse-phase HPLC with ultraviolet detection at 263 nm. A C8 reverse-phase column was used for the separation. The system includes a pump, a variable-wavelength ultraviolet detector, and an autosampler. For all assays, calibration curves were made daily from a stock solution of pure reference standard dissolved in 100% acetonitrile to a concentration of 1 mg/mL. To be accepted, values were required to have r² of the curve > 0.99 and be within 15% of the expected value. The mobile phase consisted of acetonitrile and water (50:50 vol/vol), and the flow rate was 1 mL/min. Under those conditions, the retention time was from 4 to 4.5 minutes.

For the in vitro stability study, a calibration curve was made from the stock solution of reference standard diluted in mobile phase and was linear at concentrations from 50 to 1 μg/mL. The original voriconazole solutions of 0.5%, 1.0%, and 3.0% were diluted 1:1,000 in double-distilled water to yield final concentrations of approximately 5, 10, and 30 μg/mL, respectively. Twenty-five microliters of the final solution was injected into the HPLC system. All samples were analyzed in triplicate, and mean values were reported.

Table 1—Schedule of topical ophthalmic voriconazole administration in 6 horses.

<table>
<thead>
<tr>
<th>Horse No.</th>
<th>Eye</th>
<th>Concentration of voriconazole</th>
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<tbody>
<tr>
<td>1</td>
<td>OS</td>
<td>0.5%</td>
</tr>
<tr>
<td>1</td>
<td>OD</td>
<td>0.5%</td>
</tr>
<tr>
<td>2</td>
<td>OS</td>
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<td>2</td>
<td>OD</td>
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<tr>
<td>3</td>
<td>OS</td>
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<td>3</td>
<td>OD</td>
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<tr>
<td>4</td>
<td>OS</td>
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<tr>
<td>4</td>
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<tr>
<td>6</td>
<td>OS</td>
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<td>6</td>
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OS = Left eye. OD = Right eye.
Aqueous humor samples were analyzed without extraction. Calibration curves were established each day with pooled AH (from untreated horses) to which voriconazole at concentrations of 0.05 to 5 µg/mL was added. The injection volume was 50 µL. The lower limit of quantification was the lowest concentration determined to be linear on the calibration curve (0.05 µg/mL). Statistical analysis of drug concentrations in AH by use of ANOVA was performed with statistical software. Values obtained were compared with MIC values previously determined for voriconazole against clinical fungal isolates from human patients.

Plasma samples were analyzed after solid-phase extraction. Cartridges were initially conditioned with 1 mL of methanol followed by 1 mL of distilled water by use of a vacuum manifold. Plasma samples (1 mL) were extracted, and cartridges were washed with a mixture of methanol and water (5:95 vol/vol). The sample was then extracted into clean glass tubes with 100% methanol and evaporated under compressed air at 4°C for 25 minutes. The resulting evaporate was reconstituted with 200 µL of mobile phase, and 50 µL was injected onto the HPLC system. Calibration curves were made prior to each analysis with pooled plasma from untreated horses at concentrations from 0.01 to 5 µg/mL. The limit of quantification was 0.01 µg/mL.

**Results**

**Formulation and stability of voriconazole solutions**—Voriconazole was stable in the prepared formulations for at least 28 days after reconstitution under refrigerated conditions (Figure 1). No gross abnormalities (eg, discoloration, settling, or precipitation) were observed in any of the solutions over the study period. Complete dissolution of the powder was more difficult at the 3.0% concentration and resulted in a viscous solution.

**Topical administration**—Voriconazole was detected in all of the AH samples after topical administration (Figure 2). Mean voriconazole concentration resulting from application of the 0.5% solution was significantly (P = 0.047) less than those resulting from application of the 1.0% and 3.0% solutions, but there was no significant difference in AH concentrations resulting from application of the 1.0% versus 3.0% solutions. Voriconazole was detected at low concentrations in the plasma of all horses after topical administration. A mean peak plasma concentration of 0.03 µg/mL was measured 15 minutes after administration of the final topically administered dose. Plasma voriconazole concentrations were less than the limit of quantification by 1 hour after drug administration in all 6 horses (Figure 3).

The 0.5% and 1.0% solutions were well tolerated in all eyes, with no blepharospasm, blepharoedema, conjunctival hyperemia, or epiphora observed after any administration or prior to subsequent administrations. All eyes treated with the 3.0% solution had epiphora or blepharospasm immediately after at least 50% of the administrations, ranging from 10 seconds to 2 minutes in duration. These adverse effects did not appear to be cumulative and occurred to an equal degree in response to the first and later doses. In addition, all eyes that received the 3.0% solution had mild chemosis or eyelid swelling prior to at least 25% of subsequent doses.

**Aqueous humor concentrations after oral administration**—Voriconazole was detected in the AH of all 5 horses 2.5 hours after a single orally administered dose. The mean AH voriconazole concentration was 0.86 ± 2.2 µg/mL (Figure 2). Voriconazole concentration was 38.83 ± 8.65% of the plasma concentration at the same time point (range, 30.58% to 53.1%). Concentrations of
isms.40 This enzyme is responsible for production of the cytochrome P450 enzyme system of fungal organ-
gents, which target the enzyme C-14 voriconazole concentration at that time point. Representation slightly
synthesis and accumulation of 14
Inhibition of this enzyme leads to decreased ergosterol
ergosterol, a sterol unique to fungal cell membranes.

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Voriconazole has a broader spectrum of activity than
concentrations achieved after oral
administration. No adverse effects were observed after oral administration of voriconazole.

Discussion
Results indicate that voriconazole effectively penetrated healthy equine corneas and achieved detectable concentrations in AH after topical administration and that the drug enters noninflamed equine ocular tissues after oral administration. With topical administration, significantly higher concentrations resulted from treatment with the 1.0% and 3.0% solutions, compared with the 0.5% solution, and there was no significant difference in concentrations achieved between the 1.0% and 3.0% solutions. Signs of ocular toxicosis were only observed in eyes treated with the 3.0% solution; the 0.5% and 1.0% solutions were well tolerated. This suggests that 1.0% voriconazole solution is the most appropriate concentration to use topically in future studies and clinical trials. The reconstituted voriconazole solutions remained stable for longer than 28 days. Finally, a single orally administered dose of 4 mg/kg resulted in a mean voriconazole concentration in AH of 0.86 µg/mL 2.5 hours after administration, representing slightly < 39% of the plasma voriconazole concentration at that time point.

Voriconazole is in the azole class of antifungal agents, which target the enzyme C-14α demethylase of the cytochrome P450 enzyme system of fungal organ-
isms.40 This enzyme is responsible for production of ergosterol, a sterol unique to fungal cell membranes. Inhibition of this enzyme leads to decreased ergosterol synthesis and accumulation of 14α methylsterols, disrupting cell membrane synthesis and repair.40 Voriconazole has a broader spectrum of activity than other triazole antifungal drugs and has fungicidal activity against some isolates of Aspergillus spp.40

The effectiveness of topical treatment for ocular disease is influenced by the structure of the cornea. The lipophilic epithelium and endothelium, which surround the hydrophilic stroma, create a barrier to penetration of many applied medications, greatly affecting their therapeutic potential.41 Ulcerative disease increases the ability of medications to penetrate the cornea by eliminating the epithelial barrier; however, substantial corneal disease can occur without concurrent ulceration (ie, fungal stromal abscess). In the study presented here, topically administered voriconazole effectively penetrated the intact, healthy cornea. It is therefore likely that even higher intraocular concentrations would be achieved with a loss of epithelial integrity.

Treatment with voriconazole has resulted in visual, dermatologic, and hepatic adverse effects in humans and other species,42 leading to our interest in measuring voriconazole concentrations in the plasma after topical ophthalmic administration. Drug was detected in the plasma of horses that received the topically administered medication, but the concentrations were low and persisted for < 1 hour after the final administration. Although further testing would be needed to determine the systemic effects of these low plasma concentrations, the minimal absorption after topical administration suggested that there would be no detectable adverse systemic effects, even with chronic use.

The efficacy of nontopically administered medications in horses with ocular disease depends on the ability of the drug to cross the blood-ocular barriers, specifically the blood-aqueous barrier during anterior segment disease.43 In the present study, administration of a single orally administered dose of voriconazole yielded potentially therapeutic concentrations of drug in the AH of noninflamed eyes. When compared on the basis of percentage of plasma concentrations, concentrations of the drug in AH after a single orally administered dose of voriconazole were similar to concentrations measured after multiple orally administered doses of fluconazole (38.83% vs 37.34%, respectively).40 This finding, combined with the drug’s favorable spectrum of activity, makes voriconazole an excellent choice for clinical trials on treatment for fungal keratitis in horses. Intraocular inflammation, as occurs secondary to corneal disease, compromises the blood-aqueous barrier and allows substances to enter the anterior chamber that are normally excluded. This is manifested clinically as aqueous flare, hypopyon, or hyphema.41 One consequence of this inflammation is that parenterally administered medications that may not otherwise enter the eye are able to cross the barrier and potentially exert a therapeutic effect. It is therefore reasonable to assume that the secondary uveitis accompanying fungal keratitis would allow equal or higher voriconazole concentrations to be attained in the eye.

Assessment of the potential benefit of the intraocular concentrations of voriconazole achieved in this study relies on comparison with previous in vitro analyses of the drug’s antifungal activity. Given that there are no published data regarding efficacy for treatment of fungal variants isolated from horses, the

Figure 3—Mean ± SD plasma concentrations of voriconazole after the last of 7 topically administered doses of voriconazole in the same horses as in Table 1. Notice that all concentrations of voriconazole were less than the limit of quantification (0.005 µg/mL) by 1 hour after the final dose.
voriconazole concentrations achieved in AH in this study were compared with values for MIC obtained from numerous other studies that used laboratory strains of fungi and isolates from clinical cases in human patients. Reported MIC values for voriconazole against filamentous fungi range from 0.01 to 4 µg/mL\(^2\),\(^3\),\(^4\),\(^5\) with occasional isolates, particularly of *Fusarium* spp, yielding MIC values > 8 µg/mL\(^2\),\(^3\),\(^4\),\(^5\). The MICs of voriconazole for *Candida* spp are in the range of 0.02 to 3 µg/mL\(^2\),\(^3\),\(^4\),\(^5\) with occasional isolates having MICs > 16 µg/mL\(^2\),\(^3\),\(^4\),\(^5\). In the ranges of MICs for filamentous and yeast organisms, most isolates are < 0.5 µg/mL. In the study presented here, the 0.5%, 1.0%, and 3.0% solutions yielded mean AH voriconazole concentrations of 1.42, 2.35, and 2.40 µg/mL, respectively. In addition, the single orally administered dose of 4 mg/kg yielded a mean AH voriconazole concentration of 0.86 µg/mL. These values are higher than the 0.5 µg/mL concentration typically considered the minimum concentration for potential clinical efficacy, confirming the drug's potential for topical or oral administration in treatment for equine keratomycosis.

In this study, topical administration was performed every 4 hours, a regimen commonly used in the medical management of ulcerative fungal disease in horses. It is possible that more frequent administration would result in greater intraocular concentrations, but this may not be necessary given that the concentrations achieved were in the range of expected therapeutic efficacy. It is unlikely that decreasing the frequency of administration would lessen the severity of the signs of toxicosis associated with use of the 3.0% solution because blepharospasm and epiphora were observed in all treated eyes after the first administration.

For the purposes of establishing the ocular penetrability and signs of toxicosis, the protocol used in this study adhered to a constant topical administration interval and assessed concentrations of the drug in AH after a single orally administered dose. In practice, however, topical administration of medications may initially be maintained at intervals of 4 hours or less, with the frequency gradually decreased with evidence of clinical improvement. Without evaluating intraocular voriconazole concentrations after treatment intervals longer than 4 hours, it could not be determined how long therapeutic concentrations persist. In addition, the degree to which voriconazole continues to accumulate after multiple orally administered doses could not be ascertained from this study. Nonetheless, our results provide justification for further evaluation of the ocular pharmacokinetics of voriconazole in horses and its use for treatment of fungal keratitis.

Further evaluation is also warranted to determine residual concentrations of voriconazole remaining in the corneal tissue after topical or oral administration. Detection of voriconazole in AH indicates the drug's capability to penetrate the cornea and the blood-aqueous barrier but does not definitively extrapolate to tissue residence time. Measurement of tissue concentrations would require the assay of corneal tissue samples, which were not obtained in this study.

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References


