Penetration of Voriconazole, 1%, Eyedrops Into Human Aqueous Humor

A Prospective Open-Label Study

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Objective: To determine the therapeutic efficacy of adjuvant use of voriconazole, 1%, eyedrops in the treatment of refractory fungal keratitis.

Methods: A prospective open-label trial was conducted to determine voriconazole levels obtained in human aqueous humor after administration of a 1% solution, preserved with 0.01% benzalkonium chloride, every 6 hours for 3 days, or hourly for 4 doses. Ten participants were selected among patients scheduled to undergo elective anterior segment surgery, and samples were tested using validated high-performance liquid chromatography.

Results: The mean (SD) voriconazole concentrations after hourly dosing (n=5) was 1.90 (1.12) µg/mL and after a single dosing every 6 hours (n=5) was 0.94 (1.21) µg/mL, respectively. The mean (SD) sampling times after the last administration of eyedrops were 1.1 (0.5) hours after hourly dosing and 2.1 (0.6) hours after a single dosing every 6 hours.

Conclusions: Voriconazole, 1%, eyedrops are well tolerated and penetrate into human aqueous humor when administered at hourly or 6-hourly intervals. They are effective in treating Candida and Aspergillus keratitis, are substantially more affordable than oral therapy, and have less potential to cause systemic adverse effects.

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Corneal diseases are the second most common cause of vision loss and blindness in the world. Of corneal diseases due to infections, fungal keratitis remains one of the most difficult to treat. Fungal keratitis can be slow to resolve, refractory, and may require surgical intervention if scleral invasion or corneal perforation occurs. Prognosis depends on several features such as the extent of corneal involvement, any underlying immunosuppressive disease, and the speed of establishing a definitive pathogen confirmed by laboratory culture. Although rare, patients with fungal endophthalmitis or panophthalmitis usually have a poor visual outcome. The incidence of fungal keratitis and the organisms that cause it vary considerably by geography. At the Royal Victorian Eye and Ear Hospital, Candida albicans is found in 37% of cases. Together with Aspergillus fumigatus and Fusarium species, they account for almost 70% of our cases. Despite many available agents, management of fungal keratitis remains a challenge, with no established gold standard treatment. Our most potent antifungals, the polyenes amphotericin B and natamycin, are not absorbed orally and are poorly absorbed in topical form. This limits their use, especially in an outpatient setting in which intensive eyedrop regimens are difficult to follow. Oral azoles such as ketoconazole and fluconazole are well absorbed and tend to provide reasonable penetration into eye tissues but are associated with adverse effects and provide limited spectrums of activity.

Voriconazole is a new triazole antifungal agent that is available in oral or intravenous form. It is useful in the management of fungal keratitis, has a broader spectrum of activity than amphotericin B, and is better tolerated. Voriconazole works by inhibiting cytochrome P450 demethylase to alter fungal cell membrane permeability and to arrest growth. This agent is appealing for use in ophthalmology because high liposolubility translates to high penetration through ocular tissues.

Concentrations within aqueous humor after oral administration are known, but oral administration is not without problems. Refractory cases may require...
longer-than-usual maintenance courses of voriconazole, and this often has a negative effect on hepatic function.\(^\text{16}\) In addition to its toxic effects, voriconazole is expensive. At our institution, a 6-month course of treatment will cost in excess of A$18 000 (US$15 800).

Voriconazole has low 90% minimum inhibitory concentration (MIC\(_{90}\)) values for Candida species, Fusarium, and Aspergillus and has a promisingly low MIC\(_{90}\) (0.5 µg/mL) for Paecilomyces lilacinus and Paecilomyces variotii.\(^\text{17}\) The more favorable pharmacology is attributed to its dose-dependent preferential inhibition of the P450 enzyme, sterol 14–demethylase.\(^\text{18}\)

Voriconazole exhibits nonlinear (dose- and time-independent) pharmacokinetics after oral dosing. There is wide intersubject variability in these variables.\(^\text{19}\)

Case reports of the successful adjuvant use of voriconazole, 1%, eyedrops in the treatment of refractory fungal keratitis stimulated our interest in investigating this preparation further.\(^\text{20,21}\)A literature review established that, while voriconazole eyedrops were probably safe, little was known about the achievable concentration levels within human aqueous humor.\(^\text{22}\)

We hypothesized that a 1% solution of voriconazole preserved with 0.01% benzalkonium chloride and applied by 1 of 2 dosage schedules would reach sufficient concentrations within aqueous humor to be an effective treatment for fungal keratitis. A secondary end point was patient tolerability of the formulation.

### METHODS

**SELECTION AND DESCRIPTION OF PARTICIPANTS**

This was a prospective open-label trial to determine voriconazole levels in aqueous humor following administration of a preserved 1% solution, conducted at the Royal Victorian Eye and Ear Hospital, Melbourne, Australia, and approved by its human research and ethics committee. It was also approved by the ethics committee of Monash University, Melbourne.

Ten consenting participants undergoing elective, routine, anterior segment surgery in a noninflamed eye randomly received 1 of 2 dosage regimens of the preparation at a 1:1 ratio. Randomization was completed by randomly permuted blocks and was generated using a Web site (http://www.randomization.com). All surgery was performed between October 17, 2005, and December 26, 2005.

Study exclusions were as follows: patients with renal or hepatic impairment, patients who were pregnant or breastfeeding, patients who were allergic or sensitive to voriconazole or benzalkonium chloride, and patients taking any medicine contraindicated for voriconazole.\(^\text{14}\)

**FORMULATION AND DOSE SCHEDULING OF THE STUDY DRUG**

Eyedrops were manufactured on-site by the pharmacy department as a 1% solution. They were formulated using commercially available voriconazole injection and were preserved with 0.01% benzalkonium chloride.

The 2 dosage schedules were as follows: (1) a single drop into the eye to be operated on 4 times daily for 3 days, with the last dose given approximately 1 hour before the operation, or (2) a single drop into the eye to be operated on every hour for 4 hours before the operation, with the last dose given approximately 1 hour before the start of the operation. Participants completed a diary after each administration to examine the acceptability of the formulation and any adverse effects and to confirm the precise date and time each eyedrop was administered.

During surgery, 0.1 to 0.2 mL of aqueous humor was aspirated through a paracentesis site using a 30-gauge needle attached to a syringe. This was obtained before infusion of any intraocular irrigation solution. Samples were immediately refrigerated at 4°C, the time of collection was noted, and the sample was tested within 7 days.

**TISSUE SAMPLE ANALYSIS**

Voriconazole levels were measured using high-performance liquid chromatography with diode array detection. A 100-µg/mL stock standard in methanol was prepared from pure substance voriconazole, from which working standards were made by spiking blank plasma to concentrations of 0.5, 1.0, 2.0, 4.0 and 8.0 µg/mL.

Working standards, quality controls, and samples were prepared by deproteinizing 200 µL of plasma (or 50 µL of aqueous fluid) with equivolume acetonitrile. Following vortex mixing and centrifuging, 50 µL of supernatant was transferred into a high-performance liquid chromatography vial for injection.

High-performance liquid chromatography was performed (model 1090A; Hewlett Packard, Avondale, Pennsylvania) using a 100 × 2.1-mm–internal diameter column packed with 3-µm particles (Hypersil C18; Thermo Electron Corporation, Waltham, Massachusetts), maintained at 50°C. A mobile phase (30% acetonitrile in 0.01-mol/L phosphate buffer [pH 3]) at a flow rate of 0.5 mL/min was used to isocratically elute the voriconazole, which was monitored at 255 nm. Voriconazole eluted with a retention time of 3.3 minutes.

The assay was found to be linear to at least 16 µg/mL, with a limit of detection of 0.04 µg/mL and a limit of quantification of 0.08 µg/mL at the standard monitoring conditions used. All peaks were verified for authenticity by cross-matching the UV spectral data of the peak, stored by the diode array detector against the voriconazole spectrum in the detector’s library.

Blank aqueous humor samples were run to verify that no interfering peaks were seen in the vicinity of the voriconazole peak. Spiked aqueous humor, plasma, and water were run at 8 µg/mL to confirm that there were no notable differences in recoveries.

**DATA ANALYSIS**

The mean (SD) measured drug level was obtained for each arm. The mean (SD) number of hours elapsed between the last administered dose and the time of sample collection was also calculated. The analysis of patient acceptability and adverse effects of the formulation included all data from participants who received 1 dose or more.

**RESULTS**

Participants were aged 68 to 87 years (mean [SD] age, 77.8 [4.7] years). All underwent unilateral cataract surgery in a noninflamed eye.

The mean (SD) voriconazole concentration after hourly dosing (n = 5) was 1.90 (1.12) µg/mL and after a single dose every 6 hours (6-hourly dosings) (n = 5) was 0.94 (1.21) µg/mL. The mean (SD) sampling time after the last administration of eyedrops was 1.1 (0.5) hours after hourly dosing and 2.1 (0.6) hours after 6-hourly dosings.
Table 1. 90% Minimum Inhibitory Concentrationsa

<table>
<thead>
<tr>
<th>Antifungal Agent</th>
<th>Aspergillus Species, µg/mL</th>
<th>Candida Species, µg/mL</th>
<th>Fusarium Species, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Voriconazole, 1%</td>
<td>0.5</td>
<td>0.016</td>
<td>2</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>&gt;256</td>
<td>0.5</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>&gt;256</td>
<td>0.5</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>4</td>
<td>0.022</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Fluocytosine</td>
<td>&gt;64</td>
<td>0.12</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

aValues for each drug-organism combination are calculated based on cumulative minimum inhibitory concentration data using the Yeast One test.17

A total of 80 eyedrops were administered to 10 participants. Five participants reported no adverse reaction, 3 reported 1 instance of stinging, 1 reported 2 instances of stinging, and 1 reported sneezing and coughing after the initial dose.

This study demonstrated that voriconazole, 1%, eyedrops penetrate into human aqueous humor when administered at hourly or 6-hourly intervals. The relative levels obtained seem dependent on the time of sampling in relation to the last dose, ranging from 30 to 180 minutes in this study. Because the difference in the mean sampling time after the last administered dose in each arm of the trial was statistically significant (P < .05, paired t test), it was impossible to draw any firm conclusion about which dosage regimen offered higher voriconazole levels or represented the superior treatment option.

The MIC90 reference values were exceeded for a wide spectrum of pathogens in both arms. Considering the 3 main fungal species encountered at our institution, the mean level achieved in the hourly arm (1.9 µg/mL) would adequately treat Aspergillus, Candida, and some Fusarium species (Table 1). The large SD in the 6-hourly arm does not permit any efficacy claim to be made with confidence, but its mean level (0.94 µg/mL) suggests that it will inhibit most Candida species and provide limited coverage for Aspergillus.

Because samples were obtained on average 1.1 hours after the last dose for eyedrops administered at hourly intervals, the values reported herein will be less than trough levels seen in actual clinical dosing of intensive eye drops. This is encouraging because the mean level indicates that trough levels will not dip below the MIC90 for Candida or Aspergillus infections.

The 6-hourly samples were taken on average 2.1 hours after the last dose. It is likely that, if a full 6-hour interval had elapsed, trough voriconazole levels would be below the MIC90 and the preparation would be ineffective for a period. It is possible that these levels will be higher after long-term dosing.

Relating these results directly to clinical practice has its limitations. We do not know whether they are representative of levels in actively treated eyes, we cannot quantify how the altered permeability of a diseased cornea will affect drug levels, and we are unaware whether other factors within the eye can alter the effective MIC90 in vivo.

Hariprasad et al15 found that the mean (SD) levels in the aqueous humor reached 1.13 (0.57) µg/mL when samples were obtained a mean (SD) of 2.9 (0.5) hours after two 400-mg oral doses given 12 hours apart. Comparison of this to our hourly results is of interest and provides some validity. Peak voriconazole levels after oral dosing are seen after 0.9 to 1.7 hours, whereas peaks after topical administration are likely to be within a few minutes of application. Because the mean (SD) sampling time in our trial was 1.1 (0.5) hours after administration in the hourly arm, both studies obtained samples at similar periods after the estimated time of peak concentration. On this basis, topical voriconazole, 1%, when administered every hour may achieve levels 68% higher than after oral dosing.

The formulation was well tolerated, recognizing that the study identified only patient-reported adverse effects during a short duration and was unable to identify more insidious reactions. Five subjects experienced no ill effect, and 4 subjects had transient and mild ocular discomfort. In 3 of these cases, discomfort was experienced only on initial instillation. A single patient experienced an episode of sneezing and coughing. This may be a physiologic response to the eye drop instillation or its toxicity rather than a pharmacologic reaction to voriconazole.

The total administered dose of voriconazole received by patients ranged from 2 to 6 mg, about 1% to 3% of the usual daily oral dose. This is likely the reason for the lack of any notable reported adverse effects. Because oral voriconazole treatment may be extended and the hepatic adverse effects may be potentially serious, local administration is recommended to minimize systemic adverse drug reactions.

The cost of producing the trial eyedrops was less than A$110 (US$97) per month of treatment. By contrast, treatment with oral voriconazole is slightly less than A$2900 (US$2547), making the eyedrops 96% cheaper.

The small number of subjects limited the statistical power of this study, which only a larger trial can overcome. This was evident when looking at the 6-hourly data in which the SD crossed 0 largely because the results for patient 9 (Table 2) varied greatly from those
of the others. A prospective trial of this preparation in fungal keratitis with clinical outcome as the end point would be the best way to demonstrate efficacy, but the low incidence of the disease and the need for extended treatment present difficulties. Another shortcoming was obtaining samples about an hour after the last dose. A trough level would have been more useful but harder to administer.

CONCLUSIONS

In summary, voriconazole, 1%, eyedrops are well tolerated and are capable of reaching sufficient aqueous humor levels to treat the most common causative organisms in fungal keratitis. This preparation is likely to have a valuable role in the therapeutic management of Candida and Aspergillus keratitis. It is substantially more affordable than oral therapy and has less potential to cause systemic adverse effects.

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REFERENCES