Prospective Open-Label Study of the Administration of Two-Percent Voriconazole Eye Drops[▽]

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Thirteen human subjects scheduled for elective anterior segment eye surgery received hourly 2% voriconazole eye drops 4 hours presurgery. No side effects were reported. Significantly, the voriconazole concentration in the aqueous humor of the eye was similar to that reported for the 1% voriconazole solution, suggestive of concentration-independent absorption.

Fungal keratitis accounts for 6 to 50% of all ocular infections and is one of the most difficult infections to treat (13). Seventy percent of fungal keratitis infections are attributed to *Candida albicans*, *Aspergillus fumigatus*, and some *Fusarium* species (2). At the Royal Victorian Eye and Ear Hospital (RVEEH), Melbourne, Victoria, Australia, *Candida albicans* is the causative fungus in 37% of the treated cases (2).

Voriconazole (Vfend) is effective (Table 1) against common pathogens associated with fungal eye disease (i.e., *Candida*, *Aspergillus*, and *Fusarium* species [3, 6, 8]) and some less common keratitis-causative fungi such as *Paecilomyces* spp., *Histoplasma* spp., *Scedosporium* spp., *Curvularia* spp., and *Acremonium* (3, 8). The typical corneal fungal pathogens (8) are shown in Table 1. Voriconazole is available commercially in oral and intravenous forms (Vfend package insert; Pfizer Inc., New York, NY). Case reports relating to the use of topical 1% voriconazole for ocular fungal infections are promising but limited (8). A recent study by Lau et al. reported that the concentration of voriconazole in the aqueous humor (after topical administration with 1% voriconazole solution) was sufficiently high to treat some common types of fungal infections but may be inadequate for common but less-sensitive keratitis-causative fungi (12).

To date, there has only been one case report on the topical application of 2% voriconazole solution (14). The solution was used in combination with intravenous voriconazole and was successful against *Fusarium solani* keratitis. Importantly, the extent of the role played by the 2% solution in the reported case and the extent of penetration of topically applied 2% voriconazole solution into the aqueous humor remain unknown.

The aim of the current study was to investigate the penetration of 2% voriconazole eye drops into the aqueous humor as

a potential alternative therapy for the management of ophthalmic fungal keratitis.

(Preliminary data of the work described in this paper were presented at the 13th International Congress on Infectious Diseases, Kuala Lumpur, Malaysia, June 2008.)

The current study was an open-label prospective study conducted between July 2007 and August 2008 at the RVEEH. The study was approved by the human ethics committee of RVEEH and Monash University.

Participants ≥ 18 years old scheduled to undergo routine anterior eye segment surgery were recruited. Exclusion criteria were liver or kidney failure, breast feeding, pregnant, trying to conceive, allergy to voriconazole or benzalkonium chloride, use of latanoprost or medications contraindicated for voriconazole (Vfend package insert; Pfizer Inc., New York, NY), and active ocular inflammatory conditions. Written informed consent was obtained from each participant before enrolment.

In accordance with previous studies (12, 16), for an expected standard deviation of ± 1.0 mg/liter and a ± 0.5 -mg/liter margin of error of the mean associated with a 95% confidence level, the required sample size was 13 participants.

The 2% solution was manufactured aseptically by the RVEEH Pharmacy Department. It was prepared by reconstituting Vfend IV (200-mg vial; Pfizer Australia Pty. Ltd., Sydney, NSW, Australia) with 9 ml of water for injection (Pfizer Pty. Ltd., Perth, WA, Australia) containing 0.01% benzalkonium chloride solution (benzalkonium chloride 50%; Professional Compounding Centers of America, TX; Australian Compounding Pharmacy, Australia).

A single drop (0.05 ml) of the 2% voriconazole solution was administered by a nurse to the eye to be operated on every hour for 4 hours prior to operation. The last dose was administered approximately 1 hour before surgery. A drug administration diary was used to document the date and time of administration and any side effects experienced.

At the start of surgery and before infusion of any intraocular irrigation solution, 0.1 to 0.2 ml of aqueous humor was aspirated through a paracentesis site with a 30-gauge needle attached to a syringe. Samples were immediately refrigerated at 4°C and analyzed within 7 days of collection.

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TABLE 1. In vitro MICs at which 90% of isolates are inhibited by voriconazole

Organism	MIC ₉₀ (mg/liter)	Reference
Yeast and yeastlike species		
<i>Candida albicans</i>	0.06	16
<i>Candida parapsilosis</i>	0.12–0.25	16
<i>Candida tropicalis</i>	0.25–>16.0	16
<i>Cryptococcus neoformans</i>	0.06–0.25	16
Moniliaceous molds		
<i>Aspergillus fumigatus</i>	0.50	16
<i>Aspergillus flavus</i>	0.50	16
<i>Fusarium</i> species	0.25–8	8
<i>Fusarium solani</i>	2	10
<i>Paecilomyces lilacinus</i>	0.50	16
<i>Acromonium alabamensis</i>	0.25	16
Dimorphic fungi		
<i>Blastomyces dermatitidis</i>	0.25	16
<i>Coccidioides immitis</i>	0.25	16
<i>Histoplasma capsulatum</i>	0.25	16
<i>Penicillium marneffei</i>	0.03	16
Dematiaceous fungi		
<i>Curvularia</i> species	0.06–0.25	16
<i>Scedosporium</i> species	0.5	16
<i>Scedosporium apiospermum</i>	0.5	16

Voriconazole levels in the aqueous humor were quantified by a validated high-performance liquid chromatography assay with photodiode array detection. The analytical methods have been published previously (12).

The Mann-Whitney test was used to investigate any significant difference in measured voriconazole concentrations between participants with and without diabetes mellitus. Student's *t* test was used to compare differences between means.

The 13 participants had a mean age ($\pm$ standard deviation) of 70 ± 7.7 years (Table 2). All participants had phakic lens status and required unilateral cataract surgery in a noninflamed eye. No side effects or toxicities were reported.

The mean voriconazole concentration in the aqueous humor was 1.67 ± 0.97 mg/liter, while the mean sampling time after the last eye drop administration was at 1.3 ± 0.3 h (Table 2). There was no statistical difference ($P = 0.67$) in the mean aqueous humor voriconazole concentrations between the 10 nondiabetic participants (1.72 ± 1.03 mg/liter) and the 3 diabetic participants (1.50 ± 0.91 mg/liter).

The mean aqueous humor voriconazole concentration in this study (using 2% voriconazole solution) was not significantly different ($P = 0.68$) from that reported by Lau et al. (12) using 1% voriconazole solution.

In the Lau et al. study (12), the mean aqueous humor voriconazole concentration was 1.90 ± 1.12 mg/liter and the mean sampling time after the last dose was 1.1 ± 0.5 h. Results from the current study are comparable with those of Lau et al. (12). Both studies had identical numbers and frequencies of doses administered. In both studies, the trough voriconazole levels were measured (samples were collected approximately 1 h after the last one-hour drop). Furthermore, both studies administered the same volumes (~ 0.05 ml) of eye drops at each dose (11) and used benzalkonium chloride (a transcorneal penetration enhancer [9]) as the preservative. The eye drops

TABLE 2. Patient characteristics and voriconazole concentrations in aqueous humor

Age (yr)	Sex	Diabetes mellitus	Sampling time after last voriconazole dose (h)	Voriconazole concn in aqueous humor (mg/liter)
65	Male	No	1.2	0.9
79	Female	No	1.3	1.0
69	Male	Yes	1.1	1.0
61	Female	Yes	1.3	0.9
82	Male	Yes	1.2	2.6
73	Male	No	1.3	1.3
73	Male	No	1.9	3.6
56	Male	No	1.6	0.8
67	Male	No	1.5	1.4
61	Male	No	1.6	1.0
77	Male	No	1.0	1.5
69	Male	No	0.9	3.4
75	Male	No	1.3	2.4

prepared in this study and those used by Lau et al. contained the same concentration of benzalkonium chloride (0.01%).

The results from this study are consistent with previous studies (12, 16), demonstrating that topically administered voriconazole solutions achieve therapeutic concentration in the aqueous humor for treatment of fungi in Table 1, including those encountered at our institution (RVEEH). Importantly, the concentration resulting from the 2% voriconazole eye drops is not significantly different from that reported for the 1% solution (12). It appears that the penetration of voriconazole through an intact infection-free cornea is not concentration dependent, at least for the concentration range studied (1% to 2%). This appears counterintuitive and challenges the hypothesis of the study but is consistent with observations in a recent animal study, where the voriconazole level in the corneas of horses with fungal keratitis did not change when the administered voriconazole eye drop concentration was changed from 1% to 3% (4).

While the precise mechanism of fluid/drug transport through the cornea remains obscure (5), it is important to recognize that, in this and previous studies (12, 16), the eye drops were applied to noninfected eyes. To attempt this study in patients with active fungal keratitis would be difficult, given the very low incidence of fungal keratitis and the need for long-term treatment. Nonetheless, the voriconazole concentration in the infected eye depends neither on the size of the epithelial defect nor on epithelial removal, and thus it has been suggested that epithelial damage is not necessary for voriconazole penetration (15).

We have previously demonstrated that the 2% eye drops are stable for up to 16 weeks when stored between 2 to 8°C and at 25°C (1). Furthermore, the 2% solutions have a pH range of 6.02 to 6.16 (1), which is usually well tolerated by the eye (7). It is unlikely, therefore, that any eye irritation resulting from the use of these eye drops (none reported in our study) would be a consequence of low pH. Systemic side effects resulting from the topical administration of the 2% voriconazole solution will be negligible as each administered dose (0.05 ml) contains approximately 1 mg of voriconazole, which, compared to the standard systemic daily dose of 400 mg, is unlikely to result in a systemic concentration that will cause side effects.

In conclusion, the 2% voriconazole eye drops appear to be well tolerated. The concentration of voriconazole achieved in the aqueous humor was adequate (at least theoretically) to treat typical keratitis-causative fungi. Our data suggest that the penetration of voriconazole through an intact noninflamed cornea is unlikely to be concentration dependent for the concentration range 1 to 2%.

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REFERENCES

1. Al-Badriyeh, D., J. Li, L. Leung, G. Davies, R. Fullinfaw, K. Stewart, and D. Kong. Stability of voriconazole eye drops for off-label use in the management of ophthalmic fungal infections. *Am. J. Health Syst. Pharm.*, in press.
2. Bhartiya, P., M. Daniell, M. Constantinou, F. M. Islam, and H. R. Taylor. 2007. Fungal keratitis in Melbourne. *Clin. Exp. Ophthalmol.* **35**:124–130.
3. Breit, S. M., S. M. Hariprasad, W. F. Mieler, G. K. Shah, M. D. Mills, and M. G. Grand. 2005. Management of endogenous fungal endophthalmitis with voriconazole and caspofungin. *Am. J. Ophthalmol.* **139**:135–140.
4. Clode, A., J. Davis, J. Salmon, T. M. Michau, and B. Gilger. 2006. Evaluation of concentration of voriconazole in aqueous humor after topical and oral administration in horses. *Am. J. Vet. Res.* **67**:296–301.
5. Fischbarg, J. 1997. Mechanism of fluid transport across corneal endothelium and other epithelial layers: a possible explanation based on cyclic cell volume regulatory changes. *Br. J. Ophthalmol.* **81**:85–89.
6. Gao, H., M. Pennesi, K. Shah, X. Qiao, S. M. Hariprasad, W. F. Mieler, S. M. Wu, and E. R. Holz. 2003. Safety of intravitreal voriconazole: electroretinographic and histopathologic studies. *Trans. Am. Ophthalmol. Soc.* **101**:183–189.
7. How, T. H., W. Y. Loo, E. W. Chan, L. Y. Lim, E. W. Chan, P. C. Ho, and S. Y. Chan. 1998. Stability of cefazolin sodium eye drops. *J. Clin. Pharm. Ther.* **23**:41–47.
8. Jurkunas, U. V., D. P. Langston, and K. Colby. 2007. Use of voriconazole in the treatment of fungal keratitis. *Int. Ophthalmol. Clin.* **47**:47–59.
9. Kamal, K. S., and M. Ranabit. 2007. *Essentials of ocular pharmacology and therapeutics*. Anshan Pub., London, United Kingdom.
10. Klont, R. R., C. A. Eggink, A. J. Rijs, P. Wesseling, and P. E. Verweij. 2005. Successful treatment of *Fusarium* keratitis with cornea transplantation and topical and systemic voriconazole. *Clin. Infect. Dis.* **40**:e110–e112.
11. Lau, D. 2006. Penetration of 1% voriconazole eye drops into human aqueous and vitreous humor—a prospective, open-label study. M.Clin.Pharm. thesis. Monash University, Melbourne, Australia.
12. Lau, D., M. Fedinands, L. Leung, R. Fullinfaw, D. Kong, G. Davies, and M. Daniell. 2008. Penetration of voriconazole, 1%, eyedrops into human aqueous humor: a prospective open-label study. *Arch. Ophthalmol.* **126**:343–346.
13. Ozbek, Z., S. Kang, J. Sivalingam, C. J. Rapuano, E. J. Cohen, and K. M. Hammersmith. 2006. Voriconazole in the management of *Alternaria* keratitis. *Cornea* **25**:242–244.
14. Polizzi, A., C. Siniscalchi, A. Mastromarino, and S. C. Sacca. 2004. Effect of voriconazole on a corneal abscess caused by fusarium. *ACTA Ophthalmol. Scand.* **82**:762–764.
15. Thiel, M. A., A. S. Zinkernagel, J. Burhenne, C. Kaufmann, and W. E. Haefeli. 2007. Voriconazole concentration in human aqueous humor and plasma during topical or combined topical and systemic administration for fungal keratitis. *Antimicrob. Agents Chemother.* **51**:239–244.
16. Vemulakonda, G. A., S. M. Hariprasad, W. F. Mieler, R. A. Prince, G. K. Shah, and R. N. Van Gelder. 2008. Aqueous and vitreous concentrations following topical administration of 1% voriconazole in humans. *Arch. Ophthalmol.* **126**:18–22.