Clearance of Intravitreal Voriconazole

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Purpose To investigate the elimination rate of voriconazole after intravitreal injection in rabbits.

Methods. Intravitreal injections of 35 μg/0.1 mL voriconazole were administered to rabbits. Vitreous and aqueous humor levels of voriconazole were determined at selected time intervals (1, 2, 4, 8, 16, 24, and 48 hours), and the in vitreous half-life was calculated. Four to six eyes per time point after injection were enucleated and immediately stored at −80°C. Aqueous humor samples were withdrawn before enucleation, and vitreous samples were obtained from ocular dissection and isolation at various time intervals. Voriconazole concentrations in vitreous and aqueous humor were assayed with high-performance liquid chromatography (HPLC).

Results. The concentration of intravitreal voriconazole at various time points exhibited exponential decay with a half-life of 2.5 hours. The mean vitreous concentration was 18.912 ± 2.058 μg/mL 1 hour after intravitreal injection; this declined to 0.292 ± 0.090 μg/mL at 16 hours. The mean aqueous concentration was much lower and showed a decline from 0.240 ± 0.051 μg/mL at 1 hour to undetectable levels 8 hours after injection.

Conclusions. Vitreous concentrations achieved during the first 8 hours were greater than the previously reported minimum inhibitory concentrations (MICs) of organisms most involved in fungal endophthalmitis. A rapid decline of intravitreal concentration suggests that supplementation of intraocular voriconazole to maintain therapeutic levels may therefore be required in clinical settings. Further studies are needed to determine the elimination rate of voriconazole after intravitreal injection in humans. (*Invest Ophthalmol Vis Sci.* 2007;48:2238–2241) DOI:10.1167/iovs.06-1362

Fungal endophthalmitis, a serious, sight-threatening infection, is often a complication of intraocular surgery, systemic infection, and ocular trauma. The most common organisms encountered in fungal endophthalmitis are *Candida*, *Aspergillus*, and *Fusarium* species. Intravitreal antibiotics are a mainstay of treatment for fungal endophthalmitis. In the past, amphotericin B was the only antifungal agent approved for intravitreal injection. However, amphotericin B may cause retinal necrosis at low concentrations, and a variety of fungal species showing resistance to it have been reported.[1] Voriconazole, a second-generation triazole, differs from fluconazole by the addition of a methyl group to the propyl backbone and by the substitution of a triazole moiety with a fluoropyrimidine group, resulting in a marked change in activity.[2] Voriconazole has excellent bioavailability and reaches peak plasma concentration 2 to 3 hours after oral dosing. The intraocular penetration of orally administered voriconazole in the noninflamed human eye was found to be 1.13 ± 0.57 μg/mL and 0.81 ± 0.31 μg/mL in the aqueous and vitreous, respectively.[3] Previous studies have shown voriconazole to have a broad-spectrum of activity against A*spergillus* species, *Candida* species, *Paecilomyces lilacinus*, *Cryptococcus neoformans*, *Scedosporium* species, and others. Furthermore, voriconazole has been shown to be effective as primary therapy in the treatment of invasive aspergillosis, and it is an effective salvage therapy for refractory infections caused by *Fusarium* species.[4] Recently, in an experimental study in rat, intravitreal voriconazole has been shown to be less toxic to the retina than intravitreal amphotericin B.[5] More recently, a clinically successful treatment of endogenous *Aspergillus* endophthalmitis with intravitreal voriconazole injection has been reported.[6] The purpose of this study was to determine the clearance of voriconazole after intravitreal injection and thereby the clinical relevance of intravitreal voriconazole in the management of fungal endophthalmitis.

Methods

Materials

Voriconazole (VFEND; Pfizer, Inc., New York, NY) was obtained in pure powder form and reconstituted in sterile water to obtain a concentration of 35 μg/0.1 mL. Seventeen New Zealand White rabbits, each weighing 2 to 2.5 kg, were acclimated for at least 1 week under standardized temperature (25°C–28°C), humidity (50%–60%), and light (12 hours light/12 hours dark) conditions before experimentation. All care and handling of rabbits was performed in accordance with ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and with the approval of the Institutional Authority for Laboratory Animal Care at Taichung Veterans General Hospital.

Rabbits were anesthetized with a mixture of ketamine hydrochloride (35 mg/kg; Fort Dodge Animal Health; Wyeth, Madison, NJ) and xylazine (5 mg/kg; Phoenix Scientific, Inc., St. Joseph, MO) intramuscularly in the hindquarter. Both eyes of each rabbit were included in the experiment. Anterior chamber paracentesis was performed, followed by an injection of 35 μg voriconazole in 0.1 mL sterilized distilled water at a site 3 mm posterior to the limbus. Treatment was administered using a 30-gauge needle attached to a regular insulin syringe with the bevel positioned upward in the midvitreous of the eyes, slowly and under direct visualization. A cotton tip applicator was applied to the injection site immediately after removal of the needle to prevent fluid reflux from the injection site. Mydriasis was achieved with two to three drops of tropicamide 1%, and the fundus was examined with indirect ophthalmoscopy before and after injections. Aqueous humor samples were obtained with a 30-gauge needle, and the sampling was performed on two to three rabbits at each time interval (1, 2, 4, 8, 16, 24, and 48 hours) after injection and before enucleation of the eyes. Rabbits were killed with lethal cardiac injec-

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tions of pentobarbital sodium and phenytoin sodium (Beuthanasia-D; Schering Animal Health, Kenilworth, NJ). Four to six eyes per time interval up to 48 hours and both eyes of an additional control rabbit were enucleated on the same day and immediately frozen at −80°C. The eyes were dissected while frozen, and the entire vitreous was isolated according to the technique described by Abel and Boyle.[7] Vitreous of the control eyes was isolated to obtain standardization curves for high-performance liquid chromatography (HPLC) analyses. Assays of voriconazole concentration in vitreous and aqueous humor samples were performed with HPLC.

HPLC Analysis

Analysis of the samples was performed in a masked fashion. Rabbit vitreous samples and voriconazole standard (150 μL) were each pretreated with the addition of 600 μL of 100% acetonitrile, followed by vortex mixing at high speed for 1 minute at room temperature. The mixtures were centrifuged in a microultracentrifuge (CS 120 GX; Hitachi, Tokyo, Japan) at 45,000 rpm for 30 minutes at 4°C. Supernatant (450 μL) was transferred to a clean tube and dried in a centrifugal vacuum concentrator (Speed Vac Plus SC110; Savant Instrument Inc., Holbrook, NY). Samples for injection were redissolved in 120 μL of 20% acetonitrile containing 0.1% trifluoroacetic acid (TFA) followed by 1 minute of vortex mixing. Insoluble particles were removed by ultracentrifugation at 45,000 rpm for 30 minutes at 4°C. Samples of aqueous humor (90 μL) were extracted with 450 μL of 100% acetonitrile, and 450 μL supernatant was taken for drying after ultracentrifugation. After drying, the samples were redissolved in 90 μL of 20% acetonitrile containing 0.1% TFA.

Samples were analyzed with an HPLC system including a gradient HPLC pump (Aligent 1100; Hewlett-Packard, Waldbronn, Germany) and an optical detector (UV-VIS; S-3702; Soma, Honshu, Japan) interfaced to an integrater (D-2500 Chromato-integrater; Hitachi, Tokyo, Japan). Gradient eluting system was 0.1% TFA in deionized water (buffer A) compared with 0.1% TFA in acetonitrile (buffer B) with a flow rate of 1.0 mL/min. A 20-μL volume of each sample was injected onto an RP-column (Lichrospher 100RP-18e, 250 mm × 4 mm, 5 μm; Aligent Technologies), pre-equilibrated with 20% buffer B, and voriconazole was eluted with a linear gradient of acetonitrile (20%–50% containing 0.1% TFA) in 30 minutes. Voriconazole was monitored by absorbance at 257 nm and identified by coinjection with standard. Voriconazole was found to have a t_R of 21 minutes, with no interference from the sample background. The area of the voriconazole peak after baseline subtraction was calculated and compared with the area versus mass curve for the standard to quantify the amounts of voriconazole in the samples. The standard curve was linear to 1.0 μg/mL (correlation coefficient, 0.9997; range, 0.2–10.0 μg/mL), and the detection limit was estimated to be approximately 0.1 μg/mL (signal-to-noise ratio greater than 2). Samples with higher voriconazole outside the linear range were properly diluted with 20% acetonitrile for further HPLC analysis.

Results

Indirect ophthalmoscopy of the rabbit eyes revealed no retinal damage, hemorrhage, or detachment after intravitreal injection of 35 μg/0.1 mL voriconazole. Mean voriconazole levels measured for vitreous and aqueous humor at all sampling times are listed in Table 1. The vitreous concentration declined rapidly with time. Mean vitreous concentration was 18.912 ± 2.058 μg/mL 1 hour after injection and declined to 7.406 ± 1.783 μg/mL at 4 hours and 0.292 ± 0.090 μg/mL at 16 hours, respectively. An exponential decay model was used to fit the data, and least-square regression analysis was performed. The elimination half-life was calculated from the slope of the line of log concentration versus time. The vitreous voriconazole concentration showed an exponential decay, with a half-life of 2.5 hours. Four aqueous samples were below the detection limit (0.1 μg/mL) 4 hours after injection. Aqueous concentrations showed a decline from 0.240 ± 0.051 μg/mL at 1 hour to undetectable levels 8 hours after injection.

Table 1. Measured Vitreous and Aqueous Levels of Voriconazole at Different Time Intervals after Intravitreal Injection of 35 μg/0.1 mL in Rabbits

Time (h)	Vitreous Concentration (*n*)	Aqueous Concentration (*n*)
1	18.912 ± 2.058 (4)	0.240 ± 0.051 (4)
2	13.702 ± 1.519 (4)	0.187 ± 0.066 (4)
4	7.406 ± 1.783 (6)	0.127 ± 0.008 (2)*
8	2.351 ± 0.680 (6)	0.000 ± 0.000 (6)
16	0.292 ± 0.090 (4)	0.000 ± 0.000 (4)
24	0.000 ± 0.000 (4)	0.000 ± 0.000 (4)
48	0.000 ± 0.000 (4)	0.000 ± 0.000 (4)

Concentrations of voriconazole were given in μg/mL (mean ± SD).
* Four aqueous samples were below the detection limit (0.1 μg/mL) 4 hours after injection.

Discussion

Fungal endophthalmitis is of great concern because of its rising incidence, evident severity, and ominous prognosis even with prompt treatment. The difficulty in treatment results from a combination of the growth characteristics of fungi, a scarcity of effective antifungal agents, and their poor tissue penetration. Exogenous fungal endophthalmitis results from surgery, trauma, or contiguous spread from keratitis. In contrast, endogenous fungal endophthalmitis is usually associated with immunosuppressive therapy and intravenous infusion from indwelling catheters. Successful treatment of endophthalmitis includes the prompt use of an effective antimicrobial regimen. Currently, the treatment options for fungal endophthalmitis are limited, and the susceptibility of fungus species is variable. The antifungal agent used most often to treat fungal endophthalmitis is amphotericin B. In vitro activities of amphotericin B are variable, with minimum inhibitory concentrations (MICs) ranging from less than 0.5 μg/mL to 6.73 μg/mL.[8] However, the effects created in the laboratory are not necessarily applicable to the vitreous cavity in vivo. Drug levels required in endophthalmitis to eradicate the infecting organisms may be much greater than MICs.[9] Furthermore, a variety of fungus species, including *Candida tropicalis*, *Aspergillus terreus*, *Scedosporium*, and *Fusarium* isolates, have shown resistance to amphotericin B.[1,10] These studies support the necessity of a promising and safe intravitreal antibiotic regimen to achieve adequate drug levels for the management of fungal endophthalmitis.

Voriconazole is a broad-spectrum antifungal agent that inhibits the fungal enzyme cytochrome P450 demethylase. In vitro studies have shown voriconazole MIC ranges of 0.06 to 0.25 μg/mL for *Candida* species, 0.5 μg/mL for *Aspergillus* species, and 0.5 to 8 μg/mL for *Fusarium oxysporum* and *Fusarium solani*.[8,11–14] Voriconazole exhibited non–concentration-dependent activity in an in vitro time-kill study, suggesting that maximizing the duration of exposure of a fungus to voriconazole would optimize the fungistatic activity of voriconazole.[15] Although individual case reports demonstrate the successful use of oral voriconazole, alone or combined with caspofungin, to treat fungal endophthalmitis, systemic administration achieves a vitreous concentration lower than therapeutic levels of some filamentous fungi, particularly *Fusarium* species.[16–20] It has recently been determined that an intravitreal injection of voriconazole of up to 25 μg/mL of final intravitreal concentration causes no electroretinographic

described the first successful treatment of endogenous *Aspergillus* endophthalmitis using one intravitreal voriconazole injection. Sen et al.[21] also presented intravitreal voriconazole injections for the treatment of fungal endophthalmitis caused by *Aspergillus flavus*, *Scedosporium apiospermum*, and *Fusarium* species. Because intraocular injections of voriconazole have been increasingly used in the treatment of fungal endophthalmitis, studies of the postinjection kinetics of the drug assume increasing importance. We sought to determine the elimination rate of intravitreal voriconazole in rabbits and to determine how often administration is required to maintain therapeutic levels in vitreous.

The elimination half-life of a drug in a vitreal cavity depends on two pathways, the anterior route passage into the aqueous and the posterior route by active transport across the retina. Drugs such as penicillin and β-lactam antibiotics eliminated from vitreous cavities with the use of a retinal pump mechanism have shorter elimination half-lives than drugs cleared through the anterior chamber.[22,23] Given the rapid clearance rate and the low aqueous concentrations achieved, our data suggest that voriconazole is eliminated primarily through the posterior route. Aphakia, vitrectomy, and inflamed eyes create different effects on the clearance of the drug from the vitreous cavity. In the inflamed eye, the mechanism of active transport across the retina is compromised, resulting in increased half-lives of drugs eliminated primarily through a posterior route.[24] Aminoglycoside antibiotics and vancomycin, in contrast, are thought to be cleared by passive transport by way of the anterior route and the decreased retention resulting from inflammation.[25,26] Drug elimination is faster in inflamed aphakia eyes than in inflamed phakia eyes. Similarly, more rapid clearance of drugs from eyes after vitrectomy has been demonstrated for amphotericin B and fluorouracil.[27–30]

Assuming the normal volume of the vitreous in rabbit to be 1.4 mL, the injected dose of 35 μg/0.1 mL in rabbit eyes results in a vitreous concentration of 23.33 μg/mL. Peak vitreous levels achieved were thus approximately 50 to 100 times the MICs of voriconazole to *Candida* and *Aspergillus* species. Even with *Fusarium* species, intravitreal voriconazole achieved an effective inhibitory concentration. In contrast, the voriconazole levels achieved were low in aqueous humor. Our study showed rapid decline of the vitreous concentration and exponential decay, with a half-life of 2.5 hours. In such cases, vitreous levels will be below the MICs of most fungi by 16 hours, and supplementation of intraocular voriconazole may be required in clinical settings. Fortunately, drug elimination has been noted to be slower in humans than in rabbits. Elimination of voriconazole from the serum has been reported to have a half-life of 2.5 to 3 hours in rabbits compared with 6.5 hours in humans.[31,32] The clearance of intravitreal voriconazole in our data was close to the previously reported serum elimination rate in rabbits. Additional studies are needed to determine whether the half-lives of voriconazole in human vitreous and serum are similar; if so, a slower elimination rate of vitreous voriconazole would be expected in humans.

In summary, the clearance of voriconazole after intravitreal injection was determined to have an elimination half-life of 2.5 hours. Considerably low voriconazole levels in aqueous humor after injection were shown in the study. Vitreous concentrations achieved during the first 8 hours were greater than the previously reported MICs of organisms most involved in fungal endophthalmitis. A rapid decline of intravitreal concentration suggests that the supplementation of intraocular voriconazole to maintain therapeutic levels may be required in clinical settings. Further studies are needed to determine the vitreous

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