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Penetration of Topically Administered 0.5-Percent Caspofungin Eye Drops into Human Aqueous Humor▽

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Ten participants attending elective anterior segment eye surgery received 0.5% caspofungin eye drops either 1 drop hourly for 4 h or 1 drop an hour before surgery. The eye drops were generally well tolerated. In the absence of inflammation or corneal abrasion, topical caspofungin does not achieve clinically relevant concentrations.

Fungal keratitis is a potentially blinding condition, with *Candida*, *Aspergillus*, and *Fusarium* spp. (2, 21) being the common pathogens. Current treatment for fungal keratitis is inadequate (8); thus, alternatives are needed.

Caspofungin has activity against a wide range of fungi, with MIC_{90} values ranging from 60 to 2,000 ng/ml (15, 23). Caspofungin eye drops are effective for treatment of fungal keratitis in rabbits (11, 18) and have good penetration into animal eyes with inflammation or previous corneal abrasion (10, 23). Clinically, caspofungin is administered mostly intravenously (1, 4, 6, 9, 19, 20) or intravitreally (24) for ocular mycosis. The successful use of topical caspofungin alone (13) or in combination with voriconazole (22) has been reported for two patients. However, the utility of topical caspofungin in fungal keratitis remains unknown, partly due to limited data on its penetration into human aqueous humor after topical administration. This study aimed to investigate the ocular penetration of 0.5% caspofungin eye drops into human aqueous humor.

The study was approved by the ethics committees of the Royal Victorian Eye and Ear Hospital (RVEEH) and Monash University and was registered under the Australian New Zealand Clinical Trials Registry.

Between October 2009 and January 2010, participants ≥18 years old that were scheduled for elective eye surgery at RVEEH were recruited. Exclusion criteria were as follows: inflammation of the eye to be operated on, kidney or liver failure, breast feeding, pregnancy, trying to conceive, allergy to caspofungin (Cancidas) or any of its components, or using medications known to interact with caspofungin.

Caspofungin eye drops (0.5%) (11, 13, 22) were prepared aseptically; 10.5 ml of water for injection was added to a 50-mg vial of caspofungin. All eye drops were freshly prepared and used within 24 h (7).

Participants received either 1 drop (50 μl) of eye drops to the eye to be operated on, hourly over a period of 4 h prior to surgery (Arm 1), or 1 drop an hour before surgery (Arm 2). The eye drops were administered by RVEEH nursing staff. The date and time of administration, including side effects experienced, were recorded.

During surgery, aqueous humor (50 to 150 μl) was collected through a paracentesis site using a 30-gauge needle before administration of irrigation solutions. The samples were then divided into 30-μl aliquots, stored at −80°C, and analyzed within 1 week using a validated liquid chromatography/mass spectrometry (LC/MS) assay (17).

Ten participants gave consent (Table 1). The aqueous humor caspofungin concentrations for Arm 1 and Arm 2 were 28.9 to 95.1 ng/ml and 29.8 to 34.4 ng/ml, respectively. The sampling times for Arm 1 and Arm 2 were 0.75 to 1.67 h and 0.75 to 1.92 h, respectively. The eye drops were generally well tolerated.

This study is the first to demonstrate that topically administered caspofungin penetrates into uninflamed human eyes, in contrast to results from previous animal studies (10, 23). The difference could be due to the use of a more sensitive analytical

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TABLE 1. Patient characteristics, caspofungin concentrations in aqueous humor of study participants, and side effects reported

Study arm (type of application)	Patient no.	Gender	Age (yr)	Presence of diabetes mellitus	Type of lens	Caspofungin concn in aqueous humor (ng/ml)	Postsampling time (h)	Description of side effects (duration)
Arm 1 (1 drop every hour for 4 h prior to surgery)	1	Female	66	Yes	Phakic	95.1	1.67	Tingling sensation after the first drop (a few seconds)
	2	Male	88	No	Phakic	63.8	1.17	Tingling sensation after the first drop (a few seconds)
	3	Male	62	Yes	Phakic	36.7	1.33	None reported
	4	Female	74	No	Phakic	57.1	0.75	Tingling sensation after the first drop (a few seconds)
	5	Female	59	No	Phakic	28.9	0.83	None reported
Arm 2 (1 drop 1 h prior to surgery)	1	Female	84	No	Phakic	32.6	0.75	Tingling sensation after the first drop (a few seconds)
	2	Male	74	Yes	Phakic	31.5	1.08	Tingling sensation after the first drop (a few seconds)
	3	Male	58	No	Phakic	34.4	1.58	None reported
	4	Female	59	No	Phakic	32.2	1.33	None reported
	5	Female	76	Yes	Phakic	29.8	1.92	Burning sensation (~10 min; nurse administered 2 drops of normal saline, and 5 min later the burning sensation ceased)

assay (limit of quantification [LOQ], 10 ng/ml) (17) than that used in the earlier study (LOQ, 50 ng/ml) (10).

The low concentrations, however, indicate that topical caspofungin does not have good penetration into uninflamed eyes, consistent with caspofungin being a large molecule (1,213 Da) and highly protein bound (96.5%) (5). Compounds of >500 Da experience difficulty penetrating the intact corneal epithelium after topical administration due to frictional force that reduces diffusion (16). While the concentrations observed were below the MIC_{90} for most fungal species (15, 23), topical caspofungin may still be useful for fungal keratitis. Disruption of the corneal surface or inflammation in rabbit models has resulted in higher caspofungin concentrations in aqueous humor after systemic or topical administration (10, 23). As the cornea is the primary site of infection in keratitis, high corneal concentrations of antifungal agents are desirable. An earlier study (10) reported higher mean caspofungin concentrations in the cornea than in the aqueous humor of rabbits after a single 1 mg/kg intravenous dose. The higher corneal concentrations could be due to differences in protein binding and the retention of caspofungin in the stroma (10). The results obtained from the rabbit models could be extrapolated to a human setting, given that there is no difference between the protein distribution and components in the aqueous humor of humans and rabbits (3). As fungal keratitis will generally compromise the corneal epithelium's integrity, topical caspofungin could achieve concentrations above the MIC_{90} in patients with keratitis, but this remains to be demonstrated. Clinical resolution with topical caspofungin has been demonstrated for keratitis caused by *Fusarium* species in rabbit models (18) and keratitis caused by *Alternaria* (22) or *Candida* (13) species in patients.

Arm 1 participants had higher caspofungin concentrations with greater variability than Arm 2 participants. The nature of scheduled elective surgery and access to the operating theater have no doubt contributed to the variable sampling times. For this reason, it is not appropriate to report the concentrations as means ± standard deviations. The possibility of drug accumulation upon repeated dosing and the reason for the variability in Arm 1 cannot be confirmed due to small sample sizes. Findings from animal studies have been inconsistent. Continuous topical administration (1 drop every 30 min for 6 h) with 0.7% caspofungin produced higher levels (4.94 ± 1.80 μg/ml) than a single application (1.76 ± 0.88 μg/ml) at 2 h after the last dose (23). Conversely, daily intravenous administration of 0.5% caspofungin for 7 consecutive days (10) did not lead to higher concentrations in the aqueous humor or cornea.

Minimal side effects were noted, with transient tingling sensations being the most common. A saturated caspofungin solution has a pH of 6.6 (Cancidas package insert; Merck & Co. Inc., Whitehouse Station, NJ) and should be well tolerated in the eyes (12). Side effects associated with systemic administration are unlikely, given that each topically administered dose (50 μl) of 0.5% caspofungin contains only 0.25 mg caspofungin. Following the 2-hourly regimen (22), the total daily topical caspofungin dose is 3 mg, which is 6% of a standard 50 mg intravenous dose. There was no toxicity on human ocular cells after 1 month of treatment with 75 μg/ml caspofungin (14).

In conclusion, caspofungin eye drops are well tolerated but have a low level of penetration into the uninflamed human eye.

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