Intraocular Penetration of Intravenous Micafungin in Inflamed Human Eyes

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Eight eyes of 7 patients with fungal disease received intravenous injections of 150 to 300 mg micafungin, and samples of blood, cornea, retina-choroid, aqueous humor, and vitreous humor were collected. The micafungin levels in all collected samples exceeded the MICs; however, the levels in the vitreous and aqueous humors were lower. Our findings suggest that intravenous micafungin should be given in combination with intravitreal antifungal agents after vitrectomy in severe cases of intraocular fungal diseases.

Endogenous fungal endophthalmitis is a serious inflammatory disorder that commonly occurs in immunocompromised patients. Systemic treatments for endogenous fungal endophthalmitis include the polyenes and the triazoles (1, 2). Micafungin is a water-soluble echinocandin antifungal agent, and it has efficacy comparable to that of liposomal amphotericin B with fewer side effects as a first-line treatment for candidemia and invasive candidiasis (3). Micafungin can block the formation of biofilms of *Candida* spp. (4) but has poor penetration into the vitreous after a single intravenous injection in animal models (5).

We investigated the intraocular penetration of intravenously administered micafungin in inflamed human eyes. Eight inflamed eyes of 7 patients (Table 1) who were scheduled to undergo vitrectomy or enucleation surgery were studied. The study protocol was approved by the Ethics Committees of Gifu University Hospital, and all patients gave informed consent. A 150-mg or 300-mg/dose of micafungin was infused intravenously daily. Blood samples were collected at approximately 1 h after the last intravenous injection of micafungin, and the plasma was immediately separated from the whole blood by centrifugation. Ocular samples were obtained within 30 min (cases 1 to 5) or 90 min (cases 6 and 7) after collecting blood samples. All specimens were stored at -20°C until analyses. The aqueous and vitreous samples were not diluted.

The concentration of micafungin was measured by high-pressure liquid chromatography (HPLC) according to the methods described in detail by Yamato et al. and Niwa et al. (6, 7). Briefly, after the plasma proteins were precipitated with acetonitrile containing an internal standard, they were separated on a TSK ODS-80 gel (150 mm by 4.6 mm [internal diameter]; Tosoh, Tokyo, Japan). Calibration curves were linear over the ranges of 0.01 to 2.5 $\mu\text{g}/\text{ml}$ for aqueous and vitreous humors and 0.1 to 25 $\mu\text{g}/\text{ml}$ for plasma. The inter- and intraday precisions were below 15%, and the accuracy was within 15% of the quality controls.

Parts of the vitreous and aqueous humors were cultured on Sabouraud agar, and the isolates were identified based on morphology and by sequencing the internal transcribed spacer region of the ribosomal DNA (rDNA) or the β -tubulin gene (8, 9). *Candida albicans* grew from the vitreous in cases 1 and 2, *Aspergillus*

tubingensis from the vitreous in case 5, and *Paecilomyces lilacinus* in the corneal scraping of case 6. Tests for drug susceptibility were performed on the vitreous isolates by broth microdilution according to CLSI methods (10, 11).

For the statistical analyses, unpaired *t* tests and Pearson product-moment correlation coefficients were used. A *P* value of <0.05 was considered to be significant. All statistical analyses were performed using SPSS software version 16.0 (SPSS Japan, Tokyo, Japan.).

The concentrations of micafungin (mean $\pm$ standard deviation [SD]) were 21.02 ± 4.59 $\mu\text{g}/\text{ml}$ in the plasma, 0.10 ± 0.07 $\mu\text{g}/\text{ml}$ in the vitreous humor, and 0.08 ± 0.12 $\mu\text{g}/\text{ml}$ in the aqueous humor (Table 1). The mean concentrations for 150 and 300 mg micafungin were, respectively, 17.02 and 23.30 $\mu\text{g}/\text{ml}$ in the plasma (*P* = 0.035; unpaired *t* test), 0.05 and 0.10 $\mu\text{g}/\text{ml}$ in the aqueous humor (*P* = 0.453; unpaired *t* test), and 0.09 and 0.10 $\mu\text{g}/\text{ml}$ in the vitreous humor (*P* = 0.874; unpaired *t* test). There was no significant correlations between the total dose and the concentrations of micafungin in the plasma (*r* = 0.728, *P* = 0.041), in the aqueous humor (*r* = 0.400, *P* = 0.373), or in the vitreous humor (*r* = 0.513, *P* = 0.194).

The micafungin concentration in the cornea was 5.99 $\mu\text{g}/\text{g}$ in case 6 and 1.60 $\mu\text{g}/\text{g}$ in case 7. In case 7, the drug concentration was 14.65 $\mu\text{g}/\text{g}$ in the iris, 1.20 $\mu\text{g}/\text{g}$ in the retina, and 5.81 $\mu\text{g}/\text{g}$ in the choroid.

In 2 cases, the MICs of *C. albicans* to amphotericin B, flucytosine, fluconazole, itraconazole, miconazole, voriconazole, and micafungin were 0.25 to 0.5, 0.25, 0.5 to 1, 0.125 to 0.25, 0.25, ≤ 0.015 to 8, and ≤ 0.03 $\mu\text{g}/\text{ml}$, respectively. In case 5, the MICs for *A.*

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TABLE 1 Patient demographics and micafungin concentrations in plasma and ocular tissues^a

Value or category for patient no. ^b :									
Characteristic	1 ^c	2	3	4	5	6	7 ^d	Mean ± SD or ratio	
Sex	Male	Male	Male	Male	Male	Female	Male	Male/female, 6:1	
Age (yr)	67	67	72	78	88	82	75	75.6 ± 7.8	
Side	Left	Right	Left	Left	Left	Right	Right	Right/left, 4:5	
Type of surgery	PPV, PEA, IOL	PPV, PEA, IOL	Diagnostic vitrectomy	Diagnostic vitrectomy	Paracentesis ^e	Enucleation	Left ECCE, IOL		
Isolated organism (sample)	<i>Candida albicans</i> (vitreous humor)	Negative (aqueous and vitreous humors)	Negative (aqueous and vitreous humors)	<i>Neisseria</i> spp. (vitreous humor)	<i>Aspergillus tubingensis</i> (vitreous humor)	<i>Pseudomonas fluorescens</i> (cornea)	ND	Negative (aqueous and vitreous humors)	
Final diagnosis	Fungal endophthalmitis	Fungal endophthalmitis	Endophthalmitis (<i>Noctuidia farcinica</i>)	Endophthalmitis (<i>Neisseria</i> spp.)	Fungal endophthalmitis following cataract surgery	Keratomycosis	Keratitis	Keratitis	
Final BCVA (BCVA before surgery)	20/20 (12/20)	20/20 (4/200)	20/20 (8/20)	20/20 (8/20)	10/20 (6/200) ^f	Null (LP)	6/20 (12/200)	Null (LP)	
Other medical conditions	Myelodysplastic syndrome	Pyelonephritis	DM, anemia	Chronic renal failure	DM	Pseudophakia	Cataract, glaucoma	Hypertension	
Micafungin									
Dose (mg/day)	300	300	300	300	150	150	150		
Total dose (mg)	5,700	6,300	8,700	900	1,200	2,450	450	3,662.5 ± 2,975.5	
Duration (days)	19	21	29	3	8	16	3	13.9 ± 9.1	
Concn in:									
Plasma (μg/ml)	24.77	18.31	28.37	19.12	16.78	17.80	17.03	21.02 ± 4.59	
Aqueous humor (μg/ml)	0.02 [0.08]	0.35 [1.91]	0.07 [0.25]	0.02 [0.10]	0.05 [0.30]			0.08 ± 0.12	
Vitreous humor (μg/ml)	0.06 [0.24]	0.10 [0.55]	0.24 [0.85]	0.06 [0.31]	0.05 [0.30] ^g	0.07 [0.39]	0.15 [0.88]	0.10 ± 0.07	
Cornea (μg/g)								[0.43 ± 0.66]	
Iris (μg/g)								0.10 ± 0.07	
Retina (μg/g)								[0.46 ± 0.28]	
Choroid (μg/g)								3.30 [21.52]	

^a PPV, pars plana vitrectomy; PEA, phacoemulsification; IOL, intraocular lens; ECCE, extracapsular cataract extraction; DM, diabetes mellitus; ND, not done; LP, light perception; BCVA, best-corrected visual acuity.

^b Values in brackets show the ocular tissue/plasma concentration ratio.

^c From reference 18.

^d Poor vision was detected 30 years ago.

^e After PPV and IOL removal.

^f Visual acuity before vitrectomy and IOL removal.

^g Aphakic and avitreous eye.

^h 0.1% micafungin eye drop for 9 days, 5 times per day.

tubingensis of amphotericin B, flucytosine, itraconazole, miconazole, voriconazole, and micafungin (for micafungin, minimum effective concentration [MEC]) were 1, 32, 1, 4, 1, and ≤ 0.015 $\mu\text{g/ml}$, respectively. In case 6, the MICs for *P. lilacinus* of amphotericin B, flucytosine, itraconazole, miconazole, voriconazole, and micafungin (MEC) were 2, >64 , 0.5, 2, 0.125, and 0.25 $\mu\text{g/ml}$, respectively.

The guidelines for the treatment of fungal endophthalmitis are systemic antifungal therapy combined with a monitoring of the endophthalmitis (12). For rabbits, Suzuki et al. reported that the concentration of micafungin in the retina-choroid and plasma exceeded the MICs for fungal pathogens after a single intravenous administration (5). The MIC₉₀s of micafungin for global surveillance were 0.06 $\mu\text{g/ml}$ for *Candida* spp., 0.06 $\mu\text{g/ml}$ for *Candida glabrata* and *Candida tropicalis*, and ≤ 0.008 $\mu\text{g/ml}$ for *Aspergillus* spp. (13). The MIC of micafungin against *C. albicans* was ≤ 0.03 $\mu\text{g/ml}$ and the MEC for *A. tubingensis* was ≤ 0.015 $\mu\text{g/ml}$, as isolated in our hospital.

The fungal endophthalmitis may have led to the production of proinflammatory cytokines that could have disrupted the blood-ocular barrier (14). However, the mean vitreous levels of micafungin with endogenous endophthalmitis still remained low, suggesting that even with a disrupted blood-retinal barrier, micafungin penetrated poorly into the vitreous. Groll et al. showed that the micafungin level in the aqueous humor of noninflamed rabbit eyes was low after an intravenous injection (15). The concentration in the aqueous humor was not high even in the inflamed eyes in our study, except in an eye with severe anterior inflammation. The intraocular penetration may be related to differences in the molecular weights and the solubility in ocular fluids (16–18). The micafungin level in the iris was higher than that in other ocular tissues, suggesting that the drug in the anterior chamber was bound to the melanin in the iris.

The intravenous dosing regimen for invasive candidiasis with micafungin is 100 mg/day (12). The steady-state plasma concentration of micafungin after repeated intravenous administrations is attained by 4 days, and the terminal half-life after the cessation of repetitive doses was 14 h (19). We found a significant correlation between the total dose and the concentrations of micafungin in the plasma. However, the correlations between the concentrations in the aqueous and vitreous humors after a single or total dose of micafungin were not significant. This may be due to differences in the sampling time and variations in the sampling procedures.

Our results showed that the concentration of micafungin in the cornea was high and exceeded the MICs for most *Candida* and *Aspergillus* spp. (13). The micafungin in the cornea was from the tear film, aqueous humor, or limbal vessels. However, micafungin was not detected in the tear film in a case of tunnel fungal infection. Although the reason for the accumulation of micafungin in the cornea is unclear, the differences in the degree of protein binding or retention of the water-soluble echinocandin macromolecule in the proteoglycans of the corneal stroma may allow high levels of echinocandins to accumulate in the cornea (20).

In conclusion, our results showed that the concentrations of micafungin in the cornea, the iris, and the retina-choroid were higher than the MICs for *Candida* spp. and *Aspergillus* spp. We recommend that intravenous micafungin be considered only for patients with mild endogenous fungal endophthalmitis (isolated chorioretinitis without vitreous extension) without vitrectomy. It

can also be considered as an alternative therapy for selected patients with *Candida glabrata* endophthalmitis for whom first-line fluconazole therapy cannot be used because of drug resistance. Systemic administration of micafungin has good corneal penetration. Because the level of the micafungin in the aqueous and vitreous humors is not high, micafungin may be combined with an intravitreal antifungal agent with vitrectomy for the treatment of severe endogenous fungal endophthalmitis.

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