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Case Report Open Access

Diagnostic Direct DNA Sequencing and Systemic Treatment with Voriconazole in *Scedosporium apiospermum* Keratitis? A Case Report

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Abstract

Scedosporium apiospermum keratitis is a rare but challenging infection because of its high misidentification rate and resistance to many antifungal agents. A 35-year-old immunocompetent man with severe *Scedosporium apiospermum* keratitis diagnosed by both microbiology and DNA sequencing methods, which was successfully treated with systemic voriconazole was reported. Diagnosis and drug susceptibilities were discussed.

Keywords: *Scedosporium apiospermum*; Keratitis; Voriconazole; Drug susceptibility; DNA sequencing

Case Report

A 35-year-old Chinese man was referred to our hospital due to redness, decreased vision and gritty sensation in the left eye. His symptoms deteriorated despite topical and intravenous treatments (therapeutic regimen unknown) in a local hospital for 20 days. Physical examination and laboratory work-up were negative. Predisposing factors such as recent ocular injury, contact lens use, prior keratitis or immunosuppressed status were denied. His fellow eye, however, underwent a corneal foreign body extraction one year ago when he worked in a salvage station, and soon recovered to 20/20.

At presentation, best-corrected visual acuity was hand-motion in the left eye. There was a central, ring-shaped, 6 × 6 mm dense corneal abscess with adjacent cellular infiltration and a 4 mm hypopyon. Other details were not discernible. B ultrasound examination revealed a clear vitreous and no retinal detachment. Ophthalmic examination of the right eye was unremarkable except a small nebula compatible with the corneal foreign body extraction history mentioned above.

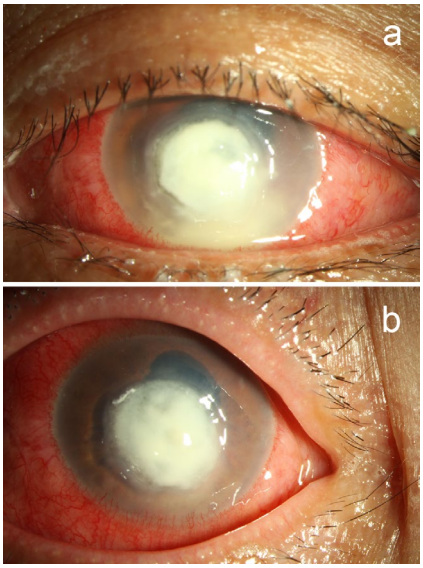

Figure 1: Clinical photograph of the left eye. (a) 2 days after admission, corneal abscess and hypopyon are demonstrated. **(b)** 5 days after admission, infiltration decreased and hypopon totally disappeared.

Fungal keratitis was diagnosed clinically. Primary microscopic examination of corneal scrapings found no bacteria or fungi. Further microbiology culture was conducted. Surgical debridement and empirical broad-spectrum treatment: intravenous voriconazole (loading dose of 6 mg/kg every 12 hours for day 1, 4 mg/kg every 12 hours from day 2-5), oral faropenem sodium 200 mg t.i.d., topical atropine t.i.d., levofloxacin 0.5%, natamycin 5% and dextran / hypromellose q.h. were commenced.

In the following days, corneal infiltration and hypopyon promptly declined (Figures 1a and 1b). On day 6, a second surgical debridement was performed; thereafter, voriconazole was shifted to orally 200 mg every 12 hours.

On day 7, the mycology culture revealed a growth of cottony whitish filamentous fungus (Figure 2a). Microscopy study revealed ovoid conidial structures at the top of conidiophores, some of which

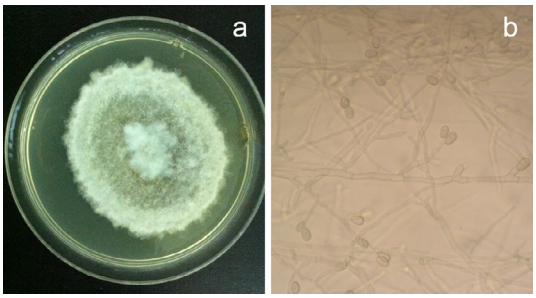

Figure 2: Microbiology photograph of the fungus. (a) 7 days after fugal culture on Sabouraud's glucose agar, cottony whitish filamentous fungus could been seen. **(b)** Microscopy (X400). Ovoid conidial structures at the top of conidiophores, with some of them clearly separated from hyphae can be identified.

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clearly separated from hyphae (Figure 2b). The isolate was submitted to a microbiology center for further identification. Meanwhile, a loopful of the colony was processed for DNA sequencing, which yielded a 99% homology with several sequences of *Scedosporium apiospermum*.

We discontinued oral faropenem sodium, and tapered topical levofloxacin 0.5% to b.i.d.. The inflammation was steadily controlled. Eighteen days later, microbiology center reported an identification of *Scedosporium apiospermum,* which was in concordance with the result of the DNA sequencing.

E-test for drug susceptibility was also performed. Minimal inhibitory concentrations (MICs) were determined after incubation at 35°C for 72 hours. MICs of caspofungin, voriconazole, fluconazole, itraconazole and amphotericin B were 0.38 μg/ml, 0.008 μg/ml, 3 μg/ml, 0.5 μg/ml and >32 μg/ml.

Since the infiltration was large and involving full-thickness corneal stroma, penetrating keratoplasty (PK) with glycerol preserved donor cornea was performed on day 13, iris neovascularization, central anterior synechia, exudative membrane and cataract were disclosed. Corneal button for microbiological examination (smear and culture) showed no growth of microorganism. Because corticosteroid was used after PK to prevent rejection, intravenous voriconazole and topical natamycin 5% were applied to prevent a relapse.

No adverse effect was encountered during the therapeutic regimen. Visual acuity of his left eye was hand-motion at discharge. Intensive follow up was recommended for further therapy like antiglaucoma surgery, cataract surgery and PK with fresh corneal graft.

Discussion

Scedosporium apiospermum, the anamorph of the ascomycete *Psudallescheria boydii*, is a world-wild saprophyte found in the soil, pounds, sewage and decaying plant. For eye affected cases, endophthalmitis patients are usually previously immunocompromised or have underlying pulmonary disease [1,2]. But for keratitis patients, predisposing factors such as contact lens wearing, ocular trauma, surgery, recurrent keratitis and multiple sclerosis are usually found [3-6]. In our case, no definite cause could be confirmed, although an unconscious injury was suspected, considering his occupation and the history of corneal foreign body extraction in his right eye.

S. apiospermum keratitis resembles other types of fugal keratitis in clinical presentations and microbiology findings [7]. The traditional identification approaches were sometimes problematic and time consuming. It was reported that when submitted to a panel of laboratories as an NEQAS quality control, a *S. apiospermum* isolate was misidentified by more than 40% of participants [8]. The delay in diagnosis and aggressive treatment makes it one of the species that most frequently lead to perforation [8], with evisceration or enucleation being the final result in 21% in 2003 [9]. In this circumstance, molecular method, which costs a shorter turnaround time but provides a result well parallel to traditional techniques [10], plays a promising role in timely diagnosis.

E-test method was proved to have a good level of agreement with the standard procedures proposed by NCCLS (the National Committee for Clinical Laboratory Standards) for the antifungal susceptibility testing. The E-test of our case indicated that the fungi was sensitive to caspofungin, voriconazole, fluconazole and itraconazole (MICs were 0.38 μg/ml, 0.008 μg/ml, 3 μg/ml, 0.5 μg/ml respectively), but resistant to amphotericin B. The results are in agreement with previous studies summarized in Table 1. These studies indicated that the most potent activity was observed with voriconazole, followed by miconazole,

drug	references	No. of strains	Method	Incubation time (hours)	MIC, μg/ml			
					Range	geometric mean	50%	90%
Albaconazole	[11]	13	M38-P	48	0.03-1	0.13	0.125	1
	[11,12]	24	M38-P	72	0.06-2	0.41-1	0.5-1	1-2
Amphotericin	[13,14]	20	E-test	48	2->32	-	>32	8, >32
	[14]	10	E-test	72	4-8	6.7	-	8
	[13,15]	37	M27-A	48	1->16	4	2-4	8->16
	[16,17]	122	M38-A	48	0.5-16	-	4.0	8.0->16
	[11]	13	M38-P	48	0.5-8	1.72	2	4
	[11,12,14,18]	51	M38-P	72	1->16	2.97-4	4	>1->16
	[19]	26	-	-	1-6	4.56	4	8
	[20]	10	Sensititre	72	2->16	-	-	4
Fluconazole	[20]	10	Sensititre	72	16-≥256	-	-	≥256
	[15]	27	M27-A	48	16->16	>16	>16	>16
	[18]	17	M38-P	72	8->64	-	16	32
Itraconazole	[16,17,21]	176	M38-A	48	0.03–>8.0	-	0.5, ≥ 8.0	1.0, ≥8.0
	[20]	10	Sensititre	72	0.5-≥16	-	-	≥16
	[19]	26	-	-	0.125-2	0.65	0.5	2
	[13,15]	37	M27-A	48	0.25-16	-	1, 8	2, 16
	[13,14]	20	E-test	48	0.25->32	-	-	0.5, >32
	[14]	10	E-test	72	0.25-0.5	0.4	-	0.5
	[11]	13	M38-P	48	0.03-2	0.35	0.5	2

	[11,12,14,18]	51	M38-P	72	0.03->16	0.78-4.5	0.5->16	1->16
	[22]	6	-	-	0.12->64	-	-	-
Ketoconazole	[20]	10	Sensititre	72	0.25-≥16	-	-	≥16
	[15]	27	M27-A	48	0.06->16	2	2	>16
	[12]	11	M38-P	72	4-16	10.07	16	>16
Liposomal nystatin	[11]	13	M38-P	48	2-16	5.99	4	16
	[11]	13	M38-P	72	4-16	11.99	16	16
Miconazole	[15]	27	M27-A	48	0.06-4	0.5	0.5	2
	[11]	13	M38-P	48	0.125	0.34	0.25	1
	[11,18]	30	M38-P	72	≤0.06-1	-	0.25-0.5	0.5-1
Micafungin	[23]	3	M38-A	24	0.25-128	-	32	-
	[18]	17	M38-P	72	16->16	-	>16	>16
Nystatin	[11,12]	24	M38-P	72	4-32	12.70-13.24	4-16	≥16
	[11]	13	M38-P	48	2-16	5.99	-	-
Posaconazole	[19]	20	-	-	0.25-1.0	0.57	0.5	1
	[17]	65	M38-A	48	-	-	1.0	8.0
	[11]	13	M38-P	48	0.125-1	0.42	0.5	1
	[11,12]	24	M38-P	72	0.03-2	0.08-0.79	0.03-1	0.25-2
Ravuconazole	[21]	54	M38-A	48	0.5->8.0	3.8	4.0	>8.0
	[12]	11	M38-P	72	0.125	0.125	0.125	0.125
Terbinafine	[19]	5	-	-	32	32	32	32
	[24]	31	M38-A	48	8->16	17.1	-	>16
	[11]	13	M38-P	48	2->32	32	32	>32
	[11]	13	M38-P	72	32->32	>32	>32	>32
Voriconazole	[16,17,21]	176	M38-A	48	0.06–4.0	-	0.25-1.0	0.25-4.0 >8.0
	[19]	26	-	-	0.06-1.0	0.22	0.25	0.25
	[15]	27	M27-A	48	0.5-2	1	1	2
	[23,25]	9	M38-A	72	0.125-1.0	-	0.125-0.5	0.25-1
	[11,12,18]	44	M38-P	72	0.01-0.5	0.06-0.17	0.06-0.25	0.125-0.5
	[11]	13	M38-P	48	0.03-0.5	0.09	0.125	0.25
	[22]	6	-	-	0.12-0.5			
	[25]	6	Sensititre	72	1	-	0.5	0.5
5-Fluorocytosine	[20]	10	Sensititre	72	4-≥64	-	-	≥64
	[15]	27	M27-A	48	>16	>16	>16	>16
	[18]	17	M38-P	72	8->64	-	16	32

Table 1: *In Vitro* Drug Susceptibilites of *Scedosporium apiospermum*.

posaconazole and albaconazole; amphotericin B, fluconazole and itraconazole showed variable antifungal activity; 5-Flucytosine did not have any antifungal activity.

Posessing a high level of *in vitro* activity, posaconazole and albaconazole might be effective therapies, but so far as we know, applications of these agents in ophthalmology have not been reported. Miconazole, which seems to be a good choice for ophthalmologists, is associated with adverse effects like hypotension, pruritus, and bone marrow / hepatic toxicity [26]. Itraconazole, which is a good alternative in some cases (including our case), is poorly absorbed with oral administration [27].

Voriconazole demonstrated a favorable MIC, as well as an optimal bioavailability. It was reported that after 12 days of oral voriconazole, the mean concentration in plasma was 3.4 μg/ml, and 1.8 μg/ml (53% of the level in plasma) in the aqueous humor, which exceeded the MIC by sevenfold [28]. Side effects consist of fully reversible mild to moderate visual disturbances and elevated liver function enzymes [29], indicating a considerable safety. The formula of voriconazole 1% eye drop expanded ophthalmological treatment regimen for this refractory disease [30-32].

We reported a case with severe *S. apiospermum* keratitis which was successfully treated with systemic administration of voriconazole. We identified the fungal species with microbiology and molecular method, and took a subsequent sensitivity test. In our opinion, repeated smear and culture are essential in microbiology examination, but DNA sequencing should be considered as an effective and efficient tool in fungal identification. As adjunctive therapy to surgical debridement, systemic and topical voriconazole are good choices.

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