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The first case of endophthalmitis due to *Rhinocladiella basitona* in an immunocompetent patient☆



Mengyang Liu ^{a,*}, Xiaowen Xin ^b, Jing Li ^a, Shengjie Chen ^a

- ^a The Affiliated Hospital of Qingdao University, Qingdao, China
- ^b Weihai Municipal Hospital, Weihai, China

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ABSTRACT

Rhinocladiella, a genus of black yeast–like fungi, is related to many infections in humans, including not only mild cutaneous lesions but also fatal brain infections. However, endophthalmitis caused by Rhinocladiella has never been reported by far. Herein, we present the first case of endophthalmitis due to Rhinocladiella basitona. The diagnosis was based on histopathology, mycology, and molecular identification. A 53-year-old female was struck by a piece of wood in her right eye. The wound in the central cornea became an ulcer and was aggravated continuously. Hyphae were found in the corneal scraping smear. Then endophthalmitis occurred and could not be controlled by the combined intravitreal antibiotic injections and vitrectomy. Finally, penetrating keratoplasty combined with retinal reattachment surgery was performed. Topical and systemic antifungal agents were administered for more than 1 month. The patient was cured, with improved visual acuity and clear corneal graft.

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1. Introduction

Fungal endophthalmitis (FE) is uncommon but may result in poor outcomes, including vision loss (Chhablani, 2011; Lingappan et al., 2012). It is usually divided into exogenous and endogenous types depending upon the mode of infection. Postocular operation (Chakrabarti et al., 2008) and trauma (Ramakrishnan et al., 2009) are the major etiologic factors of exogenous FE. For endogenous FE, however, the factors are various, such as abuse of intravenous drugs and extensive use of immunosuppressants and broad-spectrum antibiotics (Lingappan et al., 2012; Narendran et al., 2008; Zhang and Liu, 2010). The frequently encountered pathogens for endogenous FE, posttraumatic FE, and FE secondary to keratitis are Candida albicans, Aspergillus niger, and Fusarium solani, respectively (Essman et al., 1997; Sun et al., 2014; Wykoff et al., 2008; Zhang and Liu, 2010). Rhinocladiella species, mostly being Rhinocladiella aquaspersa and Rhinocladiella mackenziei, are usually seen in tropical regions. R. aquaspersa is often associated with skin infections (Badali et al., 2010a), while R. mackenziei causes brain infections in otherwise healthy individuals and leads to high mortality (Campbell and Al-Hedaithy, 1993; Didehdar et al., 2015).

R. basitona, a member of the genus *Rhinocladiella*, was first identified as a human pathogenic fungus in 1998 (Suzuki et al., 1998). It initially belonged to *Geniculosporium* and was classified to *Rhinocladiella* by de Hoog et al. (2003). The clinical case caused by *R. basitona* is extremely rare. Only 2 cases of phaeohyphomycosis due to this pathogen have been reported (Cai et al., 2013; Suzuki et al., 1998). Herein, we present

a case of endophthalmitis related to *Rhinocladiella basitona*; describe its mycological characteristics; and evaluate the activity of triazole, amphotericin B, and natamycin against this microorganism in vitro.

2. Case report

A 53-year-old female was struck by a piece of wood in her right eye and lost the vision in September 2008. The patient received corneal suture immediately and cataract extraction combined with intraocular lens implantation 15 days later at a local hospital. However, the wound in the central cornea became an ulcer and was aggravated continuously. Hyphae were found in the corneal scraping smear by microscopy. Eye drops and intravenous injection of fluconazole were given, but the infection could not be controlled. The patient was referred to our hospital on February 3, 2009. The visual acuity was finger counting/20 cm in the right eye and 20/20 in the left eye. Anteriorsegment examination showed conjunctival hyperemia and corneal edema with 2 mm × 3 mm of full-thickness infiltration in the corneal center. The iris and lens details were hazy with fibrin. Significant cytemediated inflammatory reactions were found in the anterior chamber. Lots of effusions were accrete in the ante-sac (Fig. 1A). Severe vitreous opacity and posterior vitreous detachment were observed by B-scan ultrasonography. No retinal detachment was detected. No hyphae were found by corneal scraping or confocal microscopy.

On February 5, intravitreal antibiotic therapy was performed, and 0.1 g ceftazidime was injected into the vitreous cavity. Aqueous fluid and vitreous body were observed for microbiological examination. A large number of white blood cells were seen both in aqueous fluid and vitreous body, but no bacteria or fungi were found. Tobramycin eye drops were administered, and intravenous ciprofloxacin (15 mg/kg

[☆] Conflicts of interest: None.

^{*} Corresponding author. Tel.: +86-532-82911511; fax: +86-532-82911999. E-mail address: qdliumengyang@163.com (M. Liu).





Fig. 1. Clinical features and the examination results of the patient before treatment. A. Photograph of the right eye at first presentation to our hospital showing 2 mm × 3 mm of full-thickness infiltration in the central cornea and an irregular pupil. B. Confocal microscopic image showing hyphae in the deep layer of the cornea. C. Brown hyphae in the corneal scraping smear (10% KOH, original magnification ×400).

per day) was given for a week. At 1 week after the operation, the visual acuity was counting finger/30 cm. The samples did not show any growth of microorganisms up to 7 days. The patient had steadily improved vitreous clarity and decreased inflammation in the anterior chamber after treatments.

However, the vitreous body became turbid again, and the infection became aggravated on February 26. The intravitreal antifungal therapy combined with vitrectomy was performed, and 1 mg voriconazole was injected into the vitreous cavity. The excised part was sent immediately for pathogenic examination. The sample smear and culture results were both negative. Confocal microscopic examination showed no hyphae. On March 9, the visual acuity was 20/500. The cornea was edematous with 3 mm \times 4 mm of full-thickness infiltration in the center. The vitreous cavity was clear, and no retinal detachment was found.

The patient complained the cloudiness in vision of the right eye on April 2. The cornea was edematous and cloudy with 4 mm × 5 mm of infiltration in the center. A round lesion was about 2 mm in diameter. within the substrate at the nasal side. The vitreous body was opaque. On April 6. hyphae were detected in the cornea by confocal microscopy (Fig. 1B), and brown hyphae were also found in the scraping of the cornea (Fig. 1C). The clinical specimen obtained from the scraping was incubated on chocolate agar (GC medium; Difco, Sparks, MD, USA) and 5% sheep blood agar (Oxoid, Basingstoke, Hampshire, England) at 37 °C for 72 hours and Sabouraud glucose agar (Oxoid) at 27 °C for 7 days. Eye drops of 0.5% fluconazole and oral itraconazole (200 mg per day) were administered. However, the infiltration at the nasal side of the cornea became larger 10 days later, indicating that the antimycotic treatment was not effective. Penetrating keratoplasty combined with retinal reattachment surgery was performed. The excised cornea was subjected for histopathological examination, and brown hyphae were found (Fig. 2C). After the operation, intravenous amphotericin B deoxycholate (0.6 mg/kg), oral itraconazole (200 mg), and 5% natamycin eye drops were administered daily. At 1 week after the operation, the visual acuity was 20/400, and the corneal allograft was transparent. The vitreous body became clear, the fundus was clearer than before, and the retina was flat

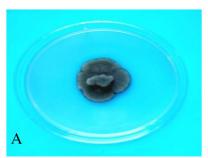
Oral itraconazole and 5% natamycin eye drops were continued for 1 month. The signs of infection were alleviated, and the visual acuity was improved. After 1 year and 6 months, the visual acuity reached 20/100. Both the corneal allograft and the aqueous fluid were clear.

3. Mycology

The excised corneal button was cut along the central line, a half for incubation on Sabouraud at 27 °C and another half for histopathological examination. The colony was 22 mm in diameter after 2 weeks, with olive-gray, smooth surface, which was black in the reverse side. Under the microscope, thick-walled, brown conidiophores were observed to arise at acute angles from creeping hyphae. The conidia were triangular, hyaline, and smooth, distributing in sympodial sequence. Conidiogenous cells were cylindrical with darkened scars. The lower conidia were shorter than the ultimate ones, with obvious scars (Fig. 2A and B). According to the morphological features observed in this isolate and the criteria put forward by De Hoog et al. (2000), the fungus was identified as *R. basitona*.

Molecular analysis of the internal transcribed spacer rRNA gene was performed by PCR using primers ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) and ITS5 (5′-TCCGTAGGTGAACCTGCGG-3′). Amplification was carried out at 95 °C for 10 min, followed by 30 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, with a final extension at 72 °C for 10 min. The PCR products were purified and sequenced (Sinogene Scientific, Beijing, China). The sequence (GenBank ID KM036370, 615 bp) was analyzed with the basic local alignment search tool for nucleotide comparison on line, showing high homology with the fragment of *R. basitona* strain (GenBank EU041806), with 88% query cover and 100% identity.

Histopathological observation confirmed the diagnosis of fungal endophthalmitis. Serially graded ethanol baths followed by xylene





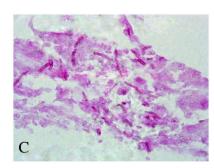


Fig. 2. The culture and histopathological examination of the excised cornea. A. The smooth, expanding, olive-gray, and wool-like colony of *R. basitona* cultures on Sabouraud glucose agar after 14 days of incubation at 27 °C. B. Microculture of *R. basitona*. Thick-walled, septate hyphae arising at acute angles and generating cylindrical cells, with slightly darkened scars and triangular, hyaline, and smooth conidia (lactophenol cotton blue staining, original magnification ×400). C. Brown septate pigmented hyphae in the basilemma (H–E staining, original magnification ×400).

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