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Case report

Successful treatment of *Beauveria bassiana* fungal keratitis with topical voriconazole

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ABSTRACT

We describe a 66-year-old woman who suffered from fungal keratitis after corneal transplantation. The causative organism was identified as *Beauveria bassiana* on the basis of morphological characteristics and the sequence of the internal transcribed spacer region of the ribosomal RNA gene. The patient was successfully treated with topical voriconazole (VRCZ) use only. We, hereby, present the first report of a case with *B. bassiana* fungal keratitis that responded to topical antifungal VRCZ treatment.

1. Introduction

Fungal infections generally occur in both animals and plants, and are often observed in immuno-compromised eyes after corneal trauma, surgery, or topical corticosteroid treatment. Fungal keratitis especially occurs in compromised eyes after corneal transplantation such as penetrating keratoplasty (PKP) and Descemet-stripping automated endothelial keratoplasty (DSAEK), and is one of the treatment refractory corneal infections because it is often difficult to diagnose and manage. The treatment can be difficult because of limited availability of antifungal agents as ocular preparations.

Beauveria bassiana is a ubiquitous fungus found in soil, and is well known as a pathogen of many insects. It is rarely found as a cause of human infection, however, several clinical cases with fungal keratitis caused by *B. bassiana* have been reported [1–9]. Because of the limited number of cases, the optimal antifungal agent and dose have not been established till date. We present the first report of a case of *B. bassiana* fungal keratitis after DSAEK, which was successfully treated by topical use of antifungal voriconazole (VRCZ) only.

2. Case report

A 66-year-old woman was referred to our hospital (Department of Ophthalmology, Keio University Hospital) because of decreased vision in the left eye 6 years earlier. Although the patient had no occupational exposure, she had a history of primary angle closure glaucoma and had undergone laser iridotomy 1 year before the first visit. The best-corrected visual acuity (BCVA) was hand motions in the left eye. Bullous keratopathy was clinically diagnosed due to persistent corneal edema. She had undergone uneventful DSAEK, phacoemulsification (PEA), and intraocular lens implantation (IOL) for bullous keratopathy and cataract in the left eye, and had been followed at our hospital with topical corticosteroids (0.1% fluorometholone). The postoperative BCVA was 30/100. The clarity of the corneal graft has been maintained in the left eye during 6 years.

Her latest ophthalmological examination of the left eye revealed hand motions as the BCVA; a corneal ulcer measuring 3.0 mm × 3.0 mm with irregular borders, stromal edema, and conjunctivociliary injection by slit-lamp biomicroscope (Fig. 1-A). We suspected fungal infectious keratitis based on the corneal findings. The focus of infectious keratitis was scanned by in vivo

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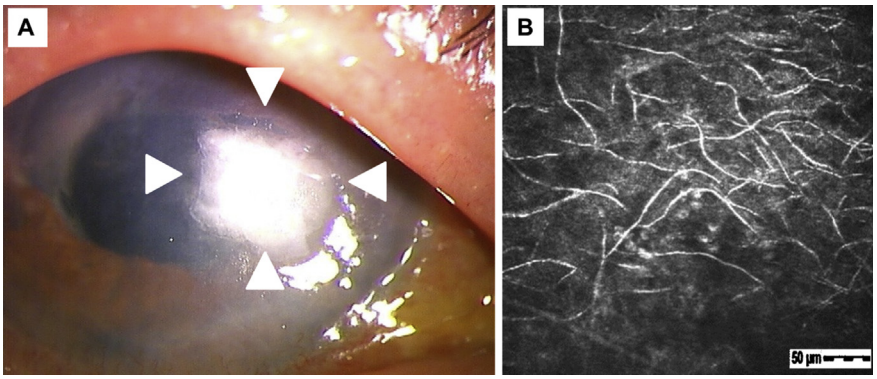

Fig. 1. Slit-lamp biomicroscopic photograph and corneal in vivo confocal microscopic image. Note the corneal ulcer with irregular borders (white arrow head), the corneal stromal edema, and the conjunctivociliary injection (A). Note the extensive filaments with branched and crossed lines (B).

laser confocal microscopy, Heidelberg Retina Tomograph II, Rostock Corneal Module [HRT II-RCM] (Heidelberg Engineering GmbH, Dossenheim, Germany), which revealed extensive filaments with branched and crossed lines (Fig. 1-B). The scraped cornea was incubated on Sabouraud glucose agar at 25 °C for seven days, and whitish powdery colonies were observed on the agar (Fig. 2-A). The Sabouraud agar media did not reveal any fungal growth at 35 °C. Numerous filamentous structures and conidiogenous cells were observed on wet specimens with lactophenol cotton blue fluid (Fig. 2-B). In order to identify the organisms by nucleotide sequencing, the isolate was sent to Medical Mycology Research Center, Chiba University. The internal transcribed spacer region (ITS1-5.8S-ITS2) of the ribosomal RNA gene was amplified by polymerase chain reaction (PCR) and sequenced, and analyzed against Basic Local Alignment Search Tool (BLASTn) at the National Center for Biotechnology Information (NCBI). The 590 bp sequence of the nuclear ribosomal ITS region from the isolate was 99.6% identical with that of *B. bassiana* (accession number AY532043) as previously reported [10].

The antifungal susceptibility of the *B. bassiana* isolate was evaluated with a microbroth dilution kit for yeast-like fungi DP Eiken trays (Eiken Chemical Co. Ltd., Tokyo, Japan), and the inhibitory concentration (IC) of the isolate was also evaluated according to the guideline of Clinical and laboratory Standards Institute (CLSI) M38-A2. The 100% IC of VRCZ against the isolate was 1 μg/mL (Table 1). After the diagnosis of *B. bassiana* fungal keratitis, topical corticosteroid was discontinued and hourly topical 1.0% VRCZ treatment was started. The corneal ulcer and conjunctivociliary injection reduced gradually after commencement of the treatment. The corneal ulcer epithelialized completely 16 weeks after commencement of the treatment. The corneal ulcer was healed by topical use of VRCZ, however, the BCVA as hand motions did not improve because of corneal edema and opacity.

Table 1
Antifungal susceptibility of the strain.

Antifungal agent	MIC (μg/mL)	MEC (μg/mL)
Micafungin	–	0.12
Amphotericin B	2 (IC_{100})	–
5-Flucytosine	>64 (IC_{50})	–
Fluconazole	16 (IC_{50})	–
Itraconazole	0.5 (IC_{100})	–
Voriconazole	1 (IC_{100})	–
Miconazole	0.5 (IC_{50})	–

MIC: minimal inhibitory concentration, MEC: minimal effective concentration, IC_{100}: 100% inhibitory concentration, IC_{50}: 50% inhibitory concentration.

3. Discussion

B. bassiana species are commonly found in soil around the world, and can infect and kill many insects (entomopathogenic). However, *B. bassiana* is an extremely rare cause of human infection. To date, only 10 cases of *B. bassiana* fungal keratitis [1–9] and only 3 *B. bassiana* fungal systemic infection cases [11–13] have

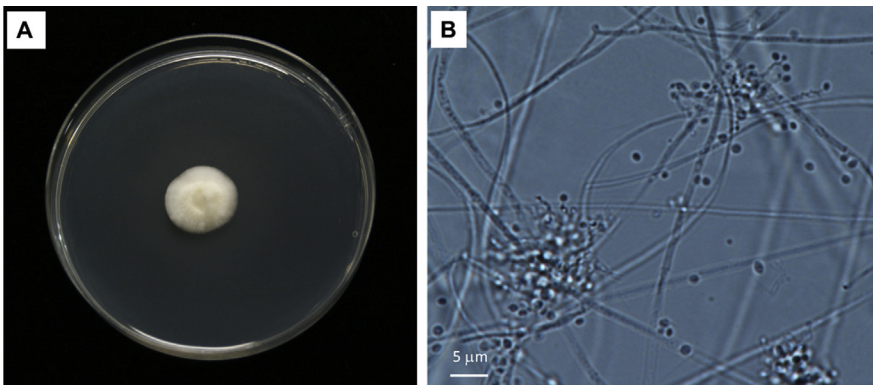

Fig. 2. Cultures of the *Beauveria bassiana* isolate from the scraped cornea. Note the whitish powdery colony grown on Sabouraud glucose agar at 25 °C for seven days (A). Microscopic photograph of the numerous filamentous structures and conidiogenous cells. Lactophenol cotton blue fluid, magnification ×500 (B).