First case of fungal keratitis caused by Pestalotiopsis clavispora

Yu Monden
Shohaku Yamamoto
Ryoji Yamakawa
Atsuko Sunada
Seishi Asari
Koichi Makimura
Yosihitsugu Inoue

1Department of Ophthalmology, Kurume University School of Medicine, Fukuoka, 2Laboratory for Clinical Investigation, 3Department of Infection Control and Prevention, Osaka University Hospital, Osaka, 4Teikyo University Institute of Medical Mycology, Tokyo, 5Division of Ophthalmology and Visual Sciences, Tottori University Faculty of Medicine, Tottori, Japan

Correspondence: Yu Monden
Department of Ophthalmology, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
Tel +81 942 317574
Fax +81 942 370324
Email you@med.kurume-u.ac.jp

Purpose: To report the isolation of Pestalotiopsis clavispora from the cornea of a patient with recurrent keratitis.

Case report: A 73-year-old male gardener presented with conjunctival injection and an oval infiltrate with feathery margins in the temporal half of the cornea in the right eye. His ocular history in the right eye included cataract surgery, five episodes of herpes simplex keratitis, three glaucoma surgeries, and bullous keratopathy. He had been treated with corticosteroids for years. Light microscopy of corneal scrapings revealed a filamentous fungus, and fungal keratitis was diagnosed. Treatment with topical voriconazole and pimaricin ointment was commenced. One month later, the infiltrate resolved. The antifungal agents were discontinued 7 months later, and keratitis relapsed 4 days after the discontinuation. The fungus was isolated and identified by molecular techniques as P. clavispora. Based on the results of antifungal susceptibility testing, treatment with topical and intravenous micafungin was initiated. The corneal infiltrate resolved 1 month after the relapse.

Conclusion: Molecular identification of the pathogen, and antifungal susceptibility testing, are useful in treating patients with fungal keratitis caused by a rare human pathogen.

Keywords: fungal keratitis, Pestalotiopsis clavispora, plant pathogen, molecular identification, antifungal susceptibility test

Introduction

Pestalotiopsis spp., filamentous fungi, are well-known plant pathogens, and have been commonly isolated in subtropical and tropical regions.1 The genus Pestalotiopsis was established in 1949 by Steyaert, following a taxonomic amendment to the genus Pestalotia. Pestalotiopsis clavispora has been reported to cause a number of plant diseases, including diseases of blueberry and avocado plants.2,3 To our knowledge, this is the first case of human infection caused by P. clavispora.

Case report

A 73-year-old male gardener presented to Kurume University Hospital in Japan in April 2012 complaining of a foreign body sensation in his right eye after sweeping up leaves and twigs on a windy day. He had a medical history of hypertension, paroxysmal atrial fibrillation, and angina. He had previously received anticoagulation therapy with warfarin. His ocular history in the right eye included cataract surgery (1990), five episodes of herpes simplex keratitis (2006, 2007, 2008 [two times], and 2010), three glaucoma surgeries (2007 [two times] and 2008), and bullous keratopathy (2011). He had been treated with corticosteroids for years (Figure 1).
At initial presentation, his visual acuity was hand motion in the right eye and 20/250 in the left eye. Intraocular pressure (IOP) was not measured in the right eye, and was 6 mmHg in the left eye. Slit-lamp examination of the right eye revealed conjunctival injection and an oval infiltrate with feathery margins in the temporal half of the cornea (Figure 2A). Corneal opacity constrained visualization of the fundus in the right eye. Light microscopy of corneal scrapings taken from the right eye at initial presentation revealed uniformly thick septate hyphae (Figure 2B). The patient was diagnosed as having keratitis caused by a filamentous fungus, and was admitted to the hospital. A foreign body was found in the infiltrate when corneal debridement was performed (Figure 2C). The foreign body could not be identified.

Topical corticosteroid treatment was discontinued, and topical treatment with voriconazole 1% hourly and pimaricin (natamycin) 1% ointment six times per day was initiated. One week after admission, the infiltrate decreased in size by about 1 mm. The patient was discharged at 1 month, after the infiltrate had resolved. At the time of discharge, visual acuity in his right eye was hand motion and IOP was 10 mmHg. Treatment with topical voriconazole four times per day, and pimaricin two times per day, was continued.

A search for the causative organism was initiated at the Central Clinical Laboratory of Kurume University Hospital, but the organism was not identified. The isolate was then sent to the Laboratory for Clinical Investigation at Osaka University Hospital, where it was examined morphologically. The isolate was suspected to be Pestalotiopsis spp., based upon light microscopy with lactophenol cotton blue staining, which revealed conidia-bearing appendages (Figure 2D).

A freshly isolated strain of Pestalotiopsis spp. from the clinical specimen was subcultured on potato dextrose agar (PDA) at 25°C for 7 days. An inoculum suspension was prepared in Roswell Park Memorial Institute (RPMI) 1640 medium and adjusted to a final inoculum of 10,000 conidia. The minimum inhibitory concentrations (MICs) for micafungin, amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, miconazole, and...
Table 1 Antifungal susceptibility testing

<table>
<thead>
<tr>
<th>Antifungal agent</th>
<th>MIC (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micafungin</td>
<td>0.03</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>0.25</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>2.0</td>
</tr>
<tr>
<td>Miconazole</td>
<td>2.0</td>
</tr>
<tr>
<td>Pimaricin</td>
<td>2.0</td>
</tr>
<tr>
<td>Voriconazole</td>
<td>2.0</td>
</tr>
<tr>
<td>Flucytosine</td>
<td>32</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>&gt;64</td>
</tr>
</tbody>
</table>

Abbreviation: MIC, minimum inhibitory concentration.
Medication should have been changed based on the results of the susceptibility testing.

Mochizuki et al reported that the corneal concentration of micafungin was 1.60–5.99 µg/g, the aqueous concentration was 0.02–0.35 µg/mL, and the vitreous concentration was 0.03–0.15 µg/mL, when the dose of micafungin was 150–300 mg/day. The MIC for micafungin against the isolate from our patient was 0.03 µg/mL. After the keratitis relapsed, our patient received treatment with topical micafungin 0.1% half-hourly, intravenous micafungin 50 mg daily, and corneal debridement weekly. Although the dose of intravenous micafungin was much lower than the dose reported by Mochizuki et al, we consider micafungin to have penetrated the cornea and the aqueous humor because of the combined use of micafungin. In addition, corneal debridement might have promoted the penetration of micafungin.

In our case, it required about 4 months to identify the pathogen as *P. clavispora*. We suspected the pathogen to be *Pestalotiopsis* spp., based upon light microscopy with lactophenol cotton blue staining that revealed conidia bearing appendages, but the species was not yet identified (Figure 2D and E). Using molecular biology techniques, the causative organism was identified as *P. clavispora*. As in our case, it is often challenging to identify an organism in human keratitis caused by rare fungal pathogens. DNA sequencing was useful in identifying the pathogen in our case. In a strict sense, epitypification with living strains is necessary before clear results can be obtained. However, it is difficult to perform epitypification in clinical or biological laboratories. The isolated organism showed 100% similarity using the Max Idnt score of the BLAST search. We therefore strongly believe that the organism was *P. clavispora*.

This is the first report of *P. clavispora* from a patient with keratitis. The results suggest that even a plant pathogen can cause human infection in immunocompromised hosts. Antifungal susceptibility testing is beneficial for the treatment of fungal keratitis caused by rare fungal pathogens, and molecular biological techniques may be the only means to identify a rare fungus causing infection in humans.

**Disclosure**

The authors have no conflict of interest to disclose.

**References**