Fungal keratitis caused by *Didymella gardeniae* (formerly *Phoma gardeniae*) successfully treated with topical voriconazole and miconazole

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**ABSTRACT**

Fungal keratitis by *Phoma* species is rare, and little information has been accumulated. We report a case of keratitis caused by *Didymella gardeniae*, formerly known as *P. gardeniae*. The patient had a history of stromal herpetic keratitis and had been treated with long-term topical betamethasone. He developed infectious keratitis in the left eye, with the causative fungus identified by ribosomal DNA sequencing. Combination treatment with topical voriconazole and miconazole proved effective.

1. Introduction

Fungal keratitis is one of the most refractory types of infectious keratitis, and can cause corneal blindness. Various genera and species of fungi can cause fungal keratitis. In Japan, *Fusarium, Candida* and *Alternaria* species are the major causes of fungal keratitis [1]. However, little information has been accumulated regarding the clinical course and appropriate treatment for rare fungal keratitis. *Phoma* species are phytopathogens that are widely distributed in the environment, most commonly found in aquatic systems and soil [2]. These fungi have the potential to be pathogenic not only in plants and animals, but also in humans. Few reports have described keratitis by these fungi, mostly requiring therapeutic corneal transplantation [3–5]. We encountered a case of keratitis caused by *P. gardeniae*, now known as *Didymella gardeniae*, and treatment by topical voriconazole and miconazole proved successful.

2. Case

The patient was a 66-year-old man with a history of bronchial asthma and duodenal ulcer. He was a golf caddy and also performed farm work. He had a history of recurrent stromal herpetic keratitis and had been treated with betamethasone eye drops for 8 years. He had been prescribed these eye drops twice per day from day -28, but stopped eye drops after he ran out of the prescription a week earlier (day -7). He was referred to our department with complaints of decreasing vision and pain in the left eye (day 0). At the initial examination, corrected visual acuity was 20/20 in the right eye and 20/50 in the left eye. Intraocular pressure was normal. Anterior segment examination of the left eye showed ciliary injection, feathery white infiltration with ulceration and endothelial plaque (Fig. 1a), while the right eye was normal. Cataracts were evident in both eyes, with no abnormalities evident on fundus examination. Corneal scrapings of the left eye were smeared and submitted to bacterial and fungal cultures (day 0). Microscopic examination of smears revealed Gram-positive bacilli (Fig. 1b) and filamentous fungi (Fig. 1c) according to Gram staining and Fungiflora Y (Trustmedical, Hyogo, Japan) [6], respectively. Fungal corneal infection was suspected and eyedrops treatment with natamycin 5% and cefmenoxime 0.5% (6 times/day respectively) and atropine 1% (once per day). Four days later (day +4), natamycin 5% eye drops were changed to natamycin 1% ointment because of eye irritation as an adverse effect. After 2 days (day +6), a black colony was grown in fungal culture, but proved difficult to identify in our hospital.

Antifungal instillation was switched to topical voriconazole 1% hourly and cefmenoxime was tapered to three times per day. Two weeks later (day +14), the isolated fungus was suspected to represent *P. gardeniae* based on sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA. Corneal epithelial defect was prolonged and corneal thinning appeared worsened after 20 days (day +34). We then added topical miconazole 0.1% at 6 times per day and topical gatifloxacin 0.3% at three times per day. Hyperemia was decreased and the lesion gradually reduced in size. The cornea became fully epithelialized within 3 months (day +96) and eye drops were stopped after 5 months (day +137). At the 8-month follow-up, corrected visual acuity was 20/200 in the left eye and no recurrence was evident (Fig. 1d).

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Later, samples of the isolated fungus were sent to the laboratory at Teikyo University for detailed species identification. Macroscopically, the colony on a potato dextrose agar plate appeared velvety and slightly powdery in texture with greenish-brown pigmentation on the surface, and the reverse side showed dark brown appearance (Fig. 2). Microscopy showed chlamydospores incorporated between hyphal cells forming long chains (Fig. 3). The strain was finally identified as *D. gardeniae*, because the species *P. gardeniae* had recently been renamed as *D. gardeniae* based on phylogenetic analysis [7]. Antifungal susceptibility testing was performed by the broth microdilution method that was based on the CLSI M38-A2 standard (Table 1). The nucleotide sequence data has been deposited in the DDBJ/EMBL/GenBank databases (accession number LC466637).
**Table 1**

Susceptibility test of antifungal agents.

| Antifungal agents | MEC/MIC (µg/mL) |
|-------------------|-----------------|
| Micafungin        | 0.06            |
| Caspofungin       | 1               |
| Amphotericin-B    | 1               |
| Natamycin         | 2               |
| Fluconazole       | 32              |
| Fluconoxidin      | > 64            |
| Voriconazole      | 0.5             |
| Itraconazole      | 1               |
| Miconazole        | 2               |
| Terbinafine       | 0.5             |

MEC = minimum effective concentration; MIC = minimum inhibitory concentration.

* Endpoints for echinocandins were defined by MEC, and those for the other classes were defined by MIC.

3. Discussion

*Phoma* species are phytopathogens generally found in soil, plants and water sources [2]. The *Phoma* genus had included over 200 species, but has recently been reorganized into 17 genera according to molecular classification [7]. The fungal strain in this report is described as *D. gardeniae* based on the new classification. Several reports have described human infection caused by *Phoma* species, including skin injuries ranging from superficial to deep trauma (69%), ocular infection (16%) and the lungs (9.4%) [2]. Most cases of *Phoma* infection have been reported in users of topical or systemic immunosuppressant drugs. This fungus may reportedly cause ocular infections such as keratitis and endophthalmitis due to either trauma or contact lens use [4,5,8].

To the best of our knowledge, three cases of *Phoma* keratitis have been reported in detail [3–5]. In these reports, keratitis did not respond to antifungal medication or required therapeutic surgery for removal of infected corneal tissues. However, which genera and species these *Phoma* fungi belong to under the current classification is not known. We therefore cannot compare the clinical course between these previous reports and our current case. In the future, molecular identification and DNA sequence databases are expected to facilitate data collection for fungal infection, especially in cases caused by rare fungi such as the present case.

**In vitro** antifungal susceptibility test is important when selecting an antifungal drug. Valenzuela et al. published a comprehensive study that reported antifungal susceptibilities in Coelomycetes, including *Phoma* species [9]. Antifungal testing showed that terbinafine and echinocandins were the most active against *Phoma* species, with a minimum inhibitory concentration (MIC) < 0.03 µg/mL. Another previous paper reported that MICs against *P. gardeniae* UM298 isolated from the nail infection, with ITS sequences showing 99.4% homology with our current strain, were reported as 1 µg/mL (amphotericin B), 0.094 µg/mL (voriconazole) and 0.094 µg/mL (caspofungin) [10]. In our strain, micafungin, voriconazole and terbinafine showed the lowest MEC/MICs, and the results are consistent with the previous reports and the observed clinical course of this case.

In conclusion, we reported a case of keratitis caused by *D. gardeniae* in a topically immunosuppressed cornea. Combination treatment using topical voriconazole and miconazole was effective. Rare fungi like *Didymella* species should be considered as a possible causative fungi when black colonies are observed in fungal culture. We realized again that DNA sequencing is helpful to identify rare fungal species, and molecular diagnosis in more patients is needed to establish effective treatments.

**Conflict of interest**

There are no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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