A refractory case of chromoblastomycosis due to *Fonsecaea monophora* with improvement by photodynamic therapy

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Chromoblastomycosis is one of the most frequently encountered mycoses in tropical and temperate regions resulting from the implantation of the etiologic agent. It is characterized by slowly expanding skin lesions caused by a specific group of dematiaceous fungi [1,2]. Several species have been reported as being involved in the disease etiology, including *Cladophialophora carrionii* [3], *Fonsecaea pedrosoi* [4], *F. monophora* [5], *F. nubica* [6], *Phialophora verrucosa* [7], and *Rhinocladiella aquaspersa* [8]. In southern China, *F. pedrosoi* and *F. monophora* are the most frequently encountered causative agents [9]. The hallmark of the disease is the observation of muriform cells in the tissue. Due to its recalcitrant nature, chromoblastomycosis is still a therapeutic challenge for clinicians [10]. Treatment success depends on many factors and the specific causal agent play an important role.

Infections caused by *F. pedrosoi* are considered to be among the most difficult to treat, while those involving *F. monophora* are treatable by many of the newly introduced antifungals [11–14]. However, this may not always be the case as we describe a refractory case caused by *F. monophora* which did not respond to many antifungal drugs and photodynamic therapy.

Keywords chromoblastomycosis, *Fonsecaea monophora*, photodynamic therapy, voriconazole

Introduction

Chromoblastomycosis is one of the most frequently encountered mycoses in tropical and subtropical regions resulting from the implantation of the etiologic agent. It is characterized by slowly expanding skin lesions caused by a specific group of dematiaceous fungi [1,2]. Several species have been reported as being involved in the disease etiology, including *Cladophialophora carrionii* [3], *F. pedrosoi* [4], *F. monophora* [5], *F. nubica* [6], *Phialophora verrucosa* [7], and *Rhinocladiella aquaspersa* [8]. In southern China, *F. pedrosoi* and *F. monophora* are the most frequently encountered causative agents [9]. The hallmark of the disease is the observation of muriform cells in the tissue. Due to its recalcitrant nature, chromoblastomycosis is still a therapeutic challenge for clinicians [10]. Treatment success depends on many factors and the specific causal agent play an important role. Infections caused by *F. pedrosoi* are considered to be among the most difficult to treat, while those involving *F. monophora* are treatable by many of the newly introduced antifungals [11–14]. However, this may not always be the case as we describe a refractory case caused by *F. monophora* which did not respond to many antifungal drugs and photodynamic therapy.
Case report

A 55-year-old male farmer residing in Panyu district of Guangzhou, China, presented to our outpatient service on 25 August 2008 complaining of an itchy, erythematous plaque surrounded with verrucous hyperplasia on the medial of his right arm (Fig. 1a). The lesion appeared and enlarged gradually after a localized trauma which occurred 13 years prior to his presentation. He had visited another hospital which resulted in a diagnosis of a deep mycosis, but for which he received no antifungal treatment. His family and past medical history was unremarkable and there was no evidence of any underlying diseases or that the patient was immunocompromised. Examination of potassium hydroxide mounts (Fig. 2a) and histopathology investigations revealed dematiaceous muriform cells (Fig. 2b, 2c) and based on these findings, the patient was diagnosed as having chromoblastomycosis. Our mycological study and DNA sequencing indicated that the etiologic agent was *F. monophora*.

From 25 August 2008 to 22 April 2010, various medication were used to treat the infection, i.e., terbinafine 250 mg/day per os (from 23 October 2008 to 16 April 2009), itraconazole 200 mg/day per os (from 18 March 2010 to 15 April 2010), these to antifungals in combination (from 25 August 2008 to 24 September 2008 and from 4 August 2009 to 25 September 2009), but without clinical or mycologic improvement (Fig. 1b). Due to these less than effective results, photodynamic therapy (PDT) employing 5-aminolevulinic acid (ALA) irradiation was initiated. The patient received ALA-PDT irradiation by itself 5 times, at weekly intervals from 22 April 2010 to 20 May 2010 (Fig. 1c). Then ALA-PDT irradiation was combined with terbinafine, 250mg/day per os, 5 times, again at weekly intervals from 9 September 2010 to 28 October 2010. In between the irradiation treatments, terbinafine 250 mg/day per os was employed. Although the lesions were obviously clinically improved, microscopic examinations revealed the presence of fungi (Fig. 1d) and new lesions developed after the withdrawal of ALA-PDT (Fig. 1e). The therapy was changed after 2 December 2010 to voriconazole 200 mg/day combined with terbinafine 250 mg/day per os. Two months later, clinical improvement could be observed (Fig. 1f) but since mycological examination was still positive, the patient is now under follow-up in the outpatient clinical.

Identification of isolates

Mycological investigation

A microscopic examination of scales from the lesion in KOH revealed the presence of muriform cells (dark brown large cells; Fig. 2a). Histopathology studies of a portion of a biopsy specimen demonstrated the presence of mild acanthosis of the epidermis, granulomatous inflammation around the entire dermis and muriform cells in microabscesses or giant cells (Fig. 2b, 2c).

DNA sequence analyses

DNA was extracted using 6% InStaGene Matrix (BioRad, Hercules, CA, USA). Ribosomal DNA ITS domains were identified using 6% InStaGene Matrix (BioRad, Hercules, CA, USA). Ribosomal DNA ITS domains were
Standards Institute (CLSI) guidelines (M38-A document) as previously described [13]. Itraconazole (Xian-Janssen Pharmaceutical Ltd, Xi’an, China), terbinafine (Beijing Novartis Pharmaceutical Ltd. [Beijing, China]) and voriconazole (Sigma, USA) were dissolved in 100% DMSO to prepare stock solutions (3,200 μg/ml). The drugs were then diluted to obtain the final concentrations, which for itraconazole and voriconazole were from 0.008 – 8 μg/ml and terbinafine concentrations of from 0.008 – 0.5 μg/ml. The isolates were subcultured and the supernatant of the colonies were collected and adjusted with saline to achieve an inoculum concentration of 10^6 conidia/ml. Each suspension was diluted 1:50–100 with RPMI 1640 to obtain the final test inoculum (0.4–5 × 10^4 conidia/ml). Suspensions of conidia of each the tested strains were inoculated into RPMI 1640 medium and incubated for 7 days at 35°C. Candida parapsilosis ATCC22019, obtained from Centraalbureau voor Schimmelcultures (CBS, The Netherlands), was used as a quality control. The final test

Antifungal susceptibility testing

The in vitro antifungal susceptibility of the clinical isolate was assessed in accord with the Clinical Laboratory and Standards Institute (CLSI) guidelines (M38-A document) as previously described [13]. Itraconazole (Xian-Janssen Pharmaceutical Ltd, Xi’an, China), terbinafine (Beijing Novartis Pharmaceutical Ltd. [Beijing, China]) and voriconazole (Sigma, USA) were dissolved in 100% DMSO to prepare stock solutions (3,200 μg/ml). The drugs were then diluted to obtain the final concentrations, which for itraconazole and voriconazole were from 0.008 – 8 μg/ml and terbinafine concentrations of from 0.008 – 0.5 μg/ml. The isolates were subcultured and the supernatant of the colonies were collected and adjusted with saline to achieve an inoculum concentration of 10^6 conidia/ml. Each suspension was diluted 1:50–100 with RPMI 1640 to obtain the final test inoculum (0.4–5 × 10^4 conidia/ml). Suspensions of conidia of each the tested strains were inoculated into RPMI 1640 medium and incubated for 7 days at 35°C. Candida parapsilosis ATCC22019, obtained from Centraalbureau voor Schimmelcultures (CBS, The Netherlands), was used as a quality control. The final test

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Table 1  The MIC and FICI of ITZ/TBF and VOR/TBF against the isolate.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>MIC (μg/ml)</th>
<th>MICs of the combination</th>
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<tbody>
<tr>
<td></td>
<td>ITZ</td>
<td>VOR</td>
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<tr>
<td>ITZ/TBF</td>
<td>1</td>
<td>—</td>
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<tr>
<td>VOR/TBF</td>
<td>—</td>
<td>0.0625</td>
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MIC, minimal inhibitory concentration; FICI, fractional inhibitory concentration index; ITZ, itraconazole; TBF, terbinafine; VOR, voriconazole. The inoculum concentration was 0.5–2.5 × 10³ conidia/ml. The results of the susceptibility testing are shown in Table 1.

**Discussion**

*F. monophora*, presently recognized as one of the agents of human chromoblastomycosis, was first described by De Hoog [5]. Our patient had a history of trauma and had lesions on exposed areas of his body. Muriform cells were observed in microscopic examinations of smears and histopathology studies of biopsy materials. The identification of the suspected etiologic agent as *F. monophora* leads to the diagnosis of chromoblastomycosis. Various antifungals have been used in treating this infection, with the best clinical outcomes obtained with itraconazole and terbinafine among the oral and systemically used drugs [11]. In the present case, the patient was initially treated with terbinafine or itraconazole or the combination of the two but without any response.

Topical PDT, which combines a non-toxic dye, termed a photosensitizer (PS), with low intensity harmless visible light to generate reactive oxygen species that are toxic to selected cells, was first applied in the field of oncology [15]. It has become a well-established treatment in dermatology for the prevention and treatment of a variety of malignant skin tumors and inflammatory diseases, including non-melanoma skin cancer, actinic keratoses, acne vulgaris, photorejuvenation, and hidradenitis suppurativa [16]. Moreover, the use of PDT has been extended to antimicrobial chemotherapy, including application in fungal infections with no reports of resistance of the etiologic agents [17]. Recently there was a description of promising results being obtained through the use of PDT (employing methylene blue as photosensitizer) in the treatment of chromoblastomycosis [18]. In the present case, PDT treatment did greatly reduce the size of the patient’s lesions, despite the fact that complete mycologic or clinical cure was not obtained. These results demonstrate that ALA was metabolized into protoporphyrin IX (PpIX) and targeted to the etiologic agent. The result of *in vitro* studies is compatible with clinical response, which showed growth inhibiting effect of ALA/ PDT on *F. monophora* (unpublished data).

Voriconazole has a broader spectrum of activity and an increased biochemical affinity to fungal 14a-demethylase, resulting in its strong antifungal activity [19]. In addition, clinical isolates of *Fonsecaea* spp. have been found through *in vitro* studies to be highly sensitive to the drug [20]. Despite the high cost, voriconazole is a safe and promising antifungal for use in treating extensive chromoblastomycosis that is refractory to the conventional therapeutic regimens. The result of the present study confirms earlier reports that voriconazole provides an effective therapy of chromoblastomycosis caused by *Fonsecaea* [12].

Our isolate of *F. monophora* was found by *in vitro* antifungal susceptibility studies to be sensitive to terbinafine, itraconazole, voriconazole, with MICs of 0.125, 1 and 0.0625 μg/ml, respectively. These results suggest that while *in vitro* susceptibility profiles may be useful to identify intrinsic microbiologic resistance to antifungal drugs, they do not predict clinical responses [21]. It must be remembered that the patient status and the lesions caused by the infection are important factors in determining the effects of treatment. Our previous study demonstrated a synergistic effect created by terbinafine and itraconazole on clinical isolates of *F. monophora* [22,23]. Voriconazole, the second generation of triazole, has a similar structure to itraconazole and its use with terbinafine may also be synergistic. Further studies are necessary to confirm the results.

Although chromoblastomycosis is associated with low cure and high relapse rates [4], most cases caused by *F. monophora* have been successfully treated. This is the first refractory case described in China. Independent to the MIC of the etiologic agent, the poor response to treatment may due to the development of fibrosis which did not allow the antymycotics to penetrate. Although complete cure was not achieved, voriconazole and PDT are promising methods in the treatment of chromoblastomycosis.

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**References**


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