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Successful treatment of primary cutaneous *Aspergillus ustus* infection with surgical debridement and a combination of voriconazole and terbinafine

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Abstract

Aspergillus ustus infections are associated with a high mortality in immunocompromised hosts, and the mold has decreased susceptibility to most antifungal drugs, especially azoles. We report primary cutaneous *A. ustus* infection in a patient who failed itraconazole therapy and was switched to voriconazole (VRC). During VRC therapy, the MICs of VRC, amphotericin B (AMB), caspofungin (CFG), and terbinafine (TBF) were 4, 2, 64, and 0.13 μg/mL, respectively. Because the MIC to VRC was high, TBF was added to VRC for synergy based on anecdotal data from other mycoses. After treatment with VRC and TBF for 5 months, MICs of VRC, AMB, CFG, and TBF of *A. ustus* were 8, 1, 64, and 4 μg/mL respectively. Although the MICs of VRC and TBF increased during antifungal therapy, the patient responded well to the combination antifungal therapy with surgical debridement. With a successful outcome despite high MICs and with limited therapeutic options currently available, we investigated the in vitro activity of posaconazole (PCZ) and VRC individually and in combination with AMB, CFG, or TBF using the fractional inhibitory concentration index (FICI) method. Combination of VRC with TBF showed synergistic activity (FICI = 0.5). Therefore, combination of VRC and TBF with surgical debridement, when appropriate, may be a viable treatment option for refractory *A. ustus* infections.
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Keywords: *Aspergillus ustus*; Voriconazole resistance; Terbinafine; Combination therapy; Immunocompromised; Cutaneous infection

1. Introduction

Invasive aspergillosis is a life-threatening infection in immunocompromised patients, especially after hematopoietic stem cell transplantation and graft versus host disease. The most common etiologic agent is *Aspergillus fumigatus*, followed by *Aspergillus flavus*, *Aspergillus niger*, and *Aspergillus terreus*. Until the early 1990s, amphotericin B (AMB) was the only agent that was available for the management of this infection. However, the significant toxicities associated with this agent made it less attractive with the introduction of newer agents such as the triazoles and the echinocandins, which are much better tolerated. Among these, voriconazole (VRC) and caspofungin (CFG) have been approved by the Food and Drug Administration for the treatment of invasive aspergillosis.

Infections caused by *Aspergillus ustus* are very rare (<1%), and less than 20 cases have so far been reported (Iwen et al., 1998; Josepa Gene et al., 2001; Panackal et al., 2006; Saracli et al., 2007; Verweij et al., 1999; Yildiran et al., 2006). Breakthrough infections with *A. ustus* in patients receiving VRC or CFG prophylaxis have been reported (Pavie et al., 2005). *A. ustus* has some unique characteristics that are clinically relevant. *A. ustus* is a multidrug-resistant pathogen with few treatment options available. Although VRC has been shown to be effective against several non-fumigatus species of *Aspergillus*, it is less active in vitro against *A. ustus* than against *A. fumigatus*, and the effect appears to be fungistatic. Terbinafine (TBF) has demonstrated good fungicidal activity against *A. ustus* and is considered to be the most active agent against *A. ustus* in

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vitro, though there is virtually no clinical experience (Garcia-Effron et al., 2004; Kantarcioğlu and Yucel, 2002; Karaarslan et al., 2004; Mosquera et al., 2002). We report the 1st case of primary cutaneous aspergillosis caused by *A. ustus* in a renal transplant patient who was successfully treated with a combination of VRC and TBF.

1.1. Clinical course

Our patient was a 56-year-old male cadaveric renal transplant recipient on tacrolimus and prednisone. The patient presented with pain, redness, and swelling in the right lower quadrant of the abdomen, near a previous peritoneal dialysis catheter site. An incision and drainage of the 2 × 3-cm erythematous lesion was performed with drainage of purulent material; subsequently, the culture grew *A. ustus*. Treatment was initially started with oral itraconazole (ITZ), but as the patient developed severe diarrhea and showed no improvement after 1 month, antifungal therapy was switched to oral VRC. He continued to be clinically and hemodynamically stable, and the lesion completely healed after 4 months of therapy with VRC. Treatment was continued because of his immunocompromised state, and 8 months into VRC therapy, the patient presented with a new tender lesion (3 × 3 cm) close to the previous abscess and associated with severe pain and erythema. Incision and drainage were performed, and the culture again grew *A. ustus*; the susceptibility test results are shown in Table 1. The isolate was resistant to ITZ, and the MIC to VRC was high (2 μg/mL). Given the clinical failure of monotherapy with VRC, the patient was started on a combination of VRC plus TBF with a gradual clinical response. Further debridement was done 5 months after therapy with this combination; the repeat cultures showed *A. ustus* but with higher MICs to both VRC (8 μg/mL) and TBF (2 μg/mL). As he continued to improve clinically, combination therapy was maintained for a total of 11 months (3 months after complete resolution of the abscess). At follow-up 6 months later, no recurrence was observed.

As the patient improved clinically despite *A. ustus* being resistant to both these agents (VRC and TBF), we evaluated the fractional inhibitory concentration index (FICI) using several drug combinations to evaluate in vitro synergy.

Table 1
Change in MIC (μg/mL) of various antifungal drugs during treatment with voriconazole and TBF

Antifungal drugs	MIC (8 months post-VRC Rx) (AU 101)	MIC (5 months post-VRC + TBF Rx) (AU 102)
AMB	4	1
VRC	2	8
PCZ	NA	4
TBF	0.13	2
CFG	1	1

NA = not applicable; Rx = treatment; TBF = Terbinafine.

2. Materials and methods

2.1. Antifungal drugs

Voriconazole, AMB, and CFG were obtained from Pfizer Pharmaceuticals (New York, NY), Sigma Chemical Company (St. Louis, MO), and Merck (Rahway, NJ), respectively. PCZ was obtained from Schering-Plough Research Institute, Kenilworth, NJ, USA. Voriconazole and AMB were dissolved in dimethylsulfoxide to make a stock solution of 1 g/L and then stored as 0.25-mL aliquots at −20 °C. Caspofungin was dissolved in sterile double-distilled water to a concentration of 10 g/L and was stored as 0.25-mL aliquots at −70 °C. Frozen stocks of the antifungal agents were thawed at room temperature and used within 24 h.

2.2. Clinical isolates

The 2 clinical isolates of *A. ustus* used in this study were obtained from the patient (Table 1). The isolates were subcultured in Sabouraud dextrose agar to check for purity and viability. Working cultures were maintained on Sabouraud dextrose agar slants at 4 °C. For long-term preservation of the cultures, conidial suspensions were prepared in glycerol 25% v/v and stored at −80 °C.

2.3. MIC determination

Conidial suspensions from 6-day-old *A. ustus* cultures were prepared, standardized by hemocytometry, and used as inocula (1×10^6) for susceptibility testing. MICs of VRC and AMB for *A. ustus* was determined by the M38-A broth microdilution technique recommended by the Clinical Laboratory and Standards Institute, and MIC was defined as the concentration that produced no visible growth.

2.4. Fractional inhibitory concentration index determination

The in vitro susceptibility of *A. ustus* to 2-drug combinations of VRC with TBF, CFG, and AMB was evaluated using the FICI method. The FICI was determined with a checker board method in a microtiter plate using the M38-A technique. Pairwise combinations of the required concentrations of antifungal drugs A and B were prepared in 2-fold increments in RPMI-1640 medium. Appropriate drug-free controls were included. To each well, 100 μL of the conidial suspension (2×10^4 conidia/mL) was added. The plate was incubated at 35 °C for 48 h, and the MIC (lowest concentration of the drug that showed no visible growth) was determined.

The FICI was then calculated using the following formula:

$$\mathrm{FICI} = (\mathrm{Ac}/\mathrm{Aa}) + (\mathrm{Bc}/\mathrm{Ba})$$

where Ac and Bc are the MICs of drugs A and B in combination, whereas Aa and Ba are the MICs of drugs A and B, respectively.