CASE REPORT

Invasive Pulmonary Aspergillosis with Hematological Malignancy Caused by *Aspergillus terreus* and *in vitro* Susceptibility of *A. terreus* Isolate to Micafungin

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Abstract

A 35-year-old man developed invasive pulmonary aspergillosis (IPA) with severe neutropenia after umbilical cord stem cell transplantation for chronic myelogeneous leukemia. Filamentous fungus isolated from his sputum was identified as *Aspergillus terreus*. Despite systemic amphotericin B (AMPH) administration, IPA progressed. However, intravenous administration of micafungin (MCFG) and oral itraconazole improved clinical data and symptoms, although he later died of massive hemoptysis. Examination of the *in vitro* susceptibility of this *A. terreus* isolate to MCFG revealed a good minimum inhibitory concentration and good time-kill assay results compared to AMPH. Thus, MCFG might be useful for IPA caused by *A. terreus*.

Key words: invasive pulmonary aspergillosis (IPA), *Aspergillus terreus*, time-kill assay, amphotericin B (AMPH), micafungin (MCFG)

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Introduction

Invasive pulmonary aspergillosis (IPA) caused by infection with the filamentous fungus *Aspergillus* is the major infectious complication in transplant recipients and patients with prolonged neutropenia (1). The approximately 200 *Aspergillus* species consist of widespread molds, of which only a few are known to be human pathogens. *Aspergillus fumigatus, Aspergillus flavus* and *Aspergillus niger* are the most commonly encountered species, but other species, such as *Aspergillus terreus, Aspergillus clavatus, Aspergillus nivus* and *Aspergillus nidulans* have rarely been reported to cause disease in humans (2). According to recent studies, effort should be made to distinguish *A. terreus* from other *Aspergillus* species, as it often has in *vitro* and in *vivo* resistance to standard treatment using amphotericin B (AMPH) (3-12).

Here, we report a case of IPA caused by *A. terreus* concomitant with chronic myelogeneous leukemia. We also evaluated the *in vitro* response of this *A. terreus* isolate to the antifungal agent micafungin (MCFG), which has a novel mechanism of action. Our results suggest that MCFG is a useful antifungal agent for IPA caused by *A. terreus*.

Case Report

A 35-year-old man was admitted to hospital in August 2003 with low grade fever and general fatigue. Physical examination confirmed non-tender marked splenomegaly. Routine complete blood count disclosed slight anemia (hemoglobin, 9.5 g/dl) and an increased leukocyte count (480,000/μl). The patient was diagnosed with accelerated phase chronic myelogeneous leukemia with the Philadelphia chromosome. Imatinib and hydroxyurea administration failed to achieve a cytogenetic response. He then underwent unrelated umbilical cord stem cell transplantation (UCSCT) in January 2004. During the 50 days after UCSCT, he showed severe neutropenia (<100/μl) and was therefore diagnosed with graft failure. He then underwent allogeneic bone marrow
transplantation (BMT) from a 2-HLA antigen-mismatched related donor in March 2004. Chest X-ray revealed a new nodular shadow in the right lung field just after BMT (Fig. 1). He was suspected of having IPA because filamentous fungi was isolated from his sputum without other pathogens and the optical density index of serum Aspergillus galactomannan antigen became positive (>5.0). Although he received systemic AMPH at 1 mg/kg per day via injection, the nodule shadow on the X-ray changed to infiltrated shadows with another new infiltrated shadow appearing in the left lung field. We therefore decided to treat this patient with intravenous administration of micafungin (MCFG) (300 mg per day) and itraconazole (ITCZ) capsules (200 mg per day), in addition to administration of AMPH. On the 22nd day after BMT, his peripheral white blood cell count had recovered to >1,000/μl. At this time, chest X-ray showed the air-crescent sign within the infiltration shadow in the right middle lung field (Fig. 2). The antifungal agents appeared to be effective as there was a decrease in C reactive protein from 28.1 mg/dl to 3.6 mg/dl, improvement in his symptoms and a decrease in the optical density index of his serum Aspergillus galactomannan antigen to 4.4. Consequently, the antifungal combination therapy of AMPH, MCFG and ITCZ was continued. However, the patient died of massive hemoptysis on the 32nd day after BMT. Autopsy was refused by his relatives.

A culture of his sputum on Sabouraud glucose agar yielded Aspergillus, which was identified as A. terreus after the patient’s death. The colonies of this fungus were cinnamon-brown in color on Sabouraud glucose agar and microscopically the colonies had long slender conidiophores and dome-like vesicles with biseriate and columnar conidial heads, consistent with A. terreus (Fig. 3).

The minimum inhibitory concentrations (MICs) of antifungal agents against A. terreus were determined by broth microdilution as described by the National Committee for Clinical Laboratory Standards (NCCLS) M-38P protocol (13). The MIC of AMPH was 1 μg/ml; flucytosine, 64 μg/ml; fluconazole: >64 μg/ml; miconazole, 0.125 μg/ml; ITCZ, 0.03 μg/ml and MCFG, 0.015 μg/ml. The A. terreus isolated from the patient was most susceptible to MCFG, and the data suggested that this isolate is also susceptible to AMPH and ITCZ in vitro.

We next performed a time-kill assay using AMPH and MCFG against this isolate according to the method of Walsh et al (3). An inoculum of $1.0 \times 10^6$ conidia/ml of A. terreus

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**Figure 1.** Chest X-ray showing pale nodular shadow in the right middle lung field on the day of allogeneic bone marrow transplantation and at 50 days after unrelated umbilical cord stem cell transplantation.

**Figure 2.** The chest X-ray on the 22nd day after bone marrow transplantation showing infiltration shadows with the air-crescent sign in the right middle lung field and infiltration shadows in the left lung field.

**Figure 3.** Microscopic findings of fungus isolated from the sputum of the patient showing long slender smooth conidiophores with dome-like vesicles at the termini. Hyphae were septate and hyaline. Conidial heads were biseriate and columnar (Lactophenol cotton blue satin, ×200).
Figure 4. Time-kill assay of *A. terreus*. *A. terreus* isolate was resistant to the fungicidal activity of amphotericin B (a), but demonstrated concentration-dependent susceptibility to the antifungal activity of micafungin (b).

isolate was incubated in Sabouraud dextrose broth plus antifungal compound using antifungal concentrations of 0.25, 1, 2, and 4 times the MIC of each antifungal agent in polypropylene tubes at 37°C. Samples were taken from each tube at 0, 6 and 24 h after incubation and these samples were then cultured on Sabouraud glucose agar at 37°C for 24 h. Colonies were counted and the calculated colony-forming units per milliliter were plotted for each time point (Fig. 4). None of the AMPH concentrations showed any fungicidal activity against the *A. terreus* isolate. Moreover, at 24 h, an increase in conidia of the isolate was observed. By comparison, for MCFG, *A. terreus* demonstrated a 100-fold reduction in viable colony forming units by 24 h at 0.015 μg/dl MCFG.

Discussion

A review of 1,477 separate *Aspergillus*-positive cultures from 24 medical centers in the United States found that *A. fumigatus* was the most frequently isolated *Aspergillus* species, causing 67% of invasive aspergillosis cases (14). *A. flavus* accounted for 16% of invasive aspergillosis followed by *A. niger* (5%) and *A. terreus* (3%). According to a report from Spain, *A. terreus* was the second most common species, after *A. fumigatus*, recovered from respiratory specimens (38 of 247 isolates; 15.4%) (15). Furthermore, a recent review of the literature revealed an increasing number of reported cases of infection caused by *A. terreus* (3-12). Walsh et al suggested that this phenomenon might be related to an increasing number of immunocompromised patients at
risk of developing invasive fungal infection (3). They also noted that empirical or prophylactic use of AMPH in high-risk patients may be another factor contributing to the selection of AMPH-resistant strains of A. terreus. Baddley et al. reported that the percentage of A. terreus isolates relative to those of other Aspergillus species had significantly increased at the University of Alabama hospital (4). However, molecular genotyping of isolates showed no evidence of nosocomial A. terreus infection in that hospital. Lass-Florl et al. suggested in their molecular epidemiological study that the onset of A. terreus infection might depend on exposure to environmental Aspergillus species, such as from in-hospital plants (5, 6). In Japan, we have found no epidemiologic reports on the incidence of infectious diseases caused by A. terreus.

Numerous previous reports have warned that A. terreus might be resistant to AMPH, the standard drug for IPA (3-12). For instance, a previous detailed study showed that AMPH had excellent in vitro activity against A. fumigatus (96% inhibited at an MIC of ≤1 μg/ml), whereas AMPH against A. terreus demonstrated poor in vitro activity (38% inhibited at an MIC of ≤1 μg/ml) (7). The antifungal clinical breakpoint of antifungal agents for filamentous fungi was not clear. However a retrospective analysis of contemporary clinical data reported that A. terreus infection was resistant to AMPH and associated with a high rate of mortality (8).

Walsh et al. showed by time-kill assay that A. terreus is more resistant to the fungicidal effects of AMPH than A. fumigatus (3). Furthermore, they demonstrated that AMPH had no effect in cleaning fungi from lung tissue in an animal model using rabbits infected with A. terreus, whereas AMPH achieved a significant reduction in the residual burden in the lungs of rabbits infected with A. fumigatus. In another study, Warn et al. showed that the treatment of A. terreus infection with MCFG significantly prolonged the survival of an experimental mouse model compared to liposomal AMPH (9). The survival rates were 20% with liposomal AMPH (5 and 25 mg/kg), but MCFG treatment showed a clear dose-dependent response with 60%, 80%, 90% and 100% survival after 1, 2, 5 and 10 mg/kg respectively. These results indicate that MCFG may play a clinically important role in the treatment of A. terreus infections.

We evaluated in vitro antifungal efficacy against our A. terreus isolate using MIC and time-kill assays. The MIC of AMPH against this isolate was 1 μg/ml, which was thought to indicate susceptibility to this agent. However, the time-kill assay demonstrated no fungicidal activity at up to 4 times the MIC concentration of AMPH against this A. terreus isolate. Thus, a reliable in vitro method of assessing the activity of antifungal agents against filamentous fungi is required to replace MIC. On the other hand, MCFG showed good antifungal activity against A. terreus on both the MIC and time-kill assays. As reported previously, the new antifungal agent ‘echinocandin’, including MCFG and caspofungin, has in vitro and in vivo efficacy against A. terreus (6, 7, 9, 10), and thus MCFG should be a useful agent against this type of infection.

In the present case, we administered AMPH immediately after diagnosis of IPA, but the IPA worsened clinically. Although the addition of MCFG and ITCZ improved the clinical data and symptoms, the patient unfortunately died of massive hemoptysis. The meniscus sign developed during the clinical course of IPA when the neutrophil count had increased to >500 cells/μl, which has been shown to be suggestive of a high risk of large scale hemoptysis (16).

New triazoles, such as posaconazole and voriconazole, have been reported to have good antifungal activity against A. terreus in vitro (3, 6-8). Clinically, voriconazole has also been reported to be effective against IPA caused by A. terreus (11); unfortunately, it was not available in Japan at the time of the present case. The results of in vitro examination, which we later performed, suggested that IPA in this case was improved by the use of MCFG. In addition, recovery from the neutropenia, which was a factor in hemoptysis, would contribute to improvement of infectious disease. Absorption of ITCZ encapsulated formulation depends on gastric acid secretion. In a patient that has not taken a meal, absorption of ITCZ into the bloodstream is low (17). After IPA onset, the present patient had no appetite. Although the plasma concentration of ITCZ was not measured in this case, it is thought that the plasma concentration of ITCZ in this patient was low. Therefore, it is doubtful that ITCZ had an effect on IPA in this case.

The present case and our study suggest the importance of identifying the exact species causing invasive aspergillosis for best selection of adapted antifungal agents. In the case of A. terreus, MCFG or new triazoles should be used, and not AMPH.

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References


778

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