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BRIEF ARTICLE

Fatal Rhizopus Pneumonia in Allogeneic Stem Cell Transplant Patients Despite Posaconazole Prophylaxis: Two Cases and Review of the Literature

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Posaconazole is a triazole with broad spectrum of activity against multiple fungi including members of the fungal order *Mucorales*. This activity has been shown both in clinical and in vitro studies, which are critically reviewed here. It has become very popular in prophylaxis in acute myelogenous leukemia (AML) induction and in the graft-versus-host disease (GVHD) settings after 2 recent prospective trials that showed advantage of posaconazole prophylaxis compared to fluconazole or itraconazole. In this report, 2 patients are presented, in whom, despite posaconazole prophylaxis, invasive and ultimately fatal *Rhizopus* pulmonary infections developed. These cases are similar to a previously reported case of *Rhizopus* infection in a stem cell transplant recipient who also received posaconazole, indicating a potential newly recognized pattern of breakthrough infections in patients receiving posaconazole prophylaxis.

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INTRODUCTION

Posaconazole [1] is a triazole similar in structure to itraconazole with broad-spectrum antifungal activity including some activity against the fungal order *Mucorales*. Its clinical efficacy was confirmed by 2 reported trials [2,3] showing advantage of posaconazole as a prophylactic agent compared to fluconazole and itraconazole in acute myelogenous leukemia (AML) and graft-versus-host disease (GVHD) settings. Voriconazole has failed to show such a significant advantage [4] compared to fluconazole in the prophylactic setting. Despite problems with its bioavailability [1], posaconazole is used widely, and reports of breakthrough fungal infections have been rare. A pattern of *Mucorales* breakthrough infection similar to that after the widespread use of voriconazole [5,6] has not been established yet.

This article reports on 2 cases of fatal pneumonias by *Rhizopus microsporus* despite posaconazole prophylaxis.

PRESENTATION OF CASES

Case 1

A 63-year-old gentleman with refractory follicular lymphoma received an allogeneic stem cell transplantation from a matched unrelated donor (MUD allo-HCT) after conditioning with busulfan (Bu), cyclophosphamide (Cy), and alemtuzumab (total dose = 60 mg.v.). He had been diagnosed with follicular lymphoma 11 years before HCT and had been heavily pretreated. After transplantation, he was never found to have progression of lymphoma, and bone marrow biopsies and autopsy materials were negative for relapsed lymphoma including a negative FISH for t(14;18) in the marrow. He developed primary cytomegalovirus (CMV) infection 1 month after transplantation despite use of irradiated leukocyte-reduced blood products. He was successfully treated with foscarnet because of ganciclovir failure. At about the same time, he was diagnosed with upper gastrointestinal and skin acute GVHD (aGVHD). Systemic steroids and tacrolimus controlled the grade II GVHD. Because of BK-hemorrhagic cystitis and the CMV infection, steroids were rapidly tapered.

Five months after transplantation, coming back from vacation, he presented with relapsed CMV viremia and skin GVHD unsuccessfully controlled

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with potent topical steroids. Systemic steroids (60 mg/day), foscarnet and concomitant prophylactic posaconazole (200 mg orally 3 times a day with food) were started. Several weeks later he presented with dyspnea and tenderness at the Port-A-Cath placement site. The port was removed and patient was admitted in the intensive care unit with respiratory distress. He deteriorated and was intubated. Despite antibacterials, liposomal amphotericin (AmBisome), posaconazole, and intravenous immunoglobulin (IVIG) (for positive parainfluenza type 2 in his respiratory secretions) he expired several weeks later from progressive pneumonia causing terminal respiratory failure. Autopsy showed diffuse angioinvasive infection in the lung parenchyma by nonseptate, right-angle branching, and irregular ribbon-like hyphal organisms morphologically consistent with *Mucorales* infection (Figure 1). The same hyphae caused mediastinal fat necrosis. Lung tissue sent for culture demonstrated no hyphal elements on direct examination and no growth was detected after 6 weeks. Premortem bronchoalveolar lavage (BAL) and routine bronchial cultures grew rare colonies of a *Rhizopus* species on inhibitory mold, sabouraud dextrose, and yeast extract agars. The fungus did not grow above 50°C. The microscopic features were consistent with a final identification of *Rhizopus microsporus* var. *microsporus* [7] (see Figure 1). No evidence of active parainfluenza or CMV pneumonia was seen. Although posaconazole levels were never checked, he and his wife had confirmed the intake of posaconazole with fatty food while he was treated with this medication prophylactically as an outpatient. Susceptibility testing using E-test strips (AB Biodisk, Solna, Sweden) demonstrated a mean inhibitory concentration (MIC) for amphotericin B of 0.064 μg/mL, posaconazole 3 μg/mL, and no zone of inhibited growth for voriconazole (MIC >32 μg/mL). Although definitive breakpoints have not been established for mold testing, the Clinical Laboratory Standards Institute (CLSI) [7] have tentatively assigned MICs of <1 μg/mL as susceptible, MIC of 2 μg/mL as intermediate, and those 4 μg/mL or above (in doubling dilutions) as resistant for all 3 of the drugs tested. In fact, posaconazole levels cannot be reliably maintained above 1 μg/mL, and, in most cases, are going to be well below 1 μg/mL for most of the dosing interval [8-10]. Using these interpretative guidelines, only amphotericin B gave a susceptible result, whereas posaconazole falls into the intermediate and voriconazole into the floridly resistant category. In vitro activities of posaconazole, voriconazole, and amphotericin have been evaluated by Sun et al. [11,12], who have found that for *Rhizopus* species, the MIC50 and MIC90 concentrations of posaconazole were 1 μg/mL and 8 μg/mL, respectively, voriconazole was >64 μg/mL for both MIC50 and MIC90, whereas those for amphotericin B were 0.125 and 0.5 μg/mL, respectively.

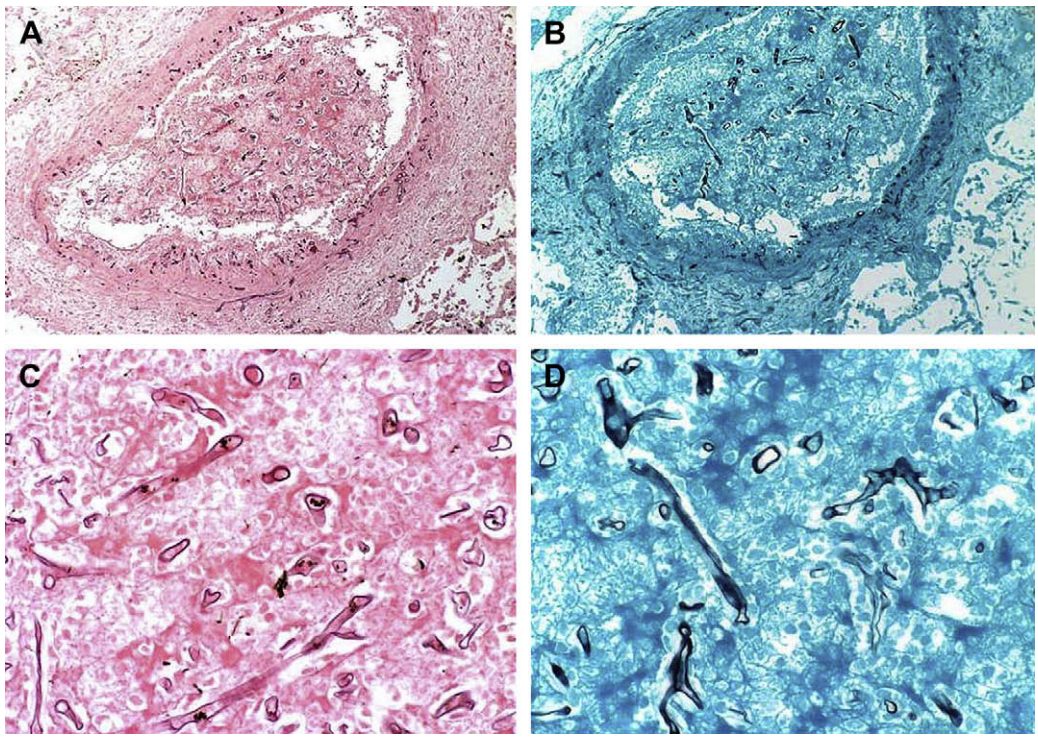

Figure 1. (A, B) Angioinvasive mucorales infection with an intraluminal infected clot in patient 1 (A, hematoxylin and eosin stain ×200; B, Gomori methenamine silver stain ×200). (C, D) High-power morphology of hyphae on patient 1 demonstrating nonseptate, ribbon-like hyphae with right-angle branches. Also note the "bubble-like" appearance of hyphae seen in cross-section. (C, hematoxylin and eosin stain ×600; D, Gomori methenamine silver stain ×600).

Case 2

A 63-year-old gentleman underwent a reduced-intensity (RIC) MUD allo-HCT after fludarabine (Flu)-melphalan (Mel)-alemtuzumab (total dose = 60 mg.v.) for refractory peripheral T cell lymphoma, not otherwise specified (NOS). He had multiple opportunistic infections posttransplant including CMV, BK-virus hemorrhagic cystitis, parainfluenza type 3, *Mycobacterium fortuitum*, recurrent *Strongyloides stercoralis* superinfections and multiple bacterial infections. In addition to the peritransplant in vivo T cell depletion with alemtuzumab, his immunosuppression had been accentuated by systemic steroids, mycophenolate mofetil (MMF), tacrolimus, and a short course of infliximab for grade III skin and gastrointestinal aGVHD, which evolved into chronic extensive persistent GVHD (cGVHD). Although he was receiving steroids and 19 days after he had been started on prophylactic oral posaconazole (200 mg orally 3 times a day with food prepared by his very caring family) he was found to have a new 1.7 cm right middle lobe nodule by both chest X-ray and high-resolution computed tomography (CT) of the chest. The lesion was not present in chest X-rays performed 11 and 13 days after the initiation of posaconazole. Biopsy of the lesion showed wide, ribbon-like, nonseptate hyphae consistent with *Mucorales* infection. Rare colonies of *Rhizopus* species were detected in primary culture on inhibitory mold, sabouraud dextrose, and brain heart infusion agars. The colonies grew well at temperatures >50°C. The microscopic features were consistent with a final identification of *Rhizopus microsporus* var. *rhizopodiformis* [7]. Posaconazole was continued and liposomal Amphotericin (AmBisome) was added. A new chest CT chest 6 days later showed enlargement of the nodule to 2.3 cm. He never suffered relapse of his lymphoma and his hematopoiesis had been consistently of donor origin. He developed significant and progressively worse respiratory distress and, after a few days of clinical deterioration, the family decided to discontinue active treatment. Susceptibility testing (performed postmortem) using E-test strips demonstrated an MIC for amphotericin B of 0.064 μg/mL, for posaconazole 3 μg/mL, and no zone of inhibited growth for voriconazole (MIC >32 μg/mL). Similar to the previous isolate, these MIC values likely reflect a lack of susceptibility to both posaconazole and voriconazole, but susceptibility to amphotericin B.

DISCUSSION

Posaconazole has clinical efficacy as a prophylactic agent for patients who undergo induction chemotherapy for AML or for those who develop systemic GVHD and require high doses of systemic corticosteroids [1]. Posaconazole is only available in an oral solution form [1], and, although its bioavailability is closely dependent on its intake with fatty meals, it has gained widespread popularity. It has also been successfully used for treatment, although most of the data being available are from prophylaxis studies because no prospective posaconazole treatment studies have been conducted except from a trial in esophageal Candidiasis in the HIV setting [13].

Its specific efficacy against *Rhizopus* and other *Mucorales* species has been documented in case reports and data from nonrandomized compassionate use of posaconazole.

Greenberg et al. [14] described the survival of 19 of 24 patients with documented *Mucorales* infection with compassionate use of posaconazole. Patients had failed or could not tolerate other antifungal agents. He emphasized the importance of surgical resection and stabilization or improvement of the underlying immunosuppressed state.

Similarly, van Burik et al. [15] conducted a retrospective study in 69 patients with proved and 22 patients with probable *Mucorales* infection. The success rate was 60%, and stable disease was observed in an additional 21% of patients after 12 weeks of treatment.

Tobon et al. [16] reported a case of amphotericin B-resistant invasive *Rhizopus* infection in a heart-lung transplant patient that responded to posaconazole. Peel et al. [17] used posaconazole as first-line agent for disseminated *Rhizopus microsporus* infection in a patient with systemic lupus erythematosus.

Early institution of posaconazole for *Rhizopus* infections is paramount as emphasized by Kok et al. [18], who reported 2 salvages of patients with rhino-orbital *Rhizopus oryzae* after early institution of posaconazole in conjunction with surgical debridement. Rutar et al. [19] described a case of periorbital *Rhizopus* infection effectively treated with posaconazole after liposomal amphotericin B had been withdrawn because of side effects and lack of improvement. The *Rhizopus* strain was highly susceptible to posaconazole in vitro. Other reports further suggest clinical efficacy of posaconazole against *Rhizopus* species [20–22].

In vitro, there are mixed data in terms of posaconazole activity against *Rhizopus*. Arikan et al. [23] reported that posaconazole was more potent in vitro against clinical isolates of *Rhizopus oryzae* compared to voriconazole, itraconazole, and amphotericin B. Perkhofer et al. [24] showed that posaconazole enhanced the in vitro activity of amphotericin B against the hyphae form of the *Mucorales* fungi, including isolates of *Rhizopus microsporus*, but not *Rhizopus oryzae*.

It seems that there is heterogeneity in in vitro susceptibility to posaconazole and amphotericin B among the different *Mucorales* species (eg, *Rhizomucor* species may have relatively lower MICs) [25].

Murine model data reported first by Rodriguez et al. [26] and then by Ibrahim et al. [27] showed that addition of posaconazole to amphotericin products

did not result in enhanced activity against *Rhizopus* species. Barchiesi et al. [28] reported only limited posaconazole activity as a prophylactic measure in a murine model of *Rhizopus oryzae* infection.

In the clinic, Vyzantiadis et al. [29] reported a fatal case of rhinocerebral *Rhizopus oryzae* infection in a patient treated with steroids for ITP. The fungus was resistant to both Amphotericin B and to posaconazole (MIC 4 μg/mL), which was later coadministered.

The 2 cases herein described raise questions as to how strong and universal is the prophylaxis against *Rhizopus* species with posaconazole. This article confirms similar concerns raised by the recent case report of breakthrough *Rhizopus microsporus* in a post-HCT patient despite posaconazole prophylaxis [30].

It is possible that some strains of *Rhizopus* are inherently resistant to, and can be selected for, posaconazole prophylaxis. The fact that there has been no pattern observed so far of breakthrough *Aspergillus* infections after the widespread use of posaconazole, but there are already 3 cases of *Rhizopus* species breakthrough infections raises the possibility that breakthrough *Rhizopus* infection could be because of limited activity of posaconazole against some *Rhizopus* species and not because of bioavailability issues of the compound. It is interesting to note that all 3 of the reported prophylaxis failures have involved varieties of the *Rhizopus microsporus* group. Furthermore, Verweij et al. [31] reported a fatal case of *Rhizopus microsporus* despite treatment with posaconazole and liposomal amphotericin B. This is significant in that the *Microsporus* group makes up about 15% of rhinocerebral infections because of the *Rhizopus* species [26].

The routine use of fungal cell wall antigens (galactomannan, beta-glucan) [32,33] has been useful in the early detection and has made the preemptive treatment of invasive fungal infections feasible, but they do not detect *Mucorales* infections. Recently, Kasai et al. [34] reported a real-time quantitative PCR assay targeting the 28S rRNA of the clinically most important agents of *Mucorales*. By using this method, early detection of infections was feasible in the blood, tissue, or BAL from infected rabbits. If this method is validated in human specimens, it may allow for an early diagnosis and treatment of human *Mucorales*.

Because of the recent reports showing improved prophylaxis with posaconazole in AML induction and steroid-treated GVHD, we believe that it should be used as the standard for prophylaxis for patients who can reliably absorb it. Nonetheless, a very low threshold should be set for CT imaging of the lungs, paranasal sinuses, or brain in the appropriate setting. This will help in the early detection of either *Rhizopus* or other breakthrough fungal infection and may improve the—disappointing so far—prognosis of those patients. The optimal routine use of serologic markers (beta-glucan or galactomannan) in addition to posaconazole is expected to help further in the detection of invasive fungal infections. Finally, documentation of compliance and bioavailability by measuring posaconazole levels are expected to be useful clinically.

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