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Multiple-azole-resistant *Aspergillus fumigatus* osteomyelitis in a patient with chronic granulomatous disease successfully treated with long-term oral posaconazole and surgery

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We describe a patient with chronic granulomatous disease and proven *Aspergillus fumigatus* osteomyelitis of the midfoot, while receiving itraconazole-prophylaxis. The isolate proved resistant to itraconazole as well as voriconazole, and showed reduced susceptibility to posaconazole. Although molecular analysis demonstrated the presence of a 53 base pair tandem repeat in the promoter region for *cyp51A*, i.e., the gene coding for the target enzyme of the azole antifungals, there were no mutations in the *cyp51A* gene. Since transformation of the promoter region into wild-type strains did not result in an azole resistant phenotype, a yet unknown mutation was suspected. The patient was treated with extensive surgery and two weeks of caspofungin therapy, followed by one year of posaconazole therapy. He made a complete recovery and did not experience any side effects. Long-term posaconazole proved to be a safe and effective treatment for multi-azole resistant *A. fumigatus* osteomyelitis in this immunocompromised patient.

Keywords *Aspergillus*, chronic granulomatous disease, osteomyelitis, posaconazole

Introduction

Chronic granulomatous disease (CGD) is a primary immunodeficiency disorder in which phagocytes fail to produce the 'oxidative burst' needed to kill certain microorganisms after phagocytosis. In the US registry of CGD-patients in 2000, invasive infections with *Aspergillus* spp. were the most common cause of mortality. In addition, 90 of 368 (25%) CGD-patients had experienced osteomyelitis, of which 20 cases (22%) were caused by *Aspergillus* [1]. In a recent European registry, *Aspergillus* spp. proved to be the most common cause of osteomyelitis in these patients (29 of 84 cases) [2]. CGD-patients usually receive an azole antifungal as oral prophylaxis, with itraconazole (ITC) the current first choice [3].

Posaconazole (PCZ) is a relatively new oral triazole with broad-spectrum antifungal activity. Long-term use is reported to be safe and effective in the treatment of febrile neutropenia or refractory invasive infections in severely immunocompromised pediatric and adult patients [4]. PCZ has been used successfully as salvage therapy for a small number of CGD-patients with invasive fungal infection of the lung after failure or intolerance of treatment with standard antifungal agents [5]. To our knowledge, no case reports of PCZ treatment for *Aspergillus* osteomyelitis in CGD-patients have been published.

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We describe a case of proven *Aspergillus fumigatus* osteomyelitis resistant to ITC and voriconazole (VRC) and with reduced *in vitro* susceptibility to PCZ in a CGD-patient. The individual was eventually successfully treated with debridement surgery and two weeks of caspofungin therapy followed by long-term oral PCZ.

Case report

The patient was an 18-year-old male who presented at his local hospital with a painful foot. At the age of three years he was diagnosed with the X-linked form of CGD after a period of prolonged fever due to multiple *Staphylococcus aureus* liver abscesses. CGD was diagnosed by NBT slide tests and absence of respiratory bursts measured by oxygen consumption and chemoluminescence. Sequence analysis revealed a missense mutation at residue 500 of the gene encoding gp91-phox, which is associated with non-functional cytochrome b558 [6]. After discharge, he received interferon gamma and trimethoprim-sulfamethoxazole as prophylaxis. ITC was added from the age of seven years, but plasma levels were not monitored. Despite prophylaxis, he had a history of relapsing and prolonged bacterial infections of the skin, gastro-intestinal and respiratory tracts. At presentation he had experienced progressive pain in his right foot and an increasing feeling of instability of four weeks duration. There was no fever. Radiological investigation, performed in the first week of his complaints, showed no abnormalities, but a second X-ray at his current presentation showed destruction of the cuneiform bones, consistent with osteomyelitis. Necrotic and infected bone was surgically removed and cultured. Because a bacterial osteomyelitis was suspected, the patient was started on gentamicin and high-dose flucloxacillin. Within one week after surgery, follow-up radiological examination showed further destruction of the cuneiform bones and navicular bone, although the patient had improved clinically. Microscopic examination had not shown signs of a fungal infection. However, *Aspergillus* spp. was recovered from the bone, after which treatment was changed to intravenous VRC at a maintenance dose of 4 mg/kg twice daily. The patient was subsequently transferred to the Academic Medical Center for further evaluation and treatment. Computed tomography of the thorax and sinuses, as well as ultrasonography of the abdomen and heart showed no evidence for fungal dissemination. Extensive debridement surgery was performed three times over the course of one month. The *Aspergillus* spp. was identified as *A. fumigatus* and susceptibility tests showed resistance to VRC (minimum inhibitory concentration (MIC) = 16 mg/l) and ITC (MIC > 16 mg/l), and reduced susceptibility to PCZ (MIC = 0.25 mg/l). The MIC values for amphotericin B and caspofungin were 1.0 mg/l and 0.5 mg/l, respectively. Treatment with VRC was discontinued and once daily treatment with 70 mg caspofungin was started. After two weeks of intravenous therapy, the patient made a fast recovery and antifungal therapy was changed to 400 mg oral PCZ twice daily. After discharge PCZ was continued for one year, along with frequent visits by the patient to the outpatient clinic. A follow-up X-ray taken one month after discharge illustrated the extensive destruction of the midfoot (Fig. 1). The patient had no complaints of pain or fever and returned to playing sports. He did not experience any side-effects. After four months of PCZ the serum creatinine slowly increased from 80 to 111 mol/l, but after adjustment of the dosage of trimethoprim-sulfamethoxazole to the standard prophylactic dose, the serum creatinine quickly dropped to 90 mol/L, suggesting a trimethoprim-sulfamethoxazole effect. After one year of follow-up there were no signs of active infection on radiological examination and the patient was

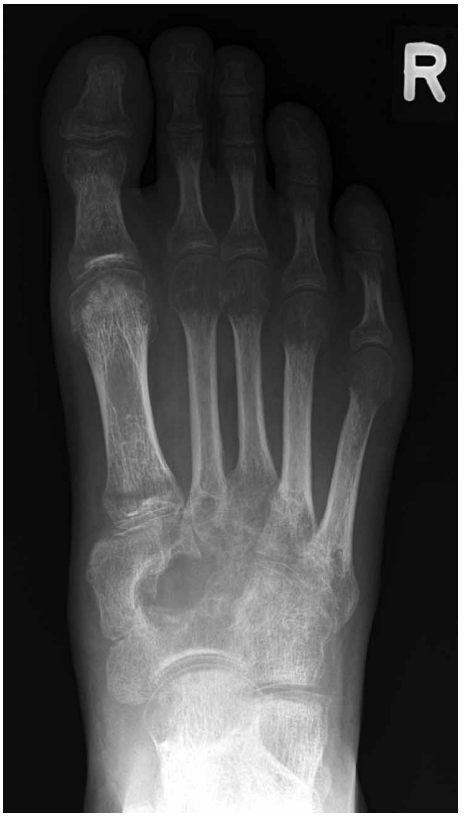

Fig. 1 Radiological examination of the right foot showing extensive ossal destruction due to *Aspergillus fumigatus* osteomyelitis.

considered cured. PCZ therapy was discontinued and ITC prophylaxis was restarted.

Analysis of the *A. fumigatus* isolate

The *in vitro* activity of amphotericin B, ITC, VRC, PCZ, and caspofungin were determined using a microdilution format according to the CLSI M38-A reference method [7]. The resistance mechanism was determined by analysis of the full sequence of the *cyp*51A gene, as described previously [8]. Molecular identification was performed by sequencing of parts of the highly conserved β-tubulin and calmodulin gene [9], in order to rule out any cryptic species within the *Aspergillus* section *Fumigati*. The morphological identification of the *A. fumigatus* isolate was confirmed and no mutations were found in the *cyp51A* gene. However, a 53 base pair tandem repeat was found in the promoter region of this gene.

Discussion

Although *A. fumigatus* osteomyelitis is relatively common in CGD-patients, the total number of cases remains small, making it difficult to compare success rates of different therapeutic regimens. A review of the literature yielded 10 cases of *A. fumigatus* osteomyelitis in 9 CGD-patients in the period between 1983 and 1999 [10]. Most of these cases were treated with a combination of surgery and deoxycholate amphotericin B, often in combination with one or more other antifungals. The new guidelines of the Infectious Diseases Society of America on the treatment of aspergillosis recommend long-term VRC in combination with surgical intervention for management of *Aspergillus* osteomyelitis in immunocompromised patients, although there is currently limited experience with VRC. A review of 20 cases of *Aspergillus* osteomyelitis treated with VRC as salvage therapy after treatment failure with deoxycholate amphotericin B and/or lipid-associated amphotericin B showed some success [11]. Of the 5 CGD-patients included in that study (age 4–14 years), one showed a complete recovery and two had a partial response. There is little experience with the use of echinocandins or PCZ in the treatment of *Aspergillus* osteomyelitis. A case report describes a 64-year-old lung transplant patient with an *A. fumigatus* empyema and disseminated aspergillosis of the ankle and adjacent bone. After surgery, he was successfully treated with 6 months of PCZ, when systemic therapy with ITC and lipid-associated amphotericin B had failed [12]. PCZ has low mean MICs (mg/l) for *A. fumigatus* compared to amphotericin B, VRC and ITC. Achievable serum levels for PCZ exceed this concentration even in multiple-azole resistant cases of aspergillosis [13]. PCZ has not been registered for use in patients under the age of 13 years. However, in a study with 12 pediatric (age 8–17 years) and 194 adult patients, PCZ concentrations in plasma were comparable and overall success rate and adverse events were similar [14]. No data are available for younger children.

In the present case, the management of *Aspergillus* osteomyelitis was further complicated by the reduced susceptibility of the *A. fumigatus* isolate to antifungal azoles, with both ITC and VCZ showing no *in vitro* activity. The prevalence of azole-resistance in *A. fumigatus* is presently unknown as a result of the fact that MIC-testing of clinical isolates is not routinely performed in the majority of clinical microbiology laboratories. However, it was recently shown that up to 6% of clinical *A. fumigatus* isolates were azole resistant in a Dutch medical centre [15]. Azoles interfere with ergosterol synthesis by inhibiting 14-α-sterol demethylase, leading to growth arrest. The most prevalent azole resistance mechanism in *A. fumigatus* appears to be related to the modification of this enzyme, which is encoded by the *cyp51A* gene. Several amino acid substitutions of *cyp51A* have been described, leading to different susceptibility profiles. A new mechanism was recently described which involved substituting the amino acid leucine for histidine at codon 98 together with a tandem repeat in the *cyp51A* promoter region. These changes result in the lack of activity of ITC and VRC and reduced activity of PCZ [16]. Furthermore, these alterations were the dominant change in the Dutch azole-resistant isolates with 96% of isolates showing this resistance mechanism [15].

The isolate from our patient had no substitutions in the *Cyp51A* gene, but did show a 53 base pair tandem repeat in the promoter region. Although expression analysis showed a five-fold increase of *cyp*51A expression, transformation of the promoter region into a wild-type strain did not result in the azole-resistant phenotype. The significance of the tandem repeat in relation to the resistant phenotype therefore remains unclear and an additional, but yet unknown, resistance mechanism is suspected [17].

The consequences of the observed phenotype for the clinical efficacy of azoles remain unclear. We anticipated that treatment with ITC or VCZ would not be successful due to the lack of their *in-vitro* activity. Although the MIC of PCZ was elevated compared with that of wild-type isolates, the MIC of the isolate was within the therapeutic range of this drug. Faced with the lack of alternative oral drugs and the necessity of long-term ambulant therapy, it was decided to treat our

patient with PCZ following primary therapy with caspofungin. Treatment with PCZ combined with extensive surgical debridement resulted in complete recovery and the drug was well tolerated without any side effects.

Conclusions

Long-term PCZ proved to be a safe and effective treatment for ITC and VRC-resistant *A. fumigatus* osteomyelitis in a CGD-patient.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1 Winkelstein JA, Marino MC, Johnston RB Jr,, *et al*. Chronic granulomatous disease. Report on a national registry of 368 patients. *Medicine* (Baltimore) 2000; **79**: 155–169.

2 Van den Berg JM, Van Koppen E, Åhlin A, *et al*. Chronic granulomatous disease (CGD): A European database. In: 13th International Congress of Immunology. Rio de Janeiro, Brazil; 2007 [abstract MS-17.2].

3 Gallin JI, Alling DW, Malech HL, *et al*. Itraconazole to prevent fungal infections in chronic granulomatous disease. *N Engl J Med* 2003; **348**: 2416–2422.

4 Raad II, Graybill JR, Bustamante AB, *et al*. Safety of long-term oral posaconazole use in the treatment of refractory invasive fungal infections. *Clin Infect Dis* 2006; **42**: 1726–1734.

5 Segal BH, Barnhart LA, Anderson VL, *et al*. Posaconazole as salvage therapy in patients with chronic granulomatous disease and invasive filamentous fungal infection. *Clin Infect Dis* 2005; **40**: 1684–1688.

6 Leusen JH, de Boer M, Bolscher BG, *et al*. A point mutation in gp91-phox of cytochrome b558 of the human NADPH oxidase leading to defective translocation of the cytosolic proteins p47-phox and p67-phox. *J Clin Invest* 1994; **93**: 2120–2126.

7 National Committee for Clinical Laboratory Standards. *Reference method for broth dilution antifungal susceptibility testing of filamentous fungi*. Approved standard M38-A. National Committee for Clinical Laboratory Standards, Wayne, PA, 2002

8 Mellado E, Garcia-Effron G, Alcázar-Fuoli L, *et al*. A new *Aspergillus fumigatus* resistance mechanism conferring *in vitro* crossresistance to azole antifungals involves a combination of cyp51A alterations. *Antimicrob Agents Chemother* 2007; **51**: 1897–1904.

9 Samson RA, Hong SB, Peterson SW, Frisvad JC, Varga J. Polyphasic taxonomy of *Aspergillus* section *Fumigati* and its teleomorph, *Neosartorya*. *Stud Mycol* 2007; **59**: 147–203.

10 Dotis J, Roilides E. Osteomyelitis due to *Aspergillus* spp. in patients with chronic granulomatous disease: comparison of *Aspergillus nidulans* and *Aspergillus fumigatus*. *Int J Infect Dis* 2004; **8**: 103–110.

11 Mouas H, Lutsar I, Dupont B, *et al*. and the Voriconazole/Bone Invasive Aspergillosis Study Group. Voriconazole for invasive bone aspergillosis: a worldwide experience of 20 cases. *Clin Infect Dis* 2005; **40**: 1141–1147.

12 Lodge BA, Ashley ED, Steele MP, Perfect JR. *Aspergillus fumigatus* empyema, arthritis, and calcaneal osteomyelitis in a lung transplant patient successfully treated with posaconazole. *J Clin Microbiol* 2004; **42**: 1376–1378.

13 Mosquera J, Denning DW. Azole cross-resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2002; **46**: 556–557.

14 Krishna G, Sansone-Parsons A, Martinho M, Kantesaria B, Pedicone L. Posaconazole plasma concentrations in juvenile patients with invasive fungal infection. *Antimicrob Agents Chemother* 2007; **51**: 812–818.

15 Verweij PE, Van der Lee HAL, Kuijpers J, *et al*. Epidemiology of multiple-triazole-resistant *Aspergillus fumigatus*. In: 47th Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, IL: American Society for Microbiology, 2007 [abstract M-2018].

16 Verweij PE, Mellado E, Melchers WJ. Multiple-triazole-resistant aspergillosis. *N Engl J Med* 2007; **356**: 1481–1483.

17 Mellado E, Alcazar-Fuoli L, Pajkrt D, *et al*. Alterations of the *cyp51A* gene promoter contribute to *Aspergillus fumigatus* multiple triazole resistance. In: 47th Interscience Conference on Antimicrobial Agents and Chemotherapy. Chicago, IL: American Society for Microbiology, 2007 [abstract M-521].

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