Case Report

Use of voriconazole in a patient with aspergilloma caused by an itraconazole-resistant strain of *Aspergillus fumigatus*

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The case is reported of a patient with cavitary sarcoidosis complicated by an aspergilloma caused by an itraconazole-resistant strain of *Aspergillus fumigatus*, who was treated with voriconazole. The authors suggest that susceptibility testing of *A. fumigatus* strains is of value during long-term therapy with itraconazole, and that voriconazole may be a good option for treatment of patients infected with itraconazole-resistant strains of *A. fumigatus*.

Introduction

Itraconazole is an azole antifungal drug with a good activity against *Aspergillus* spp., both *in vitro* and *in vivo* in animal models of aspergillosis, and this drug has been largely used for treatment of different clinical forms of aspergillosis (Stevens *et al.*, 2000). Nevertheless, itraconazole resistance in *Aspergillus fumigatus* has been reported in the clinical setting, and can occur after long-term therapy (Chryssanthou, 1997; Dannaoui *et al.*, 2001). In cases of itraconazole resistance, other azoles, such as voriconazole, may keep their activity (Mosquera & Denning, 2002; Verweij *et al.*, 2002).

We report the case of a patient with an aspergilloma caused by an itraconazole-resistant isolate of *A. fumigatus*, who was treated with voriconazole.

Case report

The patient was a 44-year-old man with a stage IV sarcoidosis, for which he received long-term corticosteroid therapy. An aspergilloma of the left upper lobe was radiologically (i.e. chest computed tomography) and microbiologically documented in 2000. An initial isolate of *A. fumigatus* was not stored or tested for antifungal susceptibility. The patient was treated with itraconazole for 3 years until May 2003. In May 2003, a bronchial aspirate again grew *A. fumigatus*. *In vitro* antifungal susceptibility testing was performed by a modification of the EUCAST

technique for yeasts [Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST) *et al.*, 2003]. A high MIC of $>8 \mu\text{g ml}^{-1}$ was found for itraconazole, whereas MICs were 0.25, 1 and $1 \mu\text{g ml}^{-1}$ for amphotericin B, voriconazole and posaconazole, respectively. The molecular mechanism of itraconazole resistance was evaluated for the isolate, and was found to be related to both an amino acid substitution in the gene *cyp51A* encoding the target enzyme of the drug (a change from methionine to lysine at position 220 in *cyp51A*) and to an increased expression of AtrG and AtrF, two multidrug transporters of the ATP-binding cassette family (M. Meneau and D. Sanglard, unpublished results). The relative increases of gene expression compared to an itraconazole-susceptible *A. fumigatus* isolate were 2.6- and 2.0-fold for AtrG and AtrF, respectively. Because the patient had persistent productive cough and scapular pain, itraconazole was stopped, and treatment with oral voriconazole at 200 mg b.i.d. was initiated. A good clinical response was observed, with decreased scapular pain and partial resolution of productive cough in the absence of antibacterial therapy. After several episodes of haemoptysis, the patient underwent two bronchial artery embolizations in May and July 2003.

Nevertheless, in mid-July 2003, a bronchial infection by *Staphylococcus aureus* developed, and the patient experienced

a new episode of haemoptysis. Then, he was referred for thoracic surgery for resection of the left upper lobe. Histological examination and fungal cultures of the pulmonary tissues were negative. Nevertheless, two bronchial aspirates, taken 3 days apart, grew *A. fumigatus*, and voriconazole treatment was continued. A new episode of bronchial infection was diagnosed in November 2003 and successfully treated with amoxycillin-clavulanic acid. Persistent diarrhoea was diagnosed in November 2003, but no specific pathogens were identified (examination of stool for bacterial pathogens and *Clostridium difficile* toxin assay were negative). Although serum levels of voriconazole were not measured, persistence of the diarrhoea, possibly due to voriconazole toxicity, led to the discontinuation of the antifungal drug in February 2004. Eventually, the patient died suddenly from a massive haemoptysis in April 2004.

Discussion

Invasive aspergillosis, chronic necrotizing aspergillosis, and aspergilloma are the most common clinical presentations of lung infections due to *Aspergillus* species (Soubani & Chandrasekar, 2002). Aspergilloma is a not uncommon complication of sarcoidosis, particularly in patients with cystic parenchymal damage (Wollschlaeger & Khan, 1984), and fatal haemoptysis is a potentially lethal complication of this infection. Moreover, sarcoidosis and steroid therapy, as found in the present case, are two factors that have been identified to be associated with a poor prognosis of the aspergilloma (Stevens *et al.*, 2000). There is no standardized therapy for aspergilloma, and different strategies have been used, including inhaled, intracavitary or endobronchial antifungal therapy, and systemic treatment with antifungal agents. Bronchial artery embolization can be tried to prevent haemoptysis, but is only temporarily effective. Surgery is the treatment of choice of symptomatic aspergilloma. However, it is associated with a high mortality and morbidity in such high-risk patients.

When surgery is not feasible, itraconazole is the drug commonly used for the medical treatment of aspergilloma (Stevens *et al.*, 2000). Nevertheless, there have been some reports of *in vitro* itraconazole resistance in *A. fumigatus*. Both *de novo* and acquired resistance during long-term therapy have been reported (Chryssanthou, 1997; Denning *et al.*, 1997; Dannaoui *et al.*, 2001). Moreover, it has been shown in animal models of aspergillosis that there is a correlation between *in vitro* resistance and lack of efficacy of itraconazole therapy (Denning *et al.*, 1997; Dannaoui *et al.*, 2001). The true frequency of itraconazole resistance in *A. fumigatus* is unknown, but acquisition of resistance during long-term therapy with itraconazole (either by true acquisition of resistance in a given strain or by replacement of the infective susceptible strain by another resistant one) has also been well reported in a limited number of patients (Chryssanthou 1997; Dannaoui *et al.*, 2001).

At least two different molecular mechanisms of resistance to itraconazole (mutation of the target enzyme of the drug and

overexpression of efflux pumps) have been reported for clinical isolates of *A. fumigatus* (Slaven *et al.*, 2002; Mellado *et al.*, 2004), and both mechanisms were found in the strain isolated in our patient. The expression of transporters AtrG and AtrF was tested in the current isolate. At present, no experimental evidence exists of a link between upregulation of these transporters and itraconazole resistance. A mutation in Cyp51a in the isolate was, however, observed. This mutation from methionine to lysine at position 220 in Cyp51A has previously been found to be associated with itraconazole resistance in clinical *A. fumigatus* isolates (Mellado *et al.*, 2004). However, no definitive evidence for the participation of this particular mutation in itraconazole resistance has yet been obtained. In the itraconazole-resistant isolate of this study, two potential resistance mechanisms existed; however, it is not yet possible to dissect their individual contribution to itraconazole resistance. The possibility of the acquisition of itraconazole resistance underlines the usefulness of *in vitro* susceptibility testing of strains in patients with aspergilloma who have had long-term therapy with an azole.

Amphotericin B, caspofungin, voriconazole and posaconazole are alternatives that can be used for treatment of *Aspergillus* spp. infections. Amphotericin B and caspofungin are only available as intravenous formulations. On the other hand, voriconazole can be given as an oral treatment. It has been demonstrated that voriconazole is an effective treatment for invasive pulmonary aspergillosis (Herbrecht *et al.*, 2002), but has been seldom used for treatment of aspergilloma. Although voriconazole possesses a similar mechanism of action to itraconazole, it has a good *in vitro* activity against most of the itraconazole-resistant strains of *A. fumigatus* (Espinel-Ingroff *et al.*, 2001; Mosquera & Denning 2002; Verweij *et al.*, 2002), and it can be considered as an alternative to itraconazole therapy. Multiresistant strains have been reported, but seem to be exceptional (Warris *et al.*, 2002). In the present case, although the patient eventually died from massive haemoptysis, the itraconazole-resistant strain was susceptible to voriconazole, and initial clinical improvement was obtained with voriconazole treatment. The occurrence of fatal haemoptysis during the course of aspergilloma is due to hyperpressure in local bronchial arteries, which may persist after medical treatment or even radical surgery.

In conclusion, long-term therapy with itraconazole in patients with aspergilloma caused by *A. fumigatus* can be associated with the development of itraconazole resistance. Susceptibility testing of strains recovered from these patients is of value to detect resistance, and voriconazole therapy may be a good alternative for treatment in cases of itraconazole resistance.

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