Azole resistance in allergic bronchopulmonary aspergillosis and *Aspergillus* bronchitis

S. J. Howard[1,2], A. C. Pasqualotto[3] and D. W. Denning[1,2]

1) The University of Manchester, The Manchester Academic Health Science Centre, Manchester, 2) Regional Mycology Laboratory Manchester, Manchester, UK and 3) Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

Abstract

Oral azole antifungal therapy is used extensively for all forms of aspergillosis, including allergic bronchopulmonary aspergillosis (ABPA). However, long-term therapy may increase the risk of resistance. Here we report itraconazole and voriconazole resistance with reduced susceptibility to posaconazole in *Aspergillus fumigatus* in two patients exposed to itraconazole. Patients were diagnosed with ABPA and *Aspergillus* bronchitis related to innate immune defects. An azole susceptible strain was initially isolated from patient 1, but later a genetically different azole-resistant strain was cultured, possibly related to sub-therapeutic itraconazole levels, which could be a trigger for selection of resistance. The mechanism of resistance identified in this case was an L98H change in Cyp51A, accompanied by a tandem repeat in the promoter region of *cyp51A* leading to increased expression. No *cyp51A* mutation was found in azole-resistant isolates recovered from patient 2. Both patients responded to posaconazole, with plasma levels of >1.0 mg/L. Subsequently, susceptible strains of different molecular types were cultured from both patients, suggesting eradication and replacement.

Keywords: ABPA, antifungal, aspergillosis, *Aspergillus fumigatus*, azole, bronchitis, resistance
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Corresponding author and reprint requests: S. J. Howard, University of Manchester, 1.800 Stopford Building, Oxford Road, Manchester, M13 9PT, UK
E-mail: susan.j.howard@manchester.ac.uk

Introduction

Allergic aspergillosis is a hypersensitivity disease manifesting in several clinical forms, including allergic bronchopulmonary aspergillosis (ABPA). ABPA is characterized by wheezing, bronchiectasis, pulmonary infiltrates and sputum containing brown plugs [1,2]. Asthmatics and cystic fibrosis patients are primarily at risk [2]. As ABPA is a long-term condition, its prevalence is higher than invasive aspergillosis (IA), but its annual incidence or rate of new diagnoses are probably lower. In addition to the conventional corticosteroid treatment, itraconazole (ITC) antifungal therapy has been shown to be advantageous in ~60% of cases [3].

Aspergillus bronchitis (or aspergillary bronchitis, as it was first called) [4] may occur in patients with underlying pulmonary or airway pathology [5], especially in the context of lung transplantation [6,7]. Antifungal therapy is probably effective if it has not evolved into pseudomembranous *Aspergillus* tracheobronchitis, which is usually fatal.

The azoles are the largest and most widely used class of antifungal drugs. Although voriconazole (VRC) is regarded as first-line therapy for invasive aspergillosis [5], ITC is still commonly used for chronic non-invasive forms of aspergillosis. Resistance to ITC in *Aspergillus fumigatus*, the species which causes the vast majority of cases of allergic aspergillosis, is well recognized. In our experience, it occurs mainly in patients with chronic forms of aspergillosis, particularly chronic cavitary pulmonary aspergillosis (CCPA) with aspergillomas. The frequency of ITC resistance in clinical *A. fumigatus* strains since the turn of the millennium (when most cases have been reported) is between 2% and 3%, and it can increase up to 6% depending on the area from which it is reported [8–10]. Cross-resistance between other azole drugs has also been reported [11,12]. To our knowledge, azole resistance has not been described among isolates caus-

ing allergic aspergillosis. In this report, we describe azole resistance in two patients with ABPA and *Aspergillus* bronchitis undergoing ITC therapy.

Case Reports

Patient 1

A 47 year-old asthmatic woman was diagnosed with ABPA. Her previous medical history was quite complex, including aortic valve replacement (twice), hypertension, hypermobility syndrome, perennial rhinitis and multiple allergies, including allergies to various houseplants, house dust, horses, dogs and pollen. The patient reported breathlessness especially on exercise. Cough was a predominant symptom, with discoloured brown sputum with hard brown plugs (which had a tree branch appearance). She had required several courses of oral corticosteroids for exacerbations. A computed tomography (CT) scan performed in January 2006 revealed some prominence and dilatation of the central bronchial tree consistent with mild bronchiectasis. There was no evidence of fibrosis or other parenchymal disease. She had normal immunoglobulin levels and eosinophil counts, with a total serum IgE of 3000 IU/mL and an anti-*Aspergillus* IgE of 94.5 IU/mL. A radioallergosorbent test (RAST) (IgE) was positive against several other fungi, including *Penicillium* (13.3), *Alternaria* (13.9), *Candida albicans* (4.4), *Saccharomyces cerevisiae* (1.3) and *Cladosporium* (1.0). Also, serum precipitins against *A. fumigatus* were at the limit of detection.

In July 2006, ITC treatment was started (capsules, 200 mg daily) while the patient was treated with omeprazole, which led to reduced cough. In October 2006, culture of her sputum yielded *A. fumigatus* susceptible to ITC (laboratory number F15767). Random serum ITC levels measured using a bioassay [13,14] in October 2006 were below the level of detection (<0.8 mg/L). She was also found to have very low mannose-binding lectin levels (0.3 mg/L; normal >4 mg/L). The patient was then treated with ITC oral solution (400 mg daily) which she found very difficult to tolerate as a result of nausea and diarrhoea. Nevertheless, an adequate, randomly tested serum ITC level (5.2 mg/L) was observed in November 2006, as determined with a bioassay [13,14].

Sputum culture performed in January 2007 revealed *Mycobacterium xenopi*. Because of drug interactions between the rifamycins and ITC, the patient was treated with ethambutol and ciprofloxacin in combination with ITC. Previous severe cholestasis under erythromycin therapy as a child precluded the use of a macrolide.

In February 2007, while being treated with a ITC solution, her sputum was abundantly yellow and thick, and the patient reported feeling very tired. Sputum culture revealed heavy growth of *A. fumigatus* (F16216), which was azole resistant (Table 1). In June 2007, posaconazole (PSC) was prescribed as salvage therapy. Over the next 6 months, her health was reasonable under PSC treatment. PSC serum levels were randomly measured using a bioassay (18th European Congress of Clinical Microbiology and Infectious Diseases, abstract P1351) between July 2007 and December 2008 and ranged from 1.01 to 1.36 mg/L. After a stormy course involving a lung abscess and a probably non-cardiogenic pulmonary oedema requiring mechanical ventilation in early and late 2008, respectively, during which VRC, amphotericin B (AMB) and PSC were used sequentially, an azole-susceptible *A. fumigatus* was isolated (F18830).

Patient 2

A 41 year-old female engineer who visited building sites suffered from intermittent haemoptysis and was diagnosed with bilateral lower-lobe bronchiectasis in 1999. She was a non-smoker and denied any constitutional upset, chest pain or shortness of breath. Since 1999 she had required antibiotics

TABLE 1. Minimum inhibitory concentration results (MIC, mg/L) and *cyp51A* mutations found in *Aspergillus fumigatus* isolates. Minimum fungicidal concentrations are also shown, unless identical to MIC values

	Susceptibility (mg/L)					
	ITC	VRC	PSC	RVC	AMB	*cyp51A*/Cyp51A alteration
Patient 1						
F15767	0.25 (0.5)	0.5	0.06	0.5	0.25 (1)	None
F16216	>8	8	2	8	0.125 (0.5)	L98H + promoter alteration
F18830	0.25	0.5	0.125	ND	0.5 (2)	ND
Patient 2						
F16311	>8	8	1 (2)	4	0.25 (0.5)	None
F16351	>8	4 (8)	0.5 (2)	4	0.25 (1)	None
F18718	1	2	0.125	ND	0.5 (>8)	ND

ITC, itraconazole; VRC, voriconazole; PSC, posaconazole, RVC, ravuconazole; AMB, amphotericin B; ND, not determined.