J Infect Chemother (2011) 17:483–486
DOI 10.1007/s10156-010-0202-1

ORIGINAL ARTICLE

Molecular typing and in-vitro activity of azoles against clinical isolates of *Aspergillus fumigatus* and *A. niger* in Japan

Moe Asano · Rui Kano · Koichi Makimura · Atsuhiko Hasegawa · Hiroshi Kamata

Received: 26 July 2010 / Accepted: 7 December 2010 / Published online: 5 January 2011
© Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases 2011

Abstract Twenty-three isolates of *Aspergillus* from Japanese transplant recipients were identified by phenotyping and subjected to detailed sequence analyses using the ITS regions and β-tubulin (*benA*). Gene sequences derived from the ITS regions and *benA* of all *Aspergillus* isolates were compared with sequences in the GenBank database for species identification. Nineteen isolates were identified as *A. fumigatus* and 4 isolates as *A. niger*. The antifungal susceptibility of the isolates to itraconazole (ITZ), voriconazole (VCZ), and posaconazole (POS) was assessed using the E-test with a 48-h incubation. Almost all the clinical isolates of the *Aspergillus* species seemed to be more susceptible to VCZ and POS than to ITZ, whereas 1 clinical isolate of *A. fumigatus* was resistant to ITZ. Sequencing of the 14α-demethylase gene (*cyp51A*) of this isolate revealed a novel mutation (F332K) in the gene.

Keywords *Aspergillus* · Antifungal susceptibility · Molecular identification · E-test · Point mutation of 14α-demethylase gene

Introduction

A. fumigatus accounts for most cases of the invasive aspergillosis (IA) that is common in transplant recipients in Japan. However, non-*fumigatus* species are now emerging as a cause of IA. Most *Aspergillus* species are susceptible to amphotericin B (AMB) and the azoles, whereas some species are considered to be resistant to these drugs [1–3]. Balajee et al. reported that the different antifungal susceptibility of the *Aspergillus* species is because of misidentification of species by phenotyping, and thus molecular confirmation of isolates is required [2, 3]. Therefore, molecular identification is important for clinical isolates from IA. However, neither detailed molecular identification of clinical isolates of *Aspergillus* species nor their antifungal susceptibility to the new azoles has been performed in Japan.

In this study, we investigated the antifungal susceptibilities of molecularly identified *Aspergillus* isolates from transplant recipients in Japan to itraconazole (ITZ), voriconazole (VCZ), and posaconazole (POS), by use of the E-test.

M. Asano · R. Kano (✉) · H. Kamata
Department of Pathobiology, Nihon University School of Veterinary Medicine, 1866 Kameino, Fujisawa, Kanagawa 252-0880, Japan
e-mail: kano@brs.nihon-u.ac.jp

K. Makimura · A. Hasegawa
Institute of Medical Mycology and Genome Research Center, Teikyo University, Hachioji, Tokyo 192-0395, Japan

Materials and methods

Strains

The 23 clinical isolates from Japanese hematological malignancy patients with proven or probable invasive aspergillosis (IA) in 2000 are listed in Table 1. They were confirmed on the basis of their purity, and identified by traditional macroscopic and microscopic techniques. They had been maintained on Sabouraud's dextrose agar and were investigated by molecular analysis and by use of antifungal susceptibility tests.

ITS regions and β-tubulin sequences for isolates

The clinical isolates were cultured in Sabouraud liquid medium at 27°C for 5 days. The mycelial samples were

Table 1 Identity, source and antifungal susceptibility of *Aspergillus* isolates

Strain no.	Specimen	Molecular identity	Identity by phenotyping	E-test MIC (mg/ml)		
				ITZ	VCZ	POS
2905	Sputum	*A. fumigatus*	*A. fumigatus*	2	1.5	0.38
2906	Sputum	*A. fumigatus*	*A. fumigatus*	4	0.38	0.38
2907	Pleural fluid	*A. fumigatus*	*A. fumigatus*	0.19	0.064	0.047
2908	Sputum	*A. fumigatus*	*A. fumigatus*	0.125	0.047	0.047
2909	Sputum	*A. fumigatus*	*A. fumigatus*	0.047	0.032	0.032
2913	Sputum	*A. fumigatus*	*A. fumigatus*	0.19	0.064	0.047
2914	Sputum	*A. fumigatus*	*A. fumigatus*	0.25	0.064	0.047
2916	Sputum	*A. fumigatus*	*A. fumigatus*	0.032	0.032	0.023
2917	TNA	*A. fumigatus*	*A. fumigatus*	1.5	0.064	0.094
2919	BALF	*A. fumigatus*	*A. fumigatus*	0.125	0.047	0.047
2922	BALF	*A. fumigatus*	*A. fumigatus*	1	0.064	0.064
2923	Lung	*A. fumigatus*	*A. fumigatus*	0.032	0.023	0.016
2924	Sputum	*A. fumigatus*	*A. fumigatus*	0.38	0.094	0.047
2926	Sputum	*A. fumigatus*	*A. fumigatus*	0.047	0.047	0.032
2927	Sputum	*A. fumigatus*	*A. fumigatus*	0.032	0.047	0.032
2928	BALF	*A. fumigatus*	*A. fumigatus*	0.19	0.032	0.094
2934	BALF	*A. fumigatus*	*A. fumigatus*	0.032	0.023	0.032
3968	Sputum	*A. fumigatus*	*A. fumigatus*	0.094	0.064	0.064
5042	Sputum	*A. fumigatus*	*A. fumigatus*	>32	0.023	0.5
2911	Sputum	*A. niger*	*A. niger*	0.006	0.016	0.032
2925	TNA	*A. niger*	*A. niger*	1	0.064	0.125
2930	TNA	*A. niger*	*A. niger*	0.032	0.008	0.012
2931	TNA	*A. niger*	*A. niger*	0.023	0.008	0.008

TNA transthoracic needle aspiration, *BALF* bronchoalveolar lavage fluid, *ITZ* itraconazole, *VCZ* voriconazole, *POS* posaconazole

collected by centrifugation at 15000×*g* and then homogenized in liquid nitrogen. The samples were lyzed with 1 mg/ml zymolyase-100T (Takara, Kyoto, Japan) in lysis buffer containing 0.1 mM EDTA, 1% sodium dodecyl sulfate (SDS), 10 mM Tris hydrochloride (pH 8.0), and 0.3% 2-mercaptoethanol at 37°C for 16 h. High-molecular-weight DNA was obtained from these mycelium samples by phenol and chloroform extraction, and then precipitated with ethanol. Extracted DNA samples dissolved in TE buffer (10 mM Tris–HCl, pH 8.0 and 1 mM EDTA) were used for polymerase chain reaction (PCR) amplification.

The ITS regions and β-tubulin sequences are usually used for molecular typing of *Aspergillus* species [6]. Universal fungal primers ITS5 (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS4 (5′-TCC TCC GCT TAT TGA TAT GC-3′) [4], and the β-tubulin primers benA-F (5′-AAT TGG TGC CGC TTT CTG G-3′) and benA-R (5′-AGT TGT CGG GAC GGA ATA G-3′), were used to amplify DNA from the *Aspergillus* species [2, 5]. PCR amplification and sequence analysis were performed as described previously [4]. Nucleotide sequences were edited using Genetyx-MAC version 7.3.1 (Genetyx, Tokyo, Japan) software and the resulting sequences were queried in the GenBank database to match the isolates to species, within the section.

Antifungal susceptibility testing against azoles

Antifungal susceptibility testing was performed using E-test gradient strips of ITZ, VCZ, and POS obtained from AB Biodisk (Solna, Sweden). Stock inoculum suspensions were prepared as described in the E-test technical guide 10 [7].

Sequence of the 14α-demethylase gene (*cyp51A*)

PCR amplification and sequence analysis of *cyp51A* of ITZ resistant strain 5402 were performed as described previously [8]. The PCR products were sequenced by the dideoxy chain termination method using an ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

Results

Molecular identification

Of the 23 *Aspergillus* isolates from transplant recipients in Japan, 19 were molecularly identified as *A. fumigatus* and 4

Download English Version:

https://daneshyari.com/en/article/6124139

Download Persian Version:

https://daneshyari.com/article/6124139

Daneshyari.com