J. Med. Microbiol. — Vol. 51 (2002), 479–483

ISSN 0022-2615

MYCOLOGY

Voriconazole and fluconazole susceptibility of *Candida* isolates

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An adapted NCCLS M27-A method was used to evaluate the activity of voriconazole (VRC) and fluconazole (FLC) against 295 *Candida* isolates collected from 189 patients (including isolates from deep sites). Isolates included 186 *C. albicans*, 54 *C. glabrata*, 27 *C. tropicalis*, 14 *C. parapsilosis*, 6 *C. krusei*, 6 *C. lusitaniae*, 1 *C. lypolytica* and 1 *C. sake*. Forty-two isolates had reduced susceptibility to FLC (MIC >8 mg/L); 83.3% of these had VRC MICs ⩽2 mg/L (9 of 11 *C. albicans*, 18 of 19 *C. glabrata*, 6 of 6 *C. krusei*, 2 of 2 *C. lusitaniae* and 0 of 4 *C. tropicalis*), including 60% of isolates collected from deep-seated infections. These results suggested that in the era of azole resistance, VRC has a promising antifungal activity for serious infections with *Candida* spp., including most species with low susceptibility to FLC and uncommonly isolated species.

Introduction

Invasive candida infection is a devastating illness [1] for which the effectiveness of initial empirical treatment is directly linked to patient outcome. Despite its universally recognised toxicity, amphotericin B was considered to be the cornerstone for successful therapy in disseminated candidosis [2]. The less toxic agent, fluconazole (FLC), offered an attractive alternative in the treatment of candidaemia and various invasive candida infections in non-neutropenic patients [3–5]. Major concerns about reduced FLC efficacy because of the increasing occurrence of *Candida* spp. potentially resistant to azole drugs [6–8] led to the development of new antifungal drugs. Voriconazole (VRC) exhibits fungicidal activity against a broad range of commonly encountered pathogenic yeasts including FLC-resistant species [9–11].

To assess the contemporary clinical value of VRC in the era of resistant yeasts, this study used an adapted NCCLS method to determine the susceptibility of recent *Candida* isolates, and compared the MICs of VRC and FLC.

Received 27 June 2001; revised version accepted 21 Nov. 2001.
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Materials and methods

Clinical *Candida* isolates collected from Feb. 1997 to July 1999 and referred to the L'Hôtel-Dieu de Québec microbiology laboratory for antifungal susceptibility determination were included in the study. Each isolate was from a different patient or represented a distinct infection site or episode in the same patient. *Candida* isolates were categorised as invasive if a sterile or surgical collection procedure was used and classed as superficial in all other situations. *Candida* isolates were subcultured on CHROMagar Candida (CHROMagar, Paris, France) to ensure species purity and for final identification of *C. albicans*. Non-*albicans* isolates were further identified by ID 32C (bioMérieux, Marcy-l'Etoile, France).

Pfizer Central Research Division (Groton, CT, USA) kindly provided voriconazole (UK-109,496) as 99.9% pure powder. Stock solution (1600 μg/ml) was first prepared by dissolving the powder in dimethyl sulphoxide (DMSO). A serial two-fold intermediary dilution was prepared by adding DMSO and RPMI 1640 (No. 04-525; Bio Whittaker, Walkersville, MD, USA) to stock solution according to NCCLS M27-A procedures [12]. After reconstitution with *Candida* suspension, VRC concentration ranged from 0.016 mg/L to 8 mg/L. DMSO concentration was exactly 1% in each well including growth control. Fluconazole (Pfizer Canada, Pointe-Claire, QC, Canada) 2560 mg/L stock solution was prepared by dissolving powder in pure water. RPMI was then used to prepare two-fold dilutions.

FLC final concentrations ranged from 0.125 mg/L to 64 mg/L when reconstituted with yeast suspension. Flat-bottomed microdilution plates (96-well tissue cell cluster, Costar 3599; Cambridge, MA, USA) were used to ensure accurate automatic reading. Wells from columns 1–10 were dispensed with 100 μl of serial dilutions of the respective antifungal drugs; 100 μl of RPMI was dispensed in column 11 to serve as the growth control and 200 μl of RPMI in column 12 for the sterility control. Individual *Candida* suspensions were prepared according to NCCLS M27-A methods and 100 μl of each was dispensed in duplicate in two consecutive rows of the microtitration plate in columns 1–11. After incubation for 48 h at 35°C, a plate shaker (DSG Titertek; Flow Laboratories) was used to achieve a homogeneous turbidity of the growth in each well. Plates were then read with a spectrophotometer (Spectra II; SLT Labinstruments, Austria) set at 492 nm. The MIC was defined as the lowest concentration of the drug that gave a 50% reduction in optical density when compared with the turbidity of the growth control well [13]. In cases where there was discrepancy between two rows of a tested isolate, the higher MIC was recorded. If there was discrepancy of more than one dilution the test was repeated. High off-scale MIC results were converted to the next higher concentration, and low off-scale results to the lowest tested concentration. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were included in each testing run as quality control and MIC results were constantly in the predicted range for both antifungal drugs [14]. GraphPad Prism 3.0 (GraphPad Software, San Diego, CA, USA) was used to test MIC data correlation (Pearson method) and to compare *Candida* spp. susceptibility to VRC and FLC (two-sided χ^2 test). A $p < 0.05$ was defined as statistically significant.

Results and discussion

Table 1 summarises the in-vitro activity of FLC and VRC against 295 *Candida* isolates collected from 189 patients, including 71 isolates (24%) from deep-seated infections. In this experiment, the MIC90 values of FLC and VRC for *Candida* spp. were consistent with data obtained by other groups [15, 16]. MICs for VRC were generally 1 $\log_{10}$ lower than MICs for FLC, indicating that *Candida* isolates that were less susceptibile to FLC still expressed low MICs for VRC [10, 17]. This was especially striking for well-recognised FLC-resistant species *C. glabrata* and *C. krusei* [18, 19]. Previously reported bimodal MIC distribution for *C. tropicalis* was more pronounced for VRC results (Table 2) [20]. This could represent a testing artifact because this effect was less marked when an end-point reading was obtained from growth after the first 24 h (data not shown). Further clinical correlation is needed to evaluate the real significance of this phenomenon. Good correlation was achieved in comparing both antifungal results ($r^2 = 0.315$, $p < 0.0001$), indicating a systematic, proportional and predictable increased VRC activity for *Candida* species. Indeed, FLC-resistant isolates tend to produce proportionally higher VRC MICs, suggesting azole cross-resistance [10, 16, 20, 21].

Based on NCCLS interpretative guidelines, 42 *Candida* isolates (14.2%) expressed reduced FLC susceptibility (MIC >8 mg/L), half of them being highly resistant (MIC ≥64 mg/L) [12]. Furthermore, 13 isolates (61.9%) would not have been identified as FLC-resistant if based on species identification only. This suggests the importance of antifungal susceptibility testing in therapeutic management. Despite reduced susceptibility *in vitro*, increasing FLC daily doses (400–800 mg/day) has proved effective for isolates with MICs of 16 or 32 mg/L [13]. From a clinical point of view, any isolate with an FLC MIC >8 mg/L has to be considered as having a high potential for non-therapeutic response to standard FLC dosage.

Although NCCLS methods can be used to determine antifungal activity of VRC, interpretation criteria have not been defined yet. At steady state, peak and trough

Table 1. In-vitro susceptibilities of *Candida* isolates (mg/L)

Species (number of isolates)	Antifungal agent	MIC50	MIC90	Range	Geometric mean
C. albicans (186)	Fluconazole	0.25	2	≤0.125–>64	5.88
	Voriconazole	≤0.0156	0.25	≤0.0156–>8	0.49
C. glabrata (54)	Fluconazole	8	32	≤0.125–>64	16.5
	Voriconazole	0.5	1	≤0.0156–8	0.70
C. krusei (6)	Fluconazole	64	>64	32–>64	64
	Voriconazole	0.5	2	0.25–2	0.88
C. lipolytica (1)	Fluconazole	2	2	2	2
	Voriconazole	≤0.0156	0.0156	0.0156	0.02
C. lusitaniae (6)	Fluconazole	0.5	64	0.5–64	16.33
	Voriconazole	≤0.0156	0.5	≤0.0156–0.5	0.14
C. parapsilosis (14)	Fluconazole	1	1	0.25–4	1
	Voriconazole	≤0.0156	0.0625	≤0.0156–0.5	0.06
C. sake (1)	Fluconazole	0.25	0.25	0.25	0.25
	Voriconazole	0.5	0.5	0.5	0.5
C. tropicalis (27)	Fluconazole	1	>64	≤0.125–>64	16.48
	Voriconazole	0.25	16	≤0.0156–>8	4.51

Table 2. *Candida* MIC distribution for voriconazole and fluconazole

Species	n	Number (%) of isolates with MIC (mg/L) of													
		0.0156	0.0313	0.0625	0.125	0.25	0.5	1	2	4	8	16	32	64	128
Voriconazole															
C. albicans	186	139	10	9	6	5	3	3	3	3	2	3			
C. krusei	6					1	2	2	1						
C. lipolytica	1	1													
C. lusitaniae	6	3		1		1	1								
C. parapsilosis	14	7	3	3			1								
C. sake	1						1								
C. tropicalis	27	1	5	5	2	2	1		2	1	2	6			
C. glabrata	54	2		6	7	10	16	10		2	1				
Total	295	153 (51.9)	18 (6.1)	24 (8.1)	15 (5.1)	19 (6.4)	25 (8.5)	15 (5.1)	6 (2.0)	6 (2.0)	5 (1.7)	9 (3.1)			
Cumulative %		51.9	58.0	66.1	71.2	77.6	86.1	91.2	93.2	95.2	96.9	100			
Fluconazole															
C. albicans	186				74	69	17	5	5	5			2	3	6
C. krusei	6												2	3	1
C. lipolytica	1								1						
C. lusitaniae	6						4						1	1	
C. parapsilosis	14					2	3	8		1					
C. sake	1					1									
C. tropicalis	27					2	9	8	2	1	1		1		3
C. glabrata	54				1			1	1	10	22	12	3	2	2
Total	295				75 (25.4)	74 (25.1)	33 (11.2)	22 (7.5)	9 (3.1)	17 (5.8)	23 (7.8)	12 (4.1)	9 (3.1)	9 (3.1)	12 (4.1)
Cumulative %					25.4	50.5	61.7	69.2	72.8	78.0	85.8	89.8	92.9	95.9	100

serum concentrations of VRC when given at 200 mg orally twice daily have ranged from 2.1 mg/L to 4.8 mg/L and 1.4 mg/L to 1.8 mg/L, respectively. Blood levels in children were consistent with those observed in adults [22]. VRC pharmacokinetics suggest that adequate serum levels are available to treat infecting fungal organisms with VRC MICs $\leqslant$2 mg/L effectively.

Thirty-five (83.3%) isolates with reduced susceptibility to FLC had VRC MIC $\leqslant$2 mg/L ($p = 0.006$). They included 9 (82%) of 11 *C. albicans* ($p = 0.02$), 18 (95%) of 19 *C. glabrata*, 6 of 6 *C. krusei*, 2 of 2 *C. lusitaniae* and none of 4 *C. tropicalis*. Furthermore, 7% of deep-seated infections were caused by *Candida* isolates with FLC MICs >8 mg/L; interestingly, 60% of these isolates demonstrated low VRC MICs ($p < 0.001$). *Candida* isolates expressing high FLC MICs and low VRC MICs were *C. glabrata* or *C. krusei*. Furthermore, three of nine *C. albicans* in this category were from clinically resistant oropharyngeal candidosis in patients who were HIV-positive.

Unlike *Candida* spp. isolated in the first years of clinical use of FLC, this study investigated the in-vitro susceptibility of isolates collected in recent years, reflecting a more clinically relevant situation with regards to azole resistance and therapeutic options [2, 23–25]. The increasing rate of invasive infection caused by *Candida* isolates that are not susceptible to FLC is a major concern for clinicians [7, 24, 26, 27]. Despite the possibility of cross-resistance, VRC has the advantages of favourable pharmacokinetics and proportionally increased potency and can overcome the therapeutic limitations of FLC.

The results of this study confirm VRC as a promising antifungal triazole with an expanded spectrum of activity against *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. lusitaniae*. They also supply some insight into the in-vitro susceptibility of less common species such as *C. lipolytica* and *C. sake*. In-vitro data, together with its pharmacokinetics, clinical efficacy and safety profile, suggest that VRC is a viable therapeutic choice for empirical therapy in severe candida infections.

This work was supported by Pfizer Canada. Results were presented in May 2000 at the ISHAM meeting in Buenos Aires.

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