

In vitro activity of voriconazole against *Candida* species isolated in Taiwan

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Received 30 September 2003; accepted 9 January 2004

Abstract

The activity of voriconazole was determined against 285 *Candida* species consisting of 53 resistant isolates, 43 susceptible-dose dependent and 189 isolates susceptible to fluconazole. The MIC₅₀ and MIC₉₀ to fluconazole were 8 and 64 mg/l, respectively. The range of minimum inhibitory concentrations (MICs) to voriconazole was from 0.0325 to 2 mg/l and the MIC₅₀ and MIC₉₀ were 0.125 and 0.5 mg/l, respectively. Only 3 of 285 tested isolates had MICs to voriconazole equal to 2 mg/l. A total of 38 isolates, consisted of 3 *Candida albicans*, 5 *Candida krusei*, 7 *Candida tropicalis* and 21 *Candida glabrata*, had MICs \geq 0.5 mg/l to voriconazole. There was correlation between the susceptibility to fluconazole and voriconazole.

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Keywords: *Candida* species; Voriconazole; Fluconazole; Resistance

1. Introduction

In the past decade, there has been a significant increase in the number of nosocomial infections caused by *Candida* species. This is probably the result of alterations in immune status associated with the acquired immunodeficiency syndrome (AIDS) epidemic, cancer chemotherapy, organ and bone marrow transplantation and invasive hospital procedures [1–3]. Infections caused by *Candida* species are becoming important causes of morbidity and mortality in immunocompromised patients. The major issues concerning currently available antifungal drugs include side effects and ineffectiveness against certain fungi. Due to broad prophylactic use and long term treatment with antifungal agents, drug resistance is an important consideration in various fungal infections [4]. During the Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) in 1999, 22 hospitals contributed 660 clinical yeast isolates [5]. Of the 632 isolates tested, 53 (8.4%) were resistant to fluconazole. Approximately 4, 8, 15 and 70% of *Candida albi-*

cans, *Candida glabrata*, *Candida tropicalis* and *Candida krusei* isolates, respectively, were resistant to fluconazole [6].

Several newly developed antifungal agents including voriconazole, candicidas, candins, nikkomycins and lipid amphotericin B have been proved to have good antifungal activity [3,7,8]. The susceptibility of *Candida* species to these recently developed drugs are not well understood. Since both voriconazole and fluconazole are triazole agents, the in vitro activity of voriconazole against different *Candida* species with various susceptibilities to fluconazole was determined in this study.

2. Materials and methods

2.1. Clinical isolates

A total of 660 yeast clinical isolates were collected from 15 April to 15 June in 1999 from 22 hospitals in Taiwan during the TSARY surveillance study [5]. The susceptibility of 632 isolates to fluconazole was determined. From these isolates, the 53 resistant isolates along with 43 fluconazole-susceptible-dose dependent isolates and a

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random selection of 189 from the 536 susceptible isolates of the major *Candida* species were further analysed for their susceptibilities to voriconazole making a total of 285. These isolates consisted of 57 of 237 (24%) *C. albicans*, 51 of 163 (31%) *C. tropicalis*, 11 of 51 (22%) *Candida parapsilosis*, 156 of 156 (100%) *C. glabrata* and 10 of 10 (100%) *C. krusei* collected from TSARY in 1999. A total of 154, 63, 22, 13, 6 and 27 isolates were obtained from urine, sputum, blood, wound, ascites and other sites, respectively.

2.2. Antifungal susceptibility testing

The minimum inhibitory concentration (MIC) to fluconazole or voriconazole of each yeast isolate was determined by in vitro antifungal susceptibility testing according to the guidelines of M27-A published in 1997 by the National Committee of Clinical Laboratory Standards (NCCLS) [9]. Both fluconazole and voriconazole were kindly provided by the Pfizer Central Research. RPMI medium 1640 (31800-022, Gibco BRL) was used for dilution and growth of yeast culture. The final growth of each isolate was measured by a Spectra MAX Plus (Molecular Devices) after incubation at 35 °C for 48 h. The interpretation of susceptibility to fluconazole was according to the guidelines of NCCLS [9]. Isolates with MICs ≥ 64 mg/l, 16–32 mg/l and ≥ 8 mg/l were defined as resistant, susceptible-dose dependent and susceptible to fluconazole, respectively. *C. albicans* (ATCC 90028), *C. krusei* (ATCC 6258) and *C. parapsilosis* (ATCC 22019) were used as control strains. The MICs of 50 and 90% of the total population were defined as MIC₅₀ and MIC₉₀.

2.3. Data analysis

Analysis was performed using Epi Info 6.04 (CDC, Atlanta, GA, USA) [10]. The significance of difference in frequencies and proportions was determined by the chi-square test with Fisher's exact correction.

3. Results

The in vitro susceptibilities to fluconazole and voriconazole of 285 *Candida* species are shown in Table 1. The range of MICs to fluconazole was from 0.125 to 512 mg/l. The MIC₅₀ of fluconazole was 8 mg/l and the MIC₉₀ was 64 mg/l. The 53 fluconazole-resistant isolates were consisted of 7 *C. krusei*, 9 *C. albicans*, 13 *C. glabrata* and 24 *C. tropicalis*. The group of 43 susceptible-dose dependent isolates consisted of 1 *C. parapsilosis*, 3 *C. krusei*, 7 *C. albicans*, 10 *C. tropicalis* and 22 *C. glabrata*. In total, 10 *C. parapsilosis*, 17 *C. tropicalis*, 41 *C. albicans* and 121 *C. glabrata* were susceptible to fluconazole. The range of MICs to voriconazole was from 0.0325 to 2 mg/l. The MIC₅₀ of voriconazole was 0.125 mg/l and the MIC₉₀ was 0.5 mg/l. Of the different *Candida* species tested, *C. parapsilosis* were very susceptible to voriconazole (MICs ≤ 0.065 mg/l). Of the 285 isolates, there were only three fluconazole-resistant *C. glabrata* isolates, one from sputum and two from urine; these had MICs to voriconazole = 2 mg/l. One fluconazole-susceptible-dose dependent *C. glabrata* isolate had a voriconazole MIC = 1 mg/l. In total, 38 isolates had MICs to voriconazole ≥ 0.5 mg/l. These were 3 *C. albicans*, 5 *C. krusei*, 7 *C. tropicalis* and 21 *C. glabrata*. There were 13 of 189 (6.9%) fluconazole-susceptible isolates that had MICs to voriconazole ≥ 0.5 mg/l. Eight of these 13 susceptible isolates had high MIC to fluconazole of 8 mg/l. Furthermore, 12 of 53 (22.6%) fluconazole-resistant isolates and 13 of 43 (30.2%) fluconazole-susceptible-dose dependent isolates had MICs to voriconazole ≥ 0.5 mg/l. Thus, isolates with higher MICs to fluconazole had a tendency to have higher MICs to voriconazole (differences between resistant isolates versus susceptible isolates and susceptible-dose dependent isolates versus susceptible isolates were significant, $P < 0.05$).

4. Discussion

Voriconazole has potent activity against a wide spectrum of both fluconazole-susceptible and resistant clinical

Table 1
Susceptibility to fluconazole and voriconazole of *Candida* species

Susceptibility to voriconazole (mg/l)	Susceptibility to fluconazole															Total	
	Susceptible					Susceptible-dose dependent					Resistant						
	cal	cgl	ctr	cpa	Subtotal	cal	cgl	ctr	cpa	ckr	Subtotal	cal	cgl	ctr	ckr		Subtotal
0.0325	33	4	3	8	48	1	2	7	1	1	12	7	1	11		19	79
0.065	2	16	5	2	25	1		2			3		2	4		6	34
0.125	4	55	4		63	2	1			1	4	2	1	7		10	77
0.25	1	38	1		40	1	10				11		3		3	6	57
0.5	1	8	4		13	2	8	1		1	12		3	2	4	9	34
1							1				1						1
2													3			3	3
Total	41	121	17	10	189	7	22	10	1	3	43	9	13	24	7	53	285

Abbreviation: cal, *Candida albicans*; cgl, *Candida glabrata*; ctr, *Candida tropicalis*; cpa, *Candida parapsilosis*; and ckr, *Candida krusei*.

isolates including *C. albicans*, *C. glabrata*, *C. krusei*, *C. parapsilosis* and *C. tropicalis*. A total of 50% *C. krusei*, 14.7% *C. glabrata*, 13.7% *C. tropicalis*, 5.3% *C. albicans* but no *C. parapsilosis* had MICs to voriconazole ≥ 0.5 $\mu\text{g/ml}$. *C. parapsilosis* was sensitive to voriconazole (MICs ≤ 0.065 mg/l), which is consistent with the observation that it is generally susceptible to fluconazole [6]. Less accumulation of fluconazole in cells and lower affinity of fluconazole to the target enzymes are the mechanisms for the primary resistance of *C. krusei* to fluconazole [11]. *C. krusei*, considered refractory to azoles [3], was also relatively less susceptible to voriconazole. Furthermore, all four isolates with MICs to voriconazole ≥ 1 mg/l were *C. glabrata*, which is also consistent with the observation that *C. glabrata* is considered less susceptible to fluconazole [3]. Though 13 fluconazole-susceptible isolates had MICs to voriconazole ≥ 0.5 mg/l , MICs to fluconazole of 8 of these 13 isolates were high (8 mg/l). Isolates with higher MICs to fluconazole tended to have higher MICs to voriconazole. Thus, resistance to voriconazole may utilise similar mechanisms, which suggests a possibility of cross-resistance between fluconazole and voriconazole. On the other hand, two *C. tropicalis* isolated from blood and urine each with low MICs to fluconazole (2 mg/l) had MICs of 0.5 mg/l voriconazole. This suggests that there may be specific mechanisms for voriconazole resistance in *Candida* species in addition to the general mechanisms mentioned above.

Triazole resistance appears to be a multi-step process [4]. The reason that both fluconazole and voriconazole are triazole agents has been used to explain the development of cross-resistance to fluconazole and voriconazole in human immunodeficiency virus infected children never treated with voriconazole [12]. Thus, cross-resistance between fluconazole and voriconazole in *Candida* species, especially *C. glabrata*, could be a potential problem when voriconazole is widely, frequently, and/or inappropriately used.

Acknowledgements

We would like to thank Pfizer Inc. for supplying fluconazole and voriconazole. We express our appreciation to all 22

participating hospitals for providing the isolates and information relating to these isolates.

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