

Comparison of Antifungal Susceptibilities to Fluconazole and Voriconazole of Oral *Candida glabrata* Isolates from Head and Neck Radiation Patients

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The antifungal susceptibilities of 79 oral *Candida glabrata* isolates to fluconazole and voriconazole were compared. The MICs at which 90% of the isolates tested were inhibited were 1 µg of voriconazole/ml and 32 µg of fluconazole/ml. Oral *C. glabrata* isolates for which the fluconazole MICs are elevated are commonly those for which the voriconazole MICs are elevated, but these increases may be transient for voriconazole, as they are for fluconazole.

Oropharyngeal candidiasis (OPC) patients undergoing radiation therapy who were infected with *Candida* species other than *Candida albicans*, particularly *Candida glabrata*, have been previously identified (11–13). *C. glabrata* is inherently more resistant to fluconazole than *C. albicans* and appears to develop resistance rapidly (6, 9, 15, 16).

Voriconazole is a new broad-spectrum triazole antifungal drug that, like fluconazole, disrupts the fungal membrane by inhibition of the cytochrome P-450-dependent 14- α -lanosterol demethylase, preventing the conversion of lanosterol to ergosterol (4). It has been shown to be active against *Candida* species and other molds, including dermatophytes, and can be administered orally or intravenously (4, 10). Numerous surveillance studies have demonstrated that, in general, voriconazole has good in vitro activity against *C. glabrata*, with the MIC at which 90% of the isolates tested are inhibited (MIC₉₀) being 1 µg/ml. The vast majority of isolates from these studies were systemic isolates, primarily from the bloodstream (2, 8, 9). Voriconazole may be a suitable alternative for treating OPC in head and neck radiation patients whose infections are due to *C. glabrata* strains which have developed resistance to fluconazole, but few data have been presented on the susceptibility to voriconazole of *C. glabrata* isolates from the oral cavity, including those causing OPC. The purpose of this study was to compare patterns of antifungal susceptibility to both fluconazole and voriconazole of oral *C. glabrata* isolates from patients receiving radiation treatment for head and neck cancer, including those of isolates that were resistant to fluconazole.

Oral isolates were obtained from 14 patients undergoing radiation therapy for head and neck cancer who were participating in a study to evaluate whether fluconazole could prevent OPC in patients with oral colonization. All patients colonized

with *C. albicans* at baseline were given fluconazole for the duration of their radiation treatment. Patients who developed OPC were treated with fluconazole as well. Oral samples were obtained weekly from patients. Two types of samples were taken, i.e., swab samples and swish samples. If cultures of both swab and swish samples were positive, isolates were taken from each. Multiple colonies were taken from a single plate if there appeared to be phenotypic differences. Isolates were considered unique if they were from different patients or were from samples taken at different times from the same patient or if different karyotypes of isolates from the same patient were displayed based on electrophoretic karyotyping using contour-clamped homogeneous electric field gel electrophoresis (1). Each isolate was evaluated by germ tube testing, plated on CHROMagar *Candida* medium (CHROMagar Company, Paris, France) (7), and subjected to metabolic evaluation by use of an API 20C system (Biomérieux, Marcy-l'Étoile, France). The isolates were then subcultured and incubated at 37°C for 24 h on Sabouraud dextrose agar to ensure purity and viability. Antifungal susceptibility testing was conducted according to methods outlined in NCCLS document M27-A2 at the Fungus Testing Laboratory at the University of Texas Health Science Center (5).

Seventy-nine unique *C. glabrata* isolates from 14 head and neck radiation patients were evaluated. Five patients developed *C. glabrata*-associated OPC, and nine patients displayed *C. glabrata* colonization. Twenty-nine different karyotypes were found among these isolates. MICs of both fluconazole and voriconazole were determined at 48 h for each isolate, as presented in Table 1. The MIC₉₀s of voriconazole and fluconazole were determined to be 1 and 32 µg/ml, respectively. The voriconazole MICs were >1 µg/ml for 8.9% of the isolates tested. The average voriconazole MIC for isolates susceptible to fluconazole (MIC, ≤8 µg/ml) was 0.257 µg/ml, the average voriconazole MIC for isolates that showed dose-dependent susceptibility to fluconazole (MIC, 16 to 32 µg/ml) was 0.965

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TABLE 1. Median fluconazole and voriconazole MICs at 48 h for 79 unique *C. glabrata* strains from 14 patients receiving radiation for head and neck tumors

Patient	No. of isolates	No. of unique isolates ^a	Median MIC (μg/ml) at 48 h ^b		FLU resistance ^c	VORI susceptibility ^d	Patient	No. of isolates	No. of unique isolates ^a	Median MIC (μg/ml) at 48 h ^b		FLU resistance ^c	VORI susceptibility ^d
			FLU	VORI						FLU	VORI		
10	5	1	32	1	DD-S				<64	4	R	NS	
12	10	4	4	0.125	S				8	0.25	S		
			8	0.125	S				8	0.125	S		
			4	0.125	S				16	0.25	DD-S		
			4	0.125	S				4	0.03	S		
13	1	1	32	2	DD-S	NS	21	26	16	16	0.5	DD-S	
14	2	1	16	1	DD-S				16	0.25	DD-S		
								8	0.125	S			
15	3	1	16	0.25	DD-S				16	1	DD-S		
18	43	23	16	0.5	DD-S				16	0.5	DD-S		
			8	0.25	S				8	0.5	S		
			8	0.125	S				8	0.125	S		
			4	0.125	S				8	0.5	S		
			8	0.5	S				8	0.125	S		
			8	0.25	S				32	0.5	DD-S		
			4	0.125	S				32	1	DD-S		
			8	0.25	S				32	0.5	DD-S		
			8	0.5	S				16	0.5	DD-S		
			8	0.25	S				8	0.5	S		
			8	0.5	S				8	0.125	S		
			8	0.25	S				16	0.5	DD-S		
			4	0.125	S				8	1	S		
			4	0.125	S				8	0.5	S		
			8	0.25	S				8	0.5	S		
			8	0.25	S				8	0.5	S		
			4	0.53	S				16	0.5	DD-S		
			4	1	S				8	0.5	S		
32	0.5	DD-S				8	0.06	S					
4	0.5	S				8	0.5	S					
8	1	S				8	0.5	S					
4	0.5	S				8	0.125	S					
4	0.5	S				8	0.5	S					
4	0.25	S				8	0.25	S					
>64	4	R	NS			4	0.25	S					
64	2	R	NS			8	0.25	S					
20	27	14	16	0.5	DD-S				8	1	S		
			8	0.25	S				8	0.06	S		
			32	0.5	DD-S				32	0.03	DD-S		
			16	1	DD-S								
			16	2	DD-S	NS							
			16	0.5	DD-S								
27	4	2	16	1	DD-S	NS	27	4	2	16	1	DD-S	
			32	2	DD-S					8	1	S	
			32	0.5	DD-S								
			32	2	DD-S	NS							
30	3	1	32	1	DD-S	NS	30	3	1	0.125	0.015	S	
			4	0.25	S								
31	5	1	32	2	DD-S	NS	31	5	1	32	2	DD-S	
			4	0.25	S								

^a Isolates were considered unique if they were from different patients or were from samples taken at different times from the same patient or if different karyotypes of isolates from the same patient were displayed based on DNA karyotyping.
^b Data are given for each unique isolate. FLU, fluconazole; VORI, voriconazole.
^c S, isolate was susceptible; DD-S, isolate showed dose-dependent susceptibility; R, isolate was resistant. Designations are based on breakpoints discussed in the text.
^d NS, isolates for which the MICs are over the proposed voriconazole susceptibility breakpoint of ≤1 μg/ml (14).

μg/ml, and the average voriconazole MIC for isolates resistant to fluconazole (MIC, >32 μg/ml) was 3.333 μg/ml. Of particular interest are the data collected for the isolates from patients 18 and 20. These two patients developed OPC and remained on fluconazole for the duration of their radiation therapy. The fluconazole MICs for both isolates showed significant increases over the course of treatment (4.0 to ≥64 and 8.0 to ≥64 μg/ml for isolates from patients 18 and 20, respectively). Voriconazole MICs showed corresponding increases when voriconazole was tested against the same isolates (0.15 to

4 and 0.5 to 4 μg/ml for isolates from patients 18 and 20, respectively). Interestingly, the MICs of fluconazole for isolates from patient 20 dropped back to pretreatment levels by 1 month after fluconazole was discontinued and were still at decreased levels 3.5 months later. The MICs of voriconazole displayed a similar pattern. The data from this in vitro study of oral isolates is in agreement with studies of systemic isolates in that, in general, voriconazole has good activity against *C. glabrata*, with an MIC₉₀ of 1.0 μg/ml (2, 8, 9). Pfaller et al. have suggested voriconazole

breakpoints based on pharmacodynamic analyses. They proposed a MIC of ≤ 1.0 $\mu\text{g/ml}$ as the breakpoint for susceptibility to voriconazole (9).

Data from our study also show that *C. glabrata* isolates for which the fluconazole MICs were significantly elevated were also those for which the voriconazole MICs were elevated. Tests of *C. glabrata* isolates from two patients (patients 18 and 20) show that the development of increases in fluconazole MICs over time is mirrored by substantial increases in voriconazole MICs over the same period. However, MIC elevations of both drugs appeared to be transient for the isolates from patient 20, suggesting that the development of resistance was not stable. Fluconazole resistance in *C. albicans* has been shown to be transient in other patient populations, and susceptibility returns when the medication is discontinued (3). It appears that constant exposure to both drugs is necessary to maintain resistance in some strains of *C. glabrata* as well.

On initial examination, these data may imply that voriconazole would not be effective in the treatment of infections caused by *C. glabrata* isolates for which the fluconazole MICs are elevated, even though no patient in this study received voriconazole. However, peak levels in blood achieved by oral dosing with voriconazole (200 to 300 mg twice a day) range from 2 to 5 $\mu\text{g/ml}$ and fall within the MICs found for the isolates from our patients (10). Also, this in vitro comparison provides no information about our hypothesis in a clinical setting. Therefore, clinical trials need to be performed to evaluate the efficacy of voriconazole against *C. glabrata* oral infections.

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