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In Vitro Activities of Voriconazole (UK-109,496) against Fluconazole-Susceptible and -Resistant *Candida albicans* Isolates from Oral Cavities of Patients with Human Immunodeficiency Virus Infection

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The susceptibility of *Candida albicans* to a new antifungal triazole, voriconazole (UK-109,496), was investigated in 105 isolates obtained from the oral cavities of patients with human immunodeficiency virus (HIV) infection to study this drug's activity against fluconazole-susceptible and -resistant isolates. MICs were determined by a broth microdilution technique according to document M27-T from the National Committee for Clinical Laboratory Standards and by using a broth microdilution technique and a synthetic high-resolution medium. These antifungal susceptibility testing methods showed high levels of agreement (93% for fluconazole and 86% for voriconazole). Data from in vitro studies showed that voriconazole has good activity against fluconazole-susceptible and -resistant *C. albicans* isolates; the MICs at which 90% of all isolates were inhibited were 0.19 to 0.39 μg/ml. We found that for isolates for which fluconazole MICs were high, voriconazole MICs were proportionally higher than those for fluconazole-susceptible *C. albicans* ($P < 0.001$). Pretreatment isolates from six patients with fluconazole-refractory esophageal candidiasis were included in the study. For these isolates the MICs were ≤0.39 μg/ml, and all patients responded to voriconazole. These results suggest that voriconazole is effective even in the treatment of fluconazole-refractory esophageal candidiasis and should be studied further to determine its clinical relevance in patients with HIV infection.

Voriconazole (UK 109,496) is a new wide-spectrum triazole with activity against *Candida* spp., *Aspergillus* spp., and *Cryptococcus neoformans*. It is more potent than fluconazole against *Candida* spp. and other fungi in vitro (3, 7) as well as in animal models (14). The excellent activity of this agent against *Candida* spp. suggests that the drug may be effective for therapy of *Candida* infections in immunocompromised patients. Oropharyngeal and esophageal candidiasis are the most frequent fungal infections in patients with human immunodeficiency virus (HIV) infection. Fluconazole has become one of the preferred antifungal agents for the treatment of these infections, but the emergence of in vitro and clinical resistance to fluconazole has focused attention on the efficacy of alternative drugs. In the present study, we used the broth microdilution procedure of the National Committee for Clinical Laboratory Standards (NCCLS) and the broth microdilution technique recommended by Pfizer Clinical Research to compare the MICs of voriconazole and fluconazole for 105 *C. albicans* isolates from the oral cavities of HIV-infected patients (9, 10).

MATERIALS AND METHODS

Antifungal drugs. Voriconazole (UK-109,496) and fluconazole were obtained as standard powders (Pfizer Central Research, Sandwich, United Kingdom). Voriconazole powder was dissolved in dimethyl sulfoxide (D-5879; Sigma), and fluconazole was dissolved in sterile distilled water. A stock solution of 1,000 μg/ml was obtained for each drug.

Isolates. One hundred five isolates of *C. albicans* were used in the study. All of the isolates were recovered from the oral cavities of HIV-infected patients. Each strain represented a unique isolate from a patient. Identification of *Candida* isolates was done by using routine microbiological techniques and the API20Caux system (Biomérieux, Freiburg, Germany). After final identification, isolates were stored at −20°C in sterile skim milk until susceptibility testing was performed. In order to obtain isolates of *C. albicans* with different patterns of in vitro susceptibility to fluconazole, we included isolates for which fluconazole MICs ranged from 0.048 to ≥100 μg/ml. In this study, isolates from six patients taken before and during treatment with voriconazole for (fluconazole-resistant) esophageal candidiasis (protocol 150-303; Pfizer Central Research) were included as well. All isolates were tested for their susceptibilities to fluconazole and voriconazole twice, on separate occasions, by a broth microdilution method, according to the recommendations provided by the NCCLS in protocol M27-T (9), and by a broth microdilution method with high-resolution (HR) medium which has been studied for in vitro testing of fluconazole (10). In order to define isolates of *C. albicans* that were fluconazole susceptible and resistant, we chose a preliminary fluconazole resistance breakpoint of ≥25 μg/ml, which was based on earlier experiences with the HR medium (13, 15). Isolates for which fluconazole MICs were <25 μg/ml were considered not resistant, and isolates for which fluconazole MICs were ≥25 μg/ml were considered resistant.

Three reference strains, *C. albicans* ATCC 90028, ATCC 90029, and Y0119, were included in the experiments.

Susceptibility testing. A broth microdilution test (M27-T-micro) was performed by following the guidelines of NCCLS document M27-T, with the spectrophotometric method for inoculum preparation (9). An inoculum of 0.5×10^3 to 2.5×10^3 cells per ml and RPMI 1640 medium buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) (M1254; Sigma) was used. Yeast inocula (100 μl) were added to each well of microdilution trays; each well contained 100 μl of the antifungal agent at double strength. The final concentrations of fluconazole and voriconazole ranged from 0.048 to 100 μg/ml, which differ from the scheme recommended in NCCLS document M27-T. The trays were incubated in air at 35°C. Drug-free and yeast-free controls were included. The MIC was defined as that concentration of the drug that produced an 80% reduction of turbidity compared with that of the drug-free control.

A broth microdilution method with HR medium (HR-micro) was performed according to earlier suggestions, with the following modifications: (i) 0.2 M phosphate buffer was used instead of 0.165 M MOPS buffer, and (ii) a technique similar to the microdilution test in the M27-T protocol was used instead of the broth macrodilution procedure (10). An inoculum of 1×10^3 to 2×10^3 yeast cells per ml was mixed in synthetic HR medium (CM845; Oxoid, Basingstoke, United Kingdom) which was prepared with 0.2 M phosphate buffer, pH 7.2. Yeast inocula (100 μl) were added to each well of microdilution trays; each well contained 100 μl of the antifungal agent at double strength. Separate dilution series of all antifungal agents were made from the stock solution by using the HR medium. The final concentrations ranged from 0.02 to 100 μg/ml for fluconazole

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TABLE 1. MICs of fluconazole and voriconazole for *C. albicans* isolates from patients infected with HIV and exhibiting mucocutaneous candidiasis

Antifungal agent, test method, and isolate group (no. of isolates)	MIC (μg/ml) at:					
	24 h			48 h		
	Range	50%	90%	Range	50%	90%
Fluconazole						
M27-T-micro	≤0.048–≥100	0.39	25	0.09–≥100	1.56	≥100
HR-micro	≤0.02–≥100	0.19	12.5	0.09–≥100	1.56	≥100
Voriconazole						
M27-T-micro (105)	≤0.048–3.12	0.048	0.19	≤0.048–3.12	0.09	0.78
Fluconazole MIC, <25 μg/ml (75)				≤0.048–0.78	≤0.048	0.19
Fluconazole MIC, ≥25 μg/ml (30)				0.09–3.12	0.39	1.56
HR-micro (105)	≤0.02–0.78	0.02	0.09	≤0.02–6.25	0.02	0.39
Fluconazole MIC, <25 μg/ml (74)				≤0.02–0.39	≤0.02	0.39
Fluconazole MIC, ≥25 μg/ml (31)				≤0.02–6.25	0.39	1.56

and voriconazole. The trays were incubated in air at 35°C. Drug-free and yeast-free controls were included. The MIC endpoints were determined visually by recording the lowest concentration of the antifungal agent that prevented the appearance of visible growth.

Discrepancies among MIC endpoints of no more than 2 dilutions were used to calculate the percent agreement.

The significance of the differences in the distributions of voriconazole MICs between isolates which were considered fluconazole resistant and fluconazole susceptible was determined by the unpaired *t* test. A *P* value of <0.01 was considered to show a statistically significant difference.

RESULTS

All yeast isolates produced detectable growth after 48 h of incubation, but not after 24 h of incubation. Three *C. albicans* isolates produced no detectable growth with M27-T-micro, and eight *C. albicans* isolates produced no detectable growth with HR-micro.

Table 1 summarizes the in vitro susceptibilities of the 105 *C. albicans* isolates to fluconazole and voriconazole as measured by M27-T-micro and HR-micro. Only data recorded after 48 h of incubation were used for the final analysis.

The MICs for the three control organisms were within the expected range for fluconazole with both methods: 0.19 to 0.78 μg/ml for *C. albicans* ATCC 90028 and ATCC 90029 and 12.5 to 25 μg/ml for *C. albicans* Y0119. Voriconazole MICs (both methods) were ≤0.09 μg/ml for *C. albicans* ATCC 90028 and ATCC 90029 and 0.19 to 0.39 μg/ml for *C. albicans* Y0119.

HR-micro yielded overall results (within ±2 $\log_2$ dilutions) that were comparable to those with M27-T-micro at 48 h (93% agreement for fluconazole and 86% agreement for voriconazole). The distribution of MICs between the two methods showed no statistically significant differences ($P > 0.01$) between the antifungal agents.

Fluconazole. As determined by M27-T-micro, 75 isolates were not fluconazole resistant (MIC < 25 μg/ml) and 30 isolates were fluconazole resistant (MIC ≥ 25 μg/ml). Similarly, as determined by HR-micro, 74 isolates were not fluconazole resistant (MIC < 25 μg/ml) and 31 isolates were fluconazole resistant (MIC ≥ 25 μg/ml).

Voriconazole. Figure 1A and B show the distribution of MICs for voriconazole versus fluconazole for 105 clinical isolates of *C. albicans* when tested by M27-T-micro and HR-micro, respectively. Voriconazole MICs ranged from ≤0.048 to 3.12 μg/ml with M27-T-micro and from ≤0.02 to 6.25 μg/ml with HR-micro, with the MICs at which 50% (MIC_{50}) and 90%

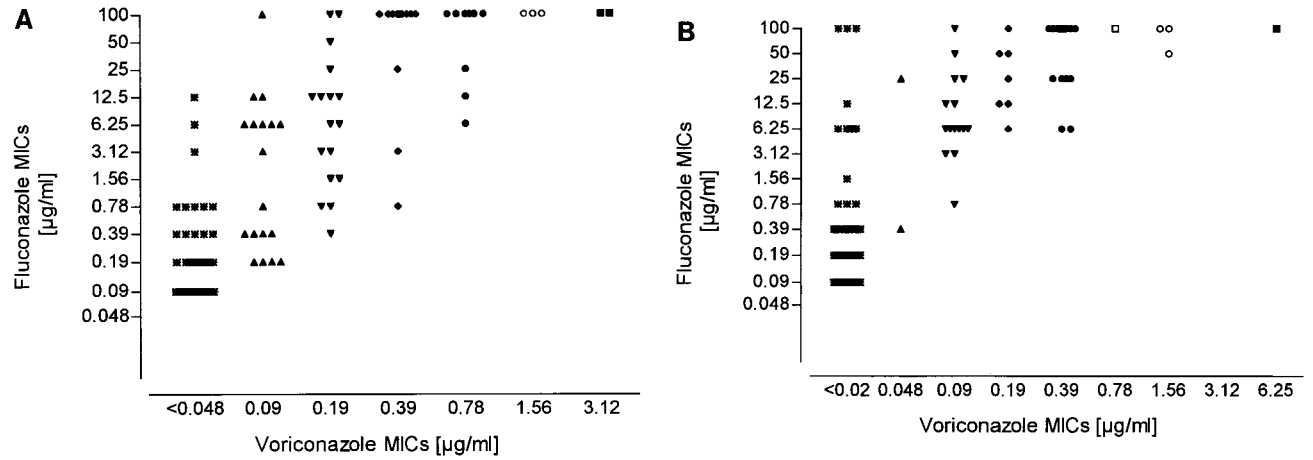

FIG. 1. Scatter plots of in vitro susceptibilities to voriconazole versus fluconazole for 105 *C. albicans* isolates tested by M27-T-micro (9) (A) and HR-micro (15) (B). Each datum point represents one experiment. (A) ✱, $n = 40$; ▲, $n = 18$; ▼, $n = 17$; ◆, $n = 14$; ●, $n = 11$; ○, $n = 3$; ■, $n = 2$. (B) ✱, $n = 62$; ▲, $n = 2$; ▼, $n = 15$; ◆, $n = 7$; ●, $n = 15$; □, $n = 1$; ○, $n = 3$; ■, $n = 1$.

(MIC_{90}) of the isolates were inhibited being 0.02 to 0.048 and 0.19 to 0.39 μg/ml, respectively. Voriconazole MICs ranged from ≤0.048 to 0.78 μg/ml (M27-T-micro) and ≤0.02 to 0.39 μg/ml (HR-micro) for the non-fluconazole-resistant isolates. For the fluconazole-resistant isolates, voriconazole MICs ranged from 0.09 to 3.12 μg/ml (M27-T-micro) and ≤0.02 to 6.25 μg/ml (HR-micro). This trend of higher voriconazole MICs in fluconazole-resistant *C. albicans* isolates was statistically significant ($P < 0.001$) regardless of the method used.

DISCUSSION

The results of the present study demonstrate an excellent level of agreement at 48 h between HR-micro and M27-T-micro for *C. albicans* isolates. The level of agreement between these two methods was good for fluconazole (93%) and somewhat lower for voriconazole (86%). Therefore, both methods may be suitable for in vitro testing of these antifungal agents. However, the definite breakpoint for resistance is still controversial for antifungal susceptibility testing methods and probably depends on the individual method used. According to earlier reports, a MIC of ≥25 μg/ml for fluconazole established with HR-micro corresponds with clinical failure (13). Data on in vitro testing of voriconazole are still rare, and breakpoints still need to be defined. Levels of voriconazole in plasma after application of 400 mg per day are expected to be from 1 to 4 μg/ml. Whether MICs higher than 6.25 μg/ml might reflect in vitro resistance to this agent needs to be substantiated by further studies.

This study confirms recent data that suggest that voriconazole is much more potent against *C. albicans* than is fluconazole in vitro (3). In testing highly fluconazole-resistant *C. albicans* isolates (MIC ≥ 100 μg/ml), we found that voriconazole MICs were in the range of ≤0.02 to 6.25 μg/ml (0.09 to 3.12 μg/ml with M27-T-micro and ≤0.02 to 6.25 μg/ml with HR-micro) (Fig. 1). We found that for isolates for which fluconazole MICs were high, voriconazole MICs were proportionally higher than those for non-fluconazole-resistant *C. albicans* isolates (MIC < 25 μg/ml), which may indicate cross-resistance. Barchiesi et al. reported similar results for the triazoles itraconazole and D0870 against fluconazole-susceptible and -resistant *C. albicans* isolates in HIV-infected patients (1, 2).

The six patients who were treated with oral voriconazole (200 mg twice a day) for fluconazole-resistant esophageal candidiasis responded to this agent, and the MICs for the corresponding pretreatment isolates were ≤0.39 μg/ml (data not shown). MICs of ≥3.12 μg/ml were found only for isolates from patients treated with voriconazole. These findings suggest that reduced susceptibility to this drug may develop during treatment, as it does with fluconazole (8, 11). However, more comprehensive studies are needed before a correlation between in vitro testing and in vivo outcome can be made for this new agent. Failure of antifungal therapy with fluconazole, which correlates with in vitro data, has been described repeatedly for mucocutaneous candidiasis but not for invasive *Candida* infections (4, 8, 11–13). Therefore, other factors may also play a role in the efficacy of an antifungal agent for the therapy of fungal infections.

Reduced susceptibility to *C. albicans* and cross-resistance between azole antifungals appears to be common, probably because all azoles have the same mechanism of action (16). The clinical consequences of these in vitro findings need to be evaluated, but recent data and our results with voriconazole indicate that other azoles are still effective for patients who do not respond well to fluconazole (5, 6).

It can be concluded from in vitro data presented in this study that voriconazole has excellent activity against *C. albicans*, even against fluconazole-resistant isolates. The procedures for antifungal susceptibility testing recommended by the NCCLS and Pfizer Central Research showed high levels of agreement for testing fluconazole and voriconazole in vitro. Preliminary data indicate good clinical efficacy even in fluconazole-refractory esophageal candidiasis and warrant further study to determine the clinical utility of this agent in HIV-infected patients.

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