In Vitro Activity of a New Antifungal Triazole, D0870, against Candida albicans Isolates from Oral Cavities of Patients Infected with Human Immunodeficiency Virus

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We investigated the in vitro activity of a new antifungal triazole, D0870, against 100 Candida albicans isolates from the oral cavities of patients infected with human immunodeficiency virus by using a broth macrodilution method following the recommendations provided by the National Committee for Clinical Laboratory Standards (document M27-P). All of the isolates were chosen from C. albicans isolates already tested for fluconazole susceptibility by the procedure of the National Committee for Clinical Laboratory Standards. Fifty isolates were considered fluconazole susceptible (MICS, ≤4 μg/ml), and 50 isolates were considered fluconazole resistant (MICS ≥8 μg/ml). The in vitro data demonstrated that D0870 had good activity against isolates tested; for 90% of all strains of C. albicans, MICs were 0.5 μg/ml. However, the D0870 MICs for the fluconazole-susceptible isolates were lower than those for the fluconazole-resistant isolates; MICs for 50 and 90% of the isolates tested were ≤0.0078 and 0.06 μg/ml, respectively, for fluconazole-susceptible isolates and 0.25 and 2 μg/ml, respectively, for fluconazole-resistant isolates (P < 0.001). Our data suggest that this new triazole could represent a valid alternative in the treatment of oral candidiasis in human immunodeficiency virus-infected patients.

D0870 is a new antifungal triazole that is the (+) enantiomer of ICI 195,739. D0870 has been reported in the literature to have good in vitro activity against different species of yeasts, molds, and dimorphic fungi (4, 8, 10, 21). No data are available about its in vitro activity against Candida albicans isolates from the oral cavities of human immunodeficiency virus (HIV)-infected patients. In the last few years the development of fluconazole-resistant isolates of C. albicans in these patients has been reported (3, 5, 11, 15, 19). The lack of standardized methods for in vitro antifungal susceptibility testing presents difficulties in recognizing isolates with reduced susceptibilities to the antifungal agents (12, 13). The Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards (NCCLS) has recently proposed a broth macrodilution reference method for the susceptibility testing of yeasts (9). In the present study, we investigated the in vitro activity of D0870 against 100 C. albicans isolates from the oral cavities of HIV-infected patients by using the proposed NCCLS procedure.

MATERIALS AND METHODS

Drug. D0870 (Zeneca Pharmaceuticals, Macclesfield, United Kingdom) was obtained as a standard powder. A stock solution of 5,000 μg/ml was prepared in polyethylene glycol 400 (Union Carbide, Danbury, Conn.) with the aid of heating at 75°C for 30 min.

Isolates. One hundred isolates of C. albicans recovered from the oral cavities of HIV-infected patients were used in the study. Each strain represented a unique isolate from a patient. All of the strains were chosen from among C. albicans isolates already tested for fluconazole susceptibility by the NCCLS procedure (9). In order to obtain isolates of C. albicans with different patterns of in vitro susceptibility to fluconazole, we included 50 isolates for which the fluconazole MICs were ≤4 μg/ml, which were considered susceptible (group A), and 50 isolates for which the fluconazole MICs were ≥8 μg/ml, which were considered resistant (group B) (Fig. 1). All of the isolates were tested for susceptibility to D0870 in a blind and random fashion by a broth macrodilution method following the recommendations provided by NCCLS (9). Two reference strains, C. albicans ATCC 90029, and Candida krusei 93-1380 (an isolate from the collection of the Fungus Testing Laboratory, Department of Pathology, University of Texas Health Science Center at San Antonio), were included in each run of the experiments.

Susceptibility testing procedure. In the present study, RPMI 1640 (American Biorganics Inc., Niagara Falls, N.Y.) medium with L-glutamine without sodium bicarbonate and buffered at pH 7 with morpholino propane sulfonic acid (0.165 M; 46.5 g/liter) was used. Drug dilutions were prepared at 10 times the strength of the final drug concentration (160 to 0.078 μg/ml) by a serial drug dilution schema for minimizing systematic pipetting errors. The drugs were stored at −70°C until they were used. The yeast inocula were spectrophotometrically prepared as described in the NCCLS document (9). Confirmation of the final inoculum size (in CFU per milliliter) was determined for all test and control organisms by subcultures on Sabouraud dextrose agar. Yeast inocula (0.9 ml) were added to 10 times the strength of the final drug concentrations in polystyrene plastic tubes (12 by 75 mm; Falcon 2054; Becton Dickinson, Lincoln Park, N.J.), bringing the drug dilutions to the final test
albicans. D0870 MICs were 0.06 and 0.125 respectively (Table 1). D0870 MICs ranged from 0.0078 to 16 μg/ml, each tube was vortexed, and its turbidity was compared with that of the drug-free growth control tube. The MIC was defined as the lowest concentration which resulted in a visual turbidity of less than or equal to 80% inhibition when compared with that produced by the growth control (0.2 ml of growth control plus 0.8 ml of uninoculated RPMI 1640) (9).

**Statistical analysis.** The significance of the differences in the D0870 MIC distributions between isolates of the A and B groups was determined by the Mann-Whitney U test.

**RESULTS**

All of the organisms tested produced detectable growth after 48 h of incubation. The inoculum sizes of the working suspensions fell within the range reported by the NCCLS document (0.5 × 10^3 to 2.5 × 10^7 CFU/ml) for all pathogenic yeasts and the two reference isolates of Candida spp. (9). D0870 MICs for C. albicans ATCC 90029 and C. krusei 93-1380 were ≤0.0078 and 2 μg/ml, respectively (Table 1). Figure 2 reveals the distribution of D0870 MICs for the 100 clinical isolates of C. albicans. D0870 MICs ranged from ≤0.0078 to 2 μg/ml, with the MICs for 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates being 0.06 and 0.5 μg/ml, respectively. However, there was a substantially different distribution of D0870 MICs between the A and B groups of C. albicans isolates (Fig. 2). D0870 MICs ranged from ≤0.0078 to 0.125 μg/ml for group A isolates, with MIC_{50} and MIC_{90} of ≤0.0078 and 0.06 μg/ml, respectively (Table 1). D0870 MICs ranged from ≤0.0078 to 2 μg/ml for group B isolates, with MIC_{50} and MIC_{90} of 0.25 and 2 μg/ml, respectively (Table 1). When the Mann-Whitney U test was applied to determine the distribution of D0870 MICs for the two groups of isolates, a statistically significant difference was found (P <0.001).

**DISCUSSION**

In the present study we investigated the in vitro activity of a new antifungal triazole, D0870, against C. albicans isolates from the oral cavities of HIV-infected individuals. Although the in vitro method for susceptibility testing of yeasts proposed by NCCLS was developed only for fluconazole, amphotericin B, ketoconazole, and fluconazole, we applied the same procedure for testing D0870. Recently, Peng and Galgiani (10) studied the effects of different assay conditions on the activity of D0870 against seven species of fungi using the broth macrodilution method proposed by NCCLS (9). Interestingly, they found that test conditions in keeping with NCCLS guidelines indeed distinguished isolates of C. albicans and Cryptococcus neoformans as susceptible when compared with the results for C. krusei and Torulopsis glabrata. Our findings corroborate those reported by those investigators. In testing 50 isolates of

**TABLE 1.** D0870 MICs for 100 clinical isolates of C. albicans and two reference control organisms

<table>
<thead>
<tr>
<th>Isolate (no.)</th>
<th>MIC (μg/ml)</th>
<th>Range</th>
<th>50%</th>
<th>90%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. albicans, group A (50)</td>
<td>≤0.0078-0.125</td>
<td>≤0.0078</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td>C. albicans, group B (50)</td>
<td>≤0.0078-2</td>
<td>0.25</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>C. albicans ATCC 90029&lt;sup&gt;a&lt;/sup&gt;</td>
<td>≤0.0078</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. krusei 93-1380&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Both of the reference control organisms were tested in 25 different sets of experiments.
fluconazole-susceptible *C. albicans*, we found that D0870 MIC$_{50}$s and MIC$_{90}$s were ≤0.0078 and 0.06 μg/ml, respectively, which are values very close to those reported by Peng and Galgiani (10). In addition, we found that for isolates for which fluconazole MICs were high, D0870 MICs were proportionally higher. The 10 isolates of the present series for which the D0870 MIC was 2 μg/ml were highly resistant to fluconazole; fluconazole MICs were ≥64 μg/ml. Interestingly, the MIC of 2 μg/ml for D0870 is very close to the susceptibility patterns reported by Peng and Galgiani (10) for *C. krusei* and *T. glabrata*, two species with frequently reported in vitro as well in vivoazole resistance (1, 18, 20). Our results confirm those previously reported from the testing of itraconazole against fluconazole-susceptible and -resistant *C. albicans* isolates in this group of patients (2). Some of the isolates tested in the present study were previously tested with itraconazole. For those isolates for which fluconazole MICs were high, MICs of itraconazole were high as well.

This phenomenon may be due to the fact that all azoles may have same mechanism of action. Thus far, two mechanisms for the reduced susceptibility of *C. albicans* toazole antifungal agents have been described. One is due to changes in the membrane sterol composition which cause a reduced level of permeability of the *C. albicans* membrane to the drugs; the second is due to a mutation of sterol 14α-demethylase, a cytochrome P450 enzyme, that results in decreased binding affinity for the azole drugs (6, 7, 14, 16). Recently, Vanden Bossche et al. studied an isolate of *T. glabrata* that was clinically resistant to fluconazole. They showed that the reduced level of susceptibility to fluconazole was due either to lower fluconazole uptake or to the increased level of P450-dependent ergosterol synthesis of the fluconazole-resistant isolate compared with that of the parental strain. Only the increased ergosterol synthesis caused itraconazole cross-resistance (17). The clinical consequences of this in vitro phenomenon are unclear. It is unknown whether treatment of patients with oral candidiasis caused by fluconazole-resistant *C. albicans* isolates with another azole fails or is resolved by an increase in the azole dosage because of the lack of clinical trials. In addition, it is difficult to correlate the in vitro data with the clinical outcome in these patients, in whom the failure of therapy for oral candidiasis may be due either to the presence of *C. albicans* isolates resistant to the drugs or to the immunodeficient state of the host. In conclusion, our findings indicate that D0870 could represent a valid alternative in the treatment of oral candidiasis in HIV-infected patients. It remains to be seen whether concentrations of this new antifungal triazole of 2 μg/ml are potentially therapeutic since there are no data available on the concentrations of the drug in the tissue and serum of humans. Additional in vivo and in vitro studies are warranted to further elucidate the potential antifungal activity of this compound in HIV-infected patients.

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REFERENCES

