In Vitro Study of Candida tropicalis Isolates Exhibiting Paradoxical Growth in the Presence of High Concentrations of Caspofungin

G. Sóczó,1 G. Kardos,1 I. Varga,2 B. Kelentey,2 R. Gesztelyi,3 and L. Majoros1*

Department of Medical Microbiology, Medical and Health Science Center, University of Debrecen, Hungary;1; Faculty of Dentistry, Medical and Health Science Center, University of Debrecen, Hungary;2; and Department of Pharmacology and Pharmacodynamics, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary3

Received 5 July 2007/Returned for modification 29 July 2007/Accepted 27 September 2007

Paradoxical growth was noted in RPMI 1640 and antibiotic medium 3 in the case of 14 and 1 of 15 Candida tropicalis strains, respectively, at a caspofungin concentration of 12.5 μg/ml using minimum fungicidal concentration tests. Time-kill assays showed that against isolates killed at lower concentrations, caspofungin at a concentration of 12.5 μg/ml was only fungistatic.

The growing body of data shows that some Candida strains, which are inhibited by a low concentration of echinocandin antifungals, exhibit growth in the presence of high concentrations of the drugs (2, 9, 10). The frequencies of this paradoxical growth (PG) were found to vary (observed for 10 to 90% of strains) among the most frequently isolated Candida species (2, 7, 9). The only species found to grow in the presence of a high concentration of all three marketed echinocandins, i.e., caspofungin (CAS), micafungin (MICA), and anidulafungin, was C. tropicalis (2).

Our aim was to examine the occurrence of PG in clinical C. tropicalis isolates in vitro by means of MIC and minimum fungicidal concentration (MFC) tests as well as by determining the killing dynamics in time-kill experiments.

(Figure 1) Six of fifteen and 7 out of 15 isolates also grew at 6.25 μg/ml after 24 and 48 h of incubation, respectively.

In AM3, isolate number 6 showed PG after the high-concentration inoculum was used but not with the standard starting inoculum. In RPMI 1640 medium, PG was detected in two cases (isolates 4 and 15) at 12.5 μg/ml CAS, using the standard inoculum. In the case of the elevated starting inoculum, partial growth was detected in all wells. In MFC tests, all isolates except number 7 grew at 12.5 μg/ml (Fig. 1).

The strains tested in the time-kill experiments together with their AMB, FLC, and 5FC MICs are listed in Table 1; representative kill curves with RPMI 1640 medium are shown in Fig. 2.

| TABLE 1. MICs and PG patterns of strains used in this study† |
|----------------|----------------|----------------|
|                | AMB (μg/ml)    | FLC (μg/ml)    | 5FC (μg/ml)    |
| Strain         | 24 h           | 48 h           | 24 h           | 48 h           |
| ATCC 750       | 1              | 0.12           | 1              | 0.12           |
| 4              | 2              | 0.5            | 0.12           | 12.5           | Both           |
| 5              | 2              | 0.25           | 0.12           | 12.5           | Both           |
| 6†             | 2              | 0.25           | 0.12           | 12.5           | Both           |
| 7              | 2              | 0.25           | 0.12           | 12.5           | Both           |
| 8              | 2              | 0.5            | 0.12           | 12.5           | Both           |
| 13             | 2              | 0.5            | 0.12           | 12.5           | Both           |
| 15             | 2              | 0.25           | 0.12           | 12.5           | Both           |

† Corresponding author. Mailing address: Department of Medical Microbiology, University of Debrecen, 4032 Debrecen, Nagyerdely krt. 98., Hungary. Phone: 36-52-417-565. Fax: 36-52-417-565. E-mail: major@dote.hu.

§ Published ahead of print on 8 October 2007.

* Strains used in the time-kill experiments are shown with their MICs for AMB, FLC, and 5FC and their PG patterns in MFC tests after 24 and 48 h in RPMI 1640 medium.
† This isolate also showed PG in AM3.
‡ MICs were determined according to the CLSI method (6).
§ Both, PG was observed at both concentrations. None, PG was observed at neither concentration.
Time-kill curves confirmed the PG of seven of the clinical isolates found to grow paradoxically by using MFC determinations in both media. Ranges of CFU changes varied between $lg0.36$ to $lg1.3$ and $lg1.51$ to $lg0.63$ CFU/ml after 24 and 48 h, respectively. Generally, CAS proved to be fungicidal at lower (≤3.12 μg/ml) concentrations, but at high concentrations (6.25 to 12.5 μg/ml), only fungistatic activity was observed (Fig. 2). When we retested the strains growing at 12.5 μg/ml, we obtained essentially the same CAS kill curves.

The killing patterns obtained using the MFC and the time-kill methods (except for isolate 4 and the ATCC 750 strain in RPMI 1640 medium) were identical for the strains tested. *C. tropicalis* ATCC 750 grew at all CAS concentrations tested in the MFC tests, after both 24 and 48 h. In the time-kill test, this strain grew only at 512 times the MIC (12.5 μg/ml), but yeasts were killed at 1 to 256 times the MIC (0.19 to 6.25 μg/ml) for CAS concentrations after 24 h. A similar discrepancy, but to a lesser extent, was found for isolate number 4.

In the time-kill assay, the presence of FLC, but not of AMB or 5FC, at 1 μg/ml, regardless of the medium used, eliminated PG even after 24 h, for all clinical isolates (Fig. 3). In contrast,
in the case of C. tropicalis ATCC 750, the combination of CAS with AMB, but not with FLC or 5FC, eliminated PG.

C. tropicalis is a species that shows PG equally in the presence of CAS, MICA, and anidulafungin (2). Moreover, Pappas et al. observed more treatment failures with daily doses of 150 mg MICA but not with daily doses of 100 mg MICA or 70 mg CAS (8), in cases of patients infected with several Candida species including C. tropicalis. They suggested that PG may contribute to this phenomenon. Similar in vivo effects were observed for infections by Aspergillus fumigatus and C. albicans (3, 11).

In our work, visible PG in RPMI 1640 medium was noted in the case of 2/15 (13%) and 14/15 (93%) isolates in the MIC and MFC tests, respectively. Similar to the results reported by Pai et al. (7), we found that the use of AM3 almost totally eliminated this phenomenon; a single isolate showed PG in the MFC tests but none in the MIC tests.

Killing curves obtained with high-concentration CAS clearly indicated that viable C. tropicalis cells were present at all times during the time-kill experiment; at very high concentrations, CAS practically became fungistatic rather than fungicidal. The time-kill method was superior to the MFC test for the ATCC strain.

In conclusion, PG could be reliably predicted by the time-kill and MFC tests but not by the MIC test. This may have future implications, because based on these and earlier data (2, 3, 11), the clinical relevance of PG cannot be excluded, suggesting that high doses of echinocandins may lead to treatment failure in certain clinical situations (8).

We thank Cecília Miszti and Erzsebet Falusi for help with isolation and identification of yeasts.

Caspofungin and fluconazole in pure powder form were kindly provided by Merck Research Laboratories and Pfizer Inc., respectively.

REFERENCES