Published in final edited form as:

Mycoses. 2011 July ; 54(4): e175–e177. doi:10.1111/j.1439-0507.2010.01867.x.

Repeated Exposure of *Candida* spp. to Miconazole Demonstrates No Development of Resistance

MA Ghannoum, J Herbert, and N Isham

Center for Medical Mycology, Case Western Reserve University, Cleveland, OH 44106.

Summary

Background—Oropharyngeal candidiasis (OPC) is a common infection among the immunocompromised population. Treatments include both systemic azoles, most commonly fluconazole (FLU), and topical agents such as miconazole (MICON). However, resistance to FLU has been reported with greater frequency.

Objective—The aim of this study was to determine the potential for development of resistance following repeated exposure of *Candida* spp. to MICON.

Materials and Methods—Two clinical isolates each of *Candida albicans*, *C. glabrata*, and *C. tropicalis* were tested. Fifteen passages of each strain were performed in concentrations of MICON at 0.5 MIC, 1 MIC, 2 MIC, and 4 MIC, with MIC determinations performed on growth obtained following each passage.

Results—There was no increase in the MIC of four of the six strains following fifteen passages in MICON. One *C. albicans* strain demonstrated a four-five dilution increase in MICON MIC at all concentrations and one *C. glabrata* strain showed a five-fold MICON MIC increase when exposed to 4 MIC. Although an increase in MIC was noted in these two isolates, the MICON MIC was still very low (0.5 µg/ml).

Conclusion—In general, there was no increase in MIC demonstrated by repeated exposure to MICON in this study.

Keywords

miconazole; susceptibility; *Candida*

Introduction

Oropharyngeal candidiasis (OPC) is common among severely immunocompromised patients as a result of HIV, chemotherapy, radiotherapy and post-transplant immunosuppression therapy. However, since the introduction of protease inhibitors as part of highly active antiretroviral therapy, the incidence in HIV-infected patients has decreased in the developed world. Left untreated, these lesions contribute greatly to the morbidity of HIV infection. [1] In patients with advanced cancer, oral candidiasis has become more and more frequent, particularly in those with haematological malignancies, those receiving systemic chemotherapy and those receiving head and neck radiotherapy due to the new standard of treatment. [2,3] Fluconazole (FLU) is often preferred to topical antifungal agents due to its convenient use compared to the multiple day dosing and poor taste of local treatments. FLU has been shown to be an effective treatment in OPC; however, studies have determined that

in late-stage HIV patients the prevalence of *Candida albicans* with decreased susceptibility to FLU has increased, probably due to the repeated use of this systemic agent. [2] Further, certain non-*albicans* strains, such as *C. glabrata* that are less susceptible to FLU, are being isolated more frequently in patients with HIV. The presence of non-*albicans* spp. has been more frequently associated with severe symptoms. [4]

We recently conducted an *in vitro* study of one hundred and fifty *Candida* strains that showed that the miconazole (MICON) was effective against all strains tested, including FLU-resistant isolates. [5] The minimum inhibitory concentration (MIC) was 0.004-1.0 and 0.06->32 µg/ml for MICON and FLU, respectively. However, the potential for the development of resistance to MICON was not established. Thus, the objective of this study was to determine whether resistance of *Candida* spp. to MICON developed following repeated exposure over time.

Materials and Methods

Recent clinical isolates obtained from the oral cavity were taken from the culture collection at the Center for Medical Mycology and included two strains each of *C. albicans*, *C. glabrata*, and *C. tropicalis*. Of these, one *C. albicans* and one *C. glabrata* strain were resistant to FLU (MIC >32 µg/ml). Initial MIC determinations were performed according to the Clinical and Laboratory Standards Institute (CLSI) M27-A2 standard for the susceptibility testing of yeasts. [6] Briefly, this microdilution method used RPMI-1640 as the medium, incubation temperature and time were 35° C for 24 hrs, and the inoculum size was 0.5-2.5 x 10³ colony forming units (CFUs)/ml. From these initial MIC determinations, the contents of the microdilution well at 0.5 MIC (a sub inhibitory concentration that was one dilution lower than the MIC) were transferred to a potato dextrose agar (PDA) plate and streaked for isolation. The yeast cells were harvested to sterile saline and adjusted to a concentration of 10⁷ CFUs/ml using a hemacytometer. One ml aliquots of this inoculum were passaged in 10 ml of RPMI-1640 containing concentrations of MICON at 0.5 MIC, 1MIC, 2MIC, and 4MIC and incubated at 35° C for 24 hrs. After incubation, tubes were centrifuged for 10 minutes at 3,000 rpm and the supernatant was decanted. Excess liquid was removed with a sterile Pasteur pipette and 0.6 ml of sterile saline was added to each tube and vortexed. For each subsequent passage, another set of MICON concentrations was prepared in microtiter plates, inoculated with 100 µl of sediment from the corresponding concentration of the previous passage and incubated for 24 hrs. Additionally, 100 µl of sediment was subcultured to PDA for MIC determination. This procedure was repeated for a total of 15 passages, with the MIC testing performed after each passage.

Results and Discussion

Our data shows that the response to repeated MICON exposure was strain- and concentration-specific. An inherent 1-dilution variation exists in MIC microdilution testing, and a 2-dilution difference meets the generally accepted criteria. [7,8] Within these parameters, it can be seen that there was no increase in the MIC of four of the six strains following fifteen passages in MICON at 0.5 MIC, MIC, 2MIC and 4MIC. (Table 1) The final MICON MICs at all concentrations against *C. albicans* 8283, *C. glabrata* 8576, and *C. tropicalis* 8679 were the same as or one-two dilutions lower than the initial MIC. The final MICON MIC against *C. tropicalis* 2395 was one dilution higher at 0.5, 1MIC, and 2MIC, and two dilutions higher at 4MIC.

Repeated exposure of *C. glabrata* 8683 to 0.5MIC, 1MIC, and 2MIC resulted in MICs that were two dilutions higher than the initial MIC, but exposure of this isolate to 4MIC resulted in a five-fold increase over the initial concentration. Finally, *C. albicans* 8479 demonstrated

a four-five dilution increase in MIC following repeated exposure to all concentrations of MICON. Following the last passage, the MIC range of all strains was 0.03- 0.5 µg/ml for all isolates, indicating that the organisms tested remained susceptible to MICON. (Table 1)

In general, there was no increase in MIC demonstrated by repeated exposure to MICON in this study. By contrast, similar studies have demonstrated that exposure of *Candida* species, including *albicans*, *dubliniensis*, and *tropicalis*, to high concentrations of FLU *in vitro* have resulted in the development of resistance as evidenced by an increase in FLU MIC. [9-11] In fact, Barchiesi et al. were able to demonstrate an increase in the FLU MIC of *C. tropicalis* ATCC 750 from 1.0 µg/ml to 8.0, 256 and 512 µg/ml after just one passage in medium containing 8.0, 32, and 128 µg/ml of FLU, respectively. [9]

The fact that two of the strains with very low initial MICON MICs of 0.008 µg/ml (*C. albicans* 8479 and *C. glabrata* 8683) demonstrated an increase in MIC up to six-fold may be due to dilution issues at the very low initial concentrations. However, with this set of test strains, a threshold seems to have been reached at 0.5 µg/ml following exposure to all concentrations; testing with a large number of strains would be needed to generalize this conclusion. Importantly, even at the highest drug concentrations (4MIC), none of the strains became resistant to MICON, with MICs remaining well below the CLSI established endpoints for FLU susceptibility (≤8.0 µg/ml).

Further, neither of the two FLU-resistant strains tested in this study developed resistance to MICON following repeated exposure, suggesting that there is no threat of cross-resistance between MICON and other azoles. Again, a much larger study would be needed to confirm this generalization.

In conclusion, this data indicates that the use of MICON as primary therapy for oropharyngeal candidiasis in the immunocompromised population has low potential to develop resistance.

Acknowledgments

This work was supported by funding to MAG from BioAlliance Pharma and NIH grant BRSACURE-Q0600136 to the Oral HIV/AIDS Research Alliance (OHARA).

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Table 1

MICON MIC values (in µg/ml) for *Candida* isolates following repeated exposure to MICON.

Isolate	Initial MICON MIC	MICON MIC following final passage			
		4 MIC	2 MIC	MIC	.5 MIC
<i>C. albicans</i> 8479	0.008	0.25	0.25	0.5	0.25
<i>C. albicans</i> 8283 *	0.5	0.25	0.12	0.25	0.25
<i>C. glabrata</i> 8683	0.008	0.25	0.03	0.03	0.03
<i>C. glabrata</i> 8576 *	0.5	0.5	0.5	0.25	0.5
<i>C. tropicalis</i> 8679	0.06	0.06	0.03	0.06	0.06
<i>C. tropicalis</i> 2395	0.12	0.5	0.25	0.25	0.25

* FLU-resistant strains