In Vitro Susceptibilities of *Malassezia* Species to a New Triazole, Albaconazole (UR-9825), and Other Antifungal Compounds

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The in vitro activity of the new triazole albaconazole (UR-9825) in comparison with those of flucytosine, fluconazole, ketoconazole, itraconazole, and voriconazole against 70 strains of *Malassezia* spp. was determined by a microdilution method using a colorimetric indicator for metabolic activity. Albaconazole showed an in vitro profile similar to those of the different antifungals tested (MIC $\leq$ 0.06 μ g/ml for all the strains).

Yeasts of the genus *Malassezia* are part of the normal mycota of the skin of humans and other warm-blooded animals, particularly in areas rich in sebaceous glands (19). *Malassezia* species may also be etiological agents of skin disorders and, uncommonly, systemic infections (3, 6, 16, 24, 30).

In 1997, the National Committee for Clinical Laboratory Standards approved a broth micro- and macrodilution method for susceptibility testing of yeasts with RPMI 1640 medium (NCCLS-M27A) (21). However, this document is not applicable to *Malassezia* species other than *Malassezia pachydermatis*, because these organisms do not grow without lipidic substances in the medium. Only a few systems for in vitro susceptibility testing of *Malassezia* species have been described. In addition to present measurements in solid media, several microdilution methods have been used, but the different liquid media used, such as modified Dixon (19, 27) and Leeming-Notman (15), are turbid; consequently, the visual and turbidimetric results are difficult to interpret. A liquid medium method has been observed to overcome the difficulties in growth reading if one uses a colorimetric indicator for metabolic activity (Alamar blue) (25). Recently, Nakamura et al. (20) described a new microdilution method based on the urease activity of *Malassezia* spp.

Albaconazole (ABC) is a new systemic triazole under development by J. Uriach & Cia S.A. (Barcelona, Spain) with both potent and broad-spectrum antifungal activity, good pharmacokinetics, and excellent bioavailability. It has demonstrated good in vitro activities against pathogenic yeasts (23), dermatophytes (10), and some filamentous fungi (4), including *Scedosporium prolificans* (5). It has also been shown to be active in the treatment of systemic aspergillosis and candidiasis in experimental animal models (2).

The aim of this study was to compare the in vitro activity of ABC with those of five antifungal drugs, namely, flucytosine (5FC), fluconazole (FLC), ketoconazole (KTC), itraconazole (ITC), and voriconazole (VRC), against 70 isolates of *Malassezia*, namely, *M. furfur* ($n = 24$), *M. pachydermatis* ($n = 10$), *M. sympodialis* ($n = 21$), and *M. slooffiae* ($n = 15$). *M. furfur*

was obtained from human skin, that of neonates with long stays in intensive care units. *M. pachydermatis* and *M. slooffiae* were obtained from healthy and diseased ears of dogs and pigs, respectively. *M. sympodialis* was isolated from normal human skin. Susceptibility testing of the drugs was initially performed with *M. restricta* ($n = 1$), *M. obtusa* ($n = 1$), and *M. globosa* ($n = 24$). However, we were unable to obtain MICs due to the slow growth of these species. Identification of the different *Malassezia* strains to the species level was done following the guidelines of Guillot et al. (11) and Mayser et al. (18). In some cases and because of ambiguity of the mentioned tests, we also identified the strains by molecular analysis as described by Gupta et al. (12). The isolates were maintained by culturing on modified Dixon agar at 32°C and were subcultured weekly more than three times before use.

All drugs were obtained as powders from single batches and were stored desiccated in the dark at 4°C and were provided by their respective manufacturers: 5FC (ICN Iberica, Barcelona, Spain), FLC and VRC (Pfizer, Madrid, Spain), KTC and ITC (Janssen Research Foundation, Beerse, Belgium), and ABC (J. Uriach & Cia S.A.). Stock solutions of KTC, ITC, VRC and ABC were prepared in dimethyl sulfoxide (Sigma, St. Louis, Mo.) at concentrations of 1,600 μ g/ml for KTC, ITC, and VRC and 3,200 μ g/ml for UR-9825. Solutions of 5FC and FLC were prepared in distilled water at concentrations of 1,280 μ g/ml. All the solutions were stored in the dark at –70°C and were used within 3 months. KTC, ITC, and VRC were tested in a concentration range between 0.03 and 16 μ g/ml. The range of ABC was 0.06 to 32 μ g/ml. FLC and 5FC were tested in a concentration range between 0.125 and 64 μ g/ml.

The method described by Schmidt and Rühl-Hörster (25) for *M. furfur* and by Palacin et al. (22) for *M. furfur* and *M. pachydermatis* was used. Inocula were prepared by growing organisms on modified Dixon's agar at 32°C for 5 days. Colonies were suspended in Leeming-Notman medium, and suspensions were adjusted to 1×10^4 to 5×10^4 CFU/ml as determined by viable counts. Fifty microliters of each working solution was pipetted into each well of a 96-well microtiter plate in U form containing 50 μ l of each prepared inoculum suspension. Growth and sterility control wells were also included. The plates were protected from evaporation by placing them in a humid chamber, and their contents were subse-

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TABLE 1. MIC ($\mu\text{g/ml}$) of 5FC, FLC, KTC, ITC, VRC, and ABC against 70 *Malassezia* isolates

<i>Malassezia</i> sp.	No. of strains	MIC ($\mu\text{g/ml}$)					
		5FC	FLC	KTC	ITC	VRC	ABC
<i>M. furfur</i>	24	>64 (24) ^a	4 (22) 2 (2)	≤ 0.03 (24)	≤ 0.03 (20) 0.06 (4)	≤ 0.03 (2) 0.06 (20) 0.12 (2)	≤ 0.06 (24)
<i>M. pachydermatis</i>	10	>64 (10)	4 (10)	≤ 0.03 (10)	≤ 0.03 (8) 0.06 (2)	≤ 0.03 (1) 0.06 (8) 0.12 (1)	≤ 0.06 (10)
<i>M. sympodialis</i>	21	>64 (21)	0.25 (8) 0.5 (7) 1 (4) 4 (2)	≤ 0.03 (21)	≤ 0.03 (21)	≤ 0.03 (21)	≤ 0.06 (21)
<i>M. slooffiae</i>	15	>64 (15)	1 (8) 2 (7)	≤ 0.03 (15)	≤ 0.03 (15)	≤ 0.03 (15)	≤ 0.06 (15)

^a Numbers in parentheses represent number of strains of a particular species with indicated MICs.

quently incubated at 32°C for 3 days. Because of the medium turbidity, the inhibition of growth or lack of growth of organisms was determined by a nonturbidimetric method. Alamar blue (AccuMed) is a colorimetric indicator of growth based on the detection of metabolic activity. It was diluted (1:4) in phosphate-buffered saline (Sigma), and 50 μl of this solution was added to each well after the last period of incubation. After another 24 h of incubation, visual reading of plates was performed. In the case of metabolic activity of the organisms, a change from blue to pink occurred. The lowest drug concentration preventing the color turning from blue to pink was defined as the MIC.

The in vitro results are shown in Table 1. All the strains were in vitro resistant to 5FC (MIC > 64 $\mu\text{g/ml}$). These results are in agreement with those obtained by Marcon et al. (17), who tested amphotericin B, 5FC, KTC, and miconazole against 15 systemic and 10 superficial *M. furfur* isolates and observed that 5FC was the least active drug in vitro (MIC > 100 $\mu\text{g/ml}$). Danker and Spector (7) reported a single *M. furfur* catheter blood culture isolate for which the MIC was > 100 μg of 5FC/ml.

KTC had MICs of ≤ 0.03 $\mu\text{g/ml}$. These data are consistent with those documented in the literature: Schmidt and Rühl-Hörster (25) measured KTC MICs for 30 strains of *M. furfur* and observed that for 29 of 30 strains the MIC was ≤ 0.06 $\mu\text{g/ml}$. Gupta et al. (13) reported that, for 95% (52 of 55) of strains tested for susceptibility to KTC, the MIC was ≤ 0.03 $\mu\text{g/ml}$ (for only three strains the MIC was 0.125 $\mu\text{g/ml}$). Hammer et al. (14) studied the in vitro activity of KTC, econazole, and miconazole against 54 *Malassezia* isolates and observed that the most potent inhibitor in vitro was KTC (0.03 to 0.25 $\mu\text{g/ml}$). These findings are similar to those of previous reports (9, 16, 28, 29).

MICs of FLC for *Malassezia* species ranged from 0.25 to 4 $\mu\text{g/ml}$, these values being similar to or lower than those reported by Strippoli et al. (MIC, 4.1 $\mu\text{g/ml}$) (26).

The MIC data for ITC are comparable with those reported in previous studies. MICs of ITC for *Malassezia* species ranged from ≤ 0.03 to 0.06 $\mu\text{g/ml}$. Ahn et al. (1) obtained MICs of ITC for *M. furfur* strains between 0.015 and 0.06 $\mu\text{g/ml}$; these values

were lower than those reported by Faergemann (MICs, 0.1 to 0.2 $\mu\text{g/ml}$) (8). Strippoli et al. (26) studied the in vitro activity of several antifungal agents (KTC, miconazole, econazole, fen-ticonazole, ITC, and FLC) against *M. furfur* isolates from pityriasis versicolor lesions and observed that ITC and KTC were the most active drugs, with a MIC of 0.8 $\mu\text{g/ml}$ for both.

Our MICs of VRC for *Malassezia* species ranged from ≤ 0.03 to 0.12 $\mu\text{g/ml}$. These results are in agreement with those obtained by Gupta et al. (13), who tested 55 strains of *Malassezia* species with VRC and obtained MICs ranging from ≤ 0.03 to 0.25 $\mu\text{g/ml}$.

To our knowledge, the present study is the first to investigate the in vitro activity of ABC against lipophilic yeasts. From our data, ABC seems to be in vitro active against *Malassezia* spp. and the clinical activity of this compound should be explored and compared in the future with those of other available drugs for the management of clinical conditions associated with lipophilic yeasts.

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