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 ≤ 0.06 μg/ml for all the strains).

Yeast 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 species to the species level was done following the guidelines of Guillot et al. (11) and Mays er 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, and VRC were prepared in dimethyl sulfoxide (Sigma, St. Louis, Mo.) at concentrations of 1,600 μg/ml for KTC, ITC, and VRC and 3,200 μg/ml for UR-9825. Solutions of 5FC and FLC were prepared in distilled water at concentrations of 1,280 μg/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 μg/ml. The range of ABC was 0.06 to 32 μg/ml. FLC and 5FC were tested in a concentration range between 0.03 to 16 μg/ml. The range of ABC was 0.06 to 32 μg/ml. FLC and 5FC were tested in a concentration range between 0.125 and 64 μg/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 × 10^5 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 μl 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-
The detection of metabolic activity. It was diluted (1:4) in phos-
bule (AccuMed) is a colorimetric indicator of growth based on
isms was determined by a nonturbidimetric method. Alamar
turbidity, the inhibition of growth or lack of growth of organ-
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The in vitro results are shown in Table 1. All the strains were
in vitro resistant to 5FC (MIC > 64 μg/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 μg/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 μg/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 μg/ml. Gupta et al. (13) reported that, for 95% (52 of 55) of
strains tested for susceptibility to KTC, the MIC was ≤ 0.03 μg/ml (for only three strains the MIC was 0.125 μg/ml). Ham-
mer 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 μg/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 μg/ml, these values being similar to or lower than those
reported by Strippoli et al. (MIC, 4.1 μg/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 μg/ml. Ahn et al. (1) obtained MICs of ITC
for M. furfur strains between 0.015 and 0.06 μg/ml; these values
were lower than those reported by Faergemann (MICs, 0.1 to
0.2 μg/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 pity-
rriasis versicolor lesions and observed that ITC and KTC were
the most active drugs, with a MIC of 0.8 μg/ml for both.

Our MICs of VRC for Malassezia species ranged from ≤0.03 to
0.12 μg/ml. These results are in agreement with those ob-
tained by Gupta et al. (13), who tested 55 strains of Malassezia
species with VRC and obtained MICs ranging from ≤0.03 to
0.25 μg/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 lipo-
philic yeasts.

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