In Vitro Comparison of Terbinafine and Itraconazole against
Penicillium marneffei

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Received 19 October 1999/Returned for modification 17 December 1999/Accepted 7 February 2000

We evaluated terbinafine and itraconazole against 30 isolates of Penicillium marneffei using a modification of
the National Committee for Clinical Laboratory Standards broth macrodilution MIC testing protocol for
yeasts. The minimal fungicidal concentration (MFC) was determined by plating 100 μl from each MIC drug
dilution having no growth onto Sabouraud glucose agar incubated at 30°C. The MFC was the dilution at which
growth was absent at 72 h of incubation. The MICs, in micrograms per milliliter, were as follows: terbinafine,
0.03 to 1.0 (geometric mean titer, 0.09); itraconazole, 0.03 to 0.5 (geometric mean titer, 0.04). The MFCs, in
micrograms per milliliter, were as follows: terbinafine, 0.03 to 8 (geometric mean titer, 2.60); itraconazole, 0.03
to 8 (geometric mean titer, 2.45). Primary fungicidal activity (MFC within 2 dilutions of MIC) was observed
with terbinafine in eight isolates and with itraconazole in four isolates. The data indicate that terbinafine is
active against P. marneffei in vitro and may have a previously unrealized role in the management of infections
caused by this fungus.

Penicillium marneffei is a thermally regulated dimorphic fun-
gus classified within the subgenus Biverticillium of the genus
Penicillium. This opportunistic pathogen is found only in
Southeast Asia, where it appears to be closely related to the
sexual fungus Talaromyces flavus (4). P. marneffei is one of
the potential indicators for patients having AIDS owing to its fre-
cuency as an opportunistic pathogen in this patient population
(2).

It has been shown that amphotericin B and itraconazole are
effective therapeutic agents to control infections caused by P.
marneffei (3, 11). Owing to the fact that itraconazole and ter-
binafine interfere with the ergosterol biosynthetic pathway and
have similar MICs for filamentous fungi (5, 6), we decided to
compare and contrast these two antifungal agents against
P. marneffei to determine whether terbinafine might have a role in
the management of infections caused by this fungus.

Twenty-six isolates of P. marneffei maintained at the University
of Texas Medical Branch culture collection and four iso-
lates kept at the Novartis Research Institute in Vienna, Aus-
tria, were tested against itraconazole (USPC) and terbinafine
(Novartis) using a protocol based upon National Committee
for Clinical Laboratory Standards reference standard M27-A
for yeasts (9). The isolates consisted of 26 strains isolated from
humans, 2 from bamboo rats, and 1 from a bamboo rat burrow.
They originated from China, Thailand, and Vietnam. Quality
control isolates included Candida albicans (ATCC 90028), C.
krusei (ATCC 6258), and C. parapsilosis (ATCC 22019).

Isolates were grown on potato glucose agar at 35°C until
adequate growth was present. Mould growth was removed
from the colony surface by adding sterile saline to the slant and
then gently disturbing the colony surface with a sterile cotton
swab to suspend the conidia and hyphae in the saline. The
solution was adjusted to a McFarland 0.5 turbidity standard.
Quality control yeasts were suspended in sterile saline and
then adjusted to 85% transmittance. The mould and yeast
suspensions were equivalent to 1 × 106 to 5 × 106 CFU/ml.
The final inoculum concentrations in the drug dilutions were
1 × 106 to 5 × 106 CFU/ml.

Terbinafine and itraconazole were dissolved in dimethyl sul-
foxide, diluted in RPMI 1640 medium (American Biorganics,
Inc., Niagara Falls, N.Y.), and dispensed into snap-cap plastic
Tubes (12 by 75 mm) to give a twofold dilution series ranging
from 0.03 to 8 μg/ml. To each drug dilution, 0.9 ml of the
inoculum was added. Appropriate solvent and growth medium
controls were prepared. These were incubated at 35°C with
each set of tests.

MICs were read on the first day that the growth control
showed good growth (24 to 48 h). Subsequent readings were
made at 24-h intervals up to an additional 72 h, which was the
endpoint. The MIC of terbinafine was the lowest concentration
being optically clear. Owing to the static activity of itracon-
azole, the MIC was the lowest concentration having a promi-
nent reduction in turbidity compared to the drug-free growth
control. This corresponded to approximately 80% or more
inhibition.

Minimal fungicidal concentrations (MFCs) were determined
after the MICs were determined. The last dilution showing
growth and all of the other dilutions showing no growth in the
MIC procedure were subcultured to Sabouraud glucose agar
plates. Using a sterile 100-μl calibrated pipette, 100 μl from each MIC drug
dilution having no growth was plated onto Sabouraud glucose agar
plates. The MFC was the last dilution
having no growth.

The MICs in micrograms per milliliter, were as follows:
terbinafine, 0.03 to 1.0 (geometric mean titer, 0.09); itracon-
azole, 0.03 to 0.5 (geometric mean titer, 0.04). The MFCs, in
micrograms per milliliter, were as follows: terbinafine, 0.03
to 8 (geometric mean titer, 2.60); itraconazole, 0.03 to 8 (geometric
mean titer, 2.45). Primary fungicidal activity (MFC within 2
dilutions of MIC) was noted with terbinafine in eight isolates and
with itraconazole in four isolates.

MIC in vitro susceptibility testing data for itraconazole may
be correlated, with caution, to patient response when patients
have mild to moderate P. marneffei infections (11). The low
MICs of terbinafine and itraconazole against the 30 isolates in
this study indicate that these isolates are sensitive to these antifungal drugs. When the MICs and MFCs are compared using the criteria for fungicidal activity, that is, MICs and MFCs within 2 dilutions of each other, terbinafine was fungicidal in eight instances, compared to four for itraconazole.

The MIC data for terbinafine and itraconazole against \textit{P. marneffei} are homogeneous and consistently low for the isolates tested. Our MIC data for itraconazole are similar to those reported by others (1, 7, 10), which collectively show that this fungus is extremely sensitive to this drug. Owing to the correlation of in vitro susceptibility testing data and clinical response for itraconazole, we believe that the low MICs (geometric mean titers: itraconazole, 0.04 \(\mu\)g/ml; terbinafine, 0.09 \(\mu\)g/ml) indicate a similar potential clinical correlation for terbinafine. In addition, our data indicate that the fungicidal values of both antifungal agents are strain dependent.

The data from this study clearly indicate that terbinafine is active in vitro against \textit{P. marneffei} at a level essentially the same as that of itraconazole. Terbinafine may have a previously unrealized role in the management of infections caused by this fungus.

We thank the Novartis Research Institute for providing us with an education grant that funded this research.

REFERENCES


