In Vitro Comparison of Terbinafine and Itraconazole against *Penicillium marneffei*

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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 fungus 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 frequency 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 terbinafine 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 isolates kept at the Novartis Research Institute in Vienna, Austria, 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 sulfoxide, 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 itraconazole, the MIC was the lowest concentration having a prominent 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 of medium was streaked onto a Sabouraud glucose agar plate, which was incubated at 30°C. 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); 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 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 *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 μg/ml; terbinafine, 0.09 μg/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 *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.

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**REFERENCES**