Sertaconazole Nitrate Shows Fungicidal and Fungistatic Activity Against

Trichophyton rubrum, Trichophyton mentagrophytes, and Epidermophyton floccosum, Causative Agents of Tinea Pedis

Alfonso J. Carrillo-Muñoz,1* Cristina Tur-Tur,2 Delia C. Cárdenes,1 Dolors Estivill,2
and Gustavo Giusiano3

1Department of Microbiology, ACIAM, Barcelona, Spain
2SPDI, CAP Manso, Barcelona, Spain; ALTHAIA Hospitals, Manresa, Spain
3Department of Micología, Regional Medicine Institute, Universidad Nacional del Nordeste, Argentina

*Corresponding author:
Dr. Alfonso J. Carrillo-Muñoz
P.O. Box 10178
E-08080 Barcelona, Spain
Phone: +3493497120
Fax: +3493497120
E-mail: acarrillo@aciam.es

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Abstract
The fungistatic and fungicidal activity of sertaconazole against dermatophytes was evaluated by testing 150 clinical isolates of causative agents of tinea pedis, 
*Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*. The overall geometric means for fungistatic and fungicidal activity of sertaconazole against these isolates were 0.26 and 2.26 µg/ml, respectively, though values were higher for *T. mentagrophytes* compared with the others. This is the first comprehensive demonstration of the fungicidal activity of sertaconazole against dermatophytes.
Dermatophytes are a subgroup of fungi that can invade keratinized tissue in mammals and fowl and subsequently cause an infection (26). Tinea pedis, also known as athlete’s foot, is caused predominately by *Trichophyton rubrum*, followed by *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (1, 26). In fact, *T. rubrum* and *T. mentagrophytes* are the most common dermatophytes in the United States, Europe, and Asia (1). There are an estimated 750,000 outpatient visits annually for tinea pedis in the United States (21), and its prevalence has been increasing in developed countries over the last several decades (1).

Treatment options for tinea pedis include topical antifungal creams and, in severe cases, oral antifungals (23, 26). The fungistatic activity of the topical azole derivative sertaconazole nitrate, \((\pm)-1\-[2,4\text{-dichloro-}\beta\text{-[}(7\text{-chlorobenzo}[\beta]thien-3yl) methoxy]\text{phenethyl}][\text{imidazole nitrate, against dermatophytes has been previously described (3-6, 10), but the only available fungicidal data against dermatophytes is limited to two strains of *T. mentagrophytes* (13). Sertaconazole has previously been shown to exhibit both fungistatic and fungicidal activity against several *Candida* species and other yeasts (2, 7-9, 11, 13, 17-20).

In this study, the susceptibility of 150 isolates of *T. rubrum*, *T. mentagrophytes*, and *E. floccosum* to sertaconazole was tested using a previously described, standardized broth microdilution method (3, 4, 14), which was adapted from the Clinical and Laboratory Standards Institute (CLSI) document M38-A for opportunistic filamentous fungi (12). Prior their antifungal susceptibility testing, isolates were subcultured on antimicrobial agent-free potato dextrose agar (Biolife Italiana, Milan, Italy) at 28°C for 7 to 15 days and re-verified by morphological (macro- and microscopic) and biochemical methods. Briefly, inocula were prepared and diluted in RPMI 1640 medium with L-glutamine and without sodium bicarbonate (Sigma-
Aldrich, St. Louis, MO, USA) buffered at pH 7.0 with 0.165 M MOPS (Sigma-Aldrich, St. Louis, MO, USA) as previously described (3, 4, 14). Sertaconazole nitrate (Grupo Ferrer, Barcelona, Spain) was dissolved to 1600 mg/ml in 100% dimethyl sulfoxide. Aliquots of sertaconazole (final concentrations ranging from 0.016 to 16 µg/ml) and inocula (final density ranging from 4.7x10^3 to 1.5x10^4 CFU/ml as recommended (12)) were combined in sterile, round-bottomed, 96-well microdilution plates (Soria-Greiner, Madrid, Spain), incubated at 28ºC, and observed for the presence or absence of visible growth. Microdilution wells were scored as follows by visual measurements at 5 days or until development of growth in the control wells: 0, optically clear; 1, slightly hazy; 2, prominent decrease in turbidity; 3, slight reduction in turbidity; and 4, no reduction of turbidity. The minimal inhibitory concentration (MIC) was determined as the lowest concentration with a score of 2. *Aspergillus fumigatus* strains NCPF 7100 and NCPF 7099 were tested as controls in each antifungal susceptibility batch. Minimal fungicidal concentrations (MFC) were determined by subculturing volumes of 10 µl from wells with score 0, spreading onto Sabouraud agar plates, and evaluating growth after 5 days at 28º C (22).

Sertaconazole was fungistatic against all three species of dermatophytes tested, with an overall geometric mean MIC of 0.26 µg/ml (Table 1). Sertaconazole was more active against *E. floccosum* and *T. rubrum* than against *T. mentagrophytes* (geometric mean MIC values of 0.12, 0.19, and 0.73 µg/ml, respectively). These trends are consistent with two previous studies that, using this method, found respective geometric mean MIC values of 0.07 and 0.08 µg/ml for *E. floccosum*, 0.19 and 0.13 µg/ml for *T. rubrum*, and 0.62 and 0.49 µg/ml for *T. mentagrophytes* (3, 4).

The results of this study also demonstrate the fungicidal activity of sertaconazole against the dermatophytes tested, with a geometric mean MFC value
of 2.26 µg/ml (Table 1). This value differs from the MFC values of ≥50 µg/ml previously reported for two strains of *T. mentagrophytes*, though it should be noted that this study utilized a different methodology (13). When the inhibition curves were compared, statistically significant differences (Student’s *t* test, *P* <0.05) were found between the concentrations of sertaconazole necessary for fungistatic and fungicidal activities (Fig. 1). The concentrations of sertaconazole needed to achieve either fungistatic or fungicidal activity are far below those reached after topical application (15). Nevertheless, the clinical advantage of fungicidal over fungistatic agents remains unclear, based on a recent examination of antifungal agents (16).

In conclusion, data obtained with 150 clinical isolates of the dermatophytes *T. rubrum*, *T. mentagrophytes*, and *E. floccosum*, causative agents of tinea pedis, demonstrated that sertaconazole exhibits a fungicidal and fungistatic profile, with greater activity against *T. rubrum* and *E. floccosum* compared with *T. mentagrophytes*. These dual fungicidal and fungistatic activities of sertaconazole are consistent with its efficacy against tinea pedis in randomized, placebo-controlled clinical trials (24, 25).

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REFERENCES


Figure Legend

FIG. 1. Inhibition curves for dermatophytes at different sertaconazole concentrations.

Exponential regression lines were fitted using GraphPad Prism 5.

MFC, minimal fungicidal concentration; MIC, minimal inhibitory concentration.
TABLE 1. In vitro fungistatic (MIC) and fungicidal (MFC) data for sertaconazole nitrate (in µg/ml) against dermatophyte fungi by means of a standardized liquid microdilution method.

<table>
<thead>
<tr>
<th>Dermatophyte</th>
<th>GMa MIC</th>
<th>MIC range</th>
<th>MIC50</th>
<th>MIC90</th>
<th>GMa MFC</th>
<th>MFC50</th>
<th>MFC90</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichophyton rubrum</em></td>
<td>0.19</td>
<td>0.02-16</td>
<td>0.25</td>
<td>1</td>
<td>1.78</td>
<td>4</td>
<td>≥16</td>
</tr>
<tr>
<td><em>(n = 100)</em></td>
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<tr>
<td><em>Trichophyton mentagrophytes</em></td>
<td>0.73</td>
<td>0.02-16</td>
<td>1</td>
<td>8</td>
<td>4.76</td>
<td>8</td>
<td>≥16</td>
</tr>
<tr>
<td><em>(n = 40)</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td><em>Epidermophyton floccosum</em></td>
<td>0.12</td>
<td>0.02-16</td>
<td>0.06</td>
<td>0.5</td>
<td>1.23</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td><em>(n = 10)</em></td>
<td></td>
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<tr>
<td>All isolates</td>
<td>0.26</td>
<td>0.02-16</td>
<td>0.25</td>
<td>2</td>
<td>2.26</td>
<td>4</td>
<td>≥16</td>
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<tr>
<td><em>(n = 150)</em></td>
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</tr>
</tbody>
</table>

aGeometric mean.

MFC, minimal fungicidal concentration; MIC, minimal inhibitory concentration.