

Sertaconazole Nitrate Shows Fungicidal and Fungistatic Activities against *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, Causative Agents of Tinea Pedis[∇]

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The fungistatic and fungicidal activities of sertaconazole against dermatophytes were 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 activities of sertaconazole against these isolates were 0.26 and 2.26 µg/ml, respectively, although values were higher for *T. mentagrophytes* than for 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, (±)-1-[2,4-dichloro-β-(7-chlorobenzo[*b*]thien-3-yl)methoxy]phenethyl]imidazole nitrate, against dermatophytes has been previously described (3–6, 10), but the only available fungicidal data against dermatophytes are limited to two strains of *T. mentagrophytes* (13). Sertaconazole has previously been shown to exhibit both fungistatic

and fungicidal activities against several *Candida* species and other yeasts (2, 7–9, 11, 13, 17–20).

In this study, the susceptibilities of 150 isolates of *T. rubrum*, *T. mentagrophytes*, and *E. floccosum* to sertaconazole were tested by 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 to the 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 reverified 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) buffered at pH 7.0 with 0.165 M morpholinepropanesulfonic acid (Sigma-Aldrich, St. Louis, MO) as previously described (3, 4, 14). Sertaconazole nitrate (Grupo Ferrer, Barcelona, Spain) was dissolved to 1,600 mg/ml in 100% dimethyl sulfoxide. Aliquots of sertaconazole (final

TABLE 1. *In vitro* fungistatic (MIC) and fungicidal (MFC) data for sertaconazole nitrate against dermatophyte fungi by means of a standardized liquid microdilution method

| Dermatophyte (<i>n</i>) | MIC data (µg/ml) | | | | MFC data (µg/ml) | | |
|---|------------------|---------|-------------------|-------------------|------------------|-------------------|-------------------|
| | GM ^a | Range | MIC ₅₀ | MIC ₉₀ | GM ^a | MFC ₅₀ | MFC ₉₀ |
| <i>Trichophyton rubrum</i> (100) | 0.19 | 0.02–16 | 0.25 | 1 | 1.78 | 4 | ≥16 |
| <i>Trichophyton mentagrophytes</i> (40) | 0.73 | 0.02–16 | 1 | 8 | 4.76 | 8 | ≥16 |
| <i>Epidermophyton floccosum</i> (10) | 0.12 | 0.02–16 | 0.06 | 0.5 | 1.23 | 1 | 8 |
| All isolates (150) | 0.26 | 0.02–16 | 0.25 | 2 | 2.26 | 4 | ≥16 |

^a GM, geometric mean.

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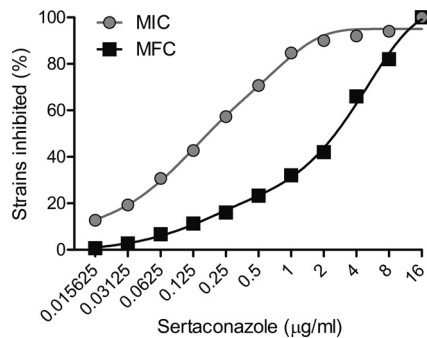


FIG. 1. Inhibition curves for dermatophytes at different sertaconazole concentrations. Exponential regression lines were fitted using GraphPad Prism 5.

concentrations ranging from 0.016 to 16 µg/ml) and inocula (final densities ranging from 4.7×10^3 to 1.5×10^4 CFU/ml, as recommended [12]) were combined in sterile, round-bottom, 96-well microdilution plates (Soria-Greiner, Madrid, Spain), incubated at 28°C, 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; 4, no reduction of turbidity. The 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 a score of 0, spreading onto Sabouraud agar plates, and evaluating growth after 5 days at 28°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*, although it should be noted that this study utilized a different methodology (13). When the inhibition curves were compared, statistically significant differences ($P < 0.05$; Student's *t* test) 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 were 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 activities against *T. rubrum* and *E. floccosum* than against *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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