Luliconazole Demonstrates Potent In vitro Activity against Dermatophytes Recovered from Patients with Onychomycosis

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The in vitro activities of luliconazole, amorolfine, ciclopirox, and terbinafine were determined against 320 dermatophyte isolates from large toenails of onychomycosis patients enrolled into an ongoing phase 2b/3 clinical study. The geometric mean MIC for luliconazole was 0.00022 µg/ml against all isolates, compared to 0.0194 to 0.3107 µg/ml for the three other agents. The in vitro potency of luliconazole was maintained regardless of the dermatophyte species.

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nychomycosis (tinea unguium) is a fungal infection of the nail bed and or plate and is associated with significant morbidity (1). Besides causing cosmetic concerns, this mycosis can result in pain or discomfort, acute bacterial cellulitis associated with spread of infection to the skin, and social stigma (2, 3). The prevalence of onychomycosis is estimated to be between 2 and 14%, with a rate of 13.8% reported in North America (1, 4). Risk factors for infection include increasing age, male gender, dystrophic nails, tinea pedis, and poor peripheral circulation (1, 3, 4). These infections are caused primarily by dermatophytes, including *Trichophyton rubrum* and *T. mentagrophytes*, and to a lesser extent by other dermatophytes (e.g., *Epidermophyton floccosum*), *Candida* species, and nondermatophytic molds (e.g., *Acremonium*, *Fusarium*, and *Scopulariopsis* species) (5, 6). Luliconazole is a novel imidazole currently under development. Pharmacokinetic and safety results from phase 1 studies in patients with onychomycosis have demonstrated high concentrations of luliconazole within the nail plates of the great toe and have shown that this agent is well tolerated when administered as a 10% solution (7). In vitro studies with a limited number of isolates have also reported potent activity of this agent against dermatophytes and other causative agents of dermatophytosis, including onychomycosis (8–10). Results from animal models and small clinical studies have also suggested in vivo efficacy against dermatophytosis (11–13). Our objective was to measure the in vitro activity of luliconazole against dermatophytes isolated at screening from toenails in an ongoing phase 2b/3 study of patients with mild to moderate toenail onychomycosis (www.clinicaltrials.gov, identifier NCT01431820). In addition, the in vitro activities of amorolfine, ciclopirox, and terbinafine, three products approved and most often used for treatment of onychomycosis in the United States and the European Union, were also determined.

Three hundred twenty dermatophytes obtained from patients with confirmed toenail onychomycosis (positive KOH and culture and clinical diagnosis) during screening were used in this study. This included 308 *Trichophyton rubrum* and 10 *T. mentagrophytes* isolates and one each of *Trichophyton tonsurans* and *Epidermophyton floccosum*. All isolates were clinical strains that had been freshly subcultured and not previously frozen. Stock solutions of amorolfine, ciclopirox, terbinafine, and luliconazole were prepared in dimethyl sulfoxide (DMSO) and further diluted in RPMI 1640 buffered to a pH of 7.0 with morpholinepropanesulfonic acid for a final DMSO concentration of 1%. Drug preparations and susceptibility testing were performed according to CLSI document M38-A2 (14), and the final inoculum was 1 × 10³ to 3 × 10⁷ cells/ml. Isolates were incubated in the presence of drugs at 30°C for 96 h. The MIC was measured as the lowest concentration of each agent that resulted in 80% inhibition of growth. *Trichophyton mentagrophytes* ATCC MYA-4439 served as the quality control organism. The concentrations that inhibited 50% and 90% of the isolates (MIC₅₀ and MIC₉₀, respectively) and the geometric mean (GM) MICs were determined for each agent.

Luliconazole had potent activity against the dermatophyte isolates collected from patients with onychomycosis in this trial. As shown in Table 1, the MICs for this agent against all isolates ranged from 0.00012 to 0.0025 µg/ml, compared to 0.008 to 0.5 µg/ml for amorolfine, 0.03 to 1 µg/ml for ciclopirox, and 0.004 to 0.25 µg/ml for terbinafine. The luliconazole GM MIC against all isolates was 0.00022 µg/ml, while those of the other agents were as follows: amorolfine, 0.0867 µg/ml; ciclopirox, 0.3107 µg/ml; and terbinafine, 0.0194 µg/ml. The MIC₅₀ and MIC₉₀ values of luliconazole were 0.00025 and 0.0005 µg/ml, respectively, and those of the other agents ranged from 0.015 to 0.25 µg/ml and 0.03 to 0.5 µg/ml, respectively.

When the data were separated by species, similar results were observed, as the potency of luliconazole was maintained regardless of the species (Table 2). Against *T. rubrum*, which made up the majority of isolates in this study (96.2%), the luliconazole GM MIC was 0.00022 µg/ml, while that of terbinafine was 0.0195 µg/ml, followed by amorolfine at 0.0883 µg/ml and ciclopirox at 0.3156 µg/ml. Similar activity was also observed against *T. mentagrophytes* isolates, with the luliconazole GM MIC at 0.000265 µg/ml, followed by MICs of terbinafine of 0.0161 µg/ml, amorolfine of 0.051 µg/ml, and ciclopirox of 0.2095 µg/ml. As observed against all isolates combined, the MIC₅₀ and MIC₉₀ values for luliconazole against either *T. rubrum* or *T. mentagrophytes* isolates were 7 to 10 dilutions lower than those observed with the other antifungals. Luliconazole demonstrated potent activity against the single isolates of *T. tonsurans* and *E. floccosum* (MIC, 0.0005 and 0.0025 µg/ml, respectively).

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studies that demonstrated potent activity for luliconazole in vitro. These results are in agreement with previous activity against dermatophyte isolates collected from patients with onychomycosis and other tinea infections (8–10). The GM MIC, MIC50, and MIC90 values reported in these studies were similar to those we measured and were significantly lower than those observed with other antifungals, including terbinafine and amorolfine. One limitation of this study is that we did not measure the in vitro activity of other azoles against these isolates. However, the luliconazole MIC range, MIC50, and MIC90 values, and GM MICs that were measured in the current study compared favorably those reported in the literature for other azoles against dermatophytes, including itraconazole, voriconazole, posaconazole, ketoconazole, and the investigational agents efi naconazole and ravuconazole (Table 3) (15–20). Although direct in vitro comparisons with newer azoles are warranted, none of these other agents, except for itraconazole, currently have an FDA-approved indication for the treatment of onychomycosis. The agents that were used in our in vitro study are approved for the treatment of onychomycosis caused by dermatophytes. In addition, previous studies that directly compared luliconazole with older azoles against dermatophytes, such as clotrimazole, bifonazole, and miconazole, reported enhanced in vitro potency of luliconazole versus these other agents (8, 9). The luliconazole MICs reported in these smaller studies against Trichophyton species (MIC range ≤ 0.00012 to 0.002) are similar to those we observed. In our study, the majority of isolates were T. rubrum. This is consistent with the species distribution previously reported in a large epidemiologic surveillance study of cutaneous fungal infections in the United States, in which the majority of toenail- and toenail-derived dermatophyte isolates were T. rubrum (5). These data, along with the recent report demonstrating high concentrations within the toenails and good tolerability in patients with onychomycosis, suggest that luliconazole may be a suitable option for the treatment of this disease.

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