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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.

Onychomycosis (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 1×10^3 to 3×10^3 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 (MIC_{50} and MIC_{90}, 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 MIC_{50} and MIC_{90} 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 MIC_{50} and MIC_{90} 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,

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TABLE 1 MICs of amorolfine, ciclopirox, terbinafine, and luliconazole against all dermatophyte isolates ($n = 320$)

Antifungal	MIC (μg/ml)[a]			
	Range	50%	90%	GM
Amorolfine	0.008–0.5	0.125	0.25	0.0867
Ciclopirox	0.03–1	0.25	0.5	0.3107
Terbinafine	0.004–0.25	0.015	0.03	0.0194
Luliconazole	0.00012–0.0025	0.00025	0.0005	0.00022

[a] MICs were measured according to CLSI M38-A2 guidelines (14) as the lowest concentration of each agent that resulted in 80% inhibition of growth compared to the growth control. 50% and 90%, MICs at which 50% and 90% of the isolates were inhibited; GM, geometric mean.

0.000125 μg/ml against each). The corresponding MICs of the other antifungals were 0.03 μg/ml and 0.125 μg/ml, respectively, for amorolfine, 0.125 μg/ml and 0.25 μg/ml for ciclopirox, and 0.03 μg/ml and 0.015 μg/ml for terbinafine.

These data demonstrate that luliconazole has potent *in vitro* activity against dermatophyte isolates collected from patients with onychomycosis. These results are in agreement with previous *in vitro* studies that demonstrated potent activity for luliconazole against a limited number of dermatophytes and other causative agents of onychomycosis and other tinea infections (8–10). The GM MIC, MIC_{50}, and MIC_{90} values reported in those 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, MIC_{50} and MIC_{90} 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 efinaconazole and ravuconazole (Table 3) (15–20). Although direct *in vitro* comparisons with newer azoles are warranted, none of these other azoles, 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 fingernail- 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.

TABLE 2 MICs of amorolfine, ciclopirox, terbinafine, and luliconazole against *Trichophyton rubrum* and *Trichophyton mentagrophytes* isolates

Organism (no. of isolates) and antifungal	MIC (μg/ml)[a]			
	Range	50%	90%	GM
Trichophyton rubrum (308)				
Amorolfine	0.008–0.5	0.125	0.25	0.0883
Ciclopirox	0.03–1	0.25	0.5	0.3156
Terbinafine	0.004–0.25	0.015	0.03	0.0195
Luliconazole	0.00012–0.0025	0.00025	0.0005	0.00022
Trichophyton mentagrophytes (10)				
Amorolfine	0.03–0.125	0.03	0.125	0.051
Ciclopirox	0.06–0.5	0.25	0.5	0.2095
Terbinafine	0.008–0.03	0.015	0.03	0.0161
Luliconazole	0.00012–0.001	0.000125	0.001	0.000265

[a] MICs were measured according to CLSI M38-A2 guidelines (14) as the lowest concentration of each agent that resulted in 80% inhibition of growth compared to the growth control. 50% and 90%, MICs at which 50% and 90% of the isolates were inhibited; GM, geometric mean.

TABLE 3 MICs of efinaconazole, itraconazole, voriconazole, posaconazole, ravuconazole, and ketoconazole

Organism and antifungal	Range (μg/ml)[a]			
	MIC	MIC_{50}	MIC_{90}	GM MIC
All dermatophytes				
Efinaconazole	0.001–0.015	0.002–0.004	0.008–0.015	0.003–0.005
Itraconazole	0.015–16	0.015–0.125	0.06–16	0.03–0.54
Voriconazole	0.001–4	0.015–0.125	0.06–2	0.03–0.29
Posaconazole	0.003–1	0.015–0.125	0.06–0.25	0.03–0.05
Ravuconazole	0.007–>8	0.015–0.125	0.06–0.25	0.03–0.22
Ketoconazole	0.031–8	0.25–0.5	2–4	0.46–1.48
Trichophyton rubrum				
Efinaconazole	0.001–0.015	0.002	0.008	0.003
Itraconazole	0.015–16	0.03–0.25	0.06–0.5	0.037–0.247
Voriconazole	0.001–4	0.015-0.0.031	0.06–0.25	0.03–0.18
Posaconazole	0.007–0.5	0.015–0.06	0.06–0.25	0.04–0.05
Ravuconazole	0.015 - >8	0.03	0.25	0.05
Ketoconazole	0.062–8	0.25	4	1.34
Trichophyton mentagrophytes				
Efinaconazole	0.001–0.003	0.004	0.015	0.005
Itraconazole	0.015–16	0.015–0.25	0.06–16	0.03–0.41
Voriconazole	0.008–0.5	NR	NR	0.125
Posaconazole	0.007–0.125	0.015	0.125	0.02
Ravuconazole	0.015–0.5	0.015	0.06	0.03
Ketoconazole	0.5–4	0.5	4	1.48

[a] MICs were determined by broth microdilution as previously reported in the literature (15–20). MIC_{50} and MIC_{90}, MICs at which 50% and 90% of the isolates were inhibited; GM MIC, geometric mean MIC. NR, not reported.

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