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CLINICAL THERAPEUTICS

Evaluation of the Ability of a Novel Miconazole Formulation To Penetrate Nail by Using Three *In Vitro* Nail Models

Luisa Christensen,[a,b] Rob Turner,[c] Sean Weaver,[c] Francesco Caserta,[c] Lisa Long,[a,b] Mahmoud Ghannoum,[a,b] Marc Brown[c,d]

Center for Medical Mycology, Department of Dermatology, Case Western Reserve University,[a] and University Hospitals Case Medical Center,[b] Cleveland, Ohio, USA; MedPharm Ltd., Surrey Research Park, Guildford, United Kingdom[c]; TDDT, School of Health and Life Sciences, University of Hertfordshire, Hatfield, United Kingdom[d]

ABSTRACT In an effort to increase the efficacy of topical medications for treating onychomycosis, several new nail penetration enhancers were recently developed. In this study, the ability of 10% (wt/wt) miconazole nitrate combined with a penetration enhancer formulation to permeate the nail is demonstrated by the use of a selection of *in vitro* nail penetration assays. These assays included the bovine hoof, TurChub zone of inhibition, and infected-nail models.

KEYWORDS miconazole, onychomycosis, topical

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Address correspondence to Mahmoud Ghannoum, mahmoud.ghannoum@case.edu.

Topical medications to treat tinea unguium have lacked efficacy (1, 2), and there has been a recent drive to improve ungual drug delivery by the development of nail-specific penetration enhancers (3–5). In this study, the ability of 10% (wt/wt) miconazole nitrate combined with a penetration enhancer formulation to permeate the nail was demonstrated by the use of a selection of *in vitro* nail penetration assays.

Three different assays were used to test permeation by enhanced miconazole nitrate solution, as described below.

Bovine hoof model. Sterile hoof discs measuring 0.5 to 1.0 mm thick, similar to the thickness of human nails, were placed on the surface of agar plates seeded with an inoculum of *Trichophyton mentagrophytes* standardized to a concentration of 2×10^5 to 5×10^5 conidia/ml as previously described (6). Three hundred microliters of a marketed 2% (wt/wt) miconazole nitrate or 8% (wt/wt) ciclopirox topical formulation was then applied to the surface of the hoof disc for 30 or 60 min. Plates were incubated for 4 days, and zones of inhibition (ZOI) were subsequently measured. As expected, the untreated controls showed no ZOI, while discs treated with miconazole nitrate 2% (wt/wt) following 60 min of exposure showed significantly larger ZOI than those exposed to 8% (wt/wt) ciclopirox nail lacquer (26.5 $\pm$ 9.7 and 2.8 $\pm$ 3.4 mm, respectively; $P \leq 0.05$).

TurChub ZOI model. The TurChub ZOI assay used a modified static diffusion cell in which sections of human nail serve as the barrier through which the drug initially penetrates prior to reaching an agar-filled receptor chamber. Three penetration-enhancing formulations, supplied by Humco Pharmaceuticals (Austin, TX, USA) and containing a novel base formulation (comprised of acetylcysteine, alcohol, camphor, EDTA, eucalyptus oil, hydroxypropyl cellulose, hydroxypropyl starch phosphate, magnesium aluminum silicate, menthol, propylene carbonate, propylene glycol, purified water, sodium hydroxide, sodium thioglycolate, strontium chloride, tea tree oil, thymol, and urea), were tested. One formulation was a placebo comprised of the base formulation only, while the other two formulations contained either 10% (wt/wt) fluconazole or 10% (wt/wt) miconazole. Two marketed products, 8% (wt/wt) ciclopirox topical solution and 10% (wt/wt) efinaconazole solution, were also investigated.

Preparation of the TurChub cells was performed as described by Traynor et al. (3). Briefly, the receiver compartment of each TurChub cell was filled with peptone-dextrose agar (PDA), ensuring complete contact with the agar in the receiver compartment and the underside of the nail. A suspension of *Trichophyton rubrum* conidia at a concentration of 1×10^7 conidia/ml was pipetted onto the agar surface and left to dry. The surface of the nail mounted in the gasket section of a TurChub cell was then dosed with 100 μl of test formulation, and the cells were incubated for 14 days.

The base formulations with 10% (wt/wt) miconazole, 10% (wt/wt) fluconazole, and 10% (wt/wt) efinaconazole were all statistically superior to the 8% (wt/wt) ciclopirox solution ($P \leq 0.05$), indicating high levels of drug permeation through the nail. There were no ZOI after dosing of the nails with either the placebo base formulation or 8% (wt/wt) ciclopirox.

Infected-nail model. Distal nail clippings were infected with a suspension of *T. rubrum* conidia at a concentration of 1×10^7 conidia/ml, mounted into a TurChub gasket system, and incubated until a fungal infection was established (3). Nails were then treated daily for 7 days with 2 μl of the test formulations described above for the TurChub model. Subsequently, the presence of viable microorganisms was measured by an ATP bioluminescence method in which the amount of luminescence is directly proportional to the ATP concentration and, in turn, the viability of the *T. rubrum* present in the infected nail. The greatest decrease in percentage ATP recovery compared to that of the infected control was observed for the 10% (wt/wt) efinaconazole solution (3.33%), compared with 4.75 and 6.57% for the base penetration-enhancing formulations with 10% (wt/wt) miconazole and 10% (wt/wt) fluconazole, respectively. In contrast, the ATP recovery following treatment with 8% (wt/wt) ciclopirox was 20.02% compared to the infected control, indicating that this test formulation was significantly less efficacious in killing the fungal cells present ($P \leq 0.05$).

In summary, the bovine hoof model assay demonstrated that miconazole can penetrate hoof material and exhibit antifungal activity. The findings of the hoof model were then further explored in more clinically relevant human nail models. Data from the TurChub ZOI and infected-nail models showed the ability of miconazole to penetrate human nail and inhibit fungal growth, as measured by ZOI and ATP recovery, respectively. In comparison to currently marketed topical nail antifungal products, our data indicate that 10% (wt/wt) miconazole in the penetration-enhancing formulation is equivalent to the 10% (wt/wt) efinaconazole solution and superior to the 8% (wt/wt) ciclopirox solution, suggesting that miconazole may be effective in the topical treatment of tinea unguium.

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