

In Vitro Evaluation of Stable Derivatives of the Chlorotaurines on Infected Human Nail Model as Potent Antifungal Agents for the Treatment of Onychomycosis

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Abstract

Background: *N,N*-dichloro-2,2-dimethylaurine (NVC-422) and *N*-chloro-2,2-dimethylaurine (NVC-612), stable derivatives of the endogenous chlorotaurines, have potent activity against viruses, yeasts, fungi, and bacteria. This study was conducted to evaluate the *in vitro* efficacy of the topical use of NVC-422 and NVC-612 on *Trichophyton rubrum* infected human nails.

Methods: MedPharm's *in vitro* ChubTur® infected nail model system was used to conduct this study. A laboratory-cultured clinical isolate of *T. rubrum* was used to infect full thickness sterile human nails. After 14-days of incubation, single applications of 2 μ L of the test formulations were applied to the surface of the nails mounted in the ChubTur® cells, opposite to where the organisms had been cultured on the nail and incubation continued for a further 7 days. The nails were then analyzed for the presence of ATP as a measure of fungal viability. Uninfected nails, infected untreated nails and placebo (vehicle) treated nails were used as controls.

Results: Single 2 μ L dosages of each test formulation applied to the surface of human toenails infected on the ventral layer with *T. rubrum* gave extremely impressive efficacy results compared to the controls. 2% NVC-422 (in 1% polymeric gel) was the most effective formulation and was statistically more effective than 2% NVC-612 (in 1% polymeric gel) ($p = 0.025$), 4% NVC-612 solution ($p = 0.004$) and 4% NVC-422 solution ($p = 0.0002$). 2% NVC-422 (polymeric gel) was not statistically significant from the non-infected controls ($p = 0.290$) indicating complete mycological kill. All of the active formulations were statistically more effective than the placebo formulation (1% polymeric gel). The placebo formulation was significantly different from the infected, un-dosed control indicating some efficacy of the vehicle.

Introduction

Distal and lateral subungual onychomycosis (DLSO) is the most common type of onychomycosis. Infection is initially a disease of the hyponychium, resulting in hyperkeratosis of the distal nail bed. It generally begins at the lateral edge of the nail rather than the central portion and spreads progressively proximally down the nail bed producing hyperkeratosis and thus onycholysis.

Onychomycosis may be caused by dermatophytes, yeasts or molds. It is accepted that dermatophytes are by far the predominant pathogens and account for the majority of the incidents of tinea pedis and onychomycosis⁽¹⁾. Of the dermatophytes, the most common cause of onychomycosis is *T. rubrum*. Causative molds include *Scopulariopsis brevicaulis* and *Scytalidium dimidiatum*. It is important to treat onychomycosis, as it is an infection and does not resolve spontaneously.

Sodium 2-(dichloroamino)-2-methylpropane-1-sulfonate (NVC-422, *N,N*-dichloro-2,2-dimethylaurine), and sodium 2-(chloroamino)-2-methylpropane-1-sulfonate (NVC-612, *N*-chloro-2,2-dimethylaurine) are stable derivatives of the endogenous chlorotaurines⁽²⁾ with potent activity against viruses, yeasts, fungi, and bacteria. The aim of this study was to conduct a full scale experiment to test the antifungal efficacy of formulations of NVC-422 and NVC-612 in simple solution or in hydrated Noveon® polymeric gel, against *T. rubrum* using the MedPharm infected nail model (TCCT™ model).

This study also compared simple solutions of these active compounds with formulations made in Noveon polymeric delivery system. The hydrated polymer adds viscosity and retention to the formulation.

Materials & Methods

This study was sponsored by NovaBay Pharmaceuticals and conducted at MedPharm Ltd, 50 Occam Road Surrey Research Park Guildford GU2 7YN, UK. MedPharm's *in vitro* infected nail model (TCCT™ model) uses *T. rubrum* infected human nail samples (Figures 2 and 3), to evaluate the efficacy of various formulations for the treatment of onychomycosis. Investigations into formulation efficacy are conducted under conditions which are closer to the clinical situation (allowing permeation of the formulation through the nail barrier where fungal cell growth is present) and thus have more practical relevance. The model uses levels of ATP recovered from viable organisms⁽³⁾ as a biological marker to demonstrate the effectiveness of different formulations in reducing the viability of fungal cells where by the lower the amount of ATP recovered, the more efficacious the formulation is against fungal cells.

Test Systems:

The ChubTur® test system is designed and trade marked by MedPharm.

Preparation of infectious organism, *T. rubrum*:

A Sabouraud dextrose agar (SDA) slope inoculated with *T. rubrum* was originally isolated from an onychomycotic patient. An SDA plate was aseptically seeded with *T. rubrum* mycelium and spores that were removed from a slope culture and transferred onto the surface of the agar. The agar plate was then incubated at 25°C for 7 days. The white spores were then washed from the surface of the plate with Ringers solution and filtered to remove mycelium and agar debris.

Preparation of the nail:

Uniform segments of the human nails were prepared to ensure their sterility and examined with a microscope to ensure their integrity.

Nail assembly, infection and testing:

The ChubTur® cells test systems were assembled according to MedPharm's standard procedures.

The prepared full thickness toenails were infected using *T. rubrum*. After 14 days, a single 2 μ L application of each test item was applied to the surface of the nail (Table 1), opposite to where the nail was inoculated with the organism suspension. The nail samples were then incubated for a period of 7 days. At this time the excess formulation was removed from the surface of the nails by light scraping of the nail surface followed by washing three times with sterile water and a sterile cotton swab. Following cleaning of the surfaces, the nails were thoroughly dried and dismantled from the ChubTur® cells. All the nails were then analyzed for the presence of ATP from the viable fungi using MedPharm's established standard procedures.

Table 1- Formulations and dosing regimen used in the study

Test item	Dose regimen
2% NVC-612 solution in hydrated Noveon pH ~ 7.5	A single dose of the formulation (2 μ L, - finite dose at t=0 only). Dose left to the end of the study 7 days of incubation. n = 6
4% NVC-612 solution in water pH ~ 7.5	
4% NVC-422 in water pH ~ 4	
2% NVC-422 in hydrated Noveon pH ~ 5	
Hydrated Noveon (water only) pH ~ 5	No formulation applied n = 3
Infected Controls	
Non-infected Controls	

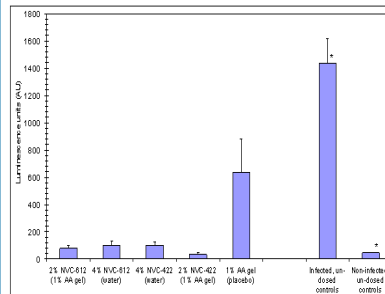


Figure 1- Comparison of ATP release (mean \pm SD, where n=6, and mean \pm range, where n=3) following one application of formulations followed by a 7-day incubation period. Six nail samples (n = 6) were used for active and placebo formulations. Three nail samples (n = 3) were used for the un-dosed controls (**).



Figure 2- Microscope pictures of *T. rubrum* (white areas) directly growing on human nail samples (x 100 magnification). Photos taken after 14 days growth. The nail is the pale pink/yellow area seen in the photograph.

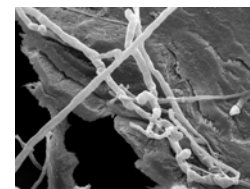


Figure 3- Example of an SEM photograph of *T. rubrum* growing directly on human nail samples following 14 day incubation in MedPharm's ChubTur® test system, prior to dosing with formulations.

Conclusions

The results of the *in vitro* infected nail study following a single 2 μ L dosage of each formulation to the surface of human toenails infected on the ventral layer with *T. rubrum* were impressive for these formulations (Figure 1) and demonstrated that 2% NVC-422 in Noveon gel was the most effective formulation and was statistically more effective than 2% NVC-612 in Noveon gel ($p = 0.025$), 4% NVC-612 ($p = 0.004$) and 4% NVC-422 ($p = 0.0002$). In fact 2% NVC-422 in Noveon gel was not statistically significant from the non-infected controls ($p = 0.290$), suggesting complete mycological kill. All of the active formulations were statistically more effective than the placebo formulation (Noveon gel). The placebo formulation was significantly different from the infected, un-dosed control indicating some efficacy for the placebo.

References

- Charif MA, Ekweski BE. "A historical perspective on onychomycosis." *Dermatol Ther.* 1997;3:43-45.
- Wang L, Khosravi B, Najafi R. "N-Chloro-2,2-dimethylaurines: a new class of remarkably stable N-chlorotaurines." *Tetrahedron Letters* 49 (2008) 2193-2195
- Yoshida T, Uchida K, Yamaguchi H. "An ATP bioluminescence assay applicable to rapid fluconazole susceptibility testing of dermatophytes." *Microbiol Immunol.* 1997;41(5):377-86.