In Vitro Evaluation of the Antifungal Activity of NVC-422 (N,N-dichloro-2,2-dimethyltaurine) using a Novel Cadaver Nail Model

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Abstract

Background. Onychomycosis is the most commonly diagnosed nail disorder1. Topical treatment of the infection is limited by the inability of currently available drugs to penetrate the human nail. NVC-422 is a hydrophilic agent with potent antimicrobial activity making it a potential candidate as an effective ungal antifungal. In this study, a novel in vitro infected human nail model was developed and used to evaluate the ability of novel NVC-422 gel formulations and nanoemulsion lacquers to penetrate and kill fungi grown on the subungual side.

Methods. Trichophyton mentagrophytes and T. rubrum were used to inoculate the ventral surface of the cadaver nails and incubated for one week at 30°C. To ensure adequate infection of the nail, scrapings were inoculated on the ventral surface of the nail and incubated for another week. Next, nails were cleaned with solvent to remove the applied formulations; ground and fungal viability determined as colony forming units (CFUs). 8% cleaned nails were then ground separately, creating suspensions of nail powder which were correspondingly positive untreated controls were weighed, sonicated and submersed in 70% isopropyl alcohol and rinsed with sterile water; 20 µl of cell suspension with a count of 2 x 10⁶ CFU/ml were placed on the ventral surface of the nail for a minimum of 2 hrs. Nail plates were then placed in a Petri dish with sterile water and incubated in a humidified incubator at 30°C for 1 week. After two weeks, the nail was placed ventral side down, and 50µl of the gel or lacquer formulations were added as a single application to the dorsal surface. Applications were performed in a biosafety cabinet protected from light. Petri plates were returned to the 30°C humidified incubator visually inspected on a daily basis for one week. One week following application of formulations, the applications were removed by rubbing the dorsal side of the nails with a cotton swab saturated with ethanol or acetone for gel and lacquer formulations, respectively, to remove any formulation residue. Treated nails and corresponding positive untreated controls were weighed, sonicated and ground separately, creating suspensions of nail powder which were cultured and CFUs quantified2.

Results. Our data showed that NVC-422 was able to penetrate the nail and effectively eradicate T. mentagrophytes and T. rubrum. The antifungal activities of the test formulations were evaluated based on: a) number of CFUs, and b) visual fungal growth on nails. The nanoemulsion lacquers (Fig. 1 & 2) and novel gel formulations (Fig. 3 & 4) demonstrated significant nail penetration and antifungal activity following nail penetration indicated by no visual growth and reduction in the CFUs to the limit of detection.

Conclusion. In this study we developed and evaluated a novel infected human cadaver nail model and used it to assess the penetration and activity of selected formulations. Our data showed that NVC-422 gel and nanoemulsion lacquer formulations were able to penetrate the nail and eradicate the two most common fungal species causing onychomycosis. Further evaluation of NVC-422 is warranted.

References


Conclusions

- Stable formulations of NVC-422 were developed in proprietary nanoemulsion (Fig. 1 & 2) and gel technologies (Fig. 3 & 4)
- Both novel formulations indicated good penetration and eradication (reduction in the CFUs to the limit of detection) of T. rubrum and T. mentagrophytes.
- We successfully established a cadaver nail model for testing our proprietary formulations.

Methods

Nail Infection and Treatment with NVC-422 formulations

T. mentagrophytes and T. rubrum were subcultured onto potato dextrose agar and oatmeal agar plates, respectively. The plates were incubated at 30°C for 10-15 days. Mature colonies were then harvested in PBS and dispersed using a sonic dismembrator. After weighing cadaver nails, they were sterilized by submersing nails in 70% isopropyl alcohol and rinsed with sterile water. 20 µl of cell suspension with a count of 2 x 10⁶ CFU/ml were placed on the ventral surface of the nail for a minimum of 2 hrs. Nail plates were then placed in a Petri dish with sterile water and incubated in a humidified incubator at 30°C for 2 weeks. After two weeks, the nail was placed ventral side down, and 50µl of the gel or lacquer formulations were added as a single application to the dorsal surface. Applications were performed in a biosafety cabinet protected from light. Petri plates were returned to the 30°C humidified incubator visually inspected on a daily basis for one week. One week following application of formulations, the applications were removed by rubbing the dorsal side of the nails with a cotton swab saturated with ethanol or acetone for gel and lacquer formulations, respectively, to remove any formulation residue. Treated nails and corresponding positive untreated controls were weighed, sonicated and ground separately, creating suspensions of nail powder which were cultured and CFUs quantified.