

Presented at the Society for Investigative Dermatology
69th Annual Meeting
May 6-9, 2009 • Montreal, Canada

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ABSTRACT

Objective: Nanoemulsions (NB-00X) are oil-in-water emulsions composed of nanometer-sized droplets, stabilized by surfactants. We previously demonstrated permeation of NB-00X into epidermal and dermal tissues following application to human cadaver skin. We incorporated terbinafine hydrochloride (TBHC) in NB-00X and determined whether nanoemulsion droplets containing TBHC could diffuse laterally from the site of application.

Methods: *In vitro* studies were carried out using excised human cadaver skin in a modified diffusion apparatus. NB-00X incorporated with 1%TBHC or Lamisil AT cream containing 1% TBHC were applied onto human cadaver skin enclosed by two concentric glass cylinders. Twenty-four hours post application, residual formulation was removed and epidermis and dermis of the dosing areas were separated, weighed and assayed for TBHC using HPLC. A punch biopsy of the inner non-dosing area (inner area) and middle non-dosing area (middle area) were processed in similar fashion. Due to apparatus design, TBHC can only reach the middle or inner tissues by permeation of test formulation into skin underlying the dosing area and traversing laterally into the non-dosing areas.

Results: Levels of TBHC found in the outer, middle and inner epidermis were 14, 35 and 310 times higher ($\mu\text{g/g}$ tissue levels) when incorporated with the NB-00X formulation as compared to the same areas (outer, middle inner) for the Lamisil AT cream. TBHC levels found in the outer, middle and inner dermis were 27, 28 and 118 times higher ($\mu\text{g/g}$ tissue levels) for the 1% TBHC/0.3 % NB-00X formulation as compared to the same areas for the LamisilAT cream. These data indicate that TBHC, when incorporated into NB-00X, can traverse laterally up to 11 mm from the dosing area.

Conclusions: These results confirm that nanoemulsion containing an antifungal agent, terbinafine hydrochloride, diffuses laterally under the stratum corneum to tissues over a centimeter away from the site of application. This suggests that, when combined with NB-00X, antifungal agents can diffuse under human nails from adjacent skin sites to kill the fungus that causes onychomycosis.

BACKGROUND

•The difficulty in treating onychomycosis results from the deep-seated nature of the fungal infection within the nail unit (nail plate, nail bed and surrounding tissue) and the inability of drugs to effectively reach all sites.

•Therapies include oral antifungal agents taken for 12-16 weeks or topical products requiring at least 48 weeks of treatment. Although oral agents are more effective, they have safety risks that limit their use in older individuals and those with co-existing medical conditions.

•Although topical treatment would be ideal for onychomycosis in order to avoid systemic side effects, they have not been effective because they do not achieve high enough concentrations in the nail bed to eradicate the organism.

•Since NB-00X has intrinsic antifungal activity and reaches the nail bed by laterally diffusing from the periungual skin, we hypothesized that incorporation of TBHC within NB-00X would result in an effective combination product for the topical treatment of onychomycosis.

METHOD OF DELIVERY

Figure 1. Proposed mechanism of delivery of nanoemulsion after topical application.

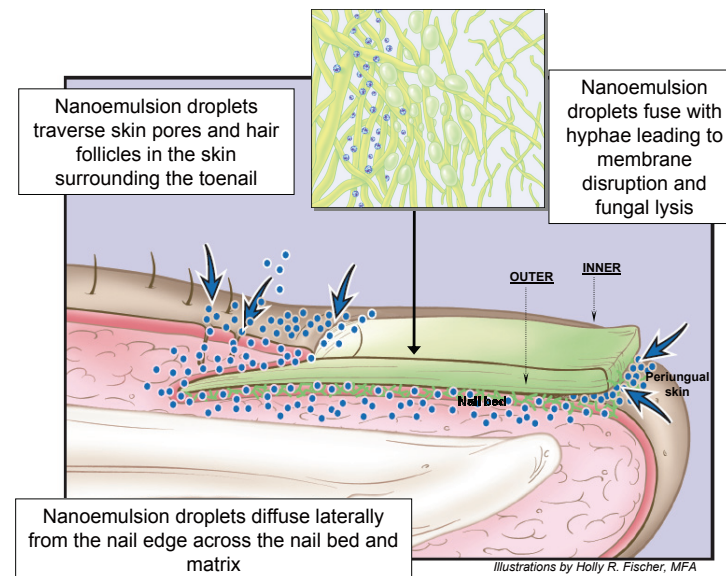
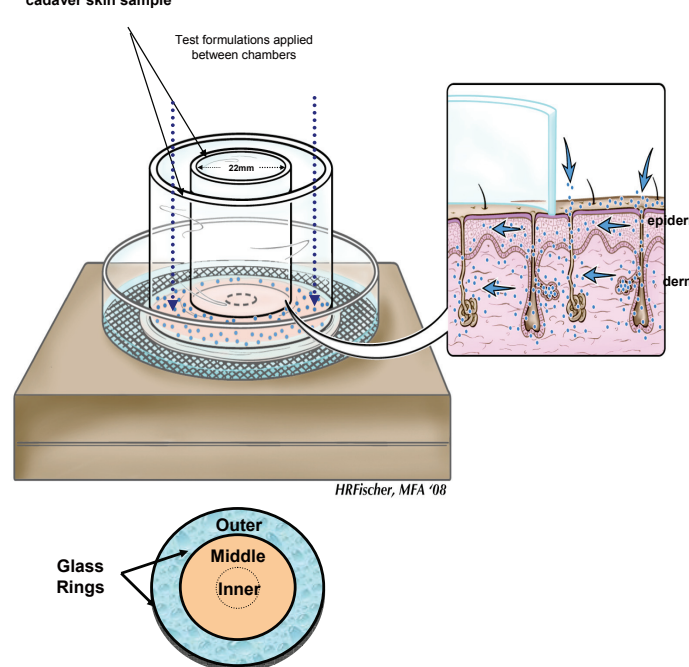


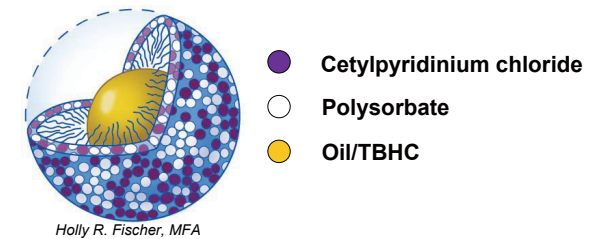
Figure 2. Schematic diagram of the lateral diffusion apparatus. Glass rings glued to cadaver skin sample



METHODS

Nanoemulsion: Nanoemulsions used in this study are oil-in-water emulsions with mean droplet diameters of ~200 nm, prepared with pharmaceutical grade excipients using a proprietary manufacturing method.

Figure 5. Nanoemulsion droplet (NB-00X).



Lateral Diffusion Method: Percutaneous absorption was measured using the *in vitro* cadaver skin finite dose technique. Parameters are described in Table 1 and the apparatus is illustrated in Figure 2.

Table 1. Experimental parameters for the lateral diffusion study.	
Apparatus	In-vitro lateral diffusion cell apparatus (Figure 2)
Membrane	Human cadaver skin, abdominal skin, 700 μm thickness
Duration	24 hours
Marker	Terbinafine Hydrochloride (TBHC)
Formulations	1% Terbinafine Hydrochloride
Dosing Volume and Frequency	527 μl of NB-00XB with 1% Terbinafine Hydrochloride at 0 hour and 8 hours (BID)
Surface Area	Outer dosing area: 5.27 cm^2 ; Middle area: 3.3 cm^2 ; Inner area: 0.5 cm^2
Dose per Surface Area	100 $\mu\text{l}/\text{cm}^2$
Cell Volume	50 mL
Receptor Solution	De-ionized water; pH 7, 37°C
Extraction Solvent	70% Ethanol solution
Assay Method	HPLC
Samples Collected	Surface wash, epidermis, dermis, and receptor samples.

RESULTS

Figure 3. Lateral diffusion of terbinafine hydrochloride in NB-00X in epidermis of human cadaver skin (CPC ($\mu\text{g/g}$): mean \pm SD).

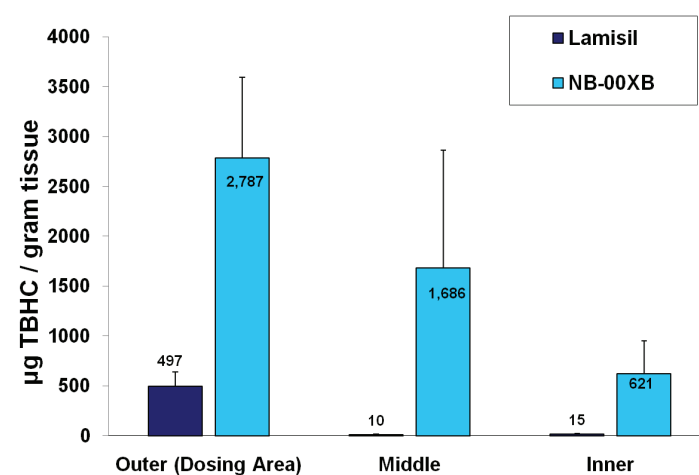
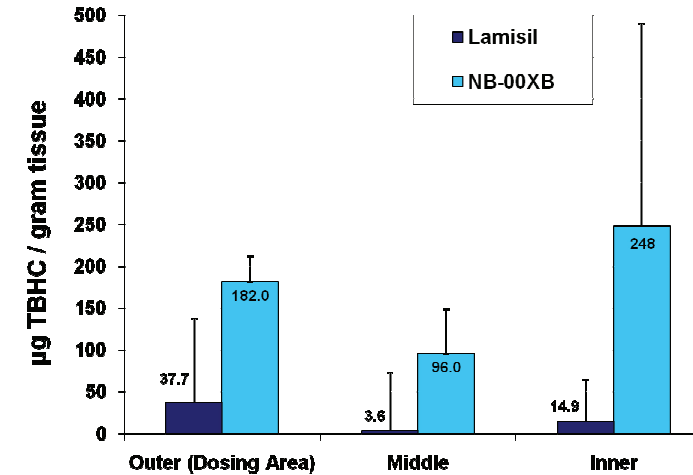


Figure 4. Lateral diffusion of terbinafine hydrochloride in NB-00X in dermis of human cadaver skin (CPC ($\mu\text{g/g}$): mean \pm SD).



CONCLUSIONS

- These data indicate that TBHC incorporated within nanoemulsion can traverse laterally over 1 cm from the dosing area.
- The nanoemulsion delivers significantly higher levels of terbinafine hydrochloride in the skin as compared to an over the counter terbinafine HCl product.
- This combination approach may result in enhanced efficacy due to:
 - 1) the dual mechanism of action
 - 2) low potential for resistance given the NB-00X "kill on contact" mechanism
 - 3) optimized delivery of the antifungal agent into skin tissues, at higher levels than oral and topical therapies