

ABSTRACT

Purpose: Nanoemulsions are topical oil-in-water emulsions composed of nanometer-sized droplets, stabilized by surfactants. We have previously demonstrated permeation of nanoemulsion into the underlying epidermal and dermal tissues following application to human cadaver skin. We performed these experiments to test whether nanoemulsion droplets could diffuse laterally to areas not directly underlying the site of application.

Methods: *In vitro* studies were carried out using excised human cadaver skin in a modified Franz diffusion apparatus. The nanoemulsion (NB-002) has a cationic surfactant cetylpyridinium chloride (CPC) oriented at the oil-water interface, used as a marker for delivery. NB-002 (0.25% (wt/wt)) at 100 µl/cm² was applied to a 5.5 cm² concentric surface area of skin enclosed by two concentric glass cylinders. Twenty-four hours post application, residual nanoemulsion was removed by swabbing from the dosing area. The epidermis and dermis of the dosing area was separated, weighed and assayed for CPC. An 8-mm (0.5 cm² surface area) punch biopsy of the inner non-dosing area (inner area) and middle non-dosing area (middle area) were processed in similar fashion. Quantification of CPC was performed by HPLC. Due to apparatus design, the only way CPC could be detected in the middle or inner tissues is through permeation of nanoemulsion into the skin underlying the dosing area traversing laterally into the non-dosing areas.

Results: Epidermal and dermal concentrations of CPC in the non-dosing area were 700 and 150 µg/gram, respectively in the middle area and in the inner area 200 and 100 µg/gram tissue, respectively. These data indicate the nanoemulsion traversed laterally up to 11 mm from the dosing area. The levels of nanoemulsion in the middle and inner area tissues were substantially higher than the previously determined concentrations of nanoemulsion that kills fungi *in vitro* (4 µg/gram).

Conclusion: These results confirm that nanoemulsion diffuses laterally under the stratum corneum to tissues over a centimeter away from the site of application. This suggests that NB-002 can diffuse under human nails from adjacent skin sites to kill the fungus that causes onychomycosis. NB-002 is currently in a multi-centered double-blinded vehicle controlled Phase 2 trial for onychomycosis.

BACKGROUND

- Onychomycosis, is a fungal infection of the nail unit (nail plate, nail bed and surrounding tissue) that is difficult to treat because nails grow slowly and receive very little blood supply. The difficulty in treating onychomycosis results from the deep-seated nature of the infection within the nail unit and the inability of drugs to effectively reach all sites.
- NB-002 is a novel topical anti-fungal nanoemulsion currently being studied in 443 subjects with onychomycosis.
- NB-002 is especially suitable for treating onychomycosis because it can achieve high levels locally with little or no systemic exposure, thereby avoiding the adverse events and drug interactions of systemic antifungal agents.
- Our novel approach delivers our anti-fungal therapeutic through the skin pores (e.g. pilosebaceous units, hair follicles and sweat glands) into the living skin strata which can laterally diffuse to the site of infection (e.g. nail matrix), hence avoiding the permeability issues associated with the nail plate.

METHOD OF DELIVERY OF NB-002

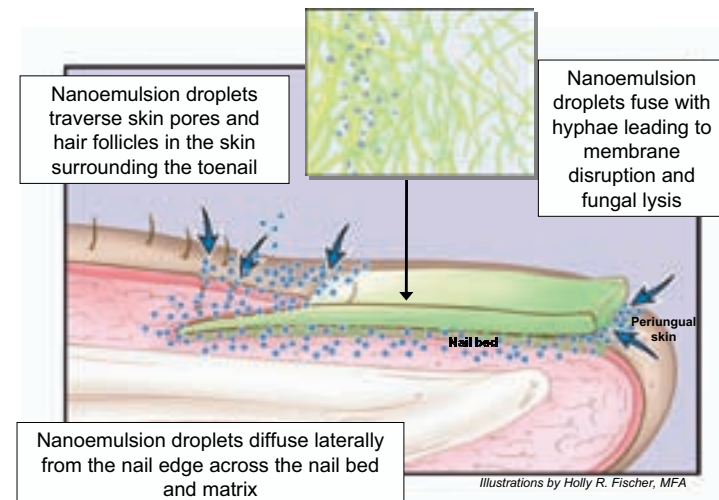


Figure 1. Proposed mechanism of delivery of nanoemulsion after topical application.

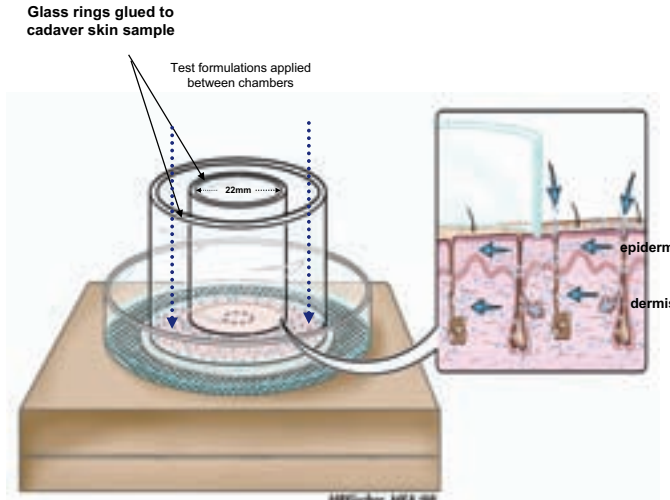
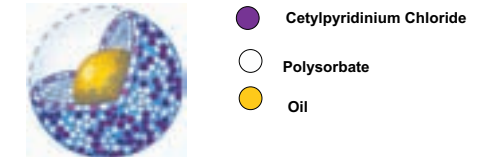


Figure 2. Schematic diagram of the lateral diffusion apparatus.

METHODS

Nanoemulsion: Nanoemulsions used in this study are oil-in-water emulsions with mean droplet diameters of ~180 nm, prepared with pharmaceutical grade excipients using a proprietary manufacturing method.

Figure 7. Nanoemulsion droplet (NB-002).



Electron microscopy: Microconidia were harvested from 7 day-old cultures of *T. rubrum* growing on potato dextrose agar using sterile distilled water and adjusted to a concentration of 106 conidia/ml. Part of the conidial suspension was pelleted and resuspended in RPMI 1640 medium and grown for 16-18 hours overnight at room temperature to allow germination of microconidia. After germination, the hyphae were collected by centrifugation and resuspended in distilled water. After mixing with different concentrations of NB-002, the samples were fixed and sputter-coated with gold and examined on an Amray 1910 FE Scanning Electron Microscope.

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	Cetylpyridinium chloride (CPC)
Formulations	0.5% aqueous CPC (5 mg/mL CPC); 0.25% NB-002 (2.5 mg CPC/mL)
Dosing Volume and Frequency	527 µl of aqueous CPC at 0 hour (QD) 527 µl of NB-002 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 µl/cm ²
Cell Volume	50 mL
Receptor Solution	pH 7, 37°C
Extraction Solvent	70% Ethanol solution
Assay Method	HPLC isocratic method for CPC
Samples Collected	Surface wash, epidermis, dermis, and receptor samples.

RESULTS

- NB-002 physically disrupts fungal hyphae (Figure 3) and spores (Figure 4)
- The levels of NB-002 achieved in the inner nailbed are 50-fold higher than the minimal fungicidal concentration

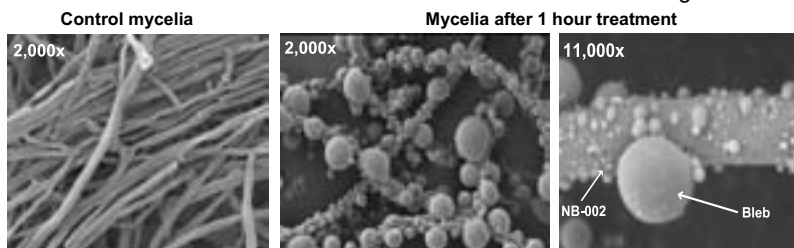


Figure 3. Scanning electron micrographs of *T. rubrum* hyphae after treatment with NB-002 (100 µg CPC/ml or 50X MIC) for 1 hour at room temperature.

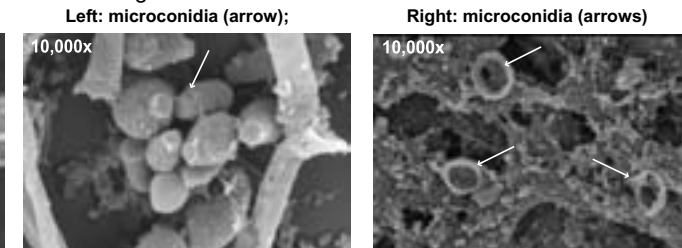


Figure 4. Scanning electron micrographs of *T. rubrum* microconidia (spores) after NB-002 treatment (12.5 µg/ml ≈ 6x MIC) for 1 hour at room temperature.

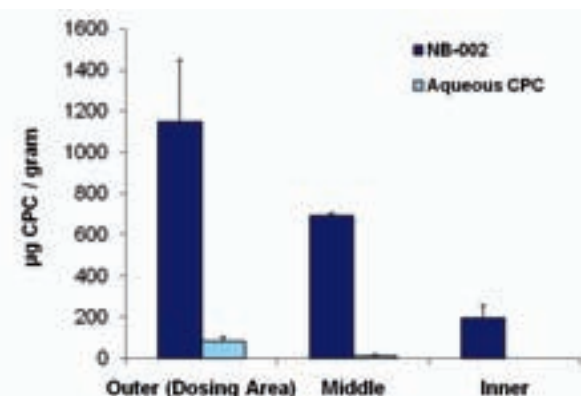


Figure 5. Lateral diffusion of NB-002 in epidermis of human cadaver skin (CPC (µg/g): mean ± SD).

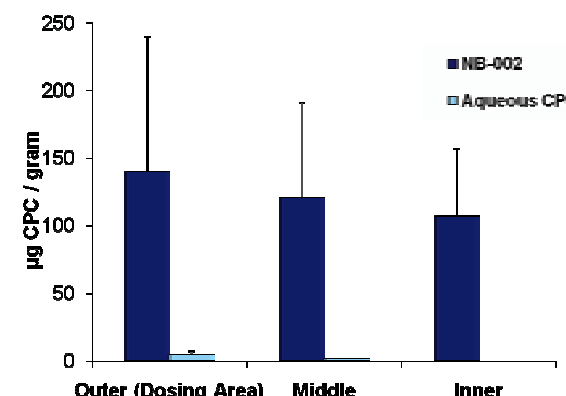


Figure 6. Lateral diffusion of NB-002 in dermis of human cadaver skin (CPC (µg/g): mean ± SD).

CONCLUSIONS

- NB-002 is substantially delivered into the epidermis and dermis.
- The nanoemulsion achieves levels 50 times the minimal fungicidal concentration (MFC=4 µg/gram), indicating this may be very effective in the treatment of onychomycosis.
- These data indicate the nanoemulsion traversed laterally 11 mm from the dosing area.
- The nanoemulsion novel delivery mechanism indicates that it can laterally traverse under the nail (avoiding a need for nail permeability), via the epidermis and dermis, to reach the site of infection.
- These data are consistent with the clinical responses observed in the Phase 2 clinical trial in onychomycosis.