

Presented at the 2008 AAPS Annual Meeting and Exposition
November 16-20, 2008; Atlanta, GA

S CIOTTI¹, R EISMA¹, L MA¹ and JR BAKER^{1,2}, JR
¹NanoBio Corporation, ²University of Michigan; Ann Arbor, MI USA

For additional information contact :
John Coffey, Jr.
Phone: (734)-302-9107
E-mail: john.coffey@nanobio.com

ABSTRACT

Purpose: NB-001 is a topical anti-viral oil-in-water emulsion composed of nanometer-sized droplets stabilized by surfactants. Due to their size and surface properties, they should diffuse into the skin via hair follicles and skin pores. The aim of this study is to investigate whether NB-001 diffuses into human skin.

Methods: *In vitro* skin permeation studies were performed with NB-001 using a Franz diffusion cell using human cadaver abdominal skin dermatomed to ~700 µm thickness. NB-001 is a nanoemulsion (NE) containing the cationic surfactant cetylpyridinium chloride (CPC) oriented at the oil-water interface. Nanoemulsions containing different concentrations of CPC (0.1%, 0.3%, 0.5%) were topically applied to human cadaver skin at various frequencies (up to 5 times in a 12 hour period) and dosing volumes (100 or 400 µl/cm²). CPC in aqueous solution was used as a control. At 24 hours, the surface was swabbed to remove residual nanoemulsion. The epidermis and dermis were separated, weighed and CPC was assayed using HPLC. Cross polar light microscopy was used to examine the cadaver skin following NB-001 applications.

Results: NB-001 delivery was substantially increased over CPC in aqueous solution. Delivery into skin was significantly higher with a dosing volume of 400 µl compared to 100 µl of NE suggesting that hydration levels may impact NE delivery into tissues. An increasing dose response was seen between 0.1% and 0.3% NB-001, with 0.3% having 6.5 times more delivery than 0.1% after one application. This was also observed after five applications, with 0.3% having 8.5 times more delivery than 0.1%. Delivery of 0.5% NB-001, however, was 3.5 and 9.3 times less than 0.3% after 1 and 5 applications, respectively. Cross polar light microscopy indicated extensive crystal formation following 5 applications of 0.5% NB-001 that appeared to block the skin pores, preventing delivery of 0.5% NB-001.

Conclusions: NB-001, a novel anti-viral NE currently in Phase 2 clinical studies for herpes labialis, achieves significant levels of dermal and epidermal delivery after a single application. The highest levels of delivery are observed with 0.3%NB-001 applied 5 times in a 12 hour period.

BACKGROUND

- NB-001 is a novel anti-viral nanoemulsion that has been shown in Phase 2 clinical trials to have efficacy equal to or greater than that reported for systemic nucleoside analogues for the treatment of herpes labialis [1].
- Nanoemulsions are high-energy oil-in-water emulsions composed of nanometer-sized droplets with cetylpyridinium chloride (CPC) at the oil-water interface, serving as a marker for nanoemulsion activity (Figure 1).
- Upon contact with herpes virus, the droplets are thermodynamically driven to fuse with and disrupt the viral envelope causing viral lysis (Figure 2).
- Since the size of the droplets (~180 nm) prevents them from intercalating into the tight junctions between epithelial cells, NB-001 does not cause skin irritation and is not absorbed into the circulation [2].
- We propose that nanoemulsions gain access to the site of infection via the transfollicular route (Figure 3) as demonstrated by the localization of NB-001 in hair follicles and sebaceous glands using fluorescent staining (Figure 4).
- Transfollicular delivery was measured using a human cadaver skin apparatus (Figure 9) that has precedence for accurately predicting *in vivo* percutaneous absorption [3,4].

[1] Jones, et al., 48th Annual ICAAC/46th IDSA, V-3771
[2] Jarratt et al., 48th Annual ICAAC/46th IDSA, V-3539
[3] Franz, T.J. Percutaneous absorption: on the relevance of *in vitro* data. *J Invest Dermatol*, 1975, 64:190-195.
[4] Franz, T.J. The finite dose technique as a valid *in vitro* model for the study of percutaneous absorption in man. In: *Skin: Drug Application and Evaluation of Environmental Hazards, Current Problems in Dermatology*, vol. 7, G. Simon, Z. Paster, M Klingberg, M. Kaye (Eds), Basel, Switzerland, S. Karger, 1978, pp 58-68.

MECHANISM OF ACTION OF NB-001

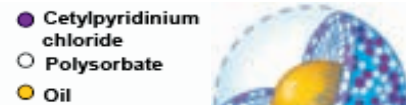


Figure 1. Nanoemulsion droplet

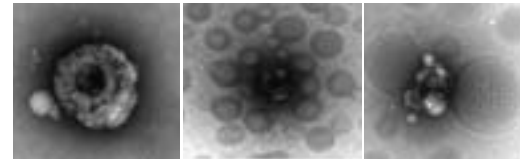


Figure 2. a) Herpes virus; b) Nanoemulsion droplets surrounding and fusing with virus; c) Disrupted viral envelope with viral lysis.

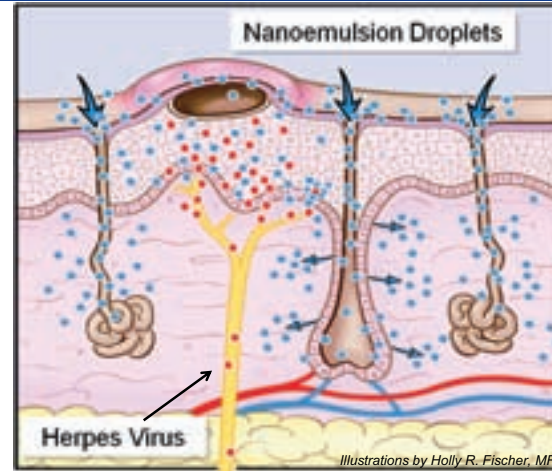


Figure 3. Delivery of Nanoemulsion into a Herpes lesion.

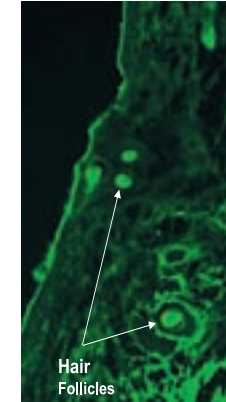


Figure 4. Fluorescein-labeled NB-001 in hair follicles and diffusion to skin tissues

RESULTS

- Delivery of NB-001 was substantially increased over aqueous CPC which produced no epidermal or dermal permeation (Figure 5).
- The skin permeation was enhanced with a larger dosage volume of 400 µL compared to 100 µL (Figure 6).

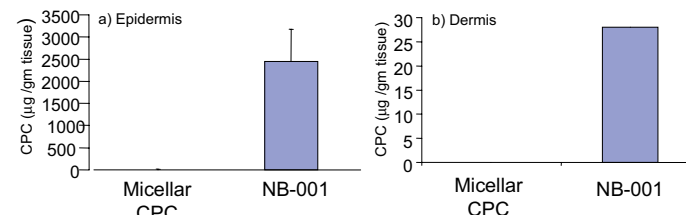


Figure 5. Micellar CPC and NB-001 delivery in human cadaver skin at 24 hours; skin epidermis (a) and dermis (b) (mean CPC (µg/cm²) ± SD)

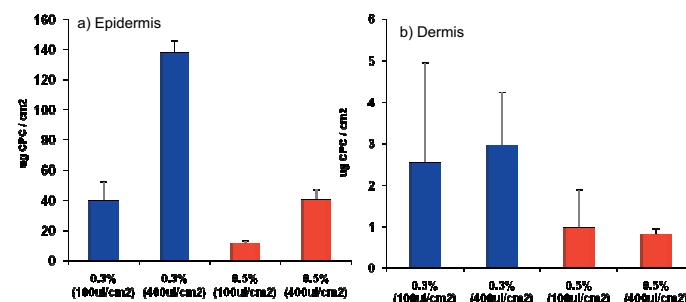


Figure 6. NB-001 in human cadaver skin epidermis (a) and dermis (b) (mean CPC (µg/cm²) ± SD) with 100 and 400 µl/cm² dose.

- There was an increasing dose response of dermal penetration of NB-001 when the concentration of NB-001 was increased from 0.1% to 0.3% and from one to five applications (Figure 7).
- The permeation decreased with five applications of 0.5% NB-001 due to precipitation of CPC on the skin surface blocking the skin pores and hair follicles (Figure 8; Table 1).

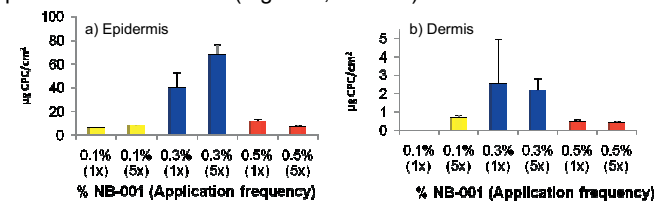


Figure 7. NB-001 in human cadaver skin epidermis and dermis after a single topical dose and 5 doses (mean CPC (µg/cm²) ± SD).

Table 1. Visual Comparisons of NB-001 Formulations.

Time (minutes)	Placebo	0.1%	0.3%	0.5%
0	-	-	-	-
10	-	-	-	-
20	-	-	-	x
30	-	-	-	xx
60	-	x	x	xxx
90	-	x	x	xxx
120	-	x	xx	xxx
240	-	xx	xx	xxx
300	-	xx	xx	xxx

-: no crystals; x: some crystals; xx: many crystals; xxx: all crystals

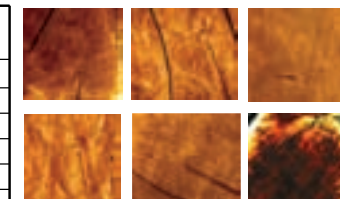


Figure 8. Cross polar micrographs of NB-001 after (a) 1x and (b) 5x applications to human cadaver skin. Darkened images depict crystallization.

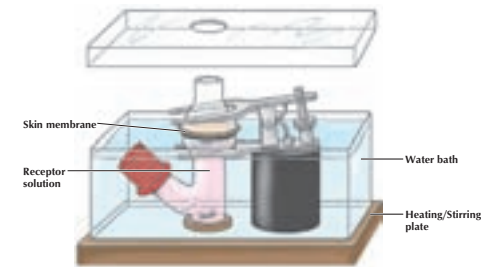
METHODS

Nanoemulsion: Nanoemulsions used in this study are oil-in-water (o/w) emulsions with mean droplet diameters of ~180 nm, made of pharmaceutical grade excipients and prepared by a proprietary manufacturing method.

In-Vitro Diffusion Method: Percutaneous absorption was measured using the *in vitro* cadaver skin finite dose technique. Parameters described in Table 2 and the apparatus is illustrated in Figure 9.

Table 2. Parameters for in-vitro diffusion apparatus.	
Apparatus	In-vitro Franz diffusion cell set up (Figure 9)
Membrane	Human cadaver abdominal skin
Duration	24 hours
Marker	Cetylpyridinium chloride (CPC)
Formulations	0.1% NB-001 (1 mg CPC/ml), 0.2% NB-001 (2 mg/ml CPC), 0.3% NB-001 (3 mg CPC/ml), 0.4% NB-001 (4 mg CPC/ml), 0.5% NB-001 (5 mg CPC/ml), 0.3% NB-001 contained a fluorescent marker
Dose and Frequency	100 µl/cm ² was applied once. For multiple applications, 100 µl/cm ² was applied every 3 hours after the initial dosing for a period of 12 hours
Cell Volume	7.7 mL
Receptor Conditions	pH 7; 37°C (receptor solution); 32°C (skin surface temperature)
Extraction Solvent	70% Ethanol Solution
Test Methods	HPLC Isocratic method for CPC; Cross polar microscopy
Samples Collected	Surface wash, epidermis, dermis, and receptor samples

Figure 9. In-vitro diffusion apparatus.



CONCLUSIONS

- The transfollicular delivery route allows NB-001 to be used for treatment of dermal and epidermal infections without causing skin irritation or requiring systemic absorption.
- Evidence of crystallization after multiple application of the 0.5% NB-001 may hinder transepithelial (follicular) delivery.
- The effect of applied volume impacts hydration levels of the nanoemulsion droplets and delivery of CPC into tissues.
- Evaporation is much slower with 400 µl verses 100 µl, hence the nanoemulsion droplets remain intact longer for sustained delivery into the tissue, as determined by CPC levels.