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ABSTRACT

Introduction: Nanoemulsion technology employs oil-in-water emulsions containing high-energy nanometer-sized droplets with active surfactants at their oil-water interface. Using this platform technology, we developed an antifungal nanoemulsion (NB-002) with potent activity against both fungal spores and hyphae.

Methods: Using previously published principles of nanoemulsion formulation, we formulated NB-002 using ingredients that are either "generally recognized as safe" (GRAS) or have a history of safe use in humans. Stability was characterized by physical appearance, droplet size, pH, and potency. Stable emulsions were tested in an in vitro fungicidal assay to determine the minimum inhibitory concentration (MIC) and minimum lethal concentration (MLC) against laboratory and clinical dermatophyte isolates associated with onychomycosis, as well as several *Candida* species. In vivo safety studies were performed in appropriate animal models.

Results: Homogenization of vegetable oil, ethanol, polysorbate, purified water, and CD-1 (cationic surfactant) at optimal ratios produced a formulation that was stable at accelerated (6 months at 40°C) and long-term conditions (3 yrs at 19°C-25°C). The MIC and MLC of NB-002 against *Trichophyton*, *Epidermophyton*, *Microsporum*, and *Candida* species ranged from 0.5 - 4 µg/mL against both fungal spores and hyphae as compared to vehicle that showed no activity. Topical administration of concentrations 1000-fold higher than the MIC showed no dermal sensitization in guinea pigs and no toxicity in a 9-month repeat dose dermal study in minipigs.

Conclusions: NB-002 is a novel nanoemulsion with potent activity against the organisms that cause onychomycosis and other dermatomycoses. NB-002 appears to be safe for topical application at doses 1000-fold higher than the minimum fungicidal concentration. A phase 1 study demonstrating the safety, tolerance, and pharmacokinetics of NB-002 has been completed, and a phase 2 study is under way in over 400 subjects with distal subungual onychomycosis.

INTRODUCTION

Onychomycosis or fungal infection of the nail bed affects 25% of adults, and the incidence rises with age such that the prevalence in adults over 50 years of age is 40%.¹ Toenail infections are caused primarily by the dermatophytes *Trichophyton rubrum* (90%), *Trichophyton mentagrophytes*, and *Epidermophyton floccosum*, while *Candida* causes 51-70% of fingernail infections.^{1,2} Onychomycosis has significant negative effects on a patient's social, occupational, and emotional functioning.³ Secondary bacterial infections, especially in immunocompromised hosts (e.g., patients with diabetes and peripheral vascular disease), is a major concern, especially in our increasingly prevalent aging and diabetic populations.⁴ Thus, a safe, effective therapy without systemic side effects would be welcomed. Topical therapy may also prove useful for the prevention of relapse or reinfection of fungal infections where environmental conditions (spores in the patient's shoes) are permissive.

METHODS

Emulsion manufacturing: The oil-in-water emulsions are composed of highly purified vegetable oil, alcohol, surfactants, and purified water homogenized using high-speed mixers. The stability of the emulsions are attributed to their ingredient ratio and the proprietary manufacturing process.

METHODS

Stability testing: The manufactured emulsions were tested for stability according to ICH guidelines. The emulsions were stored in glass vials at 40°C/75% relative humidity (RH) for 6 months or at room temperature (25°C/60% RH) for 36 months. The drug product was assessed by general appearance (white homogenous liquid with no signs of separation), pH (4-6), droplet size (<400 nm), and potency. CD-1 was used as the reporter of energized nanoemulsion droplets and was quantitated by HPLC. **NB-002 passed all criteria of the stability testing.**

Antifungal susceptibility: The MICs for the dermatophytes and *C. albicans* followed the Clinical and Laboratory Standards Institute guidances (M38-A5 or M27-A26). To determine MLC, the initial inoculation was 1-3 x 10⁴ cfu/mL to ensure that there was a 99.9% reduction in viability.

In vivo safety studies: The following animal tests were performed to confirm safety of the drug material for human use:

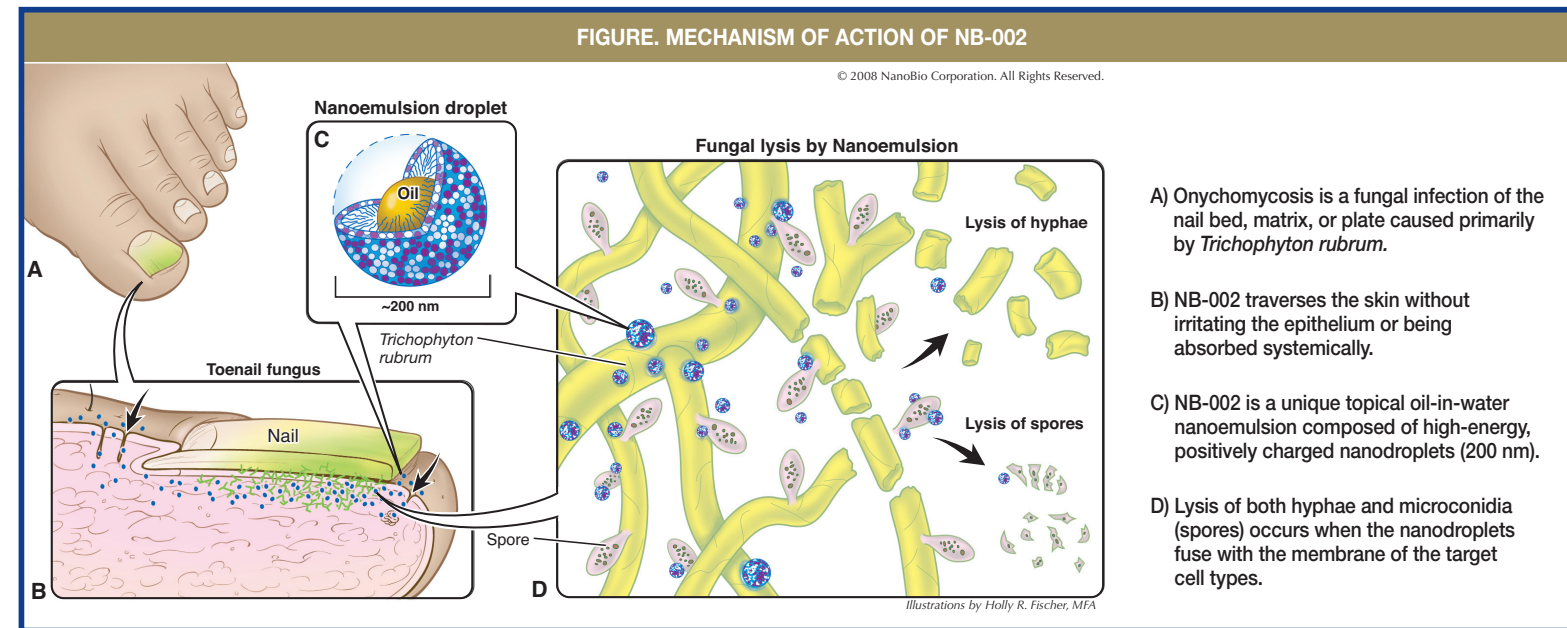
- Dermal sensitization in guinea pigs (n = 10F, 10M) given NB-002 (10 mg/mL) 3 times weekly for 3 consecutive weeks, followed by a challenge
- Dermal toxicity study in minipigs (n = 4F, 4M) given NB-002 (0.1-0.5 mg/cm²) BID for 9 months

RESULTS

TABLE 1. MIC AND MLC VALUES OF NB-002

Organism (n=5)	MIC and MLC values of NB-002 (µg/mL)			
	0.5	1	2	4
<i>Candida albicans</i> MIC	0	5	0	0
MLC	0	0	1	4
<i>Microsporum canis</i> MIC	3	2	0	0
MLC	0	5	0	0
<i>Epidermophyton floccosum</i> MIC	0	5	0	0
MLC	0	0	5	0
<i>Trichophyton mentagrophytes</i> MIC	0	1	4	0
MLC	0	0	1	4
<i>Trichophyton rubrum</i> MIC	0	5	0	0
MLC	0	0	5	0

MECHANISM OF ACTION OF NB-002



- A) Onychomycosis is a fungal infection of the nail bed, matrix, or plate caused primarily by *Trichophyton rubrum*.
- B) NB-002 traverses the skin without irritating the epithelium or being absorbed systemically.
- C) NB-002 is a unique topical oil-in-water nanoemulsion composed of high-energy, positively charged nanodroplets (200 nm).
- D) Lysis of both hyphae and microconidia (spores) occurs when the nanodroplets fuse with the membrane of the target cell types.

TABLE 2. MIC VALUES OF TERBINAFINE

Organism (n=5)	MIC values of terbinafine (µg/mL)						
	0.001	0.002	0.004	0.008	0.015	0.030	>32
<i>Candida albicans</i>	0	0	0	0	0	0	5
<i>Microsporum canis</i>	0	0	1	1	1	2	0
<i>Epidermophyton floccosum</i>	0	0	0	2	3	0	0
<i>Trichophyton mentagrophytes</i>	1	0	4	0	0	0	0
<i>Trichophyton rubrum</i>	3	2	0	0	0	0	0

TABLE 3. MIC50 AND MIC90 VALUES (µg/mL)

Organism	NB-002			Terbinafine		
	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range
<i>Candida albicans</i>	1	1	1	>32	>32	>32
Dermatophytes	1	2	0.5-4	0.004	0.015	0.001-0.030

TABLE 4. NB-002 TOXICITY STUDIES

Study	Species	Route	Dose	Test material concentration	Duration	Group size	Findings
Dermal sensitization	Guinea pig	Topical	0.3 mL per chamber	10 mg/mL	Induction: 3x weekly for 6 hrs for 3 consecutive wks; challenge for 6 hrs	10/sex/group	• No deaths occurred • No contact sensitization occurred
Chronic dermal	Minipig	Topical	0, 0.1, 0.3, 0.5 mg/cm ²	0, 1, 3, 5 mg/mL	273-274 days	4/sex/group	• No deaths occurred • NOAEL = 5 mg/mL

CONCLUSIONS

- Against 20 isolates (n = 4 species) of dermatophytes, NB-002 had a MIC₉₀ of 2 µg/mL and an 8-fold MIC range while the MIC₉₀ and range for terbinafine was 0.015 µg/mL and 30-fold, respectively
- Against the 5 isolates of *C. albicans*, NB-002 was uniformly active at 1 µg/mL while terbinafine was inactive at 32 µg/mL
- NB-002 appeared microbicidal against both *C. albicans* and dermatophytes. Although MLCs were not determined for terbinafine in this study, this compound is considered static for *C. albicans* and microbicidal for most dermatophytes⁷
- NB-002 is a safe, stable nanoemulsion with antifungal activity for use in topical treatment of onychomycosis and other dermatomycoses

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