

P2423 Antifungal Activity of NB-002, a Topical Nanoemulsion, Against Rare Fungal Pathogens of Onychomycosis

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ABSTRACT

Background: NB-002 is an oil-in-water emulsion with nanodroplets that have an average diameter of 180 nm. The size and composition allows for selective uptake into hair follicles and pores. The major pathogens in onychomycosis are the dermatophytes *Trichophyton rubrum*, *Trichophyton mentagrophytes*, and *Microsporum*. NB-002 has been shown to be uniformly fungicidal against both of these species. In this study, we assessed the antifungal activity of NB-002 against 12 genera of filamentous fungi that can be minor species causing cutaneous skin infections.

Methods: All fungi were from patients, many of which had onychomycosis or another tinea infection. The minimum inhibitory concentrations (MIC) of NB-002 and comparator compounds were determined using methodology described in the Clinical Laboratory Standards Institute M38-A2.

Results: NB-002 was the most consistently active antifungal. The MIC range was 0.5-1, 2-8, 0.5-2, 0.5-2, 0.5-1, 0.25-1, 1-2, 0.06-0.5, 0.06-1, 0.5, 0.5-1 and 0.25 µg/ml to [species (number of isolates tested)] *Aspergillus* spp. (5), *Paecilomyces* spp. (4), *Fusarium* spp. (10), *Acremonium* spp. (5), *Scopulariopsis* spp. (5), *Scedosporium* spp. (5), *Scytalidium* spp. (10), *Alternaria* spp. (3), *Epicoccum nigrum* (3), *Curvularia* spp. (3), *Phoma* spp. (3) and *Chaetomium* spp. (3), respectively. The topical nanoemulsion distinguished itself against comparators amphotericin B, itraconazole and terbinafine because of its potency against *Scopulariopsis* spp. and *Scedosporium* spp. NB-002 was superior to ciclopirox because of its activity against *Fusarium* spp. and *Paecilomyces* spp.

Conclusions: These data extend the activity of NB-002 to rare fungal species that can cause cutaneous fungal infections. The in vitro broad coverage of both the major and minor pathogens of cutaneous mycoses like onychomycosis should prove reassuring to physicians prescribing treatment regimens. NB-002 is currently in clinical trials and interim efficacy results with its overall safety profile support NB-002 as a promising option for topical treatment of onychomycosis.

BACKGROUND

• Superficial fungal infections are found in the top layers of the skin and mucous membranes, the hair, and nails. Examples of fungal infections of the skin and other external surfaces include athlete's foot, jock itch, ringworm, onychomycosis and other tinea infections. Most of these infections are caused by three major genera of dermatophytes, *Trichophyton*, *Epidermophyton* and *Microsporum*.

• Nondermatophyte filamentous fungi that are normal soil saprophytes can also cause cutaneous skin and nail infections. A number of these species are multidrug-resistant, even to systemic antifungals like amphotericin B. Many of these fungal species are opportunistic, causing cutaneous and systemic infections in immunosuppressed patients.

• NB-002 is a high energy oil-in-water emulsion composed of nanometer-sized droplets that permeate into the epidermis and dermis via the follicular route (Ciotti, et al. 2008. Abstr. 48th Intersci. Conf. on Antimicrob. Agents Chemother., abstr. A-1898) and diffuse laterally through tissue planes, reaching areas as distant as 1 cm from the site of application (Ciotti, et al. 2008. Abstr. 48th Intersci. Conf. Antimicrob. Agents Chemother., abstr. M-2135) (Figure 1).

• NB-002 physically disrupts fungi by fusing with the fungal cell surface, resulting in blebs (protrusions) of the cytoplasm, causing disruption and lysis (Figure 2).

• Clinical trials with NB-002 for treatment of onychomycosis have shown this unique topical antifungal to be safe (see P2114).

• We assessed the activity of NB-002 compared to the available antifungal drugs against 12 genera of filamentous fungi that cause cutaneous fungal infections.

RESULTS

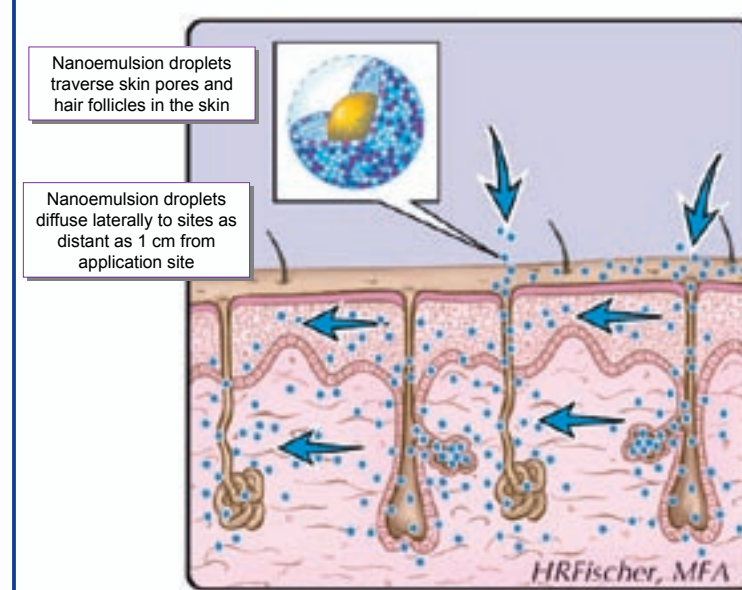


Figure 1. Uptake of NB-002 is through the transfollicular route.

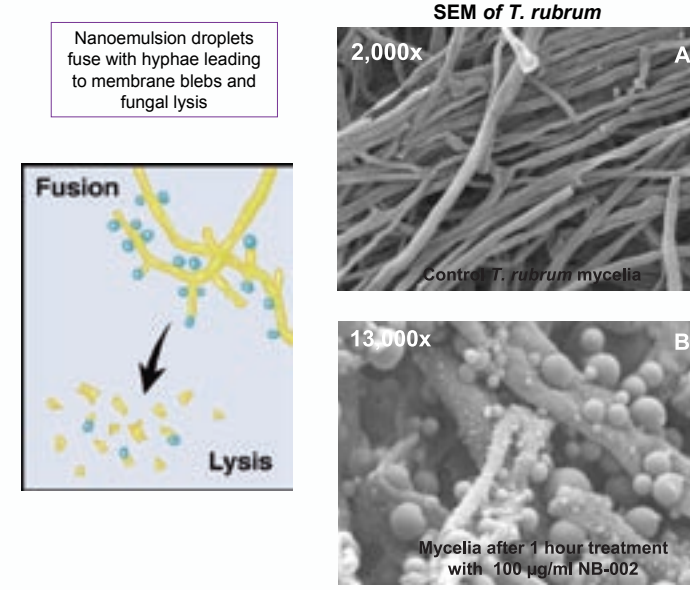


Figure 2. Fungicidal mechanism of action of NB-002.

Table 1. Antifungal activity of NB-002 and comparators against 59 isolates of filamentous fungi.

MIC Parameter (µg/ml)	Antifungal Agent				
	NB-002	Itraconazole	Terbinafine	Ciclopirox	Amphotericin B
MIC range (n=59)	0.06-8	0.06->8	≤0.004->2	0.25-16	0.125->16
MIC ₅₀ (n=33)	1	>8	1	1	2
MIC ₉₀ (n=53)	2	>8	>2	16	>16

Species	No. of Isolates	MIC range (µg/ml)				
		NB-002	Itraconazole	Terbinafine	Ciclopirox	Amphotericin B
<i>Aspergillus</i> spp.	5	0.5 -1	0.06-1	0.03-0.25	0.5-2	1-4
<i>Paecilomyces</i> spp.	4	2-8	1->8	0.25-0.5	8-16	>16
<i>Fusarium</i> spp.	10	0.5-2	2->8	2->2	1-16	4->16
<i>Acremonium</i> spp.	5	0.5-2	>8	0.125-1	0.5-4	0.5->16
<i>Scopulariopsis</i> spp.	5	0.5-1	4->8	1->2	0.5-2	>16
<i>Scedosporium</i> spp.	5	0.25-1	4->8	>2	0.5-8	>16
<i>Scytalidium</i> spp.	10	1-2	4->8	0.125-1	0.5-1	0.5-1
<i>Alternaria</i> spp.	3	0.06-0.5	0.25-0.5	1-2	0.25-0.5	1
<i>Epicoccum nigrum</i>	3	0.06-1	0.25-0.5	0.03-0.06	0.125-2	0.25-1
<i>Curvularia</i> spp.	3	0.5	0.125-0.25	0.03-1	0.5-1	0.125-1
<i>Phoma</i> spp.	3	0.5-1	0.06-0.5	0.03	0.5-1	0.5-2
<i>Chaetomium</i> spp.	3	0.25	0.5-1	1-2	0.25-0.5	0.5-4

METHODS

Emulsion manufacturing. NB-002 is an oil-in-water emulsion manufactured from ingredients that are Generally Recognized As Safe (GRAS) with a cationic detergent (cetylpyridinium chloride, CPC) that has proven safe for oral human use. The emulsion is formed from highly purified oil, ethanol, polysorbate, CPC and water. The average nanoemulsion droplet size was 180 nm as measured by dynamic light scattering. The relative activity of NB-002 can be expressed in terms of the concentration of cationic surfactant present. Thus, the antifungal activity of NB-002 is expressed in µg CPC/ml.

Source of isolates. All isolates in this study were clinical isolates from dermatological sources received in the Fungus Testing Laboratory for standard antifungal susceptibility testing and/or identification. Isolates (n=59) were confirmed to assure testing integrity.

Antifungal susceptibility. Testing was completed with a common lot of antifungal for each agent. Drug preparations were prepared according to the recommendation outlined in the Clinical and Laboratory Standards Institute (CLSI) document M38-A2. The CLSI recommended medium RPMI-1640 (Hardy Diagnostics, Santa Monica, CA) was utilized as the test medium for the azoles while testing of amphotericin B was conducted in Antibiotic Medium 3 (Difco, Detroit, MI). The inoculum size was 0.4-5 x 10⁴ CFU/ml, and incubation at 35°C for 48 hours.

Minimum inhibitory concentrations (MIC) were determined at 48 hours. The MIC for terbinafine and ciclopirox was the lowest concentration that exhibited an 80% reduction in turbidity as compared to the growth control. The MIC for amphotericin B, itraconazole and NB-002 was the lowest dilution exhibiting no visible fungal growth.

CONCLUSIONS

• NB-002 was the only antifungal consistently active against the 12 genera of filamentous fungi that cause skin mycoses.

• Other studies have shown NB-002 to have excellent fungicidal activity against dermatophytes (see P2411).

• The unique physical mechanism of action of NB-002 along with its novel transfollicular delivery route provide a more effective method of treating cutaneous skin infections that will be less prone to recurrences or resistance.

• Clinical trials with NB-002 to treat cutaneous mycoses are warranted.