In Vitro Nail Penetration of Nitric Oxide-releasing Formulations for the Topical Treatment of Onychomycosis

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SUNDAY-388

ABSTRACT

Background: Onychomycosis is a common fungal infection of the nail associated primarily with dermal and subungal changes. Nitric oxide-releasing drug candidates have been designed to deliver high levels of nitric oxide to infected nail tissue. The nitric oxide-releasing drug candidate NVN1000 (16% SB208 Lacquer) demonstrated fungicidal activity against T. rubrum in vitro. This study was designed to evaluate NVN1000’s ability to penetrate nail and human skin.

MATERIALS AND METHODS

In Vitro Time-Kill Assay: Time-Kill assays were performed in accordance with the ASTM E2783-11 Standard. Varying concentrations of nitric oxide-releasing drug substance (NVN1000 or NVN4000) were incubated with T. rubrum (ATCC 9730), and viability was measured at 4, 24, and 96 hrs.

In Vitro Nitric Oxide Release Assay: The drug substance was mixed in a 1:1 ratio with the appropriate hydrogel phase at room temperature in a custom reactor with a controlled gaseous flow of nitrogen to carry any nitric oxide released from the substance to the detector unit. The substance nitric oxide release profiles were determined using a custom designed apparatus interfacing with a nitric oxide analyzing unit (Sievers). Each substance was analyzed until no baseline level of nitric oxide was detected, or for 24 hours, whichever came first. The resulting nitric oxide release profiles were depicted in Figure 1a and the total nitric oxide release profile was plotted versus the square root of time (Figure 3a).

RESULTS

NVN1000 demonstrated fungicidal activity at the same concentration of drug substance necessary to inhibit growth (MIC = 153 µg/mL) while NVN4000 fungicidal activity (3-log reduction) was observed at eight times the minimum inhibitory concentration (MIC = 135 µg/mL previously observed for T. rubrum).

Figure 1. Nitric oxide-releasing Drug Candidates and In Vitro Nitric Oxide Release Profiles of Drug Substance (NVN1000 or NVN4000) in PBS (37°C, pH 7.4) A) Nitric oxide-releasing drug substance (NVN1000 or NVN4000) were mixed in a ratio of 1:1 with Ringer’s Hydrogel, and incubated at 37°C and 5% CO2 for 24 hrs. B) Nitric oxide release rate for NVN1000 and NVN4000 was measured as described in Methods. C) Total Nitric Oxide Release from Candidate Formulations.

Figure 2. In vitro T. rubrum Minimum Inhibitory Concentrations (MICs) and Time-Kill Assays with Nitric Oxide-Releasing Drug Substance Candidates. A) Minimum Inhibitory Concentrations. B) NVN1000 and NVN4000 were determined previously and T. rubrum cultures were inoculated with varying concentrations of either A) low-level NVN1000 or B) low-level NVN4000 drug substance in 100 µl Titr Buffer (pH 7.5-7.7) to assess fungicidal activity following a single exposure for either 4 or 24 hrs.

Figure 3. In vitro Nitric Oxide Release Profiles of Drug Product Candidate (SB208/SB218) Formulations Following Admixture at 32°C Detected via Nitric Oxide Chemiluminescence. A) The drug substance formulation was over time was assessed and B) the gaseous nitric oxide release rate was determined along with additional C) release kinetic parameters for candidate formulations.

Figure 4. In vitro Nitric Oxide-releasing Formulations Through the Nail Bed as Measured via Vertical Diffusion Cell. A) Diagram of a vertical diffusion cell and B) average nitric oxide released following application to NVN1000 or NVN4000 formulations following once daily application to nail surface. C) Mean percentage kill of T. rubrum in the ChubTurf infected nail investigated.

Figure 5. In vitro Nitric Oxide-releasing Formulations Through the Nail Bed as Measured via Vertical Diffusion Cell. A) Diagram of a vertical diffusion cell and B) mean percentage kill for various nitric oxide-releasing formulations following once daily application to the nail surface.

CONCLUSIONS

• NVN1000 demonstrated fungicidal activity at the same concentration of drug substance necessary to inhibit growth (MIC = 153 µg/mL) while NVN4000 fungicidal activity (3-log reduction) was observed at eight times the minimum inhibitory concentration (MIC = 135 µg/mL) previously observed for T. rubrum.

• NVN1000 demonstrated greater than 98.9% killing following 24 hrs of exposure to concentrations of either NVN1000 and NVN4000 demonstrating the ability of both nitric oxide-releasing candidates to exhibit fungicidal activity.

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