Nitric Oxide-Releasing Topical Therapeutic Agent for Atopic Dermatitis

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ABSTRACT

SB414, a topical cream-based nitric oxide-releasing formulation is being developed for the treatment of inflammatory skin diseases resulting from dysregulated interactions between keratinocytes and immune cells. SB414 Cream was tested in two in vivo models that probe components of atopic dermatitis (AD) pathology. The complex etiology of AD involves defects in skin barrier function, cutaneous hypersensitivity to environmental triggers, and both systemic and local immunologic responses. To assess gross anti-inflammatory activity, ear swelling was quantified in an oxazolone-induced murine model of contact hypersensitivity. Following a single topical treatment, a dose-dependent effect was observed, with the highest dose 6.0% SB414 Cream, resulting in a statistically significant (SS) 76% reduction in ear swelling as compared to untreated animals, which compared favorably to the 80% reduction achieved with 0.05% betamethasone cream.

The decreased expression of antimicrobial peptides and skin barrier defects in AD patients contribute to their enhanced susceptibility to secondary skin infections. In AD patients, *S. aureus* colonization has been shown to correlate with severity of lesions and cutaneous inflammation. The ability of topical SB414 Cream to reduce *S. aureus* bacterial counts was assessed in a porcine infected skin wound model. Dermatomed partial thickness skin defects were infected with *S. aureus* and biofilms developed for 2 days, followed by either 2 or 5 days of once daily topical dosing. Results showed a SS and dose-dependent reduction of *S. aureus* bacterial counts with SB414 as compared to untreated baseline wounds. The greatest efficacy was achieved with the highest dose after 5 days of treatment exhibiting a greater than 3-log reduction (3.46 ±0.03 Log CFU/mL) when compared to baseline.

SB414 Cream has the potential to be a promising topical therapy for atopic dermatitis given its potential ability to modulate the local immune response and reduce the *S. aureus* burden within affected skin.

MATERIALS & METHODS

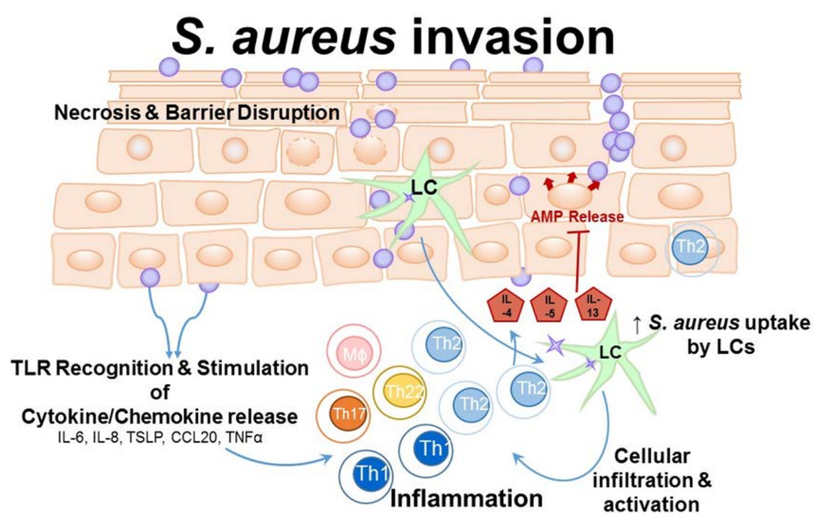
SB414 Cream – SB414 Cream is a two-component investigational drug product formulation comprised of an active ointment phase, containing the NVN1000 nitric oxide-releasing drug substance, and an inactive acetate buffered hydrogel phase that are mixed together at time of application to form a self-emulsifying cream. The aqueous hydrogel phase provides the proton source that initiates nitric oxide release from the active ointment phase.

Oxazolone-induced Murine Model of Contact Hypersensitivity – Male BALB/c mice (5/group) were sensitized with oxazolone (100 µL, 1.5% in acetone) by topical application to the shaved abdominal surface on Day 0. Seven days following sensitization, topical test articles were applied topically to the anterior and posterior surface of the right ear 30 minutes before and 15 minutes after a 2nd sensitization with oxazolone (20 µL, 1.0% in acetone). The right and left ear thickness was measured with a dial thickness micrometer gauge at 24 hours after the 2nd oxazolone application as an indicator of gross inflammation in all treatment groups. Ear swelling was calculated by subtracting the left ear (untreated control) from the right ear (treated). Percent inhibiting was calculated and one-way ANOVA followed by Dunnett's test was applied to determine statistical significance between Placebo control and treated groups. Significance was determined as $p<0.05$.

Porcine Infected Wound Model- Specific pathogen-free pigs (Looper Farms, NC) were anesthetized and 44 rectangular wounds (10 mm ×7 mm × 0.5 mm deep) were made to the paravertebral and thoracic area with an elecrokeratome. Wounds were separated by 4-5 cm of unwounded skin and individually dressed. Eight wounds were randomly assigned to each treatment group (5) and baseline. After wounding, 25µl of a methicillin-resistant S. aureus (MRSA) isolate obtained from an atopic dermatitis patient was use to inoculate each wound by scrubbing (10^5 CFU/ml) inoculums into each wound with a teflon spatula (30 seconds). All wounds were covered individually with a polyurethane film dressing (Tegaderm). The bacterial biofilms were allowed to form for 48 hours prior to treatment. Treatment groups were treated with ~300mg of test article and spread out to cover the wound and surrounding unwounded area with a sterile spatula and covered with film dressing. At the assessment time, 4 wounds per treatment group were recovered in 1 ml of neutralization solution and serially diluted. Serial dilutions were subsequently plated on selective media and incubated for 24 hours at 37°C prior to enumeration of viable colonies. Colonies were counted and the colony forming units per ml (CFU/ml), Log CFU/ml, mean Log CFU/ml and standard deviation calculated. A one-way ANOVA was used for statistical analysis. Significance was determined as $p<0.05$.

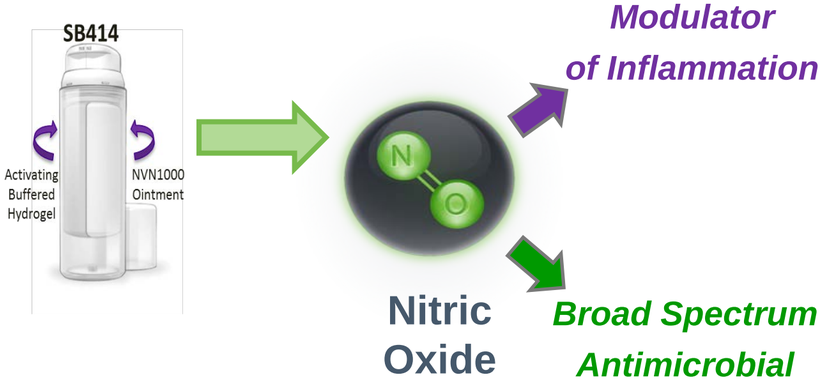
INTRODUCTION

Figure 1. Mechanisms by Which *S. aureus* Contributes to AD Pathology. *S. aureus* penetrates the epidermis via a proteolytic mechanism[1,2] that is coupled with a failure of the antimicrobial and physical barrier. *S. aureus* entry allows direct interaction with immune cells and stimulates pro-inflammatory cytokines. Th2 cytokines further inhibit AMP production and reduce the innate anti-Staph response[3].



Adapted from Hepburn L, et al. . Br J Dermatol. 2016 Oct 25 (epub ahead of print)

Figure 2. Nitric Oxide's Dual Mechanisms of Action Target AD Pathology.



References

1. *Nakatsuji T, et al. J Invest Dermatol. 2016;136(11):2192-2200.*
2. *Park KD, et al. Toxins. 2016;9(1):pii:E3.*
3. *Hepburn L, et al. Br J Dermatol. 2016 Oct 25 (epub ahead of print)*

RESULTS

Figure 3. Dose-responsive Inhibition of Ear Swelling in the Oxazolone-induced Contact Hypersensitivity Model. Percent inhibition determined versus oxazolone-only group. **p<0.05 vs. oxazolone-only.*

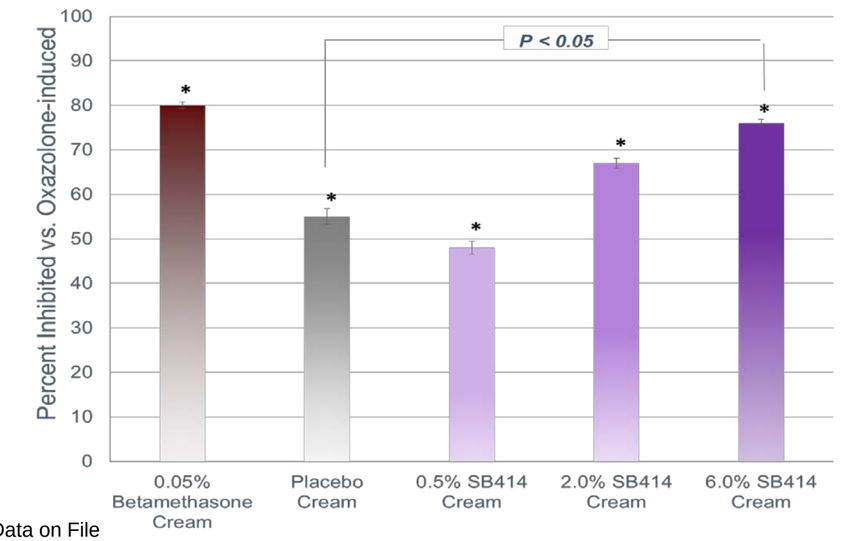


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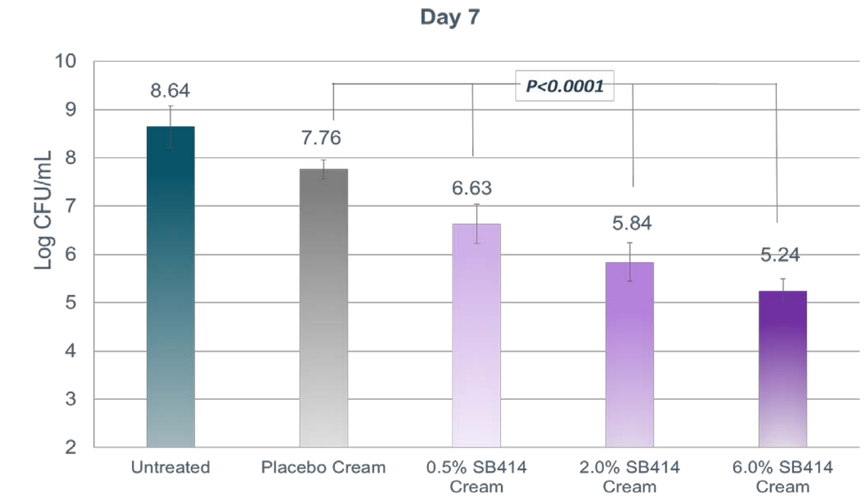
Figure 4. In Vivo Efficacy of SB414 Cream against Methicillin-resistant *S. aureus* in a Porcine Infected Wound Model. Bacterial recovery following 5 daily topical applications.



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CONCLUSIONS

- *S. aureus* invades the epidermal and dermal layers of AD skin and contributes to disease severity by activating immune cells and stimulating production of inflammatory cytokines.
- A dose-dependent inhibition of gross inflammation was observed with SB414 Cream in the oxazolone-induced contact hypersensitivity model.
- At the same strengths, SB414 Cream was observed to have potent anti-staphylococcal activity reducing MRSA counts by greater than 99.9% at the highest dose following 5 topical applications in a porcine model.
- Topical nitric oxide therapy has the potential to target 2 important aspects of AD pathology.