Effects of SB414 Cream on S. aureus and Tissue Cytokines in an Atopic Dermatitis Mouse Model

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Abstract

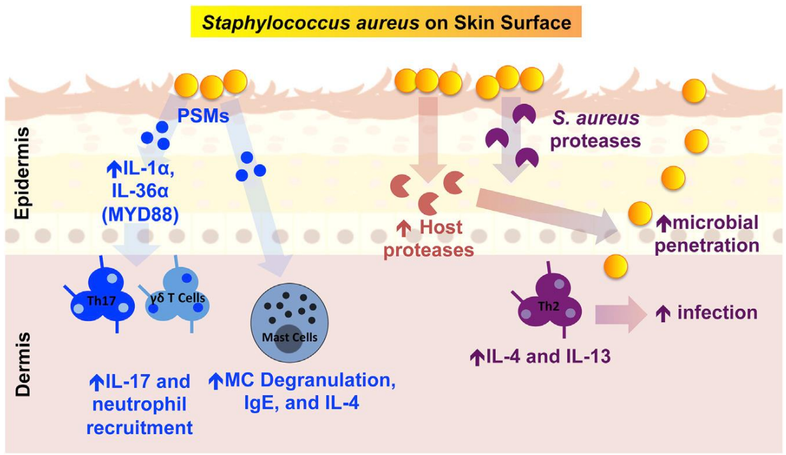
Atopic dermatitis (AD) is a chronic inflammatory skin disease where >90% of AD patients exhibit *Staphylococcus aureus* (SA) colonization of their lesional skin. The density of SA colonization has been correlated with both severity of AD lesions and the degree of cutaneous inflammation. A filaggrin-defect atopic dermatitis mouse model ($FLG^{ft/ft}$ mice) has been used previously to correlate the depth of SA skin penetration with upregulation of IL-4, IL-13 and other cytokines. Using this model where mice are first sensitized by ovalbumin and then exposed to 1 x 10^6 colony forming units (CFU) SA, a pilot study was conducted to identify the time course of peak cytokines levels (48 hrs) after cutaneous infection with SA and determine the optimal treatment window (initiate dosing at 24 hrs).

In a second study, animals were treated topically after the 24 hr SA incubation period, and again 8 hrs later, with vehicle or 6% SB414, a cream that releases nitric oxide. At 48 hrs, mice were euthanized and biopsied for SA counts and tissue cytokines levels in all treatment groups (IL-13, IL-4, TSLP, IFNɣ, IL-17a). Upregulation of individual cytokines in the untreated group ranged from 3 to 18-fold. All cytokine levels in the SB414-treated group, except IL-17a, were statistically indistinguishable from the uninfected, baseline tissue cytokine values. IL-4 was reduced by 87% and IL-13 by 76% compared to placebo treated mice. TSLP, an initiator of the atopic response and a sensitive marker of chemical irritation, was not elevated in SB414-treated infected or uninfected mice, demonstrating the local tolerability of the nitric oxide releasing cream. SA levels in the skin of SB414 treated mice were decreased to 0.5 x 10^5 CFUs, representing a >90% reduction over untreated and vehicle-treated mice. Importantly, the anti-staph effects of SB414 were not driven by increased IFN-γ tissue cytokine levels.

Collectively, these data demonstrate the ability of topically applied 6% SB414 to reduce key Th2 cytokines like IL-4 and IL-13, and to reduce SA burden in an atopic dermatitis mouse model.

Background

Skin barrier defects and bacterial dysbiosis trigger chronic inflammation and further perpetuate AD pathophysiology



S. aureus penetrates the epidermis via a proteolytic mechanism coupled with a failure of the antimicrobial and physical barrier. Entry of *S. aureus* stimulates epidermal keratinocytes to produce proinflammatory cytokines that further induce T cell and neutrophil recruitment. Induction of Th2 cytokines like IL-4 and IL-13 further reduce the innate anti-staph response through inhibition of antimicrobial peptide release while the continued barrier damage promotes infection and a sustainable Th2 response.

***S. aureus* colonization has been shown clinically to correlate with severity of AD lesions and cutaneous inflammation**

Williams, R. et al. Staphylococcus aureus: Master Manipulator of the Skin. Cell Host & Microbe. 2017; Vol22:5. Adapted with permission.
Gong, J. et al. 2006. Br. J. Dermatol. 155:680-687.
Kong, H. et al. 2012. Genome Res. 22:850-859

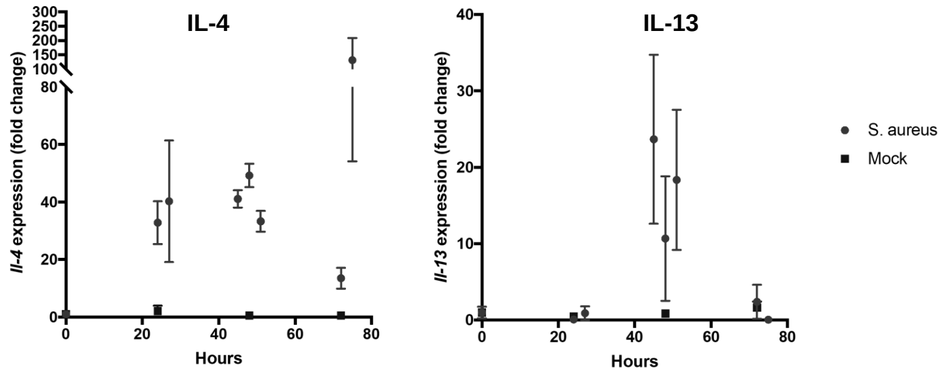
Study 1

Time course of cytokine response following *S. aureus* skin colonization to determine the appropriate timepoint following for topical intervention

Materials & Methods

1. Shaved and disinfected dorsal skin of 36 $FLG^{ft/ft}$ Balb/c mice
2. Placed agar disc containing 10^6 CFU *S. aureus* on dorsal skin and covered with wound dressing film, uninfected controls had agar disc/film only
3. Allowed *S. aureus* colonization 20 hours to occur
4. At 20 hours, removed agar disc from dorsal skin of all animals
5. Punch biopsied animals (n=3/timepoint) at timepoints 0, 20, 24, 44, 48, 52, 72 and 76 hrs for:
   - 16S rRNA species-specific primers/probe for *S. aureus* quantification
   - gDNA for cytokine qPCR analysis
6. Mock, uninfected mice received agar disc for 20 hrs and then were biopsied (n=3/timepoint) at timepoints 0, 24, 48 and 76 hrs for background cytokine levels (IL-13, IL-4, IL-17a, IL-1β) and uninfected control bacterial quantification

Results



Study 2

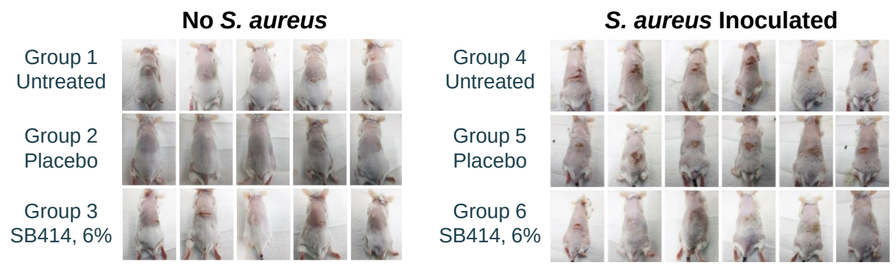
Assess the reduction in *S. aureus* colonization and key pro-inflammatory cytokines following topical intervention with 6% SB414 Cream

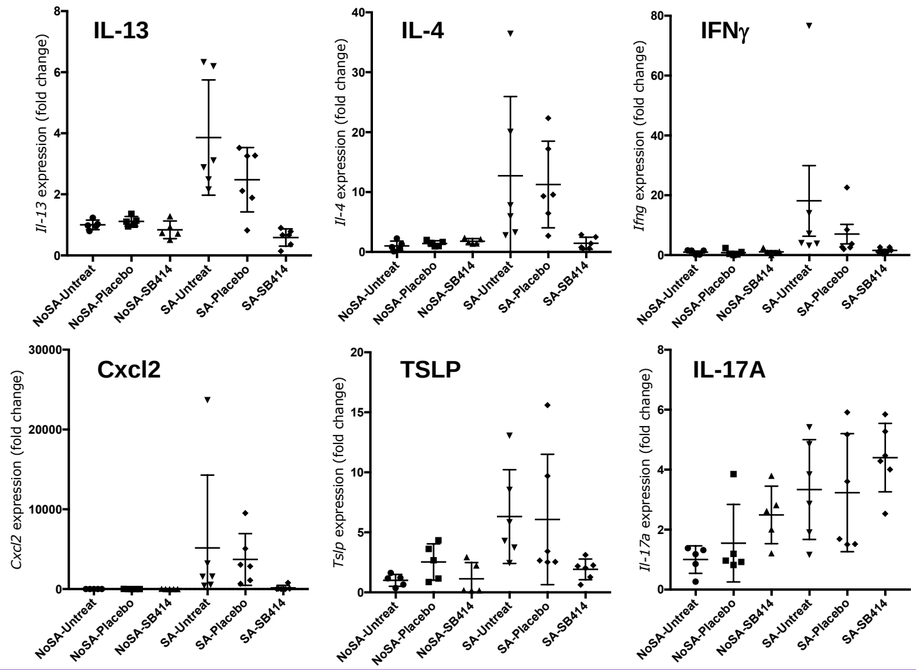
Materials & Methods

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. Based upon the results of the *S. aureus* colonization time course defined in Study 1, topical intervention occurred 24 hrs after inoculation and mice were euthanized and tissues harvested at 48 hrs post inoculation.

1. Sensitized back skin of $FLG^{ft/ft}$ mice (3-5 weeks, both sex) by OVA-patch for 1 week + 2 weeks off x 2 cycles
2. Shaved and tape-stripped mouse back 2 days after 2nd OVA patch sensitization
3. Topically applied TSB agar disc containing 10^6 CFU S. aureus (SA113) strain or agar disc only and covered with tegaderm for 24 hours
4. Removed tegaderm and TSB agar disc
5. Topically applied placebo or 6% SB414 Cream 0 hrs and 8 hrs after removing tegaderm and TSB agar disc
   - $FLG^{ft/ft}$ mice (n=33) were available for the experiment and were grouped as:
     - Group 1: No *S. aureus* + Untreated (n=5)
     - Group 2: No *S. aureus* + Placebo (n=5)
     - Group 3: No *S. aureus* + SB414 (n=5)
     - Group 4: *S. aureus* application + Untreated (n=6)
     - Group 5: *S. aureus* application + Placebo (n=6)
     - Group 6: *S. aureus* application + SB414 (n=6)
6. Obtained skin swab and skin biopsy 24 hrs after removing tegaderm and TSB agar disc for:
   - 16S rRNA species-specific primers/probe for *S. aureus* quantification
   - gDNA for cytokine qPCR analysis (IL-13, IL-4, IL-17a, TSLP, IFNɣ) and chemochine Cxcl2

Results





Conclusions

- IL-4 was reduced by 87% and IL-13 by 76% compared to placebo treated mice
- TSLP, an initiator of the atopic response and a sensitive marker of chemical irritation, was not elevated in SB414-treated, infected or uninfected mice, demonstrating the local tolerability of the nitric oxide-releasing cream
- SA levels in the skin of SB414-treated mice were decreased to 0.5 x 105 CFUs, representing a >90% reduction over untreated and vehicle treated mice and the anti-staph effects of SB414 were not driven by increased IFN-γ tissue cytokine levels
- Collectively, these data demonstrate the ability of topically applied 6% SB414 to reduce key Th2 cytokines like IL-4 and IL-13, and to reduce SA burden in an atopic dermatitis mouse model