Effects of SB204 on LPS-induced Cytokine Release in an Ex-Vivo Human Skin Model

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INTRODUCTION

NVN1000 is a macromolecular polymer with a polysiloxane backbone that contains covalently bound N-diazeniumdiolate nitric oxide donors, from which nitric oxide is released in the presence of a proton donor. SB204, a nitric oxide-releasing topical drug candidate in development for the treatment of acne vulgaris, utilizes NVN1000 as its active ingredient. SB204's potential mechanisms of action include broad-spectrum antimicrobial and immunomodulatory activity. In addition to bactericidal effects on *Propionibacterium acnes* (P. acnes), nitric oxide has known immunomodulatory effects. Nitric oxide inhibits NF-κB activity which decreases ProIL-1β and NLRP3 transcription. S-nitrosylation of NLRP3 by nitric oxide inhibits formation of the NLRP3 inflammasome assembly and cleavage of ProIL-1β to IL-1β. The purpose of this ex-vivo human skin model was to assess the anti-inflammatory effects of a nitric oxide-releasing test article (SB204) admixture formulation on lipopolysaccharide (LPS)-stimulated cytokine release in human healthy skin. Betamethasone was used as a positive control as it has been shown to decrease pro-inflammatory cytokine levels in models of inflammation and tissue injury.

BACKGROUND

Figure 1. Evidence of IL-1β in Acne Lesions Abundant IL-1β expression in papulopustular acne lesions vs. normal human skin; 50-fold increase in IL-1β mRNA vs. normal skin. Bar represents 100 µm.

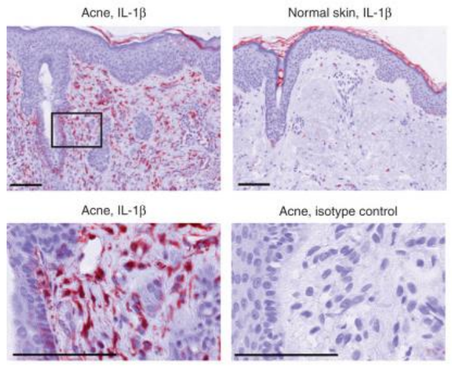

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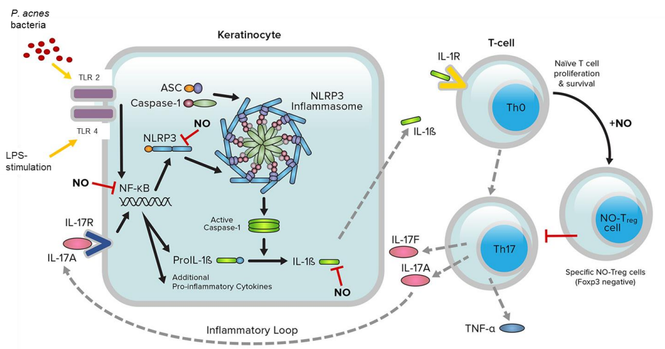
Figure 2. Nitric Oxide Affects Multiple Targets of the Inflammatory Loop in Acne.



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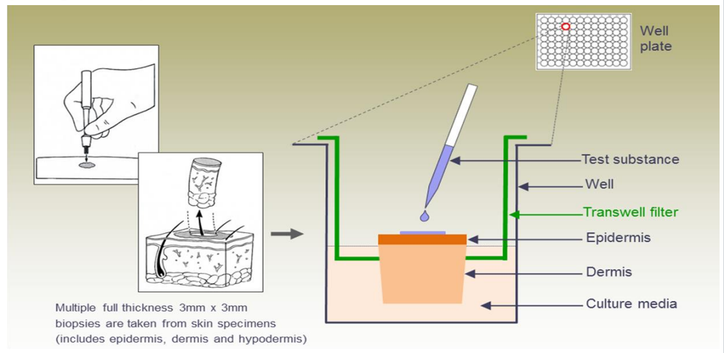
MATERIALS & METHODS

Skin was obtained from patients undergoing breast reduction, reconstruction or abdominoplasty procedure. Donors receiving anti-inflammatory treatment were excluded. Biopsies were 8 mm full thickness obtained via a biopsy punch. Test groups included: unstimulated control, stimulated (no treatment) control, placebo control, 4% SB204 gel and 0.1% Betamethasone – three biopsies/donor for unstimulated and stimulated controls and four biopsies/donor for placebo, SB204 and Betamethasone groups. Skin biopsies were randomized to treatment groups and submerged in Epilife® (supplemented with CaCl2, gentamicin and amphotericin B) for a 16 – 24 hour equilibrium period prior to start of experiment. Following the equilibrium period, biopsies were maintained in a full thickness transwell organ culture system (REPROCELL) for 24 hours. Biopsies were placed into Transwell filters with the epidermis facing upwards at the liquid-air interface and the transwell filters placed into 12-well culture plates containing 1 mL of fortified culture. LPS was spiked into the culture media at 1 µg/mL for all biopsies except in the unstimulated control group. Test articles were added topically to epidermal surface (5 mg of placebo, SB204 or Betamethasone) and then cultured at 37°C/humidified air/5% CO2. 24 hours post application of test articles, culture media was collected for cytokine analysis. Culture media samples were analyzed in duplicate for IL-6, IL-8, IL-10, IL1α, IL-1β, TNFα, MMP-1 and MMP-2 by multiplex ELISA. Luminex Magpix® system using Luminex xMAP ® compatible magnetic bead technology. Data was statistically analyzed by two methods: 1) Median values of each donor (n=6) as a percent of Vehicle or Untreated control using nonparametric ANOVA analysis and 2) Comparison between groups using all biopsies (n=18 unstimulated and stimulated controls and n=24 for placebo, SB204 and Betamethasone groups) by 2-tailed, equal variance T-test.

Figure 3. Ex-Vivo Human Skin Inflammation Model, Biopta, Ltd. (Glasgow, UK)



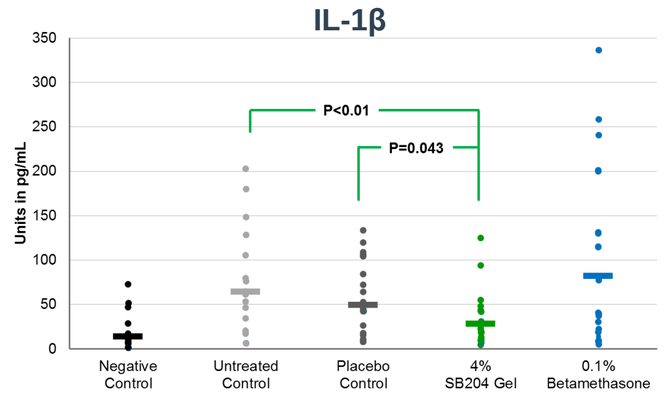
RESULTS

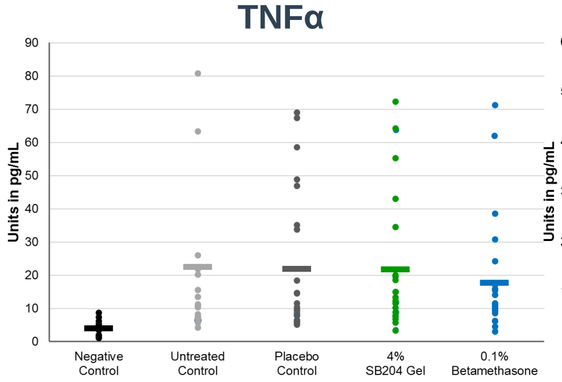
Table 1. Upregulation of Cytokines with LPS. For the 8 cytokines analyzed, five showed a statistically significant upregulation using both methods of analysis with LPS stimulation of approximately 2- to 7-fold: IL-6, IL-8, IL-10, IL-1β, and TNFα. Values are shown as Mean (SEM). P-value calculated as 2-tailed, homoscedastic T-test.

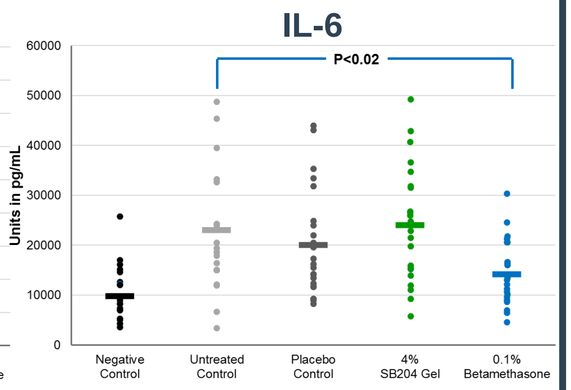
	IL-1β	IL-10	IL-6	IL-8	TNFα
Negative Control N = 18	16.3 (4.8)	12.2 (2.1)	10470 (1360)	11450 (2440)	3.9 (0.5)
Untreated Control N = 18	70 (14.3) P=0.001	56.8 (12.2) P=0.001	22470 (3000) P=0.001	21220 (3020) P=0.017	27.4 (9.9) P=0.023
Fold Increase from Negative to Untreated Control	4X	5X	2X	2X	7X

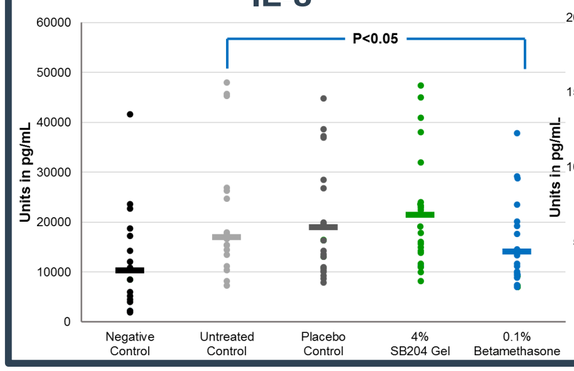
RESULTS

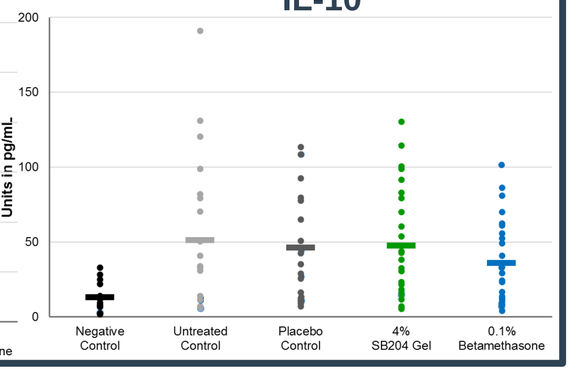
- Statistically significant inhibition of IL-1β upregulation was demonstrated with SB204 4% as compared to untreated controls and SB204 Placebo.
- Single application of 0.1% Betamethasone over 24 hours significantly inhibited the upregulation of IL-6 and IL-8 by approximately 66% and 73%, respectively, compared to untreated controls.
- Neither treatment showed significant inhibition of upregulation of IL-10 and TNFα in this human skin model.











CONCLUSIONS

- This model of LPS-induced upregulation of tissue cytokines in ex-vivo human healthy skin model showed statistically significant increases in 5 of 8 cytokines over 24 hours with TNFα showing the greatest fold (~7x) increase.
- Single application of SB204 4% over 24 hours significantly inhibited the upregulation of IL-1β by approximately 62% compared to untreated controls, P=0.045 non-parametric ANOVA.
- The inhibition in the upregulation of IL-1β supports the hypothesis that the mechanism of anti-inflammatory effect of SB204 via release of nitric oxide into the skin is mediated by the inactivation of the NLRP3 inflammasome.