The small molecule aldehyde trap NS2 exhibits potent anti-inflammatory activity in 3 murine models of inflammation

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Abstract

Free aldehydes are naturally occurring chemical species formed during a variety of biological processes, including polyamine and glucose metabolism and lipid peroxidation. Uncontrolled levels of aldehyde species lead to inflammation via activation of the NF-κβ pathway and also damage key lipids that comprise the dermal moisture barrier. Elevated levels of malondialdehyde (MDA) have been shown in a variety of inflammatory skin diseases, including psoriasis, atopic dermatitis and rosacea. Thus, pharmacological trapping of free aldehydes may represent a novel anti-inflammatory therapeutic approach.

Here, we report the effect of NS2, a novel aldehyde-binding small molecule, in reducing levels of various pro-inflammatory cytokines, and increasing levels of the anti-inflammatory cytokine IL-10, in a mouse LPS model of cytokine storm. We subsequently confirmed the anti-inflammatory effects of aldehyde trapping in a murine contact dermatitis model, in which ear swelling and thickness were decreased by prior NS2 treatment. Similarly, in a murine model of delayed type hypersensitivity dermatitis, NS2 reduced ear swelling and concomitantly lowered tissue levels of various inflammatory mediators, including G-CSF, M-CSF, MIG, MIP-1α, MIP-2 and KC.

These results suggest that NS2 could mitigate the toxic aldehyde load that is generated in various inflammatory conditions, and thus represents a new class of anti-inflammatory therapeutics.

Methods

Cytokine Storm Model: Mice first received an i.p. injection of NS2 (10, 30 or 100 mg/kg; formulated at 5 mg/mL) or vehicle. 30 mins later, lipopolysaccharide (LPS) was administered i.p. at 20 mg/kg, and 2h post LPS challenge, blood was collected by cardiac puncture, separated into plasma, and stored until cytokine analysis.

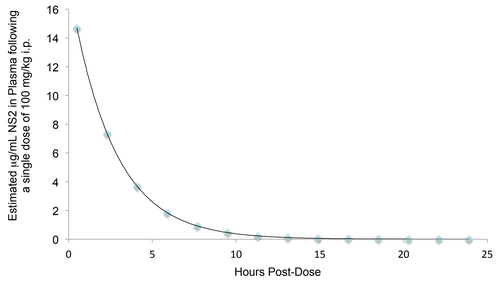
Contact dermatitis model: Mice received an i.p. injection of NS2 (100 mg/kg; formulated at 5 mg/mL) or vehicle. 30 mins later, phorbol myristate (PMA) was applied topically to the right ear at 5 μg, while the left ear received a topical application of 70% ethanol (the PMA excipient) as a control. Six hours after PMA challenge, ear thickness was determined and compared to baseline thickness.

Delayed-type hypersensitivity dermatitis model: Oxazolone (OXL) was applied topically (100 μL of 1.5% OXL in acetone) onto a shaved abdomen under light isoflurane in order to sensitize the immune system. Seven days later, mice received NS2 (100 mg/kg, i.p.) or vehicle, before being challenged 30 mins later with OXL on the right ear (20 μL of 1.0% OXL.) Left ears received topical application of acetone (the OXL excipient) as a control. 24 hours after OXL challenge, ear thickness was again determined. Both ears were immediately removed following carbon dioxide asphyxiation, with 4 mm biopsy samples snap frozen in liquid nitrogen for cytokine analysis.

Cytokine Analysis: Plasma/tissue was subjected to Multiplex LASER bead technology (Eve Technologies) to simultaneously quantify levels of 32 individual cytokines and chemokines. Results were expressed as means +/- 1 standard error (SEM). Values from OXL experimental samples were normalized to overall protein content (BCA assay).

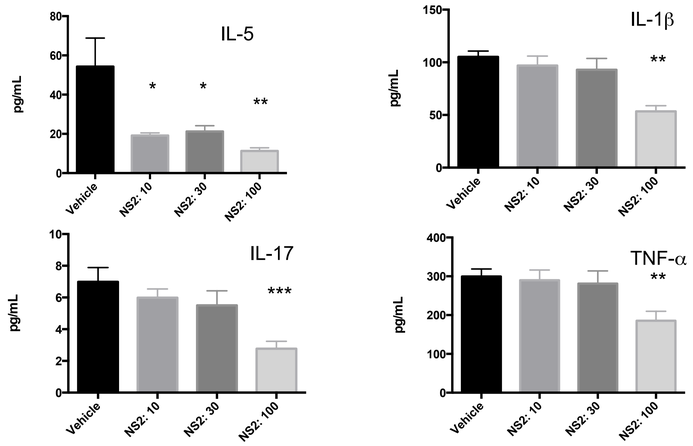
Statistical Analysis: A repeated measures 2-way ANOVA, followed by Holm-Sidak's Multiple Comparison Test was used to identify statistically significant differences in ear thickness, while 1 tailed unpaired t tests were employed to assess differences in tissue cytokine/chemokine levels. P values of ≤ 0.05 were considered statistically significant.

Fig 1. NS2 PK profile and tolerability



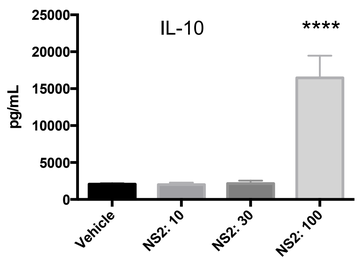
The curve above reflects an estimate of the plasma PK following a single 100 mg/kg dose administered i.p. The NS2 t½ is estimated to be ≅ 1.8h, with a T_{max} at 0.5h. NS2 was well-tolerated after a single i.p. administration of 10, 50 and 100 mg/kg, and after 7 days of dosing i.p. at 10 mg/kg.

Fig 2. NS2 decreases pro-inflammatory cytokines after LPS challenge



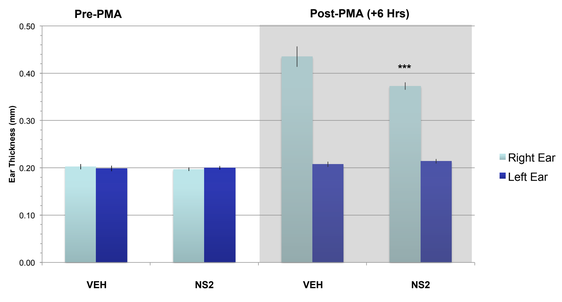
Mice (N=8-11 per group) were dosed with vehicle or NS2 (10, 30 or 100 mg/kg) i.p. 30 mins prior to exposure to LPS (20 mg/kg, i.p.). 2h post-LPS, plasma was collected and analyzed by ELISA. (*p<0.05; **p<0.01; ***p<0.001). Statistically significant reductions were also observed in LIF, MCP-1, MIP-1α, RANTES, IP-10, IL-12 (p40 and p70) and eotaxin; mean ± SEM.

Fig 3. NS2 increases the anti-inflammatory cytokine, IL-10



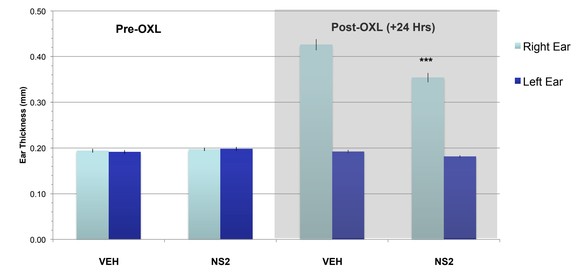
Mice (N=8-11 per group) were dosed with NS2 (10, 30 or 100 mpk) or vehicle i.p. 30 mins prior to exposure to LPS (20 mg/kg, i.p.). Two hours after LPS exposure, blood was collected for ELISA determination of IL-10 (*** p<0.001); mean ± SEM.

Fig 4. NS2 reduces ear swelling in a contact dermatitis (PMA) model



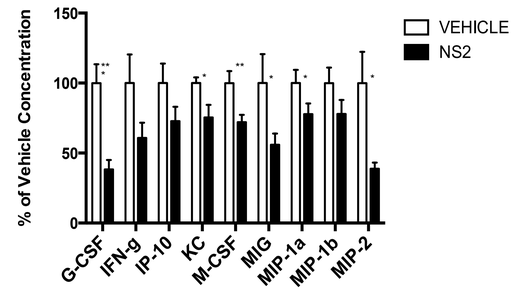
NS2 or vehicle was injected i.p. at 100 mg/kg to male mice. 30 mins later, phorbol myristate acetate (PMA) was applied topically to both the anterior and posterior portions of the right pinna (5 μg total). The left pinna received 20 μL of ethanol (PMA excipient). 6 hours after PMA application both right and left ear thicknesses were again determined. Measurements were determined at least twice from the same region of both ears, with care taken to not include hair or folded pinna. N=10 per group, mean ± SEM.

Fig 5. NS2 reduces ear swelling in a delayed hypersensitivity (OXL) model



On Day -6, abdomens were shaved and oxazolone (OXL) was applied topically (1.5%, 100 μL in acetone) to mice. On Day 1, ear thickness was measured for both right and left ears. NS2 (100 mg/kg) or vehicle was administered i.p. to mice followed by OXL (1%) applied topically (20 μL) 30 minutes later, to both the anterior and posterior portions of the right pinna. The left pinna received 20 μL of acetone (OXL excipient) to both the anterior and posterior portions. 24 hours after OXL application both right and left ear thicknesses were again determined. N=10 per group, mean ± SEM.

Fig 6. NS2 reduces inflammatory cytokines in the OXL hypersensitivity model



Mice were subjected to an OXL challenge on ear pinna, as described in methods and Fig. 5 above. 24 hours after OXL challenge, ear tissue was collected for ELISA measurement of various inflammatory cytokines. N=10 per group, mean ± SEM.

Summary/Conclusions

- **Inflammatory conditions of the skin are associated with increased aldehyde load, leading in turn to the activation of further pro-inflammatory cascades.**
- **NS2, a small molecule aldehyde trap that can irreversibly bind to free toxic aldehydes, is under development for a number of inflammatory indications.**
- **NS2 is well tolerated, making it a suitable drug candidate, and a single i.p. administration reduces inflammation in a variety of murine models despite a relatively short half-life when administered to mice i.p.**
- **In an LPS challenge model, NS2 decreased levels of various pro-inflammatory mediators, and increased levels of the anti-inflammatory cytokine, IL-10.**
- **In PMA and OXL models of contact dermatitis and delayed-type hypersensitivity dermatitis, respectively, NS2 ameliorated ear swelling, and in the OXL model, reduced tissue levels of numerous inflammatory mediators.**
- **NS2 could be a safe and effective treatment for inflammatory diseases of the skin.**