Administration of the small molecule aldehyde trap NS2 in a hamster model of radiation-induced oral mucositis.

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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 mucosal barrier. Elevated levels of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) have been shown to occur under a variety of toxic and inflammatory insults, including irradiation-induced toxicity. Thus, pharmacological trapping of free aldehydes may represent a novel prophylactic and therapeutic approach for radiation-induced tissue inflammation in cases of radiotherapy for cancer treatment.

Here, we report the ability of NS2, a novel aldehyde-binding small molecule, to trap the aldehydes MDA and 4-HNE in vitro under a variety of conditions. In vivo studies with Golden Syrian hamsters irradiated at the cheek pouch showed that sub-cutaneous administration of NS2 accelerated healing of mucositic lesions and tended to reduce the severity of resultant fibrotic scarring.

These results suggest that NS2 could mitigate the toxic aldehyde load that is generated during cancer radiotherapy, and thus represents a new class of anti-inflammatory agents for the prevention and treatment of radiation-induced mucositis.

Methods

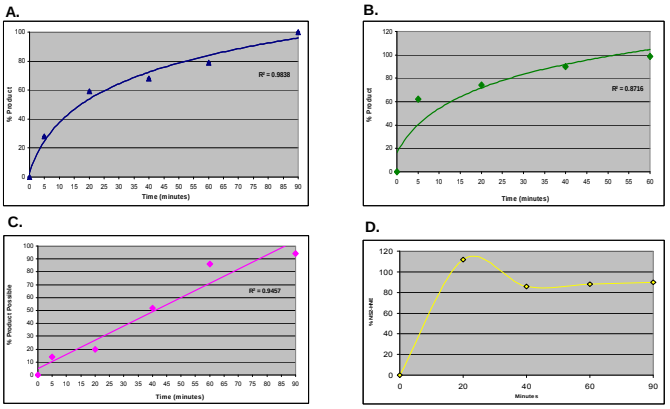
NS2-mediated aldehyde trapping: NS2 was suspended with either malondialdehyde (MDA; tetrabutylammonium salt form) or 4-hydroxynonenal (4-HNE) in phosphate-buffered saline containing various excipients (to mimic vehicle formulations for *in vivo* dosing of NS2). In the case of the reaction with MDA, 4N HCl was added to generate free-base MDA *in situ*. Reaction mixtures were stirred at room temperature and monitored by liquid chromatography mass spectroscopy (LC/MS) to determine loss of starting material and formation of the relevant adduct. In the case of the NS2-MDA adduct, adduct structure was confirmed by ^{1H} nuclear magnetic resonance (NMR) analysis. In an effort to better mimic the lipid environments found in vivo, the aldehyde-trapping reactions were repeated in the presence of linoleic acid and glyceryl trioleate.

Radiation-induced oral mucositis in hamsters: Twenty (20) male Syrian Golden Hamsters were given an acute radiation dose of 40 Gy directed to their left buccal cheek pouch. This was accomplished by anesthetizing each animal and everting the left buccal pouch, while protecting the rest of the animal with a lead shield. From day -2 until day 36, NS2 was given by subcutaneous injection (s.c.) twice daily (b.i.d.) at a dose of 10 mg/kg/injection for a total dose of 20 mg/kg/day. Mucositis was evaluated clinically in a blinded fashion starting on Day 6, using a rating scale of 0 (completely healthy with no erythema or vasodilation) to 5 (ulceration to virtually all of the pouch; pouch can be only partially everted). Clinical monitoring continued on alternate days until Day 36. On Day 36, the animals were sacrificed and the left cheek pouch was removed. Tissue was placed in 10% formalin for fixation and subsequent histopathology.

Tissue Histology: Tissues were paraffin-embedded and sectioned at 5 microns. Sections were stained with hematoxylin and eosin for routine histopathology. Masson's Trichrome stain was used for histological examination of fibrosis. Six sections/hamster were used for analysis. Fibrosis was scored on a semi-quantitative scale from 0 (no fibrosis) to 5 (severe fibrosis).

Statistical Analysis: The rank sum differences in daily mucositis scores in the control versus NS2 groups were determined. For each evaluation day, the group scores were compared using the non-parametric rank sum analysis. The difference in the number of days hamsters in each group had severe mucositis (score ≥ 3) was also analyzed, and evaluated using chi-square analysis. Fibrosis was analyzed using the Mann-Whitney test. In each case, results were considered statistically significant at $p < 0.05$.

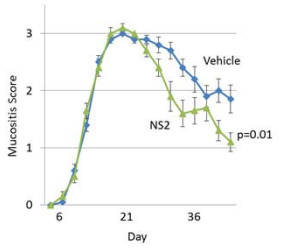
Fig 1. NS2 traps pathophysiologically-relevant aldehydes in vitro



Trapping of 2 pathophysiologically relevant aldehydes, MDA and 4-HNE, by the small molecule NS2. **A:** NS2-MDA formation as a function of time. The reaction reached 100% completion after 90 minutes and thus appears to be stable and reversible. **B**: NS2-MDA formation as a function of time, plus lipids, reaching >90% completion after 90 minutes. **C:** NS2-4HNE formation as a function of time. The reaction reached >94% completion after 90 minutes **D:** NS2-4HNE formation as a function of time, plus lipids. This reaction reached steady state equilibrium of 90% NS2-HNE adduct after 60 minutes. The presence of lipids altered the reaction kinetics by stabilizing iminium salts and allowing more efficient conversion to the stable adduct.

Fig 2. NS2 accelerates lesion healing in a hamster model of oral mucositis

A.

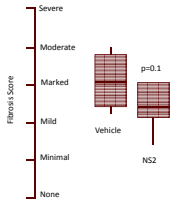


B.

Treatment	Days ≥3	Days <3	Total animal days	% Days ≥3	Chi Sq v control	P value
Control	156	164	320	48.75%	--	--
NS2	112	208	320	35.00%	12.43	0.0004

A. Mean daily mucositis scores, showing an acceleration in the resolution phase for the NS2-dosed mice. **B.** The total # days in which an animal presented with clinically significant mucositis (open ulcers; score ≥ 3) was summed and expressed as a % of the total # of days for each group. Group differences were calculated with chi-squared analysis.

Fig 3. NS2 reduces fibrotic scarring in in a hamster model of oral mucositis



Radiation-induced cheek pouch fibrosis was scored in a blinded fashion using a semi-quantitative scale from 0 (no fibrosis) to 5 (severe fibrosis). NS2 tended to decrease overall tissue fibrosis (p=0.1076).

Summary/Conclusions

- **Increased aldehyde load is implicated in a number of acute and chronic inflammatory conditions, including the sequelae of events involved in radiation-induced inflammation of the mucosal tissues.**
- **The small molecule NS2 was showed to irreversibly bind and therefore trap in situ free aldehydes (MDA, 4-HNE) which are known to be toxic in vivo.**
- **In a hamster model of radiation-induced oral mucositis, daily dosing s.c. with NS2 accelerated healing and statistically significantly reduced the amount of time animals exhibited clinically meaningful ulceration.**
- **Tissue analysis of NS2-treated hamsters showed a noticeable trend towards a reduction in fibrotic scarring.**
- **NS2 could be a safe and effective treatment for radiation-induced mucositis and other sequelae caused by cancer radiotherapy.**