

Abstract

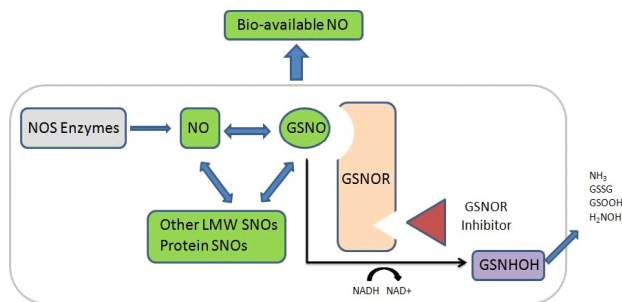
INTRODUCTION: S-nitrosogluthione reductase (GSNOR) is a Class III alcohol dehydrogenase (ADH) that represents a novel therapeutic target in inflammatory bowel disease (IBD). GSNOR may contribute to IBD through dysregulated activity and thus altered metabolism of its substrate, S-nitrosogluthione (GSNO), an abundant low molecular weight S-nitrosothiol (SNO). GSNO serves as a reservoir for nitric oxide (NO), exerts potent anti-inflammatory actions, and maintains epithelial barrier functions in the gut. These studies determined the efficacy of small molecule inhibitors of GSNOR (N6022 and N6547; Table 1) in mouse models of dextran sulfate sodium (DSS) colitis.

METHODS: Efficacy of GSNOR inhibitors was determined in both an acute preventative and chronic interventional treatment DSS model using video endoscopy and histopathology (Figure 1 and Table 2).

RESULTS: Oral N6022 and N6547 significantly decreased colon injury severity with effects comparable to prednisolone. In the acute model, N6022 and N6547 attenuated injury at doses of 1 or 10 mg/kg/day when given prior to and throughout the DSS exposure (Figures 2-4; Table 3). N6547 attenuated DSS elevations of circulating cytokines (Table 4). In the chronic model, N6547 attenuated injury at a dose of 10 mg/kg/day when given after DSS (Figure 5). DSS caused significant body weight loss, an effect that was less pronounced in mice treated with GSNOR inhibitors compared to prednisolone (Figures 4-5).

CONCLUSION: The ability of GSNOR inhibitors to attenuate disease severity and inflammatory mediators in mouse models of colitis points to the utility of targeting GSNOR in IBD to restore the anti-inflammatory and related NO mediated signaling influences regulated by this enzyme.

Introduction



GSNO provides a reservoir for NO and exists in equilibrium with NO and other SNOs. This pool of bioavailable NO functions to maintain smooth muscle tone, improve blood flow, and exert anti-inflammatory influences.^{1,2}

GSNO is catabolized by GSNOR which depletes GSNO, thus reducing the bioavailable NO pool.³ GSNOR dysregulation in respiratory, cardiovascular, and gastrointestinal diseases may lead to increased GSNO catabolism with the ensuing loss of NO and NO mediated functions.^{4,5}

We developed novel small molecule inhibitors of GSNOR as potential therapies to treat diseases characterized by dysregulated GSNOR by restoring GSNO, bioavailable NO, and NO mediated activities.⁶

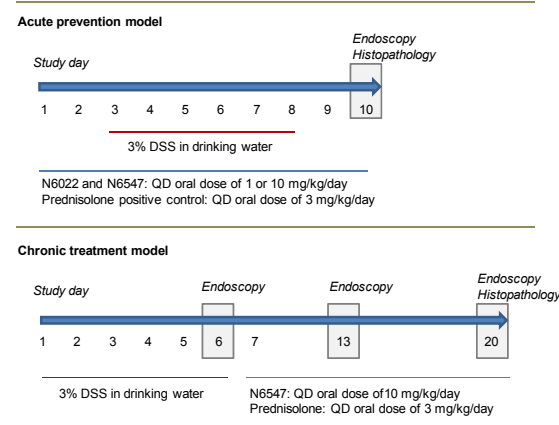
In the GI tract, NO and GSNO maintain normal intestinal physiology via anti-inflammatory actions and maintenance of the intestinal epithelial cell barrier. In IBD, lowered levels of GSNO and NO disrupt epithelial barrier function via dysregulation of proteins involved in maintaining epithelial tight junctions.^{7,8} Loss of epithelial barrier function, entry of microorganisms, and lowered anti-inflammatory actions due to the lowered GSNO and NO levels, are key events in IBD progression that can be potentially influenced by targeting GSNOR. Therefore, we explored the efficacy of GSNOR inhibitors for the prevention and treatment of colitis in mouse models of this disease.

Table 1. GSNOR Inhibitors

	N6022	N6547
GSNOR IC ₅₀	20 nM	55 nM
Other ADHs IC ₅₀	≥ 135 nM	≥ 20 μM
Bioavailability (mouse)	≤ 2.5%	2.7%

Study Design

Figure 1. Acute and Chronic DSS Models



Colitis was induced by giving DSS (35-60 kD) in the drinking water of male C57Bl/6 mice for 6 days (Figure 1). In the acute model, N6022 or N6547 were dosed orally (QD) at 1 or 10 mg/kg/day for 10 days starting 2 days prior to and continuing 2 days post-DSS. In the chronic model, N6547 was dosed orally (QD) at 10 mg/kg/day for 14 days starting one day post-DSS. Mice exposed to DSS and treated orally with phosphate buffered saline as vehicle (DSS) or 3 mg/kg/day prednisolone (Pred.), and mice not exposed to DSS (Control) served as controls. Efficacy was assessed in a blinded fashion via video endoscopy and histopathology (Table 2).^{9,10} Plasma cytokines were determined via ELISA (RBM, Austin, TX). Body weights were measured daily. Significant ($p < 0.05$) differences between treatment and DSS were determined with One-way ANOVA, Dunnett's (body weights, cytokines) or Chi-Square, Pearson's (endoscopy, pathology; SAS-JMP). N = 8-12 mice (DSS groups); N=5 (Control).

Table 2. Colitis Scoring Scale

Endoscopy (Mucosal Layer)		Histopathology (Epithelium, Connective Tissue, Submucosa)	
Score	Description	Score	Description
0	Normal	0	Normal
1	Loss of vascularity	1	Minimal inflammation, edema, necrosis
2	Loss of vascularity; friability	2	Mild inflammation, edema, necrosis
3	Friability; erosions	3	Moderate inflammation, edema, necrosis
4	Ulcerations and bleeding	4	Marked inflammation, edema, necrosis

Results

Figure 2. N6547 Preserves Colon Vascularity and Mucosal Integrity

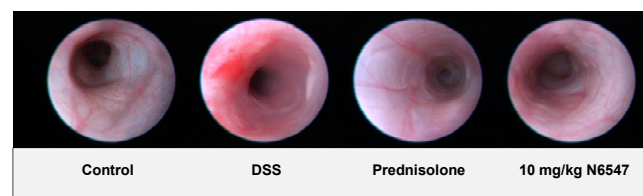
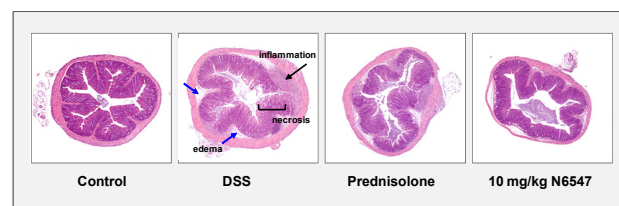


Figure 3. N6547 Attenuates IBD Pathology



Acute Model Results

Figure 4. GSNOR Inhibitors Decrease Colitis Severity

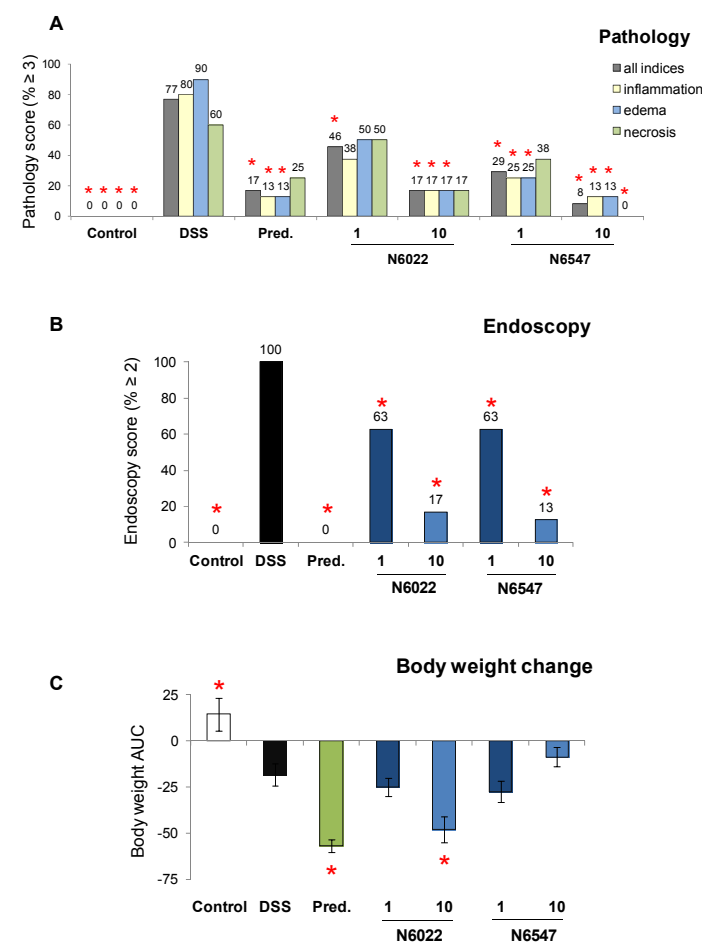


Table 3. N6547 Efficacy Across Multiple Studies

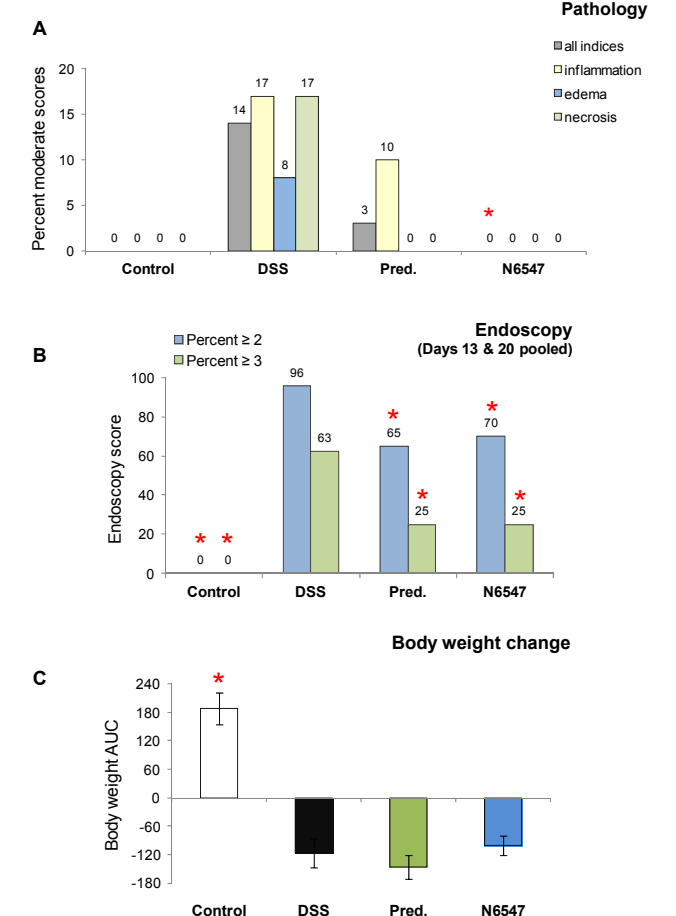
Treatment	mg/kg	% Severe Injury Scores					
		Endoscopy			Pathology		
Control	---	0*	0*	0*	0*	0*	0*
DSS	---	80	100	90	60	77	60
Pred.	3	10*	0*	0*	10*	17*	46
N6547	0.1	---	---	50	---	---	42
	1	---	63*	63	---	29*	50
	10	20*	13*	38*	33*	8*	17*

Table 4. N6547 Lowers Inflammatory Cytokines

Analyte (ng/mL)	Control	DSS	10 mg/kg N6547
IL-1β	7.136 ± 0.853	9.545 ± 1.045	7.754 ± 0.605
IL-6	0.006 ± 0.001	0.417 ± 0.157	0.130 ± 0.041
IL-10	0.073 ± 0.015	0.231 ± 0.085	0.081 ± 0.022
IL-17A	0.010 ± 0.004	0.014 ± 0.003	0.008 ± 0.001
KC/Groα	0.154 ± 0.028*	2.431 ± 0.560	1.255 ± 0.355*
TNFα	0.048 ± 0.004*	0.085 ± 0.010	0.055 ± 0.011

Chronic Model Results

Figure 5. N6547 Decreases Colitis Severity



Summary and Conclusions

N6022 and N6547 significantly attenuated colitis severity in a dose-dependent manner when administered via a preventative regimen in the acute DSS mouse model. N6547 also lowered cytokines associated with inflammatory pathways in IBD. Comparable significant effects were observed for N6547 in three separate studies.

N6547 attenuated colitis severity when administered via a treatment regimen in the chronic DSS mouse model.

The GSNOR inhibitors demonstrated effects comparable to the positive control, likely at doses that allow a higher therapeutic index relative to corticosteroids.

Targeting GSNOR with small molecule inhibitors may represent a promising new approach to IBD therapy.

Literature Cited

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