

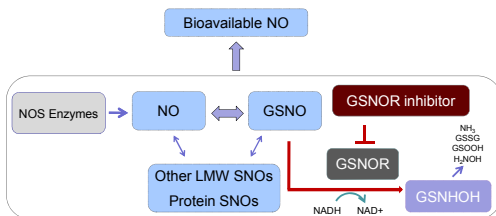
Oral S-Nitrosoglutathione Reductase Inhibitors Attenuate Pulmonary Inflammation and Decrease Airspace Enlargement in Experimental Models of COPD

Joan P. Blonder¹, Sarah C. Mutka¹, Dan Drolet¹, Bassam Damaj², Dianne Spicer³, Vince Russell³, Xicheng Sun¹, Gary J. Rosenthal¹, Charles Scoggins¹
¹N30 Pharmaceuticals, Boulder, CO; ²Bio-Quant/Apricus Biosciences, San Diego, CA, ³Argenta Discovery, Harlow, Essex, UK

Abstract

RATIONALE Chronic obstructive pulmonary disease (COPD) is an inflammatory disease often associated with cigarette smoking and characterized by progressive airflow limitation and lung damage. In the lung, endogenous S-nitrosothiols (SNOs) including S-nitrosoglutathione (GSNO) mediate nitric oxide (NO)-based signaling, inflammatory status, and smooth muscle function. S-nitrosoglutathione reductase (GSNOR) inhibition represents a novel approach to treating COPD via restoring GSNO and GSNO-mediated anti-inflammatory and bronchodilatory activities. The efficacy of small molecule inhibitors of GSNOR was evaluated in two murine models of COPD, a porcine pancreatic elastase (PPE) instillation model and a sub-chronic tobacco smoke (TS) inhalation model. **METHODS** Efficacy of the GSNOR inhibitors, N6338 and N91115, were determined as described in Figures 1 and 3. **RESULTS** N6338 or fluticasone+CXCR (positive control) attenuated protease induced lung pathology, with significant effect on Lm after 14 days of dosing. N6338 also decreased methacholine (MCh)-induced airway hyper-responsiveness (AHR) and bronchoalveolar lavage fluid (BALF) cells. N91115 significantly inhibited the TS-induced increase in BALF cellular infiltrate and demonstrated similar efficacy as the positive control, roflumilast. N91115 also attenuated the TS-induced increase in BALF matrix metalloproteinase 9 (MMP-9). **CONCLUSION** Oral GSNOR inhibitors demonstrated efficacy in murine models of protease or smoke induced COPD. Beneficial effects of GSNOR inhibitors on parameters of pulmonary function, inflammation, and emphysema were evident, and represent a promising new therapeutic approach to COPD.

Introduction



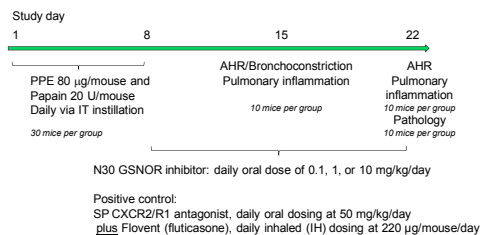
We have developed first-in-class molecules that are potent and selective inhibitors of GSNOR, a Class III alcohol dehydrogenase (ADH) enzyme (Table 1).^{1,2} GSNOR inhibitors may increase bioavailable NO by inhibiting the breakdown of GSNO, an abundant low molecular weight SNO, reservoir for NO, and main substrate for GSNOR.^{3,4} Restoring the levels of NO in diseases characterized by lowered NO, leads to maintenance of smooth muscle tone, improved blood flow, and anti-inflammatory influences. The therapeutic value of these effects has been well described in many organ systems including the lung, cardiovascular system, and gastrointestinal tract.⁵ GSNOR dysregulation and subsequently, reduced levels of GSNO and NO, have been implicated in respiratory diseases^{6,7} in which the lowered levels of GSNO and NO may contribute to pathophysiology via reduced bronchodilatory and anti-inflammatory actions. These activities are key events in disease progression that may be influenced by targeting GSNOR. Studies were performed to explore the utility of GSNOR inhibitors (N6338 and N91115) to modulate inflammatory, smooth muscle, and emphysema parameters in mouse models of COPD.

Table 1. GSNOR inhibitors

	N6338	N91115
GSNOR IC ₅₀	70 nM	16 nM
Other ADH classes IC ₅₀	> 250 μM	> 250 μM
Bioavailability (mouse)	18%	45%

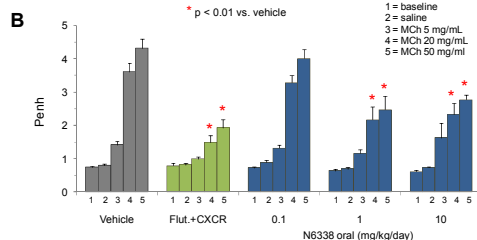
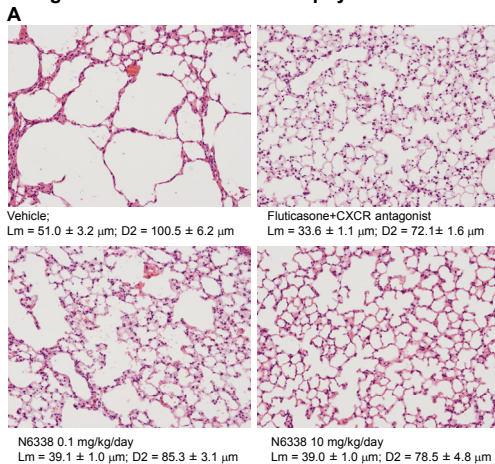
Elastase Model

Figure 1. PPE model experimental design



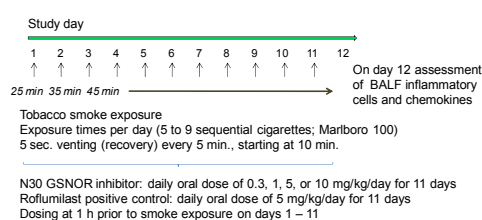
The PPE model of COPD was conducted at Bio-Quant. COPD was induced in 8 week old female C57Bl/6 mice (Harlan) via 8 day intratracheal (IT) dosing of PPE and papiain, and then the GSNOR inhibitor (N6338) was dosed orally for 7 to 14 days (Figure 1). MCh-induced AHR was measured using a Buxco chamber (enhanced pause; Penh) (Figure 2B). BALF cells were counted via light microscopy. Airspace enlargement was determined via lung histopathology and morphometric analysis of mean linear intercept (Lm) and average equivalent diameter (D₂)⁸ from two random fields of two sections from each lung (Figure 2A). Body weights were measured twice weekly. Statistical differences were evaluated with One-way ANOVA and Dunnett's tests.

Figure 2. N6338 attenuates emphysema and AHR



Smoke Model

Figure 3. Smoke model experimental design



The 11 day smoke-induced inflammation model of COPD was conducted at Argenta. Lung inflammation was induced in 16 g female C57Bl/6 mice (Charles River) via whole body inhalation exposure to tobacco smoke while the GSNOR inhibitor (N91115) was dosed orally for 11 days at one hour prior to smoke exposure (Figure 3). The PDE4 inhibitor, roflumilast, was used as the positive control and naive mice exposed to air served as negative controls. BALF cells were counted via light microscopy (Figure 4, study b & Table 2). Post study analyses of inflammatory mediators in BALF (Figure 5, study c) were assessed via ELISA (R&D Systems). Body weights were measured twice weekly (Figure 6, study c). Statistical differences among groups were evaluated with One-way ANOVA and Bonferroni's tests.

Figure 4. N91115 inhibits BALF cells

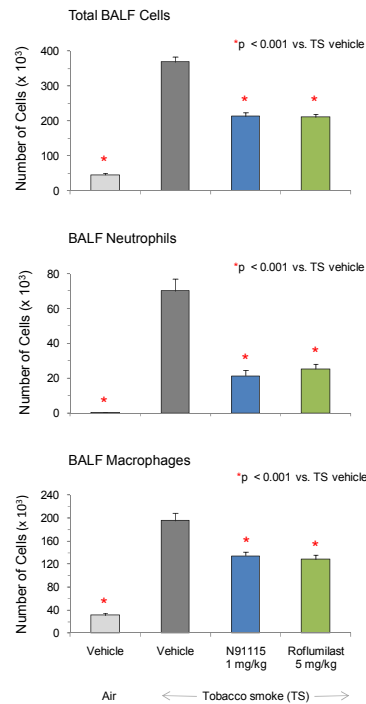


Table 2. N91115 efficacy across multiple studies

study	% Inhibition of Smoke-induced Increase							
	a	b	c	c	d	b	c	d
	N91115				Roflumilast			
dose (mg/kg)	0.3	1	1	5	10	5	5	5
Endpoint								
Total cells	34	48	35	36	44	49	37	44
Neutrophils	37	70	55	55	54	64	47	61
Macrophages	31	37	37	36	43	41	40	42
Lymphocytes	41	65	64	58	50	71	53	40
Eosinophils	65	95	---	---	69	83	---	86
MMP-9	---	---	77	62	71	---	49	51

Figure 5. N91115 inhibits BALF MMP-9

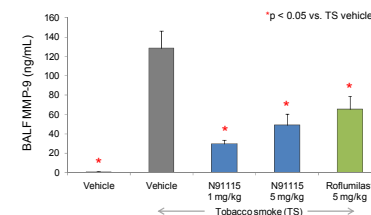
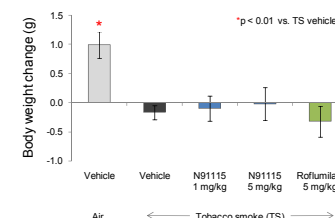


Figure 6. N91115 does not effect body weight



Summary & Conclusions

Oral GSNOR inhibitors demonstrated efficacy in mouse models of elastase or tobacco smoke induced COPD. GSNOR inhibitors showed significant benefit toward parameters of pulmonary function (AHR), inflammation (BALF inflammatory cells and mediators), and emphysema (Lm, D₂). Efficacy was observed at low doses (0.1 or 0.3 mg/kg), with similar potency, and likely, at a higher therapeutic index, when compared to positive controls. Targeting GSNOR with small molecule inhibitors may represent a promising new approach to COPD therapy.

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