Toxic Effects of Hyperbaric Oxygen in S-nitrosoglutathione Reductase Null Mice

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Background

- Pathogenesis of CNS and pulmonary O_2 toxicity involves neuronal excitation

- Potential mechanism
  - Imbalance between excitatory (glutamate) and inhibitory (GABA) synaptic transmission

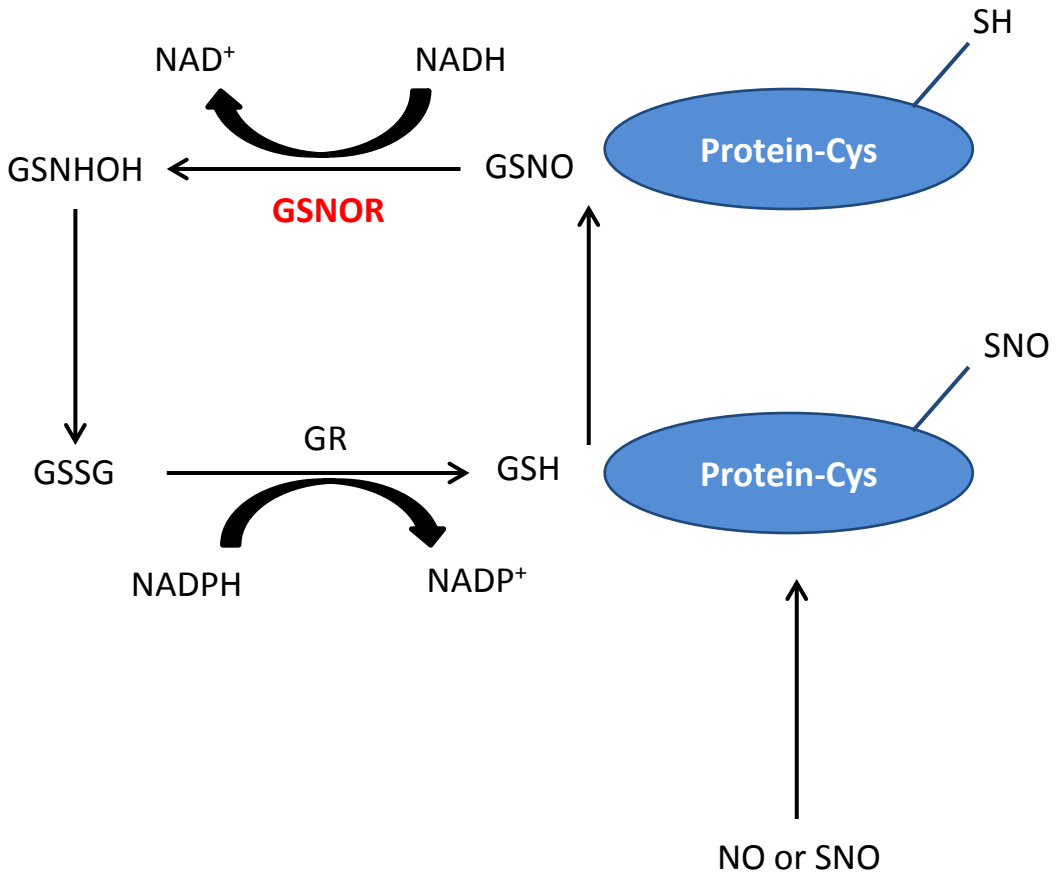
    (Demchenko IT and Piantadosi CA. Undersea Hyperb Med 2006)

  - May result from S-nitrosylation of enzymes that catalyze the synthesis of inhibitory neurotransmitters, i.e., GABA

    (Demchenko IT et al. Undersea Hyperb Med [abstract] 2007)

GSNOR

- Physiological control:
  - Ventilation
    (Lipton et al. Nature 2001)
  - Brochodilation
    (Choudhry S et al. Parmacogenet Genomics 2010)
  - Vascular tone and contractility
    (Beigi F et al. PNAS 2012)



GSSG, glutathione disulfide; GSH, glutathione; GSNHOH, S-hydroxysulfenamide; GSNO, S-nitrosoglutathione; GSNOR, S-nitrosoglutathione; GR, glutathione reductase; NAD, nicotinamide adenine dinucleotide

Objective

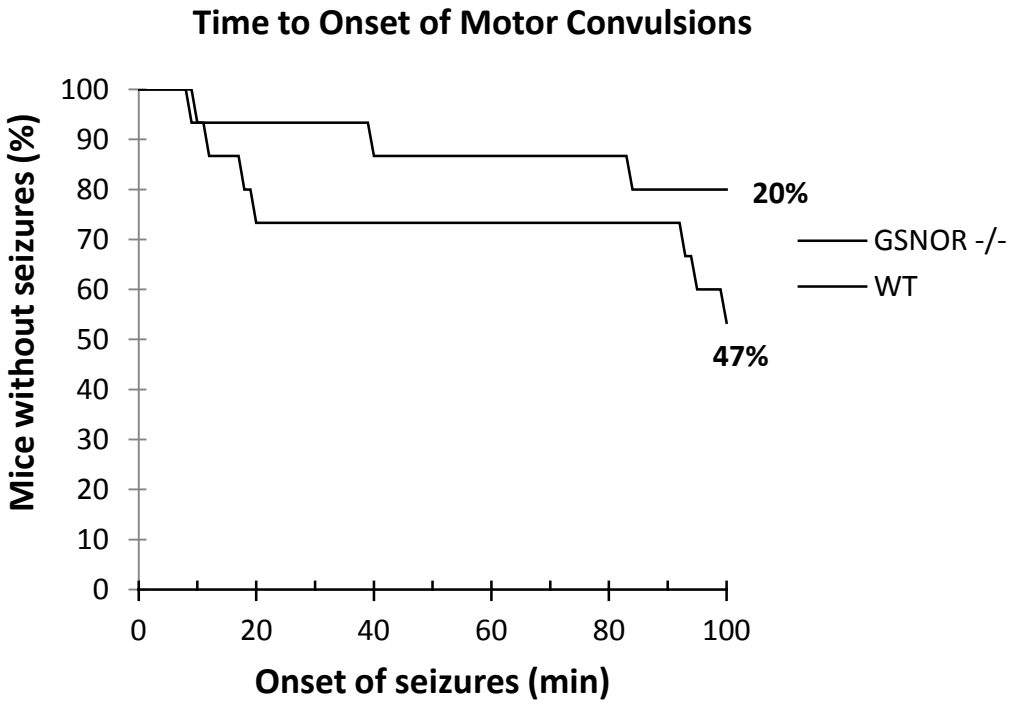
- Determine whether ability to reduce protein S-nitrosothiols protects against HBO_2 seizures and acute lung injury in mice at 4 ATA.

- ***Hypothesis:*** GSNOR -/- mice will have shortened seizure latency and more severe pulmonary injury than WT mice.

Methods

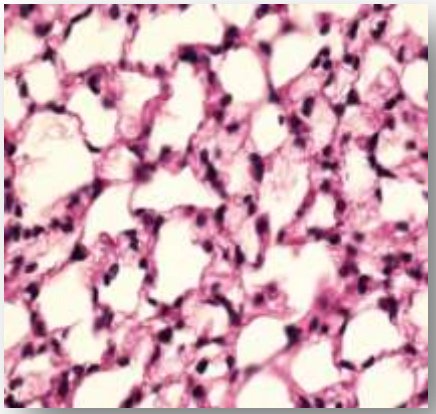
- Animals: C57BL/6 & GSNOR -/- mice aged 11-25 weeks
- HBO_2 exposure: 4 ATA for 100 min
  - CNS O_2 toxicity
    - Seizure latency
  - Acute lung injury
    - H&E stained lung sections assessed by light microscopy
      - alveolar edema
      - interstitial and septal thickening
      - intra-alveolar cells and debris
      - Extent: 0 -4 (in quartiles)
      - Severity: 0-3 scale (absent to severe)
      - ALI score: Sum of extent x severity for each category
    - BALF:
      - LDH
      - Total protein
      - Total NOx
  - Mechanism
    - Protein S-nitrosation by biotin switch assay

Results

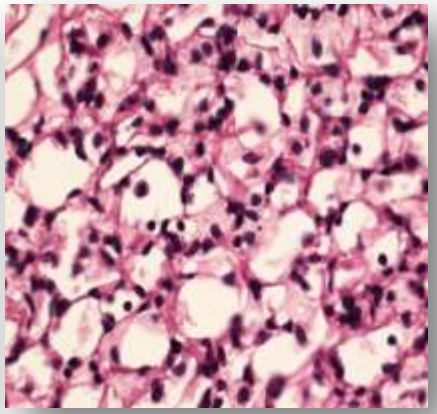


- *n* = 15 for WT and GSNOR -/-

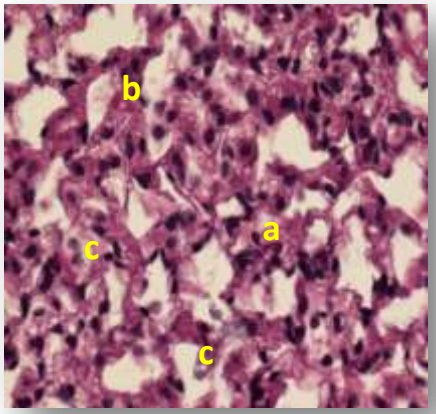
Acute lung injury



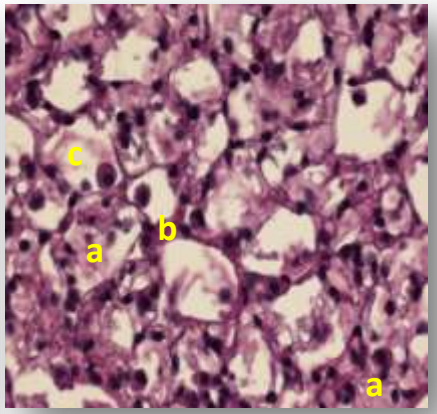
WT Control



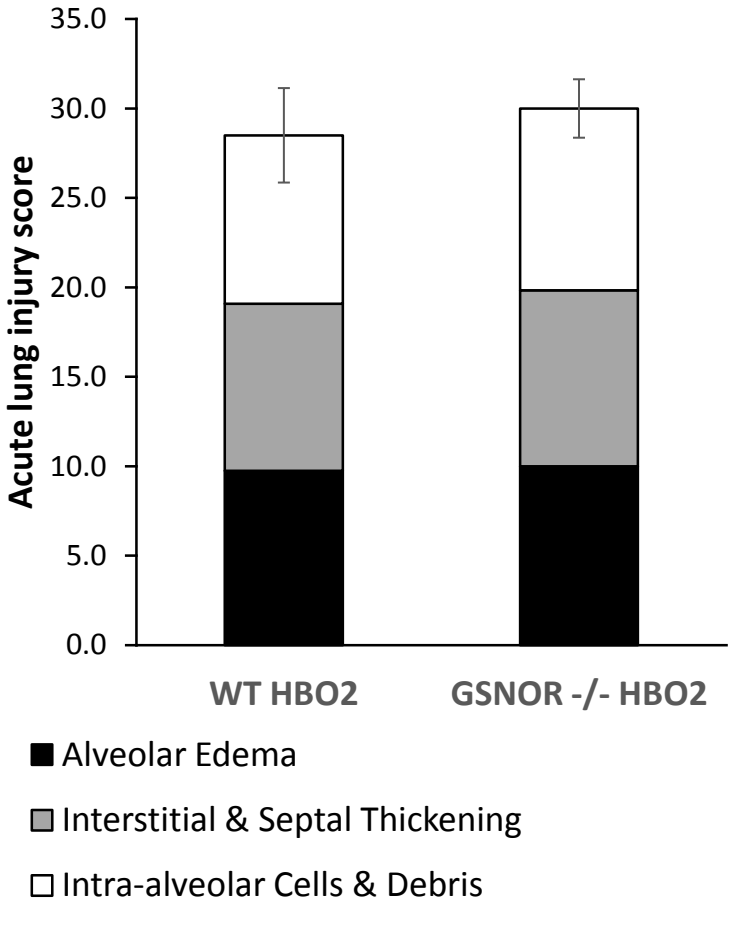
GSNOR -/- Control



WT HBO_2



GSNOR -/- HBO_2



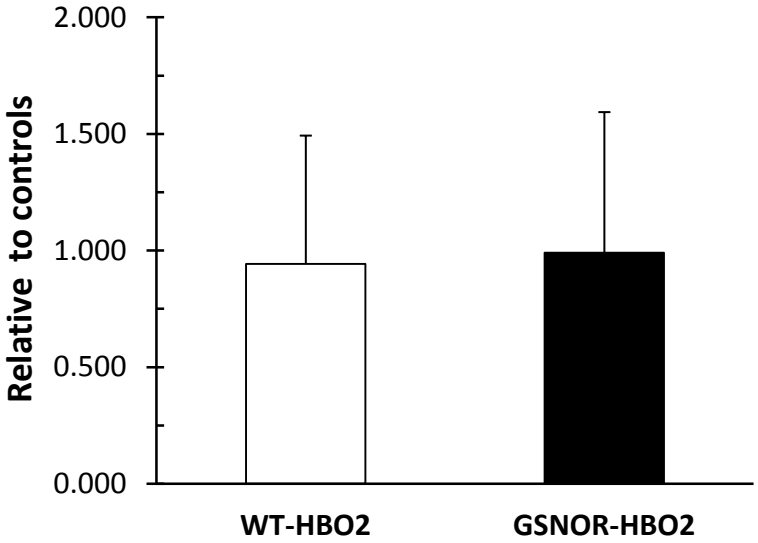
BALF biomarkers of lung injury/Inflammation

	Control		HBO_2		
	WT	GSNOR -/-	WT	GSNOR -/-	*p*-value
LDH (U/L)	25.3 ± 7.3	22.6 ± 2.6	28.4 ± 10.1	22.4 ± 5.6	0.201
Total Protein (mg/mL)	0.13 ± 0.10	0.14 ± 0.04	0.81 ± 1.1	0.21 ± 0.1	**0.018**
NOx (µmol/L)	6.0 ± 2.4	5.9 ± 1.0	10.8 ± 7.0	14.4 ± 11.2	0.155

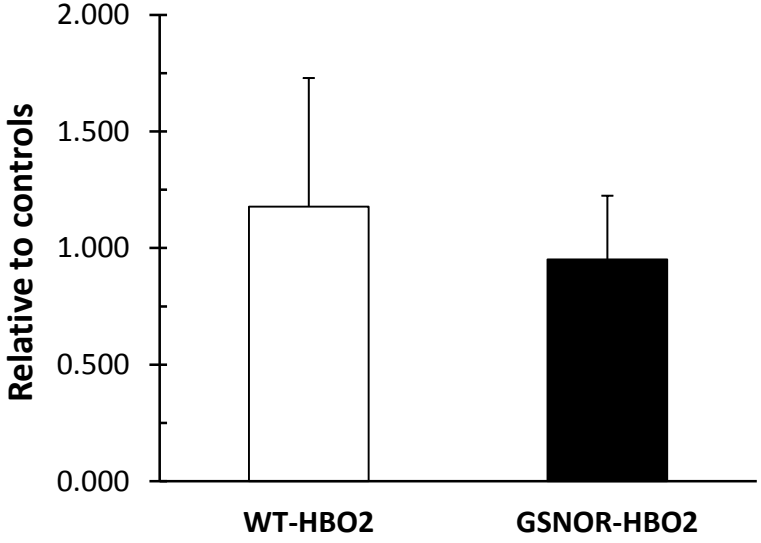
Values are mean ± SD. One-way ANOVA for between group comparisons.

Total protein S-nitrosation

Forebrain



Hindbrain



WT (*n* = 9) and GSNOR -/- (*n* = 9) mice. Values are mean ± SD and expressed relative to each strains control group (non-HBO_2).

Summary/Conclusions

- In contrast to our hypothesis, mice with unregulated protein S-nitrosylation displayed similar signs of neurotoxicity and acute lung injury with HBO_2 at 4 ATA.

- Does not preclude S-nitrosylation of “specific proteins” playing a pathogenic role in HBO_2-mediated excitotoxicity.

- Future work directed at S-nitrosylation of specific proteins in candidate regions of the brain to determine the site and cause of neurotoxicity and pulmonary damage.

Acknowledgements

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