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

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Background

Oxygen toxicity is a known complication of hyperbaric oxygen exposure (HBO_2), especially above 3 atmospheres absolute (ATA), and can cause seizures and acute lung injury. The pathogenesis involves nitric oxide (NO)-mediated neuronal excitation (1), although precise molecular details remain elusive. One hypothesis is that there is an imbalance between excitatory (glutamatergic) and inhibitory (γ-aminobutyric acid, GABAergic) synaptic transmission (2), potentially occurring as a result of protein S-nitrosylation (covalent attachment of NO into cysteine-SH side chains of proteins) of enzymes that catalyze the synthesis of inhibitory neurotransmitters, i.e., GABA (3). The S-nitrosylation of proteins is in equilibrium with S-nitrosoglutathione (GSNO), with GSNO levels being regulated by GSNO reductase (GSNOR), an NADH dependent enzyme that removes NO from protein S-nitrosothiols (4). In addition to the brain, proteins in the lung and cardiovascular system also undergo protein S-nitrosylation resulting in brochodilation (5), vasodilation (6) and an increase in cardiac output (7). Since HBO_2 ultimately increases NO production that mediates neurogenic pulmonary damage (1), we examined the role of protein S-nitrosylation with HBO_2 by using mice deficient in GSNOR. The ***objective*** of this research was to determine whether the ability to reduce protein S-nitrosothiols protects against HBO_2 seizures and acute lung injury in mice at 4 ATA. Our hypothesis was GSNOR -/- mice will have shortened seizure latency and more severe pulmonary injury than WT mice.

Methods

Animals: C57BL/6 & GSNOR -/- aged 11-25 weeks
HBO_2 exposure: 4 ATA for 100 min
CNS O_2 toxicity: Seizure latency
Acute lung injury:

- Hematoxylin and eosin stained lung sections assessed by light microscopy
  - Categories:
    a) alveolar edema
    b) interstitial and septal thickening
    c) and intra-alveolar cells and debris
  - Extent: 0 (absent), 1 (< 25% involved), 2 (25-50% involved), 3 (50-75% involved), 4 (>75% involved
  - Severity: 0 (absent), 1 (mild), 2 (moderate), 3 (severe)
  - Acute lung injury score calculation: Sum of extent x severity for each category.
- Bronchoalveolar lavage fluid (BALF):
  1. Lactate dehydrogenase (LDH)
  2. Total protein
  3. Total nitrites and nitrates (NOx)

Mechanism: Total protein S-nitrosylation (fore- and hindbrain)

- Biotin switch assay (cysteine and S-nitrosylated cysteine derivatized by MMTS and biotin-HPDP).

Results

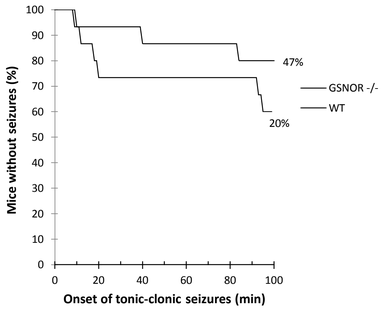

Fig. 1. Time to onset of motor convulsions in WT (n = 15) and GSNOR -/- (n = 15) mice exposed to 4 ATA > 99% O_2 for 100 min. 93% of animals in both groups displayed symptoms of CNS O_2 toxicity, and 47% of WT and 20% of the GSNOR -/- mice exhibited tonic-clonic seizures ($p > 0.05$; Log-rank test).

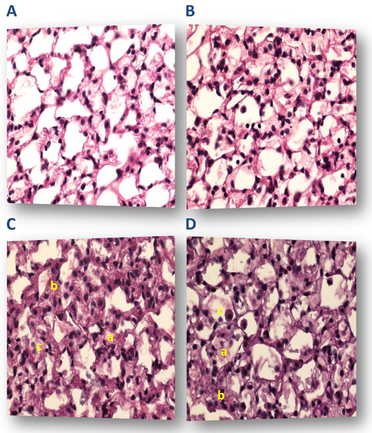

Fig. 2. Lung light micrographs (40x) from (A) WT control, (B) GSNOR -/- control, (C) WT HBO_2 (C), and (D) GSNOR -/- HBO_2 mice. In HBO_2 exposed mice, (a) alveolar edema, (b) interstitial and septal thickening, and (c) intra-alveolar cells and debris.

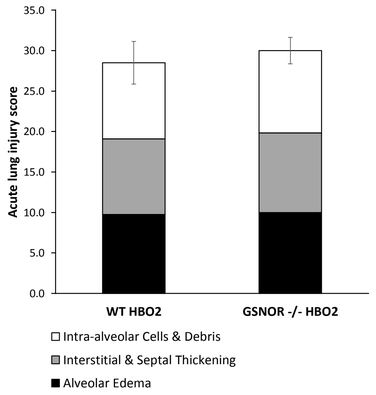

Fig. 3. Acute lung injury scores. Indices of lung injury (alveolar edema, interstitial and septal thickening, and intra-alveolar cells and debris) derived from semiquantitative analysis of light micrographs were combined to yield a complete measure of HBO_2 damage in WT HBO_2 ($n = 6$) and GSNOR -/- HBO_2 ($n = 6$) mice. Values are mean ± SD. Mann-Whitney U test to determine group differences, $p > 0.05$.

	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

Table 1. BALF LDH, total protein and NOx levels in control mice (WT, $n = 5$ and GSNOR -/-, $n = 5$) and mice exposed to 4 ATA > 99% O_2 for 100 min (WT, $n = 15$ and GSNOR -/-, $n = 13$-14). Values are mean ± SD. One-way ANOVA for between group comparisons.

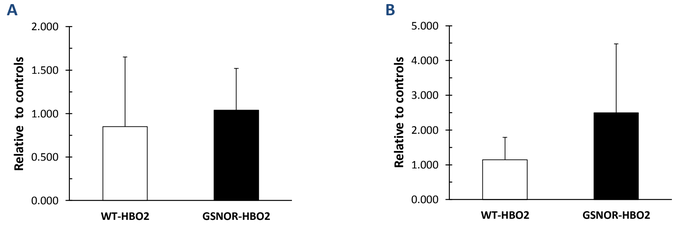

Fig. 4. Total protein S-nitrosation in the forebrain (A) and hindbrain (B) of WT ($n = 9$) and GSNOR ($n = 9$) mice exposed to 4 ATA > 99% O_2 for 100 min. Although no significant between group differences were observed, there was a trend for a GSNOR-HBO_2 increase in total protein S-nitrosylation ($p = 0.069$; t-test). 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 at 4 ATA > 99% O_2.
  - Possibly protective? (seizure latency, BALF total protein and hindbrain protein S-nitrosation).
- 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.

References

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