Glutathione / Thioredoxin
Nrf2 & Hyperoxia

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Oxygen is Good

- GENERATION OF USABLE ENERGY FROM FOOD
- METABOLISM OF ENDOGENOUS INTERMEDIATES
  - STEROID HORMONES
  - PROSTAGLANDINS
- GENERATION OF OXIDANTS FOR PROTEIN FOLDING
- IMMUNE DEFENSE FUNCTIONS, CELL KILLING
- GENERATION OF •NO
- METABOLISM OF DRUGS AND XENOBIOTICS
Bronchopulmonary Dysplasia
A disruption of normal vascular and alveolar development

- O₂ toxicity
- Infection
- Barotrauma
- Volutrauma
Birth: A Hyperoxic Challenge

• The evolutionary adaptation to extrauterine aerobic existence required the development of efficient cellular electron transport systems to produce energy.

• Biochemical defenses, including antioxidant enzymes, evolved to protect against oxidation of cellular constituents by ROS.

• There is increased transfer of antioxidants during the last days of gestation.
**BREATHING:**

- Fetus transfers from an *intrauterine “hypoxic” environment* (PaO$_2$ of 20-25 mm Hg) to an *extrauterine “normoxic”* (yet relatively hyperoxic) environment (PaO$_2$ of 100 mm Hg)

- Immediately prior to birth there is an up ramping of antioxidant enzyme activity

- Upon exposure to oxygen, newborn lungs of many species increase their normal complement of protective antioxidant enzymes
Review Article

REACTIVE OXYGEN SPECIES, ANTIOXIDANTS, AND THE MAMMALIAN THIOREDOXIN SYSTEM

JONAS NORDBERG and ELIAS S. J. ARNER
Medical Nobel Institute for Biochemistry, Department of Medical Biochemistry and Biophysics, Karolinska Institute, Stockholm, Sweden

\[
\begin{align*}
\text{O}_2 & \xrightarrow{e^-} \text{O}_2^- \\
\text{H}_2\text{O}_2 & \xrightarrow{e^-} \cdot\text{OH} + \text{OH}^- \\
2\text{H}^+ & \xrightarrow{e^-} 2\text{H}_2\text{O}
\end{align*}
\]
REACTIVE OXYGEN SPECIES, ANTIOXIDANTS, AND THE MAMMALIAN THIOREDOXIN SYSTEM

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Sources of Radicals

- UV Light
- Ionizing Radiation
- Smoking
- Metabolism
- Air Pollution

Tissue Injury

- $\text{O}_2^-$ → OH$	ext{-}$
- $\text{O}_2^-$ → NO$	ext{-}$
- O$_3$ + UV → OH$	ext{-}$
- $\text{O}_2^-$ → H$_2$O$_2$ → OH$	ext{-}$
Oxidative Stress - Simplified

Oxidants
Reactive-Oxygen Species
- Superoxide ($O_2^-$)
- $H_2O_2$
- Peroxynitrite (ONOO$^-$)
- Hydroxyl Radical (HO$^-$)

Antioxidants
- Superoxide Dismutases
- Heme Oxygenase
- Glutathione
- Thioredoxin
Oxidative Stress - Simplified

**Oxidants**
- Reactive-Oxygen Species
  - Superoxide ($O_2^-$)
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**Antioxidants**
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Oxidative Stress - Simplified

Oxidants
- Reactive-Oxygen Species
  - Superoxide (O$_2^\cdot$)
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Antioxidants
- Superoxide Dismutases
- Heme Oxygenase
- Glutathione
- Thioredoxin
Glutathione (GSH) Redox Cycle
Glutathione (GSH) & Thioredoxin (Trx) Redox Systems

Transcription Factor & DNA Binding
Ribonucleotide Reductase
Protein Binding
Reduction of Protein Disulfides

\[
GSH + O_2 \rightarrow SOD \rightarrow H_2O_2 \rightarrow \text{SOD} \rightarrow O_2 + e^- \]

\[
GSSG + NADP^+ \rightarrow \text{GPx} \rightarrow \text{GSH} \rightarrow \text{GR} \rightarrow PSSG \rightarrow \text{NADPH}
\]

\[
\text{Trx(SH)_2} \rightarrow \text{TrxR} \rightarrow \text{Trx-S}_2 \rightarrow \text{Prx} \rightarrow \text{Trx(SH)_2}
\]

Pentose Shunt

\[
\text{NADP}^+ \rightarrow \text{NADPH} \rightarrow \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O}
\]

Plasma Lymph Bile

Bile

PSH

PSH

Trx(SH)_2

Trx-S_2
Neu Mice

- Glutathione reductase (GR) knock-outs
  - Reduction of GSSG would seem essential to GSH-dependent antioxidant defense mechanisms
    - Not more susceptible to hyperoxia than were wild-type mice

- Observations suggest compensatory responses

Rogers LK. *Toxicol Sci.* 2004 Dec;82(2):367-73
Trx as an e\textsuperscript{-} shuttle between reduced TrxR and GSSG

\[ \text{NADPH} + \text{TrxS}_2 + H^+ \xrightarrow{\text{TrxR}} \text{NADP}^+ + \text{Trx(SH)}_2 \]

\[ \text{Trx(SH)}_2 + \text{GSSG} \xrightarrow{} \text{TrxS}_2 + 2\text{GSH} \]

\[ \text{NADPH} + \text{GSSG} + H^+ \xrightarrow{\text{TrxR/Trx}} \text{NADP}^+ + 2\text{GSH} \]

Adapted from: Kanzok et al. \textit{J Biol Chem.} 22-Dec-2000; 275(51)
Aurothioglucose (ATG) & Auranofin (AFN)

- Anti-inflammatory gold compounds FDA approved to treat rheumatoid arthritis
- Potent inhibitor of selenium and cysteine-containing enzymes including TrxR

Fig. 2. TrxR, GSHPx, PH-GSHPx and GR activities in 18,000 × g liver supernatants from mice that were treated with ATG (100 mg/kg b.w., 8 h, black bars) or left untreated (open bars). Bars indicate means ± S.E.M., n = 3.

Human Placenta Thioredoxin Reductase

ISOLATION OF THE SELENOENZYME, STEADY STATE KINETICS, AND INHIBITION BY THERAPEUTIC GOLD COMPOUNDS*

(Received for publication, April 15, 1998, and in revised form, May 27, 1998)

Stephan Gromer‡§, L. David Arscott¶, Charles H. Williams, Jr.¶¶, R. Heiner Schirmer‡§¶¶, and Katja Becker‡
Hypothesis: ATG-mediated TrxR inhibition will enhance hyperoxic susceptibility of GR-KO mice
Methods

- 6 wk-old GR-KO (Neu) and C3H/HeN mice
- Administered 25 mg/kg ATG or saline vehicle control
- Placed in hyperoxia (>95% O₂) or room air for 72 or 96 hours
ATG potently inhibits lung TrxR activity in adult mice

ATG inhibits TrxR activities in Neu lung and liver up to 96 h
GR-KO (Neu) Mice: Increased basal Trx levels & TrxR-dependent GSSG Reduction

TABLE 1. HEPATIC GLUTATHIONE DISULFIDE REDUCTASE ACTIVITY (mU/mg PROTEIN)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Activity (mU/mg Protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>a1Neu</td>
<td>1.2 ± 0.1&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>23.5 ± 1.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Auranofin (μM)

A

![Image of Trx1 protein levels comparison between a1Neu and C3H/HeN strains.]

B

![Image of Trx2 protein levels comparison between a1Neu and C3H/HeN strains.]

Diagram:

- GSH → GPx → H<sub>2</sub>O<sub>2</sub> → GSSG → GR → NADPH
- H<sub>2</sub>O
ATG exacerbates hyperoxic lung in GR-KO (Neu) mice and attenuates lung injury in control mice
ATG exacerbates hyperoxic lung in GR-KO (Neu) mice and attenuates lung injury in control mice
Table 1  Enhanced sensitivity of Nrf2-disrupted compared with wild-type mice following acute and chronic toxicological challenges

<table>
<thead>
<tr>
<th>Challenge</th>
<th>Site</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetaminophen</td>
<td>Liver</td>
<td>Increased lethality and greater severity in hepatic damage, as demonstrated by increased plasma alanine aminotransferase activity, and centrilobular hepatocellular necrosis</td>
<td>(74, 75)</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>Lung</td>
<td>Lethality coupled with extensive lung injury: enlarged and hemorrhagic, with pulmonary infiltrates and destruction of the alveolar architecture</td>
<td>(97)</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>Lung</td>
<td>Increased lung weight, epithelial cell death, inflammation, and pulmonary fibrosis</td>
<td>(98)</td>
</tr>
<tr>
<td><strong>Hyperoxia (&gt;95% O₂)</strong></td>
<td>Lung</td>
<td>Enhanced pulmonary damage characterized by increased protein permeability, macrophage inflammation, and epithelial injury</td>
<td>(50)</td>
</tr>
<tr>
<td>Tobacco smoke</td>
<td>Lung</td>
<td>Earlier onset and more extensive emphysema with pronounced bronchoalveolar inflammation</td>
<td>(49, 105)</td>
</tr>
<tr>
<td>Elastase</td>
<td>Lung</td>
<td>Enhanced inflammation and emphysema</td>
<td>(106)</td>
</tr>
<tr>
<td>Ovalbumin</td>
<td>Lung</td>
<td>Enhanced asthmatic response</td>
<td>(94)</td>
</tr>
<tr>
<td>Bacterial endotoxin</td>
<td>Lung</td>
<td>Increased pulmonary inflammation, edema, septic shock</td>
<td>(69)</td>
</tr>
<tr>
<td>Diesel particles</td>
<td>Lung</td>
<td>Severe hyperplasia and accumulation of the oxidative DNA adduct 8-hydroxy-deoxyguanosine in bronchial epidermis</td>
<td>(107)</td>
</tr>
<tr>
<td>Carrageenan</td>
<td>Pleural cavity</td>
<td>Enhanced inflammation coupled with persistent invasion by neutrophils and delayed recruitment of macrophages</td>
<td>(68)</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>Stomach</td>
<td>Increased multiplicity and tumor volume in neoplasia of the forestomach coupled with higher levels of carcinogen-DNA adducts</td>
<td>(78, 108)</td>
</tr>
<tr>
<td>Hydroxybutyl-nitrosamine</td>
<td>Bladder</td>
<td>Increased incidence of transitional cell carcinomas</td>
<td>(80)</td>
</tr>
<tr>
<td>Malonate, 3-Nitropropionic acid</td>
<td>Brain</td>
<td>Increased sensitivity to morphological and behavioral neurotoxicities by inhibition of mitochondrial complex II</td>
<td>(100, 109)</td>
</tr>
</tbody>
</table>
Cytoprotective Nrf2 Pathway Is Induced In Chronically Txnrd 1-Deficient Hepatocytes

Elena S. Suvorova¹, Olivier Lucas¹, Carla M. Weisend¹, MaryClare F. Rollins¹, Gary F. Merrill², Mario R. Capecchi³, Edward E. Schmidt¹,₄,*

¹ Veterinary Molecular Biology, Montana State University, Bozeman, Montana, United States of America, ² Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon, United States of America, ³ Howard Hughes Medical Institute (HHMI), University of Utah, Salt Lake City, Utah, United States of America, ⁴ Center for Reproductive Biology, Washington State University, Pullman, Washington, United States of America
Loss of Thioredoxin Reductase 1 Renders Tumors Highly Susceptible to Pharmacologic Glutathione Deprivation

Pankaj Kumar Mandal¹, Manuela Schneider², Pirkko Kölle³, Peter Kuhlencordt³, Heidi Förster¹, Heike Beck², Georg W. Bornkamm¹, and Marcus Conrad¹,⁴
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Chem. Res. Toxicol., Just Accepted Manuscript • DOI: 10.1021/bx4001013 • Publication Date (Web): 22 May 2013
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**A**

![Graphs showing expression levels of Gsta1, Gsta2, and Gpx2](image)

![Western blot images of various proteins](image)
Enhanced Nrf2 activation in the airway epithelium of conditional TrxR1-KO mice
The Nrf2 system

Net Cellular Outcome:
- Net increase in TrxR activity
- Cytoprotective effect

Reflection...
Hypothesis: TrxR1 inhibition protects against the effects of hyperoxia via Nrf2-dependent mechanisms
AFN, DNCB, and Sulforaphane Inhibit TrxR & Induce Nrf2 Nuclear Accumulation
AFN and DNCB Induce Nrf2-dependent Gene Transcription
TrxR1 knockdown induces Nrf2 activation in mtCCs
AFN enhances GSH levels in mtCCs
The effects of AFN on mtCC viability are prevented by BSO.
ATG enhances Nrf2 activation in adult murine lungs
Objective: Test the hypothesis that ATG administration would be protective in a murine model of acute respiratory distress syndrome.

-12 h

- Intratracheal administration of 0.375 µg/g LPS

0 h

- Intraperitoneal injection of 0 or 25 mg/kg ATG and/or 800 mg/kg BSO

72 h

- Exposure to >95% O₂
ATG decreases lung injury in a murine model of ARDS
ATG enhances lung GSH levels

**A**

Lung GCLM Expression
Fold Change $\Delta \Delta C_t$ (Relative to Saline)

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ATG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>

**B**

Lung GSH (µmol/g)

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ATG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>15</td>
</tr>
</tbody>
</table>

**C**

Lung GSSG (nmol/g lung)

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ATG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75</td>
<td>100</td>
</tr>
</tbody>
</table>

**D**

Lung GSH/GSSG

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>ATG</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>
ATG improves survival in a murine model of ARDS

ATG/BSO

% surviving

0 50 100

Hours

0 50 100 150

ATG * $

BSO $ saline $
Summary

At homeostasis:
• GSH/Trx systems detoxify ROS
• GSH or Trx disruption well-tolerated
  – Nrf2 activation (Keap1)
  – Compensatory upregulation of GSH or Trx system.

In hyperoxia:
• Enhanced free radical production
  – Nrf2 activation
  – Compensatory upregulation of GSH-dependent responses
  – Unable to prevent fatal injury
• Therapeutic TrxR inhibition
  – Well-tolerated
  – Nrf2 activation (Keap1)
  – Upregulation of GSH-dependent responses
  – Pulmonary cytoprotection
  – Decreased lung injury
Hypothesized role of Nrf2 in lung development & BPD