Oxygen-Induced Pulmonary Injury in γ-Glutamyl Transpeptidase-Deficient Mice

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Abstract. We used mice with a targeted disruption in γ-glutamyl transpeptidase (GGT-deficient mice) to study the role of glutathione (GSH) in protection against oxygen-induced lung injury. These mice had reduced levels of lung GSH and restricted ability to synthesize GSH because of low levels of cysteine. When GGT-deficient mice were exposed to 80% oxygen, they developed diffuse pulmonary injury and died within eight days. Ten of 12 wild-type mice were alive after 18 days. Administration of N-acetylcysteine (NAC) to GGT-deficient mice corrected GSH values and prevented the development of severe pulmonary injury and death. Oxygen exposure induced an increase in lung GSH levels in both wild-type and GGT-deficient mice, but induced levels in the mutant mice were <50% of those in wild-type mice. Cysteine levels were ~50-fold lower than GSH levels in the lungs of both wild-type and GGT-deficient mice. Levels of lung RNA coding for the heavy subunit of γ-glutamyl cysteine synthetase rose three- to fourfold after oxygen exposure in both wild-type and GGT-deficient mice. In contrast, oxygen exposure failed to provoke increases in glutathione synthetase, glutathione peroxidase, glutaredoxin, or thioredoxin.

Key words: Glutathione—Hyoxia—Oxidative stress—Acute lung injury—Respiratory distress syndrome—Antioxidants.

Abbreviations/Acronyms: glutathione (GSH); N-acetylcysteine (NAC); γ-glutamyl transpeptidase (GGT); adult respiratory distress syndrome (ARDS); γ-glutamyl cysteine synthetase (γGCS).

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Introduction

Tissue injury resulting from free radical and oxidative damage has important implications in biology and medicine [10, 16, 48]. The lung is commonly a target of oxidative damage as a consequence of supplemental oxygen administration to patients with lung disease to reverse hypoxemia and to ensure adequate delivery of oxygen to tissues [18, 27]. A frequent consequence of treatment with supplemental oxygen is pulmonary oxygen toxicity, which may be manifested in adults as adult respiratory distress syndrome (ARDS) [39], and in children it may contribute to the development of bronchopulmonary dysplasia [8, 25]. The relationship among treatment with high concentrations of supplemental oxygen, free radicals and bronchopulmonary dysplasia has been observed in experimental animal models [5, 6, 11, 12, 13, 22, 46, 52]. Exposure to elevated concentrations of oxygen causes extensive lung injury in all mammalian species studied to date [2, 10]. Wild-type rats die ~70 hours after exposure to 100% oxygen. Lung damage resulting from hyperoxia is characterized by morphologic and physiologic alterations at the level of the alveolocapillary barrier, which increases permeability to solutes and results in interstitial and intra-alveolar edema and respiratory failure [15]. The process of lung injury in animals exposed to 100% oxygen goes through an initiation phase during which there is no significant evidence of morphologic injury but in which the biochemical processes associated with the development of lung injury are set in motion. The initiation phase is followed by an inflammatory/destructive phase which is rapidly followed by death. In exposures to adaptive doses of hyperoxia (~85% oxygen), the inflammatory phase is followed by survival and the development of interstitial fibrosis [15].

The underlying mechanism of tissue injury from exposure to high concentrations of oxygen appears to be the increased generation of highly reactive oxygen compounds [10]. Oxygen radicals can react with and damage many cellular components, including lipids, proteins, and nucleic acids [37, 40]. A number of mechanisms have evolved to protect cells from injury from free reactive oxygen compounds. One of the most important of these is the glutathione (GSH) pathway. This tripeptide (γ-glutamyl-cysteinyl-glycine) is the major source of non-protein intracellular reducing equivalents and functions as an antioxidant by reducing hydrogen peroxide and other peroxides to water and alcohols, respectively, in a reaction catalyzed by glutathione peroxidase. This pathway and the superoxide dismutase–catalase pathway constitute the principal known systems for protection against reactive oxygen compounds in the lung [43, 51].

In addition to the GSH pathway, thioredoxin, a potent protein disulfide reductase, is involved in antioxidant defense and appears to overlap at least in part with GSH in its protective effects [4, 26, 31]. To date there has been no analysis of the role of the thioredoxin system in protection against hyperoxia-induced lung injury.

In response to oxidative stress, cells increase GSH synthesis. This increase is achieved by induction of γ-glutamyl cysteine synthetase (γGCS), the rate-limiting enzyme in GSH synthesis [47]. Studies suggest that increased susceptibility to hyperoxic lung damage may result from reduced GSH content [28, 29]. Other