Accumulation of Lung Tissue Oxidized Glutathione (GSSG) as a Marker of Oxidant Induced Lung Injury*

Carl W. White, M.D.; Robert F. Minnack, B.S.; and John E. Repine, M.D.

Oxidants have been implicated recently in diverse models of interstitial lung disease (ILD) including injuries caused by bleomycin, asbestos, and anthracyclic drugs. These oxidants may be produced endogenously by lung cells (anthracyclic drugs) or exogenously by inflammatory cells that have been recruited and/or activated in the lung. If oxidants do have primary and/or facultative roles in causing ILD, then markers of lung tissue oxidation are needed to help determine basic mechanisms of ILD and purported interventions.

The importance of the glutathione redox cycle as a defender of lung cells against hydrogen peroxide and other oxidative injuries is increasingly well recognized. It seems likely that a metabolite of the glutathione system (oxidized glutathione, GSSG) could provide a marker of oxidant induced injury. More specifically, since optimal function of the pentose phosphate shunt and glutathione reductase systems is needed to maintain cell glutathione in the reduced state (GSH), and since very little lung glutathione is present in the oxidized (GSSG) form under normal conditions, GSSG may be a useful marker of oxidative stress in lung tissue (Fig. 1). Our hypothesis was that exposure to hyperoxia would promote excessive production of oxygen metabolites and lead to accumulation of oxidized glutathione (GSSG) in the lung. To test this premise initially, we chose hyperxia-induced lung injury, which is similar to some models of accelerated ILD in that an early phase of endogenous oxidant production occurs and is followed by a stage of chronic alveolar inflammation, which may facilitate additional lung injury.

MATERIALS AND METHODS

Following injection with saline or antioxidant enzymes (polyethylene glycol [PEG]-attached superoxide dismutase [SOD] and catalase [CAT]), male Sprague-Dawley rats (350–400 g) were exposed to hyperoxia (>99% O2, 1 atm) or to normoxia. After 54 hours of exposure, rats were anesthetized (pentobarbital, 110 mg/kg) and tracheotomies and thoracotomies were performed. Following heparin (150 U) administration via the pulmonary artery, catheters were placed and lungs were rapidly perfused blood-free and freeze-clamped at liquid nitrogen temperature. Measurement of oxidized and total glutathione in lung tissues was done using recently described tissue preparation and assay methods. In additional rats' pleural effusion volumes were measured, and lungs were lavaged with saline for measurement of albumin concentrations.

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RESULTS

Exposure of rats to hyperoxia for 54 hours caused significant (p<0.05) increases in their lung tissue oxidized glutathione (GSSG) contents (33±2 nmol/2 lungs) when compared to normoxia-exposed controls (5.0±0.4). In contrast, total lung glutathione (GSH+2GSSG) contents increased only slightly following hyperoxic exposure (3.2±0.2 μmol/2 lungs vs 2.6±0.1 μmol/2 lungs for air controls). In addition, increases in lung GSSG contents were decreased in lungs of hyperoxia-exposed rats pretreated with PEG-SOD and PEG-catalase (19±3 nmol/2 lungs) compared to untreated rats which had been exposed to hyperoxia for 54 hours. No difference was seen in total lung GSH contents of PEG-SOD + PEG-catalase treated rats that had been exposed to hyperoxia compared to those of normoxia-exposed rats.

Pleural effusion volumes and lung lavage albumin concentrations also increased in rats exposed to hyperoxia for 54 hours and paralleled increases in lung GSSG contents. Furthermore, decreases in these lung injury parameters paralleled decreased GSSG contents from lungs of hyperoxia-exposed rats that had been treated with PEG-SOD and PEG-catalase. PEG-SOD and PEG-CAT-treated rats also survived longer in hyperoxia than saline-injected control rats.

DISCUSSION

Exposure of rats to normobaric hyperoxia caused marked accumulations of GSSG in blood-free lung tissues that paralleled increases in lung injury and mortality. These increases in lung tissue GSSG contents clearly represent an alteration of the lung's redox status, especially because there is usually very little GSSG present normally in the lung. In addition, these increases in lung GSSG contents appear to be related to O₂ metabolites, since they were decreased in lungs of rats that were protected by treatment with PEG-SOD and PEG-catalase.

This study demonstrates that the lung accumulates GSSG during chronic, physiologic oxidant stresses. Most of the important reactions resulting in GSSG formation occur due to direct or indirect sulfhydryl oxidation of GSH. Thus, the accumulation of GSSG by the lung is an important finding with respect to oxidant injury. However, it should be noted that handling of GSSG may vary somewhat in different tissues. For example, although careful studies have demonstrated a parallel rise in intracellular hepatic GSSG, the liver expels GSSG into both bile and blood under oxidant stress. Under pharmacologic oxidant stress, the lung also ejects GSSG into the circulation but also accumulates tissue GSSG. On the other hand, RBCs are extremely efficient at actively ejecting extremely low concentrations of GSSG. Thus, even though the specificity of GSSG as an indicator of oxidant stress is accepted, more specific measurements of oxidants are needed to help determine their contribution to lung injury.

Table 1—Effect of Hyperoxia on Lung GSSG and GSH Contents, Lung Lavage Albumin Concentrations, Pleural Effusion Volumes, and Survivals

<table>
<thead>
<tr>
<th>Test Conditions</th>
<th>Pretreatment</th>
<th>Exposure</th>
<th>Lung GSSG Contents (nmol/2 Lungs)</th>
<th>Lung GSH Contents (μmol/2 Lungs)</th>
<th>Lung Lavage Albumin Concentrations (mg/dl)</th>
<th>Pleural Effusion Volumes (ml)</th>
<th>Survival (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>Normoxia</td>
<td>5.0±0.4 (6)*</td>
<td>2.6±0.1 (6)</td>
<td>28±2.1 (13)†</td>
<td>0±0 (19)†</td>
<td>&gt;1,000 (10)†</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>Hyperoxia</td>
<td>33±2 (12)</td>
<td>3.2±0.2 (12)</td>
<td>183±10 (13)</td>
<td>7.2±0.3 (48)</td>
<td>60±0.9 (18)</td>
<td></td>
</tr>
<tr>
<td>PEG-SOD + PEG-CAT</td>
<td>Hyperoxia</td>
<td>19±3 (6)*</td>
<td>2.4±0.1 (6)</td>
<td>57±10 (12)†</td>
<td>1.4±0.4 (18)</td>
<td>88±16 (6)†</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SEM (number of determinations).
†Value significantly (p<0.05) different from value obtained for saline-injected, hyperoxia-exposed animals.
REFERENCES

2 Smith AC, Boyd MR. Preferential effects of 1,3-bis-(2-chloroethy1)-1-nitrosourea (BCNU) on pulmonary glutathione reductase and glutathione/glutathione disulfide ratios: possible implications for lung toxicity. J Pharmacol Exp Ther 1984; 229:658-63

Proteolysis by Neutrophils While in Contact with Substrate*

Incomplete Inhibition by α1-Proteinase Inhibitor and Effect of Substrate Opsonization

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Remodeling of extracellular matrix components may accompany lung injury and repair in interstitial lung diseases. Inflammatory cells may exclude proteinase inhibitors from zones of contact with extracellular matrix macromolecules and thus create microenvironments that permit proteolysis.

We used a solid-phase proteinase assay utilizing 35S-labeled human plasma fibronectin (FN). Peripheral blood neutrophils (PMN), stimulated with PMA, were incubated in 35S-FN-coated wells containing various concentrations of α1-proteinase inhibitor (α1-PI). The observed FN proteolysis was due to PMN elastase. FN proteolysis by 2 × 10⁶ PMA-stimulated PMN was inhibited ≤87% by amounts of α1-PI as great as 200 μg/assay, but purified PMN elastase (20 ng, with equivalent proteolytic activity) was inhibited ≥98% by 250 ng α1-PI. Addition of antioxidants (catalase, 1,000 U/ml; DMSO, 1%; and superoxide dismutase, 50 μg/ml) did not increase this maximal inhibition by α1-PI.

To enhance PMN-surface interaction, we next overlaid 35S-FN-coated wells with anti-FN IgG or FAB' fragments, then added varying concentrations of α1-PI and unstimulated PMN. Representative data (CFM = 5Đ, n = 4, 12.5 μg α1-PI) were FN, 157 ± 64; FN-IgG, 570 ± 61; and FN-FAB', 158 ± 19, while FN proteolysis without added α1-PI was 1,427 ± 204, 1,700 ± 222, and 1,379 ± 80, respectively. Spreading of the PMN on FN-IgG was greatly enhanced (surface contact area 263 ± 9 μm² in contrast to 175 ± 6 and 122 ± 4 μm² for FN and FN-FAB', respectively). FN proteolysis occurred at zones of contact of cells with substrate, as revealed by indirect immunofluorescence microscopy.

The results indicate that: (1) when PMNs are in contact with a susceptible substrate, a portion of their proteolytic activity is protected from inhibition by α1-PI; and (2) PMN interaction with IgG on the surface, via Fc receptor binding, enhances cell spreading and proteolysis at zones of contact with substrate. Similar mechanisms may permit matrix proteolysis in vivo.

"In Neutrophil Influx into Lungs of Bleomycin-treated Rabbits Assessed Noninvasively by External Scintigraphy*

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In the pathogenesis of idiopathic pulmonary fibrosis (IPF) and other forms of chronic lung disease, it is thought that an inflammatory stage associated with neutrophil migration into the lung precedes the development of fibrosis. The neutrophil has many systems that are potentially injurious to tissues, but a specific role for this cell in the pathogenesis of chronic lung injury and fibrosis has not yet been identified. It has been reported that large numbers of neutrophils in bronchoalveolar lavage (BAL) fluid from patients with IPF indicate severe, progressive disease, whereas in experimental bleomycin-induced lung fibrosis, it has been suggested that neutrophils may protect against the development of fibrosis. To define more clearly the role of the neutrophil in lung disease, we must identify stages associated with neutrophil influx, study factors influencing neutrophil migration into the lung, and assess the cellular response to therapy. Another stimulus for this study is the general assumption that identification of inflammatory stages of disease will lead to therapeutic intervention before irreversible lung fibrosis occurs. Attempts have been made to identify inflammatory stages of IPF by noninvasive means, but pulmonary function testing, serology, and gallium-67 scintigraphy have proved generally unhelpful. The cell profile in BAL fluid has been used as an index of lung cellularity, but whether there is a

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