stances, oxygen metabolite lung injury may result in the development of interstitial fibrosis.

REFERENCES


Hydrogen Peroxide Causes Permeability Edema and Hypertension in Isolated Salt-Perfused Rabbit Lungs*

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Reduction products of oxygen, including superoxide anion ($O_2^-$), hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical (•OH), have been implicated in the pathogenesis of oxygen toxicity,•• drug-induced lung injury,••• and experimental and clinical ARDS.†• These toxic $O_2$ species are referred to informally as $O_2$ radicals, and can be generated in the lung from many sources including release from neutrophils and macrophages,• metabolism of certain drugs, and inhalation of oxidant gases. Since $O_2$ radicals are known to interact with many substances, including lipids and proteins that are vital to cell structure and function, we became interested in the hypothesis that $O_2$ radicals might affect the permeability of the alveolar-capillary membrane and thereby contribute to the pathogenesis of the permeability pulmonary edema characteristic of the above disorders. In this study, $O_2$ radicals are generated by either xanthine oxidase or glucose oxidase and their effects on isolated lungs are determined.

METHODS

All experiments used New Zealand white rabbits. Cannulas were placed in the main pulmonary artery and left ventricle, and the pulmonary circulation was flushed with 500 ml of albumin-containing balanced-salt perfusate. The lungs and heart were then dissected free and suspended in a 37°C, humidified chamber from a force displacement transducer which continuously monitored changes in lung weight. Mean pulmonary artery pressure was monitored. The lungs were ventilated with room air—5% CO$_2$ at 2 cm H$_2$O PEEP. After a stable baseline period, purine and xanthine oxidase or β-D glucose and glucose oxidase were introduced into the perfusate with or without the prior introduction of $O_2$ radical scavengers. After 30 minutes, lung edema was assessed by changes in lung weights and lung lavage albumin concentrations. Lavageable albumin was used as a reflection of flow of albumin across the air-blood barrier. In additional experiments, lung perfusion pressures were controlled by papaverine and alveolar-capillary membrane permeability was assessed by the edema formation in response to a venous pressure challenge (mechanical elevation of outflow venous pressure from 0 mm Hg to 10 mm Hg for 10 minutes).

RESULTS

Injection of purine plus xanthine oxidase into isolated lung perfusates caused marked pulmonary vasoconstriction that could be inhibited by catalase (H$_2$O$_2$ scavenger), dimethylthioureas (•OH scavenger), or papaverine (non-specific smooth muscle relaxant) (Fig 1). Protein-rich pulmonary edema was associated with this $O_2$ radical-induced pulmonary hypertension, and was characterized by frothy edema fluid emerging from tracheas, lung weight increases (35±7g), and increased lung lavage albumin concentrations (88±157 mg/dl). Again, pulmonary edema formation was inhibited by catalase or dimethylthiourea, but not by superoxide dismutase (SOD, $O_2^*$scavenger). To determine if the aforementioned hydrostatic changes were the sole cause of the pulmonary edema or if a change in permeability of the alveolar-capillary membrane (ACM) had occurred, we maintained baseline perfusion pressures with papaverine during $O_2$ radical exposure and after 30 minutes of assessed ACM permeability by measuring edema formation in response to a venous pressure challenge. We found that isolated lungs exposed to purine plus xanthine oxidase responded to the venous pressure challenge with marked increases in lung weights and lavage albums compared to papaverine controls (Fig 2). Again, this purine-xanthine oxidase induced response was blocked by pre-addition of catalase. These findings suggested that H$_2$O$_2$ or an H$_2$O$_2$-derived product caused 1) pulmonary vasoconstriction, 2) increased ACM permeability, and 3) protein-rich pulmonary edema.

To confirm that H$_2$O$_2$ and not $O_2^*$ was the toxic $O_2$ species in...
this model, we performed additional experiments with glucose oxidase and β-D glucose, a system that produces 
H₂O₂ but not O₂⁻. Glucose oxidase-exposed lungs developed pulmonary vasoconstriction (perfusion pressure increase 32 ± 10 mm Hg) and protein-rich pulmonary edema (weight gains, 48 ± 9 g; lavage albumin concentrations, 1119 ± 119 mg/dl). Increased ACM permeability was again demonstrated by blocking the pulmonary vasoconstriction with papaverine and then challenging the lung with a venous pressure of 10 mm Hg for 10 minutes. Glucose oxidase exposed lungs showed dramatic weight gains (53 ± 2 g) and lavage albumin accumulations (1316 ± 53 mg/dl) compared to papaverine controls (5 ± 3 g and 42 ± 9 mg/dl). All of the above effects of glucose oxidase were blocked by catalase, but not by heat-inactivated catalase.

**DISCUSSION**

This investigation demonstrates that H₂O₂ and/or H₂O₂-derived products can cause pulmonary vasoconstriction and increased permeability of the alveolar-capillary barrier in isolated salt-perfused rabbit lungs. The result of these effects in this model is protein-rich pulmonary edema. An understanding of the effects of O₂ radicals on lung tissue has become relevant to many pulmonary disorders. Our findings relate most closely to examples of neutrophil-dependent lung injury, since neutrophils are potent producers of O₂ radicals, and could well exert some of their effects in acute lung injury by release of toxic O₂ radicals, granular enzymes, and/or arachidonate metabolites. In addition, this investigation supports the possibility that O₂ radicals are involved in the pathogenesis of O₂ toxicity and certain drug-induced lung injuries, including those of paracetamol and bleomycin.

Evidence from investigations in other model systems supports similar conclusions. For instance, Johnson and Ward have shown that O₂ radicals can cause acute lung injury in rats. Several investigators have shown that O₂ radicals are toxic to various cellular components of the lungs.

Finally, Flick and Staub have shown that SOD protects against increased vascular permeability after microembolism in sheep. Our studies complement these and further support the possibility that O₂ radicals may play important roles in the genesis of acute permeability pulmonary edema.

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Hamster tracheal epithelial cells were incubated in Ham's F12 medium (10% calf serum, 50 μg/ml gentamicin, 25 U/ml nystatin) containing 2.5 μCi/ml L-[35Se] Selenomethionine (specific activity 0.6-4 Ci/mmol, Amer sham Corp., Arlington Heights, IL). After 24 hours, the [35Se]-labeled medium was removed and cells were rinsed 3 × in phosphate-buffered saline (pH 7.4) before trypsinization and plating in multwell dishes (2 × 10³ cells/35 mm wells). At this juncture, asbestos containing a diversity of fiber lengths (Rhodesian crocidolite, UI C C reference sample; Canadian chrysotile, UI C C reference sample) and sized chrysotile, (Johns Manville, Denver) was added in medium (0.36-2.9 μg/cm² surface area of dish) alone and with addition of SOD (100 μg/ml, Orgentec, Diagnostic Data Inc., Mt. View, CA), catalase (50 μg/ml, Sigma Chemical, St. Louis) or DABCO (5 × 10⁻⁴ M, Aldrich Chemical, Milwaukee) to individual wells (N = 3 per group, duplicate experiments). After 24 hours, medium was removed, centrifuged to remove cell debris, and 100 μl aliquots assessed using a gamma counter. Adherent cells were washed 3 × in PBS, dissolved in 0.5 N NaOH and the radioactivity in 100 μl aliquots determined.

Assay for Determination of Endogenous SOD

At 24, 48, 72 and 96-hour intervals after addition of minimally toxic amounts of UI C C chrysotile (0.36 μg/cm²) or crocidolite (1.8 μg/cm²), the activity of SOD was determined in cell-free extracts using the procedures of McCord and colleagues and Beauchamp and Fridovich. The presence of SOD in monolayers of cells was confirmed also by the histochemical technique of Hoidal et al.

Importance of Oxygen Free Radicals in Asbestos-Induced Injury to Airway Epithelial Cells*

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The biologic events important in mineral-induced cell injury are undefined. Although the toxic effects of a variety of minerals are attributed to damage of membranes and the release of cytoplasmic and lysosomal enzymes, it is unknown how and why injury occurs. We hypothesized an important role of oxygen-free radicals, reactive species which cause peroxidation of lipids and damage to macromolecules such as DNA. To test this hypothesis, tracheal epithelial cells were exposed in vitro to crocidolite and chrysotile asbestos. Cell injury then was evaluated quantitatively at intervals using release of ⁷⁷Se as an index of cytotoxicity. To determine whether damage to epithelial cells could be prevented by inhibitors of oxygen-free radicals, superoxide dismutase, the enzyme converting superoxide (O₂⁻) to H₂O₂ and O₂, catalase, the enzyme converting H₂O₂ to H₂O and O₂, or 1,4-diazobicyclo[2.2.2] octane (DABCO), a scavenger of singlet oxygen (¹O₂), was added to cultures exposed to asbestos. The activity of endogenous SOD also was measured after addition of fibers.

A number of studies suggest that long, thin asbestos fibers are more pathogenic and cytotoxic than short fibers or particles. The availability of sized preparations of chrysotile, long (>10 μm) vs short (<2 μm), allowed us to examine the relationship between fiber length and cell damage.

Materials and Methods

Assay for Release of ⁷⁷Se

The objective of these experiments was to develop a reproducible assay for quantification of mineral-induced cell injury. Accordingly,