Glutathione peroxidase levels in the rats with excess selenium were not higher than in the animals with normal selenium levels. We conclude that survival with exposure to hyperoxia in selenium deficient rats is correlated with lung glutathione peroxidase levels and that increases in both survival and glutathione peroxidase activity in lungs following selenium supplementation lags behind the return of selenium to normal levels in the lung. Glucose-6-phosphate dehydrogenase and glutathione reductase are not affected by changes in dietary selenium levels. Cross et al have also reported reduced glutathione peroxidase levels in Se deficient rat lungs. They showed that superoxide dismutase levels were not affected and that the ability to tolerate 80% oxygen was reduced. Thus, our data corroborate this work and confirm that the reduction in glutathione peroxidase levels alone is sufficient to increase oxygen susceptibility.

REFERENCES


Pulmonary Arterial Wall Injury and Remodelling by Hyperoxia*

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It is well established that breathing high oxygen concentrations damages the alveolar wall, including the capillary bed. Early lung injury includes damage to endothelial cells and type I pneumocytes, and is followed by edema, increase in the concentration type II pneumocytes, forma- tion of hyaline membranes, cellular infiltration and, ultimately, fibrosis. We have recently reported that breathing high oxygen concentrations for 7 days (87-90%) at normobaric pressure, in the rat, causes pulmonary hypertension, increase in pulmonary vascular resistance and remodelling of the wall of pulmonary precapillary arteries. We describe here the effect on pulmonary arterial wall structure of breathing hyperoxia for several weeks, and then changes after weaning from breathing hyperoxia and recovery in air.

MATERIAL AND METHODS

Adult male Sprague-Dawley CD-SPF rats breathed 87% oxygen

Table 2—Relation of Lung Glutathione Peroxidase Levels to Dietary Selenium

<table>
<thead>
<tr>
<th>Diet</th>
<th>Units/g dry lung*</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Se+ (40-45 days)</td>
<td>17.31 ± 1.46 (13)</td>
<td>100</td>
</tr>
<tr>
<td>Se− (40-45 days)</td>
<td>3.69 ± 0.60 (10)</td>
<td>21.3</td>
</tr>
<tr>
<td>Se− (45 days, 3 days)</td>
<td>9.49 ± 0.34 (5)</td>
<td>54.8</td>
</tr>
</tbody>
</table>

*Units are expressed as µmol NADPH oxidized/min ± SEM. (Number of rats is in parentheses.)
for 28 days. At the end of this period, the lungs from some animals were examined; all other animals were weaned to air over a 7-day period (≈10% O2 daily). Lungs from these animals were examined either at the end of weaning or after the animals had an additional period of recovery in air of 2, 4 or 8 weeks. Pulmonary arteries in excised lungs were distended by injection of barium-gelatin (at 60°C), at constant pressure (76 mm Hg). Using this technique, arteries >15 microns in external diameter (ED) are filled with the medium, but capillaries or veins are not. In tissue sections stained to demonstrate elastic fibers and muscle cells (Miller elastic van Gieson)6 the arterial population was analyzed by quantitative morphometric techniques.4 Arteries were characterized by their wall structure as muscular, partially muscular or nonmuscular and by their position in the lung, accompanying bronchioli or terminal bronchioli (ie, pre-acinar vessels) or accompanying respiratory ducts, alveolar ducts or alveolar walls (ie, intra-acinar vessels). The ED and medial thickness (MT) (ie, the distance between the internal and external elastic laminae of vessels) was measured and the percentage of medial thickness (%MT) calculated (2 × MT/100 × ED). Additional tissue was processed for transmission electron microscopy.

RESULTS AND COMMENT

Hyperoxia reduced the concentration of arteries (number per 100 alveoli) by two thirds. Many occluded small intra-acinar vessels and vessel remnants were seen. An example of a muscularized alveolar wall artery with an occluded lumen is shown in Figure 1. During weaning and recovery, the vessel concentration increased somewhat in some animals, but within any 1 group it did not significantly increase.

Muscle appeared in the arterial wall further to the lung periphery than normal, ie, muscular arteries were found in the alveolar wall where they are normally absent. Within the acinus as a whole, hyperoxia increased the proportion of muscularized arteries (ie, muscular and partially muscular arteries) at the expense of nonmuscular ones (Fig 2). Although weaning had little additional effect, during recovery, additional intra-acinar arteries were muscularized. Increase in the concentration of muscularized vessels occurred during the first 2 weeks of recovery, then regression started, but even after 8 weeks, still more vessels were muscularized than after hyperoxia alone.

The mean MT (±SE) of all muscularized (ie, muscular and partially muscular) pre-acinar and intra-acinar arteries (1.5±0.3 and 2.0±0.2 in control rats) was significantly increased by hyperoxia (6.2±0.5 and 9.6±0.5 respectively). During weaning and recovery, the values for both pre-acinar and intra-acinar vessels fell significantly and by 8 weeks were close to normal (2.8±0.2 and 4.1±0.6 respectively).

Hyperoxia caused right ventricular hypertrophy: the ratio of the left ventricle + septum (LV + S) to the right ventricle (RV) was significantly decreased. Weaning further increased hypertrophy of the RV reducing the ratio to its lowest value (LV + S/RV in controls = 4.20±0.10 and in weaned rats 1.46±0.12). After 2 and after 4 weeks' recovery in air, the ratio was similar to that at the end of hyperoxia; after 8 weeks, it was significantly greater than at the end of hyperoxia, but significantly below the value for the control.

The generation of free oxygen radicals during breathing of hyperoxia causes endothelial cell injury leading to striking remodelling of the pulmonary arterial bed. Vascular changes are associated with right ventricular hypertrophy. There is a) loss of small pre-capillary arteries, b) muscularization of vessels in the alveolar wall, c) increase in wall thickness of muscularized arteries. All of these changes contribute to reduced vascular volume. Paradoxically, weaning and return to air are associated with regression of some features and progression of others. Whereas the thickness of the muscle
Alveolar Injury by Oxygen Metabolites Alters the Composition of Extracellular Matrix


The structure and functional integrity of the alveolus are determined by the connective tissue components of the alveolar wall. The basal lamina determines the orientation of cells and is necessary for the restoration of normal alveolar architecture after injury.1 The interstitium largely determines the tensile and elastic properties of the lung.2 Injury that results in alterations of either component may lead to a functionally abnormal alveolus.

Recently, evidence has accumulated to suggest that oxygen radicals are important mediators of lung injury.4,5 In one model,6 alveolar damage induced by the enzymatic release of oxygen metabolites results in pulmonary fibrosis. We used this model to investigate the changes in alveolar connective tissue that occur as lung injury proceeds to fibrosis. Our immunohistologic studies of injured tissue reveal sequential alterations in matrix during injury and repair, and the accumulation of interstitial collagens in alveolar connective tissue.

MATERIALS AND METHODS

The animals used were 250 g pathogen-free male Long-Evans rats. Endobronchial instillation of either glucose (1 mg), glucose oxidase (35 μ) and lactoperoxidase (5 μ) in saline, or saline alone, was performed as described by Johnson et al.4 Eight experimental animals were sacrificed from 1 through 20 days after injury. The lungs of 2 saline control and 2 untreated rats were also examined. All lungs were fixed by inflation with Carnoy’s solution and embedded in paraffin. Four micron sections were deparaffinized, rehydrated, and examined by the indirect immunofluorescent antibody technique. Sections were also examined by routine light histologic techniques.

Antibodies to types I and III rat skin collagen were made in rabbits.6 Antibodies to human skin type I collagen and human placenta type IV collagen were also prepared from immunized rabbits. Antibodies to types III and IV human kidney collagen were made in sheep.7 At least one of the antibodies to each collagen type was affinity purified. Specificity was tested by ELISA and radioimmunoprecipitation. Antibody to laminin was generously provided by Dr. G. R. Martin, National Institute of Dental Research, Bethesda, MD.

RESULTS

Light microscopic examination of injured lungs revealed changes similar to those previously described.4 At day 1, interstitial and intra-alveolar edema was prominent and was associated with a polymorphonuclear leukocytic infiltrate. By day 5, the areas of most severe injury had become necrotic with loss of all recognizable alveolar architecture. In less severely injured areas, alveolar septa were hypercellular and contained mononuclear leukocytes. By day 20 necrotic regions had become organized, and thickened alveolar walls contained large quantities of connective tissue.

The immunohistologic appearance of normal and saline-instilled lungs was similar. A small amount of type I collagen antigen could be identified in some alveolar walls, but was prominent in vessel and bronchial walls (Fig 1A) and pleura. Type III collagen antigen was consistently identified in alveolar walls, but was most concentrated in the connective tissue surrounding the orifices of individual alveoli (Fig 1B). Type IV collagen and laminin appeared in a similar linear distribution along the basal laminae of alveoli and capillaries. Both antibodies to each collagen type produced identical results and blocking experiments confirmed the staining specificity.

One day after injury no change was seen in type I or type III collagen antigens. However, in some areas the intensity of staining of type IV collagen and laminin was reduced. By day 5 only minimal amounts of connective tissue antigens were detected in regions of necrosis. Beginning at day 5, in areas of less severe injury, hypercellular septa contained increased quantities of types III and IV collagen and laminin antigens. As regions of necrosis became organized, type IV collagen and laminin were identified in the capillary basal laminae of granulation tissue, and subsequently, types I and III collagen were identified in the interstitium. By day 13, the antigens of collagen types I (Fig 1C) and III (Fig 1D) were abundant in fibrotic alveolar walls and in organized scar.

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