these animals have markedly different responses to oxidant gases, but the amount of vitamin E in their lungs is quite similar (nursing pups, 160 μg/g; 90-day-olds, 175 μg/g; nursing mothers, 140 μg/g; 2-year-olds, 120 μg/g). In contrast, the lung tissue of animals of the same age maintained on formulated diets containing 0, 200, and 3,000 mg of vitamin E/kg of food respond very similarly during the first 72 hr (Fig 4) in spite of the fact that the vitamin E level in the lungs of these animals was 5.3, 146, and 325 μg/g of tissue, respectively (Fig 6). (We regard vitamin E levels of less than 15 μg/g of tissue as depleted.) While some animals in each group showed less injury and repair than others, it was impossible to distinguish significant differences between the groups in the “over-all” response.

In conclusion, therefore, it appears that the response to injury and repair in the lung is independent of the level of vitamin E in the tissue.

REFERENCES


Changes in O$_2$ Toxicity and Glutathione Peroxidase Levels in Selenium Deficient Rats*

Susan M. Deneke, Ph.D.;† Stanley N. Gershoff, Ph.D.;‡ and Barry L. Fanburg, M.D.*

Patients treated for acute or chronic conditions requiring oxygen administration are often also suffering from a variety of nutritional deficiencies which could affect their ability to tolerate hyperoxia. We have previously reported that enzymes in the glutathione pathway and glutathione itself play an important role in the protection of the lung against hyperoxia. We have also shown that glutathione levels in oxygen exposed animals can be modified by protein deprivation and supplementation with cysteine. In the present study, we have produced selenium deficiency in rats to further test the role of glutathione reactions in the susceptibility of the lung to hyperoxia.

MATERIAL AND METHODS

Male Charles River COBS-CR rats were placed on selenium-free diets formulated in our laboratory (Table 1). This diet contained <2.0 ppb selenium.

Paired groups were fed the same diet supplemented with 2.0 ppm sodium selenite, which is an excess selenium diet (Se group). The diets were continued for 45-50 days at which time lung glutathione peroxidase levels in animals on the selenium deficient diets (Se group) were <25% of the lung levels of control animals given selenium supplementation.

At this time, rats were either exposed to >98% O$_2$ and the time of death determined or were sacrificed for lung enzyme assays. Animals used for enzyme assays were killed with sodium pentobarbital. The lungs were perfused in situ with cold isotonic saline solution, homogenized in 5 mM potassium phosphate buffer pH 7.8, and centrifuged at 300 x g for 10 minutes. Enzyme activity was measured in the supernatant. Glutathione peroxidase was assayed by a modification of the method of Little et al at pH 7.5 using cumene hydroperoxide as a substrate. Glucose-6-phosphate dehydrogenase (G6PDase) and glutathione reductase (GRase) were assayed as previously described. Lung and diet selenium levels were assayed by ESA Laboratories, Bedford, MA using atomic absorption spectrosocpy. Some groups of rats with demonstrated selenium and glutathione peroxidase deficiency were re-fed the high selenium diets for 3 days. Enzyme levels and oxygen susceptibility of these animals were also determined. This group will be designated Se++.

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Table 1—Low Selenium Diet

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torula yeast</td>
<td>35%</td>
</tr>
<tr>
<td>Corn oil (Toopherol stripped)</td>
<td>10%</td>
</tr>
<tr>
<td>Hegsted's salt mixture</td>
<td>4%</td>
</tr>
<tr>
<td>Sucrose</td>
<td>50.7%</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.3%</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td></td>
</tr>
</tbody>
</table>

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RESULTS

Figure 1 shows the composite of death times of the Se⁻, Se⁻⁻ and Se⁺⁺ animals. The SE⁺⁺ group was more susceptible to oxygen exposure than the SE⁺ group. The mean ± SEM survival time in oxygen for the SE⁺⁺ group was 56.9 ± 1.1 hr (34 rats). The SE⁺⁺ group survived 50.3 ± 1.5 hr (19 rats). The SE⁺⁺ group survived an intermediate time of 53.2 ± 1.1 hr (18 rats).

Glutathione peroxidase levels in these groups of rats were related to the survival times in hyperoxia as can be seen in Table 2.

We also examined levels of GRase and G6PDase in these rats lungs. The mean ± SEM for G6PDase were: SE⁺⁺, 10.05 ± 0.45 (23 rats), Se⁺⁺, 10.54 ± 0.89 (18 rats) and Se⁺⁺, 8.92 ± 0.15 (15 rats), (expressed as μmol NADP⁺ reduced per min per g dry lung). There were no significant differences between these values. GRase levels were SE⁺⁺, 6.94 ± 0.45 (31 rats), Se⁺⁺, 8.04 ± 0.89 (28 rats), Se⁺⁺, 7.34 ± 0.55 (18 rats) (expressed as μmol NADPH oxidized per min per g dry lung). These values were also not statistically different from each other.

Selenium levels measured in rat lungs varied with the age of the animal at the beginning of the diet. When 90 g rats were placed on the low and high selenium diets for 48 days, the levels of selenium in the lungs of deficient animals were <20 ppb and those in rats fed high Se diets were >856 ppb. (Normal tissue values have been reported to be between 80-200 ppb.) When larger rats (~150 g) were placed on the same regimen, the selenium levels in the Se⁺⁺ group were 40 ppb and 124 ppb in the Se⁺⁺ group. Selenium levels were in the normal range after 3 days of refeeding the high Se diet.

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.)

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*

Rosemary Jones, Ph.D.; W. M. Zapoi, M.D., FCCP;† and Lynne Reid, M.D.

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, formation of hyaline membranes, cellular infiltration and, ultimately, fibrosis. We have recently reported that breathing high oxygen concentrations for 7 days (85-90%) 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.

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