BIOCHEMICAL AND PATHOLOGICAL CHANGES IN RESPONSE TO HYPEROXIA AND PROTECTION BY ANTIOXIDANTS IN RATS

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Abstract: A significant decrease in blood haemoglobin, reduced glutathione and protein in lung and liver, without any change in blood reduced glutathione, was observed in rats exposed to 80% oxygen. Hydrogen peroxide induced erythrocyte haemolysis was significantly increased following exposure to hyperoxia. The lungs of rats exposed to hyperoxia showed perivascular edema. Simultaneous treatment with antioxidants, vitamin A, C, or E, protected the animals against oxygen toxicity.

Key words: hyperoxia, RBC, reduced glutathione, lung, haemoglobin, antioxidants

INTRODUCTION

The toxic effects of increased oxygen tension in tissues have been recognised for many years (1,2), with the most toxic effects being exerted on erythrocytes and lung parenchyma (3). To combat these toxic effects, aerobic organisms have protective mechanisms by way of enzymes, thiol containing compounds, as well as antioxidants (vitamin A, C and E) (4, 5). Since the use of oxygen is increasing in space capsules and hospitals, this study was carried out to observe the biochemical changes resulting from hyperoxia, and their protection by antioxidants.

METHODS

Adult male albino rats of Wistar strain (100-120 g), maintained on standard pellet diet (Lipton India Ltd.) and water ad libitum, were divided into five groups (n=5). Group I served as control. Group II to V were exposed to 80% oxygen and 20% nitrogen for 7 hr daily in a dynamically operated whole body exposure chamber (6), for 5 days a week for 2 weeks. Fifteen minutes prior to exposure the animals of group III, IV and V were given daily, vitamin A (60 J.1g kg^-1, i.m.), vitamin C (10 mg/kg^-1, i.m.) and vitamin E (10 mg/kg^-1, i.m.), respectively (7, 8), while groups I and II were only given normal saline.

Twentyfour hours after the last exposure (13th day) the animals were lightly anaesthetised with ether and maximum blood was withdrawn from ocular plexus in heparinised vials. These animals were then sacrificed by cervical dislocation and their lungs and liver quickly removed, cleaned and weighed. A small portion of the lung was preserved in 10% buffered formalin for histopathological studies (9). The blood was used for estimation of haemoglobin (10), reduced glutathione (11), and hydrogen peroxide induced erythrocyte haemolysis (12). Known quantities of lung and liver were used for estimation of reduced glutathione and protein (13). The data were analysed by Student’s 't' test.

RESULTS

The changes in the level of haemoglobin, hydrogen peroxide induced erythrocyte haemolysis, re-
duced glutathione and protein induced by hyperoxia, and protection provided by antioxidants are shown in Table I. The blood haemoglobin was decreased

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood haemoglobin (g 100 ml⁻¹)</th>
<th>%H₂O₂ induced haemolysis</th>
<th>Reduced glutathione (μmole/ml or g⁻¹)</th>
<th>Protein (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Blood</td>
<td>Lung</td>
<td>Liver</td>
<td>Blood</td>
</tr>
<tr>
<td>1. Control</td>
<td>13.29</td>
<td>±0.46</td>
<td>31.35</td>
<td>±4.02</td>
</tr>
<tr>
<td>2. Oxygen exposed</td>
<td>11.22b</td>
<td>±0.38</td>
<td>33.05c</td>
<td>±1.55a</td>
</tr>
<tr>
<td>3. Oxygen exposed + Vit. A</td>
<td>12.60c</td>
<td>±0.35</td>
<td>34.07c</td>
<td>±1.57</td>
</tr>
<tr>
<td>4. Oxygen exposed + Vit. C</td>
<td>11.92c</td>
<td>±0.49</td>
<td>39.50c</td>
<td>±2.04</td>
</tr>
<tr>
<td>5. Oxygen exposed + Vit. E</td>
<td>11.80c</td>
<td>±0.15</td>
<td>32.30c</td>
<td>±1.45</td>
</tr>
</tbody>
</table>

Dose of Vit. A = 60 μg/kg⁻¹ of retinol, Vit. C = 10 mg/kg⁻¹ and Vit. E = 10 mg/kg⁻¹

Significant from group 1 : a - P<0.05, b - P<0.01.
Significant from group 2 : c - P<0.05, d - P<0.01.

Histopathological changes in lungs exposed to hyperoxia can be best appreciated when compared with normal lungs (Fig. 1). The hyperoxia-exposed lungs showed perivascular edema, hydropic degeneration in the endothelial cells of the pulmonary arteries, and mild pulmonary congestion (Fig. 2). Majority of pulmonary veins showed eosinophilic

![Fig. 1: Normal lung tissue of rat showing homogenous alveolar pattern with normal alveolar septa, airducts and bronchiole.](image1)

![Fig. 2: Lung tissue from rat exposed to hyperoxia showing perivascular edema, hydropic degeneration in the endothelial cells of pulmonary arteries and mild pulmonary congestion.](image2)
degenerative changes. Animals exposed to hyperoxia treated with vitamin A also showed mild pulmonary congestion and inflammatory reaction, but there was no perivascular edema (Fig. 3). Treatment with vitamin C showed not only pulmonary congestion and inflammatory reaction but there was perivascular edema also (Fig. 4). Treatment with vitamin E showed degeneration of endothelial lining of blood vessels of few areas but the histoarchitecture was normal in majority of the areas when compared to vitamin C or A (Fig. 5).

DISCUSSION

It is widely accepted that structural and functional changes occur in the lungs of animals exposed to increased oxygen tension (14). It has been shown that there is an enhanced production of superoxide ions and hydrogen peroxide in lung mitochondria and microsomes (15, 16). Since RBC and lung cells are exposed to oxygen tension, tissue changes are expected (3). In the present study haemoglobin was significantly decreased in animals exposed for 11 days to 80% oxygen, indicating damage to RBC. The hydrogen peroxide induced RBC haemolysis was also significantly increased. Crapo and Tierney (17) had observed an increase in lung protein after exposure to oxygen. However, in the present study, total lung and liver protein was reduced, which could be due to an inhibition of protein synthesis (17).

Hyperoxia also decreased GSH level, while animals treated with antioxidants before hyperoxia had GSH level close to that of control. This is probably a result of oxidation of red cell components, membrane damage, or haemolysis, as it has been reported previously that GSH is required for maintenance of membrane (18). Vitamin E deficiency has been reported to increase hydrogen peroxide induced eryth-
rocyte haemolysis (19). In the present study, hyperoxia induced increase in erythrocyte haemolysis has been corrected by vitamin A or E. Histopathological examination of lung reveals that vitamin E is a better protective agent than vit. A.

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