PROTECTIVE EFFECTS OF SUPEROXIDE DISMUTASE AGAINST OXYGEN TOXICITY IN RAT'S HEART LUNG PREPARATION

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Oxygen toxicity in the non-ischemic and non-hypoxic heart has not been reported. In an experiment on isolated rat heart lung preparation, the effects of superoxide dismutase (SOD) on oxygen toxicity during hyperoxic perfusion were evaluated with intramyocardial high energy phosphates and the release of creatine phosphokinase (CPK) in the perfusate blood. Although there were no significant differences in high energy phosphates between SOD-treated and untreated hearts, the CPK release from the SOD-treated hearts was significantly less than from the untreated hearts. SOD increased the oxygen pressure of perfusate blood, too. These results indicate that hypoxia induced cardiac and lung cell damage which was protected by SOD.

Cytotoxic oxygen metabolites such as superoxide anion, hydrogen peroxide and hydroxyl radical have been implicated as participants in ischemic cardiac damage.1–5 Cell damage due to oxygen radicals also occurs when the non-ischemic perfused heart is deprived of oxygen and then reoxygenated.6–10 This phenomenon is known as the oxygen paradox. However, oxygen toxicity in the non-ischemic and non-hypoxic heart has not been reported.

The purpose of the present study was to determine whether oxygen toxicity in the hyperoxic perfused heart exists or not and whether superoxide dismutase (SOD) reveals protective effects on it. An isolated rat’s heart lung preparation was chosen to evaluate the direct effects of oxygen and SOD on the heart through the lung.

MATERIALS AND METHODS

Male Wistar rats (300–350g) were anesthetized with 50 mg/kg of pentobarbital intraperitoneally. A tracheostomy was performed, and constant volume (1.5 ml) intermittent positive pressure ventilation was instituted at a rate of 80 breaths/min with 100% oxygen. The chest was opened and flooded with ice-cold saline and the heart was arrested during the preparation. Cannulae were inserted into the aorta and the superior and inferior venae cavae. A pacing wire was placed on the right atrium. The cannula of the superior vena cava was used to monitor right atrial pressure. A heart lung preparation was perfused with perfusate blood (25 ml), containing red blood cells which were collected from another rat, and Krebs Ringer bicarbonate buffer, with hematocrit and pH of 25% and 7.4, respectively. The concentrations (mM) of the buffer constituents were as follows: NaCl 127, KCl 5.1, CaCl2 2.2, KH2PO4 1.3, MgSO4 2.6, NaHCO3 15, glucose 5.5 and heparin. The perfusate blood pumped from the aorta, passing through a pneumatic resistance, was collected in a reservoir that was warmed at 37°C throughout the experiment by means of a water jacket and then returned to the inferior vena cava. No other organs except heart and lung were perfused (Fig. 1). The heart rate was recorded with a

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Nihon Kohden’s bioelectric amplifier AB-621G and the cardiac output was measured with an electromagnetic blood flow meter MFV-1200. The arterial pressure and the right atrial pressure were measured with carrier amplifiers AP-621G using transducer TP-101T and LPU-0.1A, respectively.

All hearts were perfused at a heart rate of 250 beats/min, a cardiac output of 30 ml/min and a mean arterial pressure of 80 mmHg. Five minutes after the perfusion with 100% oxygen, 2 mg of SOD (5000 U/mg, Boehringer Mannheim 567680) was administered in the perfusate blood in group S (n = 7). No drug was given in group C (n = 7).

Thirty minutes after the start of perfusion, hearts were freeze-clamped by liquid nitrogen. Blood gas analyses were made and the perfusate blood was collected and analyzed for creatine phosphokinase (CPK) activity with the tetrazolium method. Subsequently, the heart tissue was freeze-dried for 6 days. A part of the freeze-dried sample was extracted with perchloric acid and centrifuged at 3000 rpm. High energy phosphates (ATP: adenosine triphosphate, ADP: adenosine diphosphate, AMP: adenosine monophosphate) were determined spectrophotometrically by standard techniques according to Bergmeyer.\textsuperscript{11} The values of high energy phosphates were expressed as micromoles per gram of dry weight.

Significant differences were determined by the nonpaired t test. A probability of p < 0.05 was regarded as statistically significant. The data were given as means ± SEM.

**TABLE I CARDIAC ENERGY METABOLITES**

<table>
<thead>
<tr>
<th></th>
<th>ATP</th>
<th>ADP (μ mole/g.d.t.)</th>
<th>AMP</th>
<th>EC</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>19.50 ± 0.47</td>
<td>3.55 ± 0.09</td>
<td>0.600 ± 0.118</td>
<td>0.900 ± 0.008</td>
</tr>
<tr>
<td>S</td>
<td>18.72 ± 0.58</td>
<td>3.55 ± 0.13</td>
<td>0.696 ± 0.170</td>
<td>0.892 ± 0.011</td>
</tr>
</tbody>
</table>

ATP = Adenosine triphosphate; ADP = Adenosine diphosphate; AMP = Adenosine monophosphate; EC = Energy charge.
RESULTS

There were no significant differences in high energy phosphates and the calculated energy charge (EC: [ATP + 0.5 × ADP]/[ATP + ADP + AMP]) between groups S and C (Table I). However, the CPK release from the hearts in group S was significantly less than from the hearts in group C (S vs C: 21.1 ± 3.1 vs 45.4 ± 2.8 mU/ml, Fig. 2). The CPK release from the hearts perfused with air was 20.4 ± 2.3 mU/ml (n = 4; unpublished data). Although there were no significant differences in pH and PCO₂ between the two groups, PO₂ in group S was significantly higher than that in group C (Table II).

DISCUSSION

The findings of the present study show that hyperoxia induced cell damage resulting in CPK release, and it may be mediated in part by cytotoxic oxygen metabolites because SOD reduced the CPK release from the heart. Superoxide dismutase dismutates the superoxide anion into hydrogen peroxide and oxygen. Hydrogen peroxide, itself a moderately strong oxidant, is dismutated into water and oxygen by catalase. Therefore, a protective effect of SOD and catalase on the heart has been demonstrated. In regional ischemia-reperfusion models, this enzyme combination has reduced myocardial infarct size. It is possible that one or both of these enzymes would confer protection. The administration of SOD alone appears to be sufficient for myocardial salvage. However, these results are not in agreement with the findings of Myers et al. which demonstrated that SOD alone did not significantly reduce creatine kinase loss in reoxygenated hearts. Variable results may be attributed to differences in species and methods. In this experiment, hearts were normally perfused which was indicated by the data of high energy phosphates.

The mechanism for oxygen radical-related myocardial injury are not yet completely understood. A question whether production of oxygen metabolites is greatest during hypoxia or during subsequent oxygen repletion has been debated. Myers et al. have suggested that important damage due to oxygen-derived metabolites occurred during hypoxia, based on their observations. On the other hand, free radicals might be expected to be produced during repfusion. We expect from this study that free radicals are more or less produced at any time. Under normal conditions, tissues and cells are protected from their damaging effects by the various antioxidant systems in the body. Hypoxia or severe ischemia induces a reduction of the protective mechanisms against oxygen toxicity. When natural defenses are overwhelmed, free radical-related cell damage occurs.

The primary target of injury for normobaric hyperoxia is the lung. Hyperoxia increases oxygen radical production in rat lungs and lung mitochondria. Therefore, the administration of SOD may be protective against oxygen toxicity in the lung. Maclellan et al. have demonstrated

*TABLE II BLOOD GAS ANALYSIS AT THE END OF PERFUSION*

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>PCO₂ (mmHg)</th>
<th>PO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>7.333 ± 0.028</td>
<td>13 ± 1</td>
<td>301 ± 17</td>
</tr>
<tr>
<td>S</td>
<td>7.356 ± 0.042</td>
<td>12 ± 1</td>
<td>375 ± 23*</td>
</tr>
</tbody>
</table>

C = Control group; S = SOD-treated group
*p < 0.025 (non-paired t test)
that continuous intraperitoneal infusion of SOD confers some protection against pulmonary oxygen toxicity in the rat. Recently, Turrens et al.\(^2\) have reported that survival of rats exposed to 100% oxygen was increased when liposomes containing catalase and SOD were injected intravenously before and during exposure. In this experiment, SOD increased the oxygen pressure of perfusate blood. It may have improved the oxygen delivery in the lung which had been damaged by oxygen radicals.

Although oxygen toxicity in the lung has been reported and reviewed\(^2\),\(^4\) it has not been reported in the normal perfused heart. It is interesting in the present study that only 30 minutes, perfusion with hyperoxia induced cardiac cell damage which was protected by SOD. The tolerance of oxygen toxicity in various species is different.\(^2\) Therefore, the results obtained from the rats cannot be extrapolated directly to humans. However, we conclude that the administration of 100% oxygen, even in a short time, may exert a deleterious effect on the heart and lung.

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