Saturated hydrogen saline protects rats from acute lung injury induced by paraquat

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INTRODUCTION
Paraquat (PQ) is an effective herbicide used widely, harmless to surrounding environment,[1] but it is life-threatening when absorbed.[2] Most patients may die from lung injury caused by PQ, with a reported mortality rate of 25%-76%.[3] Excessive production of oxygen free radicals as well as lipid peroxidation are contributing factors for PQ-induced acute lung injury as shown by several studies.[4,5] However, effective detoxification therapy for PQ poisoning has not been found yet. A recent report suggested that hydrogen dissolved in liquid can selectively remove toxic oxygen radicals and thus play a role in antioxidant therapy.[6,7] The present study aimed to explore protective effects of saturated hydrogen saline on lung injury of rats after PQ poisoning.

METHODS
Experimental animals and grouping
Thirty healthy male Sprague-Dawley (SD) rats,
weighing 200 to 300 g, were provided by the Experimental Animal Center of Medical College of Nanchang University, Jiangxi, China. The SD rats were randomly divided into three groups: control group, intoxication group and intervention group, with 10 rats in each group.

**Agents and equipments**

The agents and equipments used in this study were as follows: 20% PQ solution (Shanghai Xian Zhen Da Pesticide Company, Shanghai), 2% hydrogen saturated saline (Diving Department of Second Military Medical University, hanghai), alondialdehyde (MDA) assay kits (Nanjing Jiancheng Biological Engineering Research Institute, Nanjing), an 8-hydroxy deoxyguanosine (8-OHdG) ELISA kit (Wuhan China-US Science and Technology Co. Ltd), an Allegra 64R low speed centrifuge (Beckman Company, USA), an ELX-800 microplate reader (PET Company, USA), a BS 400S Electronic Libra (Sarrorius Company, USA), a water bath (Beijing Dongcheng District Medical Machinery Factory, Beijing), a 722 spectrophotometer meter (Shanghai Third Analytical Instrument Factory, Shanghai).

**Animal models and treatment**

The rats in the intoxication and intervention groups received intragastric administration of PQ at a dose of 50 mg/kg and the rats in the control group were fed with an equal volume of saline. After one hour of intoxication, the intervention group accepted an intraperitoneal injection of 5 mL/kg of hydrogen saturated saline two times a day for 3 days. On the third day, arterial blood was took from the abdominal aorta for gas analysis after an intraperitoneal injection of 150 mg/kg of 3% pentobarbital. Then the rats were exsanguinated by the abdominal aorta. The animal lungs were taken out for wet/dry ratio, MDA content, expression of 8-OHdG, and pathological changes.

**Detection of lung wet/dry weight ratio**

The upper lobe of the right lung was taken out for detecting wet/dry weight ratio. Moisture and blood on the tissue surfaces were dried with absorbent filter paper. Then electronic balance was used to weigh the wet tissues immediately and dry weight was measured after the tissues dried in an 80 °C oven for 24 hours. Finally, the wet/dry weight ratio (W/D) of the lung was calculated.

**Detection of 8-OHdG in the lung**

The middle lobe of the right lung was taken and rinsed in cold saline to remove blood. Then it was dried with filter paper, saved in the vial, and placed in a refrigerator set at -80 °C for 8-OHdG detection. ELISA was also performed to detect 8-OHdG.

**Detection of MDA in the lung**

The posterior lobe of the right lung was taken for MDA detection. The tissue of the lobe was mixed with cold saline at a weight/volume ratio (g/mL) of 1:9. Frozen lung tissues were homogenized after centrifugation at 3000 r/min for 15 minutes. The supernatant was dislodged to measure MDA with the thiobarbituric acid (TBA) assay method.

**Histopathological analysis**

The left lung was removed and then transferred to 4% paraformaldehyde solution for 24 hours. The tissues of the lung were paraffin-embedded, sectioned in a butterfly-shape for 5-mm thickness, and placed on slides stained with hematoxylin-eosin (HE) for histopathological analysis.

**Statistical analysis**

Data were analyzed with SPSS11.5 software, and expressed as mean±standard deviation. The data of the groups were compared by one-way analysis of variance (ANOVA), and multiple samples were compared with the SNK-q test. The data on time were measured by repeated ANOVA. The difference was statistically significant when the P value was less than 0.05.

**RESULTS**

**Changes of arterial blood gas**

Arterial partial pressure of oxygen (PaO_2) was decreased more significantly in the intoxication group than in the control group (P<0.01). However it was increased more significantly in the intervention group than in the intoxication group (P<0.01). However, there was no significant difference in arterial PaO_2 between the intervention group and control group (P>0.05) (Table 1).

**Lung W/D changes**

The W/D ratio of lung tissue increased more significantly in the intoxication group and intervention group than in the control group (P<0.01). But the degree of the increase was lesser in the intervention group (P<0.05) (Table 1).

**Changes of MDA content in lung tissue**

The MDA content of lung tissue was increased more
significantly in the intoxication group ($P<0.01$) and intervention group than in the control group ($P<0.05$); whereas it was lower in the intervention group than in the intoxication group ($P<0.05$) (Table 1).

### 8-OhdG content of lung tissue

8-OhdG expression increased more significantly in the intoxication group and intervention group than in the control group ($P<0.01$ and $P<0.05$ respectively); whereas 8-OhdG expression was lower in the intervention group than in the intoxication group ($P<0.01$) (Table 1).

### Pathological changes in lung tissue

Morphological changes in HE stained lung tissue were observed under a light microscope (Figure 1). The control group demonstrated clear structures in lung tissue, thin alveolar wall, widened alveolar septa without congestion, and no inflammation or bleeding of cells. However, hemorrhage, edema, alveolar septal thickening, influx of inflammatory cells, and fibrin deposition were observed in the intoxication group. Similar changes were found in the intervention group but in lesser degree, suggesting an alleviation of lung damage after use of saturated hydrogen saline.

### DISCUSSION

When PQ is absorbed into the body, PQ accumulates in lung tissue mainly via alveolar epithelial cells and bronchioles of the membrane polyamines transport system.\(^8\) Pulmonary toxicity is due to its activated reactive oxygen species (ROS), which could cause oxidative stress to the lung tissue.\(^9\) Hydrogen as a good scavenger of toxicity oxygen free radicals can protect against oxidative stress disease.\(^10\) Compared with other anti-oxidants, it only scavenges strong toxic hydroxy radicals and nitrous acid, without affecting the physiological activity of other free radicals.\(^11\) As the smallest non-polar molecules in nature, hydrogen can rapidly penetrate the membrane and enter cells and organelles such as mitochondria.\(^12\)

Lung injury after PQ poisoning leads to hypoxemia, which is a major cause of death in such patients. The mechanism of hypoxemia may be due to impaired alveolar cells, severe pulmonary congestion and edema, widened alveolar septum, alveolar collapse, and ventilation flow disorder.\(^13,14\) In this study, we found decreased arterial PaO$_2$, severe bleeding and edema of lung tissue, infiltration of numerous inflammatory cells, and widened lung interstitial space. With the intervention of hydrogen saturated saline, arterial PaO$_2$ was improved, and pathological injury of the lung was mitigated more significantly than that in the poisoning group, indicating that hydrogen saturated saline can improve the level of arterial PaO$_2$ and reduce pathological damages in lung tissue.

The wet/dry weight ratio of the lung is a good indicator for pulmonary edema. In this experiment, the wet/dry weight ratio of toxic lung tissue was significantly increased after PQ poisoning, indicating the presence of pulmonary congestion and edema. After the intervention with hydrogen

### Table 1. Changes of arterial PaO$_2$, W/D, MDA, 8-OhdG in the three groups (mean±SD)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>PaO$_2$ (kPa)</th>
<th>Lung W/D</th>
<th>MDA</th>
<th>8-OhdG expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>11.87±0.42</td>
<td>4.34±0.21</td>
<td>2.55±0.26</td>
<td>7.71±1.74</td>
</tr>
<tr>
<td>Intoxication</td>
<td>10</td>
<td>9.34±0.47$^*$</td>
<td>5.24±0.25$^*$</td>
<td>4.48±0.59$^*$</td>
<td>23.48±3.4$^*$</td>
</tr>
<tr>
<td>Intervention</td>
<td>10</td>
<td>10.30±0.27$^*$</td>
<td>4.97±0.26$^*$</td>
<td>3.16±0.52$^*$</td>
<td>9.49±2.17$^*$</td>
</tr>
</tbody>
</table>

Compared with control group, $^*P<0.01$; compared with intoxication group, $^P<0.01$.  

**Figure 1.** Pathological changes in lung tissue (HE×100).
saturated saline, water content of the lung in the intervention was high, but it was significantly lower than that in the poisoning group, indicating that hydrogen saturated saline can reduce pulmonary edema after PQ poisoning.

MDA as the final metabolite of lipid peroxidation, also reflects the degree of lung tissue injured by ROS.[15,16] PQ poisoning is closely related to lipid peroxidation.[17] In the present study, the MDA level of lung tissue in the poisoning group was significantly higher than that in the control group, whereas it was decreased after use of hydrogen saturated saline. This suggested that toxic oxygen radicals were selectively removed by hydrogen saturated saline, thus to some extent palliating the PQ-induced oxidative stress damage and reducing the levels of MDA and other lipid peroxidative products.

8-OhdG is a marker of exogenous and endogenous factors indicating oxidative damage of nuclear DNA or mitochondrial DNA.[18-20] When PQ is absorbed into the body, ROS is produced to attack DNA bases and in turn to form a variety of modified bases such as 8-hydroxyguanine, 8-hydroxyl adenine, cytosine glycol, and thymine glycol. Guanine molecules contain high orbital energy, resulting in accelerating generation of 8-hydroxyguanine and thymine glycol. Guanine molecules contain high orbital energy, resulting in accelerating generation of 8-hydroxyguanine and thymine glycol. Guanine molecules contain high orbital energy, resulting in accelerating generation of 8-hydroxyguanine and thymine glycol. Guanine molecules contain high orbital energy,

In this study the MDA level and 8-OhdG expression in the lung were increased markedly after PQ intoxication, illustrating that PQ induces excessive production of ROS especially OH, that leads to serious oxidative damage to DNA. Moreover MDA level and 8-OhdG expression were significantly lower in the intervention group than in the poisoning group, indicating that hydrogen saturated saline is able to selectively remove toxic oxygen radicals and therefore reduce oxidative DNA damage caused by ROS to some extent.

In conclusion, saturated hydrogen saline could inhibit lung tissue damage induced by oxidative stress and oxygen free radicals, reduce pulmonary edema, mitigate the pathological changes of lung tissue, and improve blood PaO2 after PQ poisoning. This saline is also considered to prevent acute lung damage. But little is known about its adverse effects on the body so far.

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