Effects of propofol pretreatment on lung morphology and heme oxygenase-1 expression in oleic acid-induced acute lung injury in rats

Zelong Tan, Huaizhou Wang, Jing Sun, Mingsheng Li

Abstract

Purpose: To investigate the effects of propofol pretreatment on lung morphology and heme oxygenase-1 expression in oleic acid-induced acute lung injury in rats.

Methods: A total of 32 male Sprague-Dawley rats (250–300g) were randomly divided into the following four groups (n=8/group): group C, group OA, group OA+PR, and group OA+IX to compare related parameter changes.

Results: $\text{PaO}_2$, $\text{PCO}_2$, and $\text{PaO}_2/\text{FiO}_2$ were significantly different among the four treatment groups ($P<0.05$ or $P<0.01$). Lung wet/dry weight ratio and HO-1 protein expression also significantly differed among the groups ($P<0.01$). Immunohistochemistry showed that the expression of HO-1 in group OA+PR was stronger than those in groups OA, OA+IX, and C. Light microscopy revealed that pathological changes in lung tissues in group OA+PR were milder than those in group OA and group OA+IX. Electron microscopy showed that alveolar type II epithelial cell ultrastructure in group OA was relatively irregular with cell degeneration and disintegration and cytoplasmic lamellar bodies were vacuolized. Changes in group OA+PR were milder than those in group OA; however, they were more severe in group OA+IX than in group OA.

Conclusion: Propofol significantly increases the expression of HO-1 in the lung tissue and prevents changes in lung morphology due to ALI in rats.

Key words: Propofol. Heme Oxygenase-1. Acute Lung Injury. Morphology. Rats.
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Introduction

Acute lung injury (ALI) is a critical condition characterized by severe hypoxemia and respiratory distress. Conventional treatment measures do not effectively improve prognosis in such patients. ALI is associated with innate immunity in ischemia-reperfusion injury, traumatic brain injury, and septic shock. Systemic inflammation is the fundamental cause of ALI. Because the prevention of oxidative stress is reported to be beneficial in ALI, the expression of heme oxygenase-1 (HO-1) is also presumed to be associated with ALI.

Propofol is a fat-soluble intravenous anesthetic that exerts good antioxidant effects; its lung-protective roles have also been verified. It can regulate innate immunity and the expression of proinflammatory signals in sepsis. It can also reduce the expression of toll-like receptor 2/4 in sepsis, attenuate the expression of NF-κB in sepsis, and upregulate the expression of HO-1. Although propofol is known to increase the expression of HO-1 in ALI, its effects on morphological changes in the lungs remain unexplored. This study investigated the impact of propofol on the histomorphology of the lung in oleic acid (OA)-induced ALI in rats and explored its possible mechanisms of action, aiming to provide evidence for clinical treatment.

Methods

The study was approved by the ethics committee of Tai’an Central Hospital, Tai’an, Shandong, China.

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol has been reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Tai’an Central Hospital. All procedures were performed in accordance with the Declaration of Helsinki of the World Medical Association.

Sample collection

Arterial blood (0.5ml) was withdrawn from the internal carotid artery, and dissolved gases in the blood were analyzed by a GEMpremierTM3000 blood gas analyzer (US Instrumentation Laboratory Company). After blood withdrawal, all rats were euthanized by cervical dislocation. Further, the left lung of each rat was isolated. The wet (W) and dry (D) weights (followed by 72h drying to a constant weight at 80°C) were noted, and the W/D weight ratio was calculated. The median lobe of the right lung of each rat was cut into a 0.3×0.3×0.5cm portion and fixed in 10% neutral formalin (pH 7.2–7.4) for 24h for immunohistochemical and HE staining. Histopathological findings were reported and double-checked by three different blinded pathologists.

Immunohistochemistry results

Immunohistochemistry was performed...
using a ready-to-use two-step (non-biotin) test kit. Rabbit anti-rat primary antibodies, goat anti-rabbit secondary antibodies, and DAB chromogenic agent were purchased from Beijing ZSGB-Bio Origene Co., Ltd. A Leica DM1000 microscope (LeicaDMCT6.5.0 Switzerland Ltd.) was used to obtain images, which were analyzed for the integral optical density of HO-1 protein using the image-Pro Plus 6.0 optical analysis system. Each tissue section was sampled at five different fields and the average value was calculated.

Nine right lung lobes (1×1×1mm) were also randomly sampled, followed by fixation in 2.5% glutaraldehyde. After rinsing twice with 0.1M PBS (10min/rinse), they were post-fixed with 1% osmic acid for 2h, treated with acetone gradient dehydration, and embedded by epoxy Resin 618. Ultrathin sections were prepared and stained stepwise with uranium dye and lead; the ultrastructure of the alveolar type II epithelial cells (AEC-II) was then observed using a Philips TECNAI10 electron microscope (the Netherlands Philips company).

Statistical analysis

All data analyses were performed using the SPSS13.0 statistical software; data are expressed as the mean±standard deviation. Inter groups differences were analyzed using one-way ANOVA followed by the LSD (when the variance was homogeneous) or Dunnett T3 (when the variance was non-homogeneous) post hoc test, with P<0.05 considered as statistically significant.

Results

\[ \text{PaO}_2, \text{PCO}_2, \text{and PaO}_2/\text{FiO}_2 \]

\[ \text{PaO}_2 \text{ and } \text{PaO}_2/\text{FiO}_2 \text{ in all ALI groups were significantly lower than those in group C (P<0.01); however, PCO}_2 \text{ was significantly higher (P<0.01). PaO}_2 \text{ and } \text{PaO}_2/\text{FiO}_2 \text{ in group OA+PR were significantly different from those in group C (P>0.05). PaO}_2 \text{ and } \text{PaO}_2/\text{FiO}_2 \text{ in group OA+PR were significantly higher than those in group OA (P<0.01); however, PCO}_2 \text{ was significantly lower (P<0.01). PaO}_2 \text{ and } \text{PaO}_2/\text{FiO}_2 \text{ in group OA+IX were significantly lower than those in group OA (P<0.01 or P<0.05) (Table 1).} \]

<table>
<thead>
<tr>
<th>Group</th>
<th>PaO2</th>
<th>PCO2</th>
<th>PaO2/FiO2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>92.15±1.23</td>
<td>39.65±1.77</td>
<td>438.65±5.89</td>
</tr>
<tr>
<td>OA</td>
<td>56.15±3.46</td>
<td>60.62±4.01</td>
<td>266.08±20.56</td>
</tr>
<tr>
<td>OA+PR</td>
<td>91.20±1.21</td>
<td>46.25±1.99</td>
<td>431.02±5.50</td>
</tr>
<tr>
<td>OA+IX</td>
<td>50.48±1.69</td>
<td>58.23±2.23</td>
<td>239.78±8.01</td>
</tr>
</tbody>
</table>

Compared with group C, ★★P<0.01; compared with group OA, ★★P<0.01, ★P<0.05.

Lung W/D weight ratio, lung injury score, and HO-1 protein expression

Lung W/D weight ratio, lung injury score, and HO-1 protein expression in all ALI groups were significantly higher than those in group C (P<0.05 or P<0.01). Lung W/D weight ratio and lung injury score in group OA+PR were significantly lower than those in group OA (P<0.05 or P<0.01); however, HO-1 protein expression was significantly higher (P<0.01). Group OA+IX exhibited significantly increased lung W/D weight ratio and lung injury score (P<0.05) but significantly decreased HO-1 protein expression (P<0.01) (Table 2).
Table 2 - Comparison of W/D, lung injury score and IOD of HO-1 protein expression among different groups (\(\bar{x} \pm s, n=8\)).

<table>
<thead>
<tr>
<th>Group</th>
<th>W/D</th>
<th>Lung injury score</th>
<th>HO-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>3.66±0.05</td>
<td>0.86±0.43</td>
<td>3.55±0.14</td>
</tr>
<tr>
<td>OA</td>
<td>5.79±0.20▲▲</td>
<td>2.65±0.55▲</td>
<td>4.77±0.04▲▲</td>
</tr>
<tr>
<td>OA+ PR</td>
<td>4.76±0.15▲▲ ▲▲</td>
<td>1.53±0.51▲★</td>
<td>5.15±0.06▲▲ ★★</td>
</tr>
<tr>
<td>OA+ IX</td>
<td>5.96±0.16▲▲ ★★</td>
<td>2.84±0.53▲★</td>
<td>4.28±0.12▲▲ ★★</td>
</tr>
</tbody>
</table>

Compared with group C, ▲▲ \(P<0.01\), ▲ \(P<0.05\); compared with group OA, ★★ \(P<0.01\), ★ \(P<0.05\).

Light microscopy of the lung tissue

The gross specimen images are shown in Figure 1. A clear lung structure was seen in group C; the alveolar septal capillaries were mildly expanded and congested, and the partial alveolar septa and bronchial stroma exhibited a small degree of lymphocyte infiltration. The lung tissue in group OA was damaged; the alveolar septal capillaries were markedly expanded and congested, scattered small focal bleeding was observed, and coagulated lung necrotic foci of different sizes were present.

The structure of the lung tissue in group OA+PR was still clear; the alveolar septal capillaries were expanded and congested with only mild edema in the alveolar cavity. The structure of the lung tissue in group OA+IX was damaged; the alveolar septal capillaries were markedly expanded and congested, the alveolar cavity was partially filled with edema fluid, extensive lymphocyte infiltration (with few neutrophils) was observed in the partial alveolar septum, and coagulated lung necrotic foci of different sizes were observed (Figure 1).

![Figure 1 - A: Clear lung structure can be seen, the alveolar septal capillaries mildly expand and congest, and partial alveolar septum and bronchial stroma exhibit a small amount of lymphocyte infiltration; B: The structure of lung tissue is unclear, the alveolar septal capillaries expand and congest, together with scattered small focal bleeding and coagulated lung necrotic foci with different sizes; C: The structure of lung tissue is still clear, the alveolar septal capillaries expand and congest, and a small amount of edema fluid can be seen in the alveolar cavity; D: The structure of lung tissue is unclear, the alveolar septal capillaries exhibit high-degree expansion and congestion, partial alveolar cavity is filled with edema fluid, partial alveolar septum have the infiltration of a large number of lymphocytes and a small amount of neutrophils, together with coagulated lung necrotic foci with different sizes (HE staining x200).](image)

HO-1 protein expression in the lung tissue

HO-1 protein in group OA+PR was strongly expressed in the bronchial cells, AECs, and alveolar macrophages; however, it was weakly expressed in groups OA, OA+IX, and C (Figure 2).
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**Figure 2** - The expression of HO-1 protein: OA+PR group was strong positive; OA group and OA+IX group, the expression was positive with OA+IX group was weaker; the expression in C group was weakly positive.

*AEC-II ultrastructure*

Electron microscopy revealed regular AEC-II shape in group C; the cell surface was covered with microvilli of different lengths and thicknesses, the nuclei were conspicuous, and the cytoplasm contained various lamellar bodies in different numbers, sizes, and maturation stages. Group OA exhibited relatively irregular AEC-II shape, cell degeneration, and disintegration; the microvilli on the cell surface were reduced in number, and the cytoplasmic lamellar bodies were emptied (partially into the alveolar cavity). Group OA+PR exhibited relatively regular AEC-II shape, the microvilli were reduced in number, the nuclei were conspicuous, and the cytoplasm contained various lamellar bodies in different numbers and sizes. Group OA+IX exhibited irregular AEC-II, cell degeneration, and disintegration; no microvilli were seen on the cell surface, and the cytoplasmic lamellar bodies were emptied and vacuolized (Figure 3).

**Figure 3** - A: Regular AEC-II shape in group C, the cell surface is covered with microvilli with different lengths and thickness, the nuclei are obvious, and the cytoplasm contains various lamellar bodies with different numbers and sizes, different maturation stages of which can also be seen; B: Group OA exhibits relatively irregular AEC-II shape, cell degeneration, or even disintegration; the microvilli on the cell surface are reduced, the cytoplasmic lamellar bodies are emptied (partially into alveolar cavity); C: Group OA+PR exhibits relatively regular AEC-II shape, the microvilli are reduced, the nuclei are obvious, the cytoplasm contains various lamellar bodies with different numbers and sizes; D: Group OA+IX exhibits irregular AEC-II, cell degeneration, or even disintegration, no microvillus can be seen on the cell surface, and the cytoplasmic lamellar bodies are emptied and vacuolized. Ultrastructure of AEC-II (red arrow: microvilli, blue arrow: lamellar bodies).

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**Discussion**

Results of this study show that OA injection induced ALI in rats in a dose- and time-dependent manner. OA-induced ALI presents an early phase of necrosis and microvascular thrombosis, followed by a repair phase with the proliferation of type II cells.
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and fibrotic foci in subpleural areas. The analysis of arterial blood gas 
PaO₂ in group OA showed a progressive decline in respiratory capacity and significant cyanosis with the 
PaO₂/FiO₂ < 300mmHg. The lung W/D weight ratio increased, and pathological damage was evident in the lung tissue, which also indicated alveolar space telangiectasia with scattered small focal bleeding without coagulation necrosis, alveolar collapse, and atrophy. These changes indicate ALI.

In this study, we evaluated the impact of propofol on OA-induced ALI in rats. Consistent with results of previous studies, results of this study show that propofol can significantly reduce OA-induced ALI. We also found that HO-1 was strongly expressed in the propofol-pretreated lung tissue, which can significantly improve PaO₂, significantly decrease lung W/D weight ratio, lung injury, and apoptosis of AT-II in OA rats. Zinc porphyrin IX also exhibited this effect, suggesting that HO-1 is involved in the anti-apoptotic action of propofol. Propofol pretreatment significantly reduced edema of the lung tissue.

Previous studies have shown that propofol can inhibit inflammatory response by inhibiting reactive oxygen species-regulated Akt/IKKβ/NF-κB signaling and reduce endotoxin-induced ALI. Because the etiology of ALI is complex, the protective effect of propofol on OA-induced ALI needs to be further investigated. HO-1 plays a key role in ALI. HO-1-based treatment strategies in ALI may be more meaningful, and drug discovery in this direction will increase our treatment options. Therefore, we aimed to identify the process by which propofol upregulates HO-1 expression to relieve OA-induced lung morphological changes in ALI.

ALI is a dynamic pathophysiological process that injures the lung tissue. Although currently used treatment measures to control or eliminate causes of ALI, including pulmonary protective ventilation, the rational application of antibiotics, liquid intake control, and continuous blood purification, exist, the prognosis of ALI/acute respiratory distress syndrome (ALI/ARDS) remains a challenge. Therefore, there is a need to further investigate the occurrence and development mechanisms of ALI/ARDS and possible effective interventions. Based on previous studies, we presumed that propofol has a protective effect on OA-induced ALI. This is the first study performed to compare the effects of propofol and zinc porphyrin IX pretreatment on ALI in terms of HO-1 expression and lung histomorphology. We found that PaO₂ and PaO₂/FiO₂ in group OA+PR were significantly higher than those in group OA (P<0.01); however, lung W/D weight ratio and lung injury score were significantly lower (P<0.01 and P<0.05, respectively). The expression of HO-1 protein was significantly higher in group OA+PR than in group OA (P<0.01), indicating that propofol induces the expression of HO-1. Zinc porphyrin IX (specific inhibitor of HO) was found to reduce HIF-1α expression and inhibit hypoxia-mediated VEGF release and cell proliferation in group OA+IX. PaO₂ and PaO₂/FiO₂ were significantly lower in group OA+IX than in group OA (P<0.05), whereas the lung W/D weight ratio and lung injury score were significantly higher than in group OA (P<0.01 and P<0.05, respectively). The expression of HO-1 was significantly lower in group OA+IX than in group OA (P<0.01), which showed that HO-1 expression was inhibited by zinc porphyrin IX. Consequently, the high expression of HO-1 in group OA+PR was related to propofol, which is consistent with related reports.

This effect was confirmed by lung histomorphology analysis by light microscopy and AEC-II ultrastructure...
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analysis by electron microscopy. The results of this study have high clinical importance in the diagnosis and treatment of ALI patients; however, because the results are mainly based on animal experiments, further human trials are necessary.

■ Conclusion

The pretreatment with propofol promotes the expression of HO-1 in the lung tissue and significantly prevents changes in lung morphology due to ALI.

■ References

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