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Effect of captopril on serum TNF-α level in acute lung injury rats induced by HCL

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ABSTRACT

Objective: To observe the effect of captopril on the tumor necrosis factor-α (TNF-α) level and arterial blood gases in acute lung injury (ALI) induced by HCL in rats, and to analyze its protective mechanism. **Methods:** Fifty Wistar rats were selected and randomly divided into three groups, with 20 rats in Group Ⅰ and Ⅱ, respectively and 10 animals in Group Ⅲ. ALI model was constructed by intratracheal injection of diluted hydrochloric acid (pH=1.25, 1.2 mL/kg). Group I rats received not any treatment after construction of ALI model. Group Ⅱ rats were treated with captopril (5 mg/kg, *i.p.*) 5 min after induction of ALI. Group Ⅲ served as normal control without any treatment. Ninety minutes after construction of ALI model, all the rats were sacrificed. Blood was withdrawn for detection of TNF-α level and arterial blood gases index. And lung tissue slices of the three groups were prepared for observation of pathologic histology changes. **Results:** TNF-α level in serum of Group Ⅰ and Ⅱ rats was significantly higher than that in Group Ⅲ ($P<0.05$), while TNF-α level in serum of Group Ⅱ was significantly lower in Group I ($P<0.05$). $PaCO_2$ level was significantly higher ($P<0.05$), while PaO_2 was significantly lower ($P<0.05$) in Group I and Ⅱ rats than those in Group Ⅲ. $PaCO_2$ was significantly lower ($P<0.05$) and PaO_2 was significantly higher ($P<0.05$) in Group Ⅱ than those in Group I. Histological observation showed diffuse congestion and severe edema of lung tissue, obvious thickening and structure damage of alveolar walls and a large amount of neutrophil infiltration in Group I rats. Group Ⅱ rats showed mild edema of lung tissue; only a small portion of alveolar walls showed thickening and only a few of neutrophil infiltration could be observed. The degree of injury was remarkably slighter than that of Group I rats. Group Ⅲ rats showed clear lung tissue structure and normal morphology; alveolar walls were uniform and the margin was smooth and few neutrophil could be observed. **Conclusions:** Captopril can significantly reduce serum TNF-α level, elevate PaO_2 and reduce $PaCO_2$ in rats with ALI. It has a protective effect on ALI rats.

1. Introduction

Acute lung injury (ALI) caused by aspiration of acidic gastric content is a common complication in perioperative period and anesthesia. Aspiration in anesthesia is also an important reason that leads to acute respiratory distress syndrome (ARDS)[1-3]. According to statistics[4], the incidence rate of aspiration in the process of anesthesia can reach to 1/10 000-7/100 000. Therefore, effective prevention and treatment of ALI resulted from reflux and aspiration is an important problem to be solved during perioperative period and anesthesia. At present, the main dispute on the clinical treatment of aspiration is whether to preventively apply antibiotics and corticosteroids or not[5-7]. However, some studies have confirmed that[8-11], the prophylactic use of antibiotics and high doses of corticosteroids for short-term initially has no obvious effect for the treatment of aspiration. On the contrary, it can increase the deposition of collagen, influencing the repair of alveolar epithelial cells.

In recent years, with the constant and deeper clinical research on rennin-angiotesin system (RAS), it was found that angiotensin Ⅱ can effectively stimulate DNA synthesis

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in lung fibroblasts; promote the proliferation of fibroblasts, resulting in the lung tissue fibrosis[12]. Captopril is an angiotensin converting enzyme inhibitor which can reduce the permeability of blood vessels, remove a variety of free radicals, thus reducing tissue edema and inflammatory exudation. It has remarkable effect on anti-inflammatory and anti fibrosis[13]. Based on this theory, the authors assume that captopril has certain protective effect against ALI induced by aspiration of gastric acid. In the present study, ALI rat model was constructed by aspiration of hydrochloric acid so as to simulate the ALI caused by gastric acid. And captopril was applied to observe its protective role in ALI rats.

2. Materials and methods

2.1. Animals

Fifty healthy adult Wistar rats of clean level, weighing 200-250 g [average weight of (225±12) g], regardless of male and female, were selected and purchased from laboratory animal center of Southern Medical University. The rats had free access to water, and were fed at class Ⅱ level. The management of the animal during the experiment was strictly followed according to the relevant provisions in Regulations for the Administration of Affairs Concerning Ex-Perimental Animals.

2.2. Reagents and apparatus

Captopril was purchased from Guangzhou Bai Yun Shan Pharmaceutical General Factory (Guangzhou, China). Tumor necrosis factor alpha (TNF-α) kits were procured from Boster Biological Engineering Co., Ltd. (Wuhan, China). 25% Urethane were provided by Beijing Zhongshan biotechnology company (Beijing, China). Rapidpoint 405-blood gas analyzer and high-speed centrifuge SG-850 (Siemens, German), Optical microscope (BH-2) (Olympus, German), IDA-2000 digital microscopic image analysis system (Beijing Kong Hai Science and Technology Development Co., Ltd., Beijing, China) were used in the present study.

2.3. Modeling

ALI model was constructed by instillation of distilled hydrochloric acid into the trachea[14]. Animals were anaesthetized by intraperitoneal injection of 25% urethane (2.0 mL/kg). Skin preparation was done at the throat of rats. After regular disinfection, the throat was incised longitudinally with the rat head at higher position. The skin and subcutaneous tissue were separated layer by layer, until the trachea was exposed. A 1-mL syringe was inserted into the trachea, through which hydrochloric acid (pH=1.25, 1.2 mL/kg) was dripped at a constant speed. Modeling was considered successful if the rats showed fast shallow breathing, acute coughing, acropodium and lip empurpling. And then the incision was closed.

2.4. Grouping of animals and treatments

Fifty Wistar rats were randomly divided into three groups, with 20 rats in Group Ⅰ and Ⅱ, respectively and 10 animals in Group Ⅲ. Group Ⅰ rats received not any treatment after construction of ALI model. Group Ⅱ rats were treated with captopril (5 mg/kg, *i.p.*) 5 min after induction of ALI. Group Ⅲ served as normal control without any treatment.

2.5. Observation

Ninety minutes after modeling, venous blood was withdrawn from the tails of all the rats. Serum TNF-α level was determined by strictly following the instructions of the kit. And arterial blood of the rats was withdrawn from the neck for determination of blood gas indexes, including pH value, PaO_2, $PaCO_2$. And then all the rats were sacrificed. Biopsy was prepared from posterior lobe of the right lung tissue for observation of histopathology changes in each group. The detailed preparation of biopsy was as follows. The lung tissue was cut into size of 3 mm ×3 mm, fixed in 10% formalin for 24 h, conventionally dehydrated with gradient ethanol. The specimens were soaked in xylene until they are transparent. Then the specimens were embedded in paraffin, cut into 4 μm section, dewaxed by dimethyl benzene, stained with Harris's hematoxylin for 10 min, separated by 0.5% hydrochloric acid and alcohol, washed by water for 1 h, dehydrated with gradient ethanol, and then dyed in 0.5% saturated alcohol eosin solution for 1 min. The slices were then mounted in alcohol, neutral gum for microscopic observation.

2.6. Statistical analysis

The obtained data were analyzed using SPSS13.0 statistical software. And results were expressed as mean±SD. One-way ANOVA was used for comparison between groups. $P<0.05$ was regarded as statistically different.

3. Results

3.1. TNF-α levels and blood gas indexes in different groups

TNF-α level in serum of Group Ⅰ and Ⅱ rats was significantly higher than that in Group Ⅲ ($P<0.05$), while TNF-α level in serum of Group Ⅱ was significantly lower in Group Ⅰ ($P<0.05$). $PaCO_2$ level was significantly higher ($P<0.05$), while PaO_2 was significantly lower ($P<0.05$) in Group Ⅰ and Ⅱ rats than those in Group Ⅲ. $PaCO_2$ was significantly lower ($P<0.05$) and PaO_2 was significantly higher ($P<0.05$) in Group Ⅱ than those in Group Ⅰ (Table 1, Figures 1 and 2).

3.2. Histopathology observation

Histological observation showed that lung tissue of Group Ⅲ rats showed clear lung tissue structure and normal morphology; alveolar walls was uniform and the margin was

smooth and few neutrophil could be observed. While Group Ⅰ rats showed diffuse congestion and severe edema of lung tissue, obvious thickening and structure damage of alveolar walls and a large amount of neutrophil infiltration. Group Ⅱ rats showed mild edema of lung tissue; only a small portion of alveolar walls showed thickening and only a few of neutrophil infiltration could be observed. The degree of injury was remarkably slighter than that of Group Ⅰ rats (Figure 3).

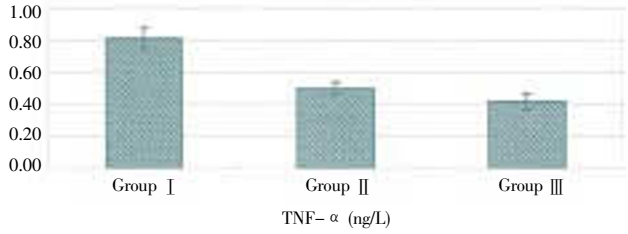

Figure 1. TNF-α levels in the three groups.

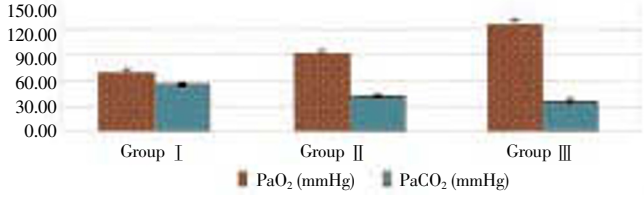

Figure 2. blood gas indexes in the three groups.

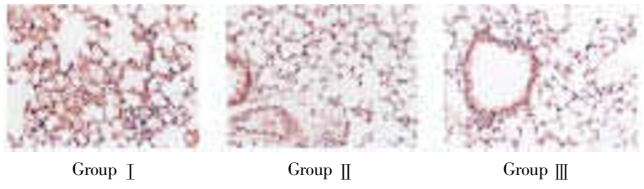

Figure 3. Histopathology observation (×200).

4. Discussion

ALI is the damage of alveolar epithelial cells and capillary endothelial cells caused by a variety of pathogenic factors. Acute pulmonary edema is resulted from accumulation of a large number of protein liquid, leading to serious acute respiratory failure[15]. The first stage of ALI is the early exudation stage with the main manifestations of alveolar injury, necrosis of type I alveolar epithelial cells, pulmonary edema after capillaries injury, aggregation of inflammatory cells and the release of inflammatory mediators. The second stage is the fibrogenesis stage and the main manifestations are proliferation of type Ⅱ alveolar epithelial cells and fibroblasts, and the repair and remodeling of lung tissue[16-19]. Aspiration of vomitory reflux contents is often encountered in the process of anesthesia, and aspiration in the process of anesthesia is also an important cause of ALI and ARDS[20-22]. The severity of lung injury caused by aspiration depends largely on the amount of acid inhalation. Gastric acid aspiration can firstly cause chemical damage of airway and alveoli, followed by more serious secondary inflammatory reaction. The release of TNF-α and IL-8 mediated by acid results in the accumulation of a large number of neutrophils, causeing extensive damage of lung[23]. Therefore, effective prevention and control of ALI resulted from reflux and aspiration is of great significance to secure the safety of anesthesia and perioperative period. In the present study, ALI model was constructed by tracheal drip of diluted hydrochloric acid. The alveolar walls of Group Ⅰ rats was obviously thickening, the lung tissue was diffuse hyperemia and edema, and numerous neutrophils were infiltrating; arterial blood gas analysis showed reduction of PaO_2 and increase of $PaCO_2$, suggesting the successful construction of ALI model using this method.

ALI is a common clinical severe acute disease with lung inflammation by the interaction of a variety of inflammatory cells as one of the pathological features, eventually leading to damage to the pulmonary capillaries. At the onset of ALI, due to the spread of the uncontrolled systemic inflammatory response, a large number of inflammatory cytokines are released, causing outbreak of inflammation[24]. Studies have confirmed that[25], the more serious of ALI, the higher the IL-1β, TNF-α and IL-6 content in the serum, suggesting that accumulation of neutrophils in the lung play an important role in the progression. Hence, inhibiting inflammatory response of the body plays a key role in the treatment and prognosis of ALI. Captopril is an angiotensin-converting enzyme inhibitor. It has some effects such as reducing degradation of bradykinin and inhibiting synthesis of angiotensin Ⅱ. It can also reduce the hypertension of pulmonary arterial, prevent pathological refactoring of cardiovascular. Captopril can obviously screen oxygen free radicals, inhibit lipid peroxidation, promote the recovery of pulmonary vascular endothelial dysfunction, reduce vascular permeability, alleviate tissue edema and inflammatory exudation, and has certain anti-inflammatory and anti-fibrosis effects[26]. Based on the above theory, the author consider that the captopril has a therapeutic effect on ALI. A study of Xiang *et al* shows that[17], TNF-α is one of the most important cell pro-inflammatory factor that causes ALI. It can directly damage endothelial cells and increase their permeability. TNF-α can activate the release of protease and oxidation substances; inhibit the active substance on the alveolar surface, leading to the reduction

Table 1
Comparison of TNF-α content and blood gas indexes among the three groups.

Group	*n*	TNF-α (ng/L)	pH	PaO_2 (mmHg)	PaO_2 (mmHg)
Ⅰ	20	0.82±0.06*	7.21±0.04	68.38±4.06*	54.74±2.68*
Ⅱ	20	0.51±0.03*#	7.33±0.04	91.30±3.67*#	41.26±2.43*#
Ⅲ	20	0.42±0.04	7.36±0.03	124.20±8.58	35.05±2.94

*P<0.05 compared with Group Ⅲ, #P<0.05 compared with Group Ⅰ.

of pulmonary compliance and pulmonary edema. In the process of ALI TNF-α content can reflect the severity of the injury of lung tissue. In this study, the serum TNF-α levels in Group Ⅰ and Ⅱ rats were significantly higher than that in group Ⅲ 90 min after modeling ($P<0.05$), suggesting that TNF-α participated in the progress of ALI, and its content in serum was positively correlated with the severity of ALI, thus, the more serious the disease, the higher the content of TNF alpha. Results also showed that after treated with captopril the serum TNF-α content in Group Ⅱ rats was significantly lower than that in Group Ⅰ; and histological observation showed that the degree of lung injury in Group Ⅱ rats was obviously lighter than that in Group Ⅰ rats, indicating that the severity of ALI can be reflected by the serum TNF-α content in rats, which is in consistency with the previous study[19]. In this study, after treated with captopril, $PaCO_2$ of Group Ⅱ rats was significantly lower ($P<0.05$) and PaO_2 is significantly higher ($P<0.05$) than those in Group Ⅰ respectively, suggesting that after treatment with captopril, lung function of ALI rats was obviously improved and captopril certain treatment effect on aspiration ALI.

It can be concluded that captopril can significantly reduce serum TNF-α level, elevate PaO_2, reduce $PaCO_2$ in rats with ALI, and effectively alleviate the severity of ALI. Captopril can be used as a potential therapy for ALI related inflammation.

Conflict of interest statement

We declare that we have no conflict of interest.

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