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Protective effect of Astragaloside IV against sepsis-induced acute lung injury in rats

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KEYWORDS

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Cecal ligation and puncture;
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Abstract The study aimed to explore the protective effects of AS-IV against sepsis-induced ALI. Sepsis was induced by cecal ligation and puncture (CLP) method in Sprague Dawley rats. Rats were randomly assigned into five groups: animals undergoing a sham CLP (sham group); animals undergoing CLP (CLP group); animals undergoing CLP and treated with AS-IV at 2.5 mg/kg bw (low-dose AS-IV [L-AS] group), at 5 mg/kg bw (mid-dose AS-IV [M-AS] group), and at 10 mg/kg bw (high-dose AS-IV [H-AS] group). At 6 h, 12 h and 24 h post-CLP surgery, six rats were respectively sacrificed to collect blood and lung tissue samples. The levels of arterial blood gas index, lung water content, protein level and leukocyte counts (total amount, neutrophils and lymphocytes) in bronchoalveolar lavage fluid (BALF) and cytokines such as TNF-α and IL-6 in BALF were measured at each time point in different groups. HE-staining and optical microscopy were performed to examine the pathological changes in lungs. The 72 h-survival rate of each group was also recorded. PaO_2 was decreased significantly, while the lung water content, BALF protein level, cell numbers, BALF cytokine TNF-α and IL-6 levels were increased significantly for CLP group as compared with sham group. Moreover, pathological injury was observed in lung tissue indicating the successful sepsis-induced ALI model. Speaking of the effect of AS-IV, we founded that, compared with the CLP group, the AS-IV treatment groups could significantly alleviate all the above negative changes exited in the CLP group in a dose-dependent manner. What's more, the pathological injury was also gradually improved by AS-IV treatment compared with the CLP rats. AS-IV exerts its protective effect against sepsis-induced ALI in rats via improving pulmonary ventilation function, decreasing the permeability of alveolar epithelium and capillary as well as repressing lung inflammation.

1. Introduction

Sepsis, a common complication of burn, trauma, hypoxia, and post-surgery, is a systemic inflammatory response syndrome caused by infection (Tjardes and Neugebauer, 2002; Lever and Mackenzie, 2007). With relatively high morbidity mortality, sepsis is considered to be the leading cause of death of patient in the intensive care unit (ICU) (Tjardes and Neugebauer, 2002; Levy et al., 2001; Angus and van der

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Poll, 2013). Multiple organ function impairment may occur at severe sepsis and ultimately develop to multiple organ dysfunction syndrome (Marshould, 2001). Lung is one of the most vulnerable organs at sepsis with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS) which occurs at early stage and is with high morbidity (Hudson et al., 1995; Husak et al., 2010; Angus et al., 2001). The severe pulmonary inflammation, vascular permeability, diffuses infiltration in both lungs, and pulmonary alveoli edema, hypoxemia and lung compliance decrease are the characteristics for ALI (Villar et al., 2011). Both intra pulmonary factors and extrinsic pulmonary factors make contribution to the pathogenesis of ALI/ARDS. The intra factors include aspiration pneumonia, severe diffuse lung infection, pulmonary contusion, and extrinsic factors include sepsis caused by extrinsic pulmonary infection, wound shock and burn (Brun-Buisson et al., 2004; Gattinoni et al., 1998; Sheu et al., 2010). Among these, sepsis is the most common cause of ALI (Suntharalingam et al., 2001; Rocco and Zin, 2005). Currently, there are still no effective drugs and therapies for the treatment of sepsis-associated ALI/ARDS. The main method is supportive treatment such as mechanical ventilation for respiration support. However, increasing evidence has demonstrated that mechanical ventilation brings damage to organs when improving the oxygenation for the patients. Mechanical tension caused by excessive mechanical ventilation, is an important cause for lung injury (Rocco and Zin, 2005; Martin et al., 2003) and could often cause inflammation in lungs. Therefore, development for new method of curing ALI caused by sepsis other than mechanical ventilation is imperative.

Astragaloside IV (AS-IV) is a saponin separated from astragalus with wide biological and pharmacological activity. Pharmacological experiments showed that AS-IV has protective effects, such as anti-inflammation (Zhang et al., 2003; Gui et al., 2013), antioxidant (Gui et al., 2012) anti-brain infarction, neuro-protection (Luo et al., 2004; Cheng et al., 2006), anti-hypertension (Zhang et al., 2006), and myocardial protection (Li and Cao, 2002). However, there are only a few researches about the effect of AS-IV on lung injury. Xiong et al. (2010) found AS-IV is protective for rat pulmonary ischemia–reperfusion lung injuries. Chen et al. (2016) thought AS-IV exerted the protective effect for paraquat induced rat lung injuries by restraining Rho/ROCK/NF-κB signal pathway. This study aimed to seek the effect of AS-IV on ALI through further research on the sepsis ALI rat model.

2. Materials and methods

2.1. Reagent and animals

Astragaloside IV was purchased from Shrqbio Co. Ltd. (Shanghai, China, purity ⩾98%). BCA Protein Assay Kit, rat TNF-α and IL-6 ELISA kits were purchased from Wuhan Boster Bio-Engineering Co. Ltd. (Wuhan, China).

Healthy male Sprague Dawley rats (200 ± 20 g) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China). All animals were raised at SPF laboratory animal room with a 12 h light/dark cycle at 24 ± 2 °C and 40–70% humidity. Animals were allowed to have free access to water and food during experiment period. All animal experiment operations were conducted according to nursing and use guidance for animal experiment operation of National Institutes of Health.

2.2. Animal groups

After a period of adaptation, ninety SD rats were selected and divided into five random groups (n = 20 per group): sham group, CLP control group, low-dose AS-IV (L-AS) group, middle-dose AS-IV (M-AS) group, and high-dose AS-IV (H-AS) group. The AS-IV was diluted into three different concentrations with 1% carboxymethyl cellulose (CMC). The L-AS, M-AS and H-AS groups were administrated with AS-IV by gavage at the dosages of 2.5 mg/kg bw, 5 mg/kg bw and 10 mg/kg bw, respectively, while the sham group and sepsis model group were gavaged with 1% CMC at the same volume. The indicators determined in this study were observed at three time points (6 h, 12 h and 24 h post-CLP), and each time six rats were used. Another fifty SD rats were distributed into the same five groups as above for the observation of survival rate.

2.3. Acute lung injury molding

Sepsis was introduced by CLP technique as described previously (Ritter et al., 2003; Sener et al., 2005). Briefly, after a 12-h deprivation of food but not water, the rats were generally anesthetized with chloral hydrate anesthesia (0.3 ml/100 g bw), and then a midline abdominal incision was made to expose the cecum. After a cecal ligation treatment, an 18-gauge needle was used to puncture through the central segment of ligation, and a small amount of cecal contents was squeezed out through the puncture wound. Then, the cecum was restored into the abdominal cavity and the surgical incision was sutured layer by layer. In sham group rats, the cecum was exposed and the bowel was massaged as described above, but it was not ligated or punctured.

2.4. Aortic blood-gas analyses

Six animals were sacrificed 6 h, 12 h and 24 h after CLP, respectively, by intraperitoneal injection of chloral hydrate (0.3 ml/100 g bw) for general anesthesia. Blood samples of 2 ml were obtained from aorta abdominals for arterial blood gas analyses by automatic blood gas analyzer (America Nova Company, Nova-K type).

2.5. Dry/wet ratio of lung

Right upper pulmonary lobe was excised, blotted dry and weighed, and then placed in an oven at 70 °C for 48 h to obtain the dry weight. The ratio of the wet lung to the dry lung was calculated to assess tissue edema.

2.6. Histopathology observation

Right lower lobe was taken from the animals which had been executed after 6 h, 12 h and 24 h of CLP respectively. After being washed wash with saline solution, the organ was fixed by formalin to make paraffin section. The pathological