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Original

Expression of Hsp70 in Acute Lung Injury Induced by Lipopolysaccharide

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Abstract: We examined the expression of HSP70 in acute lung injury induced by lipopolysaccharide (LPS), in order to explore the mechanism of HSP70 in acute lung injury. Using an acute lung injury model in Wistar rats, we observed the in vivo HSP70 expression by immunohistochemistry and Western blot. After exposure to LPS, the level of HSP70 increased above the control level at 1 hour, reached the maximum level at 2 hours, and returned to the control level by 6 hours. Our study suggested that the expression of HSP70 is involved in the protection of acute lung injury induced by LPS.

Key words: lipopolysaccharide, acute lung injury, HSP70, rat

Introduction

The heat shock proteins (HSP) are a family of stress proteins that are highly conserved evolutionally. These proteins play an pivotal role in the protection against stressed factor stimulated by many stressors such as high temperature, ischemia, hypoxia-reperfusion, infection, injury and drug, through increasing the expression of the HSP genes. Using immunohistochemical method and Western blot technique, we detected the expression of HSP70 in the lung in an animal model of acute lung injury induced by lipopolysaccharide (LPS), and explored its correlation with acute lung injury to provide a theory for the prevention and cure of acute lung injury

Materials and Methods

Animals and LPS administration

The experiments were conducted on twenty-four Wistar rats weighing 200 to 250 g obtained from the animal center of China Medical University. Animals were randomized into experimental groups and control groups. Both were divided into 4 groups of 1, 2, 4 and 6 hours after induction of acute lung injury. In the experimental groups, LPS was injected intravenously via the internal jugular vein at a dose of 5 mg/kg body weight. In the control groups, the vehicle physiological saline was injected in the same manner as in the experimental groups. At the designated time after LPS injection, rats were anesthetized with 30% trichlormethane and killed. The left lungs were fixed in 10% formaldehyde and then embedded in paraffin. Serial sections 5 μm in thickness were prepared for immunohistochemistry. The right lungs were removed and frozen at -70°C until Western blot analysis.

Lipopolysaccharide was purchased from Sigma Corporation (USA). Rabbit polyclonal antibodies to HSP70 and SABC immunohistochemical kits were obtained from Wuhan Boster Biological Technology Ltd. (China). 3,3'-diaminobenzidine was purchased from Zhongshan Golden Bridge Biotechnology Co, Ltd, (China).

Immunohistochemical staining

Sections were dewaxed and endogenous peroxidase was quenched with 3% hydrogen peroxide in methanol for 15 minutes at room temperature, followed by heat treatment in 10 mM citrate buffer for antigen unmasking. Normal goat serum was used to block nonspecific antibody binding. Sections were then incubated with primary antibody diluted in the blocking serum overnight at 4°C. After thorough washing in PBS, the sections were incubated with the secondary antibody for 20 minutes at room temperature followed by incubation with the SABC complex for 20 minutes at room temperature. Sections were then incubated with diaminobenzidine (DAB) until the brown color developed, rinsed in distilled water, counterstained with Harris's hematoxylin, rinsed in water, dehydrated and mounted. As controls for the staining procedure, sections were incubated with PBS instead of the primary antibody. No nonspecific staining was observed in all the sections used as negative controls for the staining procedure.

Western blot analysis

Rat lung tissue was lysed in a RIPA buffer (0.15 M Nacl, 0.05

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M Tris-HCl, pH 7.2, 1% Triton X-100, 1% sodium deoxycholate, 0.2% SDS) in the presence of a protease inhibitor cocktail (leupeptin, pepstatin and aprotinin) for 1 hour at 4°C. After ultracentrifugation at 4°C (12,000 ×g), supernatants were diluted, boiled for 3 min, and loaded on an SDS/PAGE gel (15%; 20 µg per lane). The transferred membranes were incubated with an affinity purified polyclonal antibody against HSP70 overnight at 4°C. Then the membranes were incubated with secondary antibody for 2 hours at room temperature followed by coloration by AEC. The HSP 70 on the gel was analyzed by the Image Analysis Software of Motic Advanced 3.1.

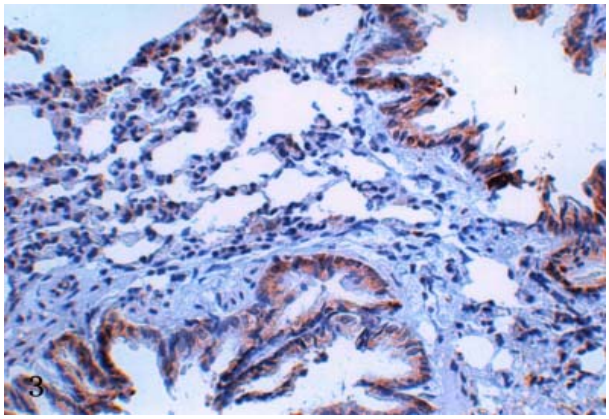

Fig. 3: Expression of HSP70 in the lung 4 h after LPS injection, ×200.

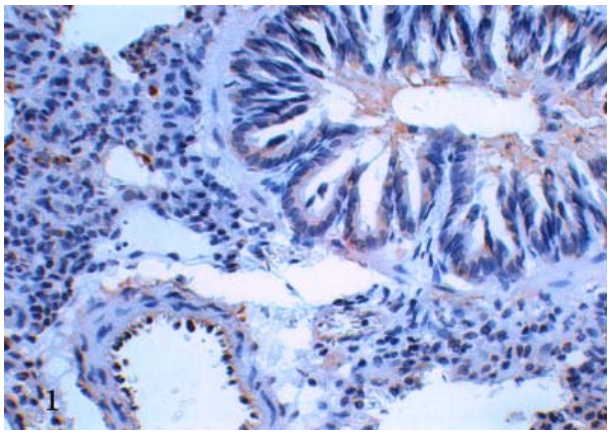

Fig. 1: Expression of HSP70 in the lung 1 h after LPS injection, ×200.

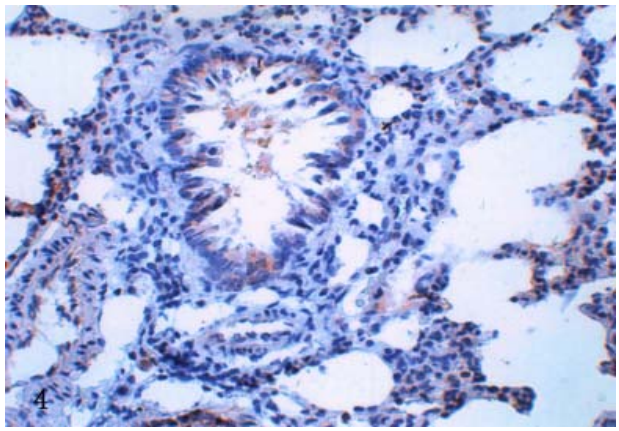

Fig. 4: Expression of HSP70 in the lung 6 h after LPS injection, ×200.

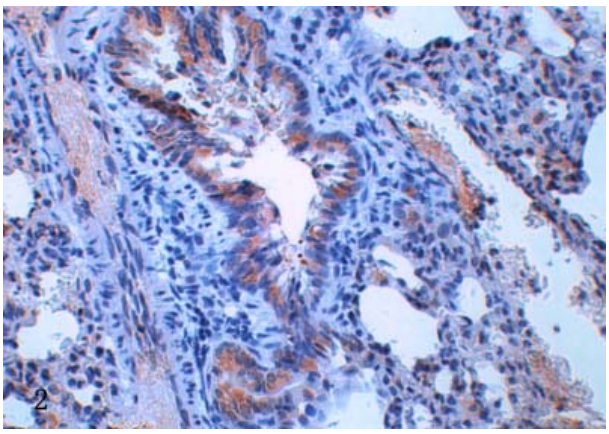

Fig. 2: Expression of HSP70 in the lung 2 h after LPS injection, ×200.

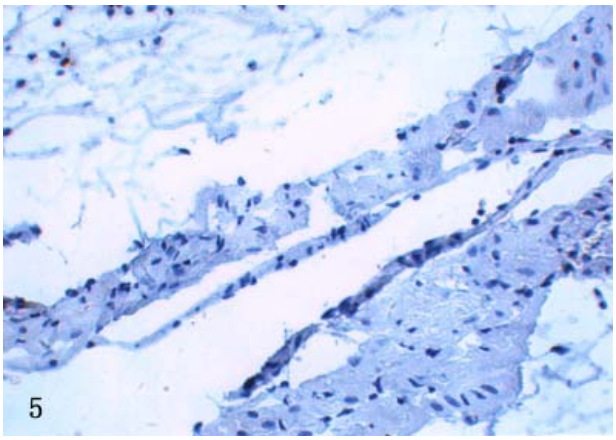

Fig. 5: Expression of HSP70 in the lung in the control group, ×200.

Statistics Analysis

The data of gray scale of HSP70 protein are presented as mean ± SE Statistical comparisons were performed by one-way ANOVA followed by Student's t test using the software of SPSS 10.0 for windows. $P<0.01$ was considered statistically significant.

Results

Results of immunohistochemical staining of HSP70

HSP 70 was expressed in tracheal epithelium. Alveolar epithelial cells and macrophages were weakly stained. The positive substance was brown and granular, and distributed in cytoplasm. At 1 h after LPS injection, the HSP70 immunostaining in tracheal epithelium and alveolar epithelial cell was stronger compared to

The expression of HSP70 protein in lung tissue treated by LPS at different times (Western blot).

the control group (Fig.1, Fig.5). At 2 h and 4 h after LPS injection, the staining of positive cells was markedly more intense than in the control groups (Fig.2, 3). The staining intensity reached a peak at 2 h. There was no significant difference between the experimental and control
groups at 6 h after injury (Fig.4).

Results of Western blot analysis

The expression of HSP70 protein in lung tissue was significantly higher in the experimental groups at 2 h and 4 h after the LPS injection compared to the control groups (Fig.6, Table 1, $P<0.01$). No significant differences were observed at other time points.

Table 1: The expression of HSP 70 in LPS-induced acute lung injury in rats

Time after treatment(hour)	Control group	Experimental group
1	210.5±6.09	191.50±13.23
2	214.67±7.20	183.50±5.68*
4	206.33±9.87	192.33±5.24*
6	193.83±5.08	195.00±3.74

* compared with control group $P<0.01$

Data are the gray scale of HSP70 bands on Western blots. *: group $P<0.01$ compared with control

Discussion

The lung is a target organ often affected by infectious diseases, and pyemia leads to acute lung injury and then develops into adult respiratory distress syndrome and multi-organ distress syndrome. The pathological characters of these diseases are the aggregation of neutrophils in the lung, intravascular coagulation, augmented pulmonary capillary permeability, followed by lung edema and hypoxemia. Many studies have shown that blood components such as plasma protein and neutrophils reach the alveolar space through demolished alveolar epithelial cells and vascular epithelial cells, and that increases in protein level, ratio of neutrophils in the alveolar washing, and even the wet to dry weight ratio of the lung can be induced by lipopolysaccharide. The pathological changes induced by LPS are consistent with acute lung injury[1-2]. In addition, LPS activates and enhances the biological effects of biological factors, and aggravates lung injury and the accompanying clinical symptoms.

Heat shock proteins are a family of stress protein and an endogenous protective protein. The heat shock protein family consists of six groups; HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs[3-6]. HSP70 is the most conserved and important member with a wide distribution, and is expressed under the condition of stress-related damages[4]. The potential protective mechanisms of HSP70 lie in four aspects. First, its antiinflammatory function decreases the inflammatory mediators and increases the expression of antiinflammatory cytokines such as IL-10. Second, HSP70 stimulates the production of antioxidant and increases the level of liberated iron and cGMP through exhausting the oxidized hemopexin. Third, its ability to regulate the repair and synthesis of protein prevents the synthesis of other proteins and plays a role as molecular chaperons. Finally, HSP70 is an important apoptotic inhibitor. HSP70 reacts with the signal transduction pathway of c-JunN-terminal kinase and inhibits its function to mediate apoptosis[7]. Furthermore HSP70 inhibits apoptosis through regulating and protecting the cytoskeleton. In a study in which rats were given heat pretreatment before intravenous LPS injection to induce HSP expression, rats given LPS after heat pretreatment had significantly decreased lung leakage index and myeloperoxidase (MPO) level 5 hours after the injection compared with the group without heat pretreatment, while the expression of HSP72 was increased[8]. This study showed that induction of HSP protects lung tissue and attenuates mortality in rats. Animals given glutamine exhibited marked reduction of end-organ damage such as heart, lung and colon, and significant reduction of preinflammatory molecules from peripheral blood mononuclear cells. These data suggest that glutamine may protect against mortality and attenuate end-organ injury in endotoxemic shock via enhanced HSP72 expression[9]. Another study showed that HSP70 dramatically increased the pulmonary arterial oxygen content and predominately decreased the lung wet to dry weight ratio and MPO level in HSP70-transfected graft lung compared with the control group[10].

We observed the expression of HSP70 in bronchiolar epithelial cells and pulmonary epithelial cells in LPS-induced acute lung injury animal models, using immunohistochemical and Western blot techniques. Our data showed that the level of HSP70 was increased in the experimental groups and suggested that HSP70 may participate in the protection of organs after acute lung injury.

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