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Effect of dexamethasone on the content of pulmonary surfactant protein D in young rats with acute lung injury induced by lipopolysaccharide

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Abstract: **Objective** Pulmonary surfactant protein-D (SP-D) is regarded as a valuable biomarker in acute lung injury (ALI) and acute respiratory distress syndrome (ARDS). This study was to explore the changes of SP-D content in lung tissue following ALI and the effect of dexamethasone (Dex) on the SP-D content in young rats. **Methods** One hundred and forty-four 21-day-old Sprague-Dawley rats were randomly assigned into control, ALI and Dex-treated groups. ALI was induced by intraperitoneal injection of lipopolysaccharide (LPS) (4 mg/kg) in the rats from the ALI and Dex-treated groups. Normal saline was given for the control group. Dex (5 mg/kg) was administered 1 hr after LPS injection in the Dex-treated group. At each time interval of 6, 12, 24, 36, 48 and 72 hrs after LPS injection, eight rats of each group were randomly chosen and sacrificed. Western blot was employed to detect the content of SP-D in lung tissues. **Results** The pulmonary SP-D content decreased significantly at 36, 48 and 72 hrs after LPS administration in the ALI group, and reduced to a nadir (0.92 ± 0.11 vs 3.27 ± 0.52) at 48 hrs compared with that of the control group ($P < 0.01$). The SP-D content in the Dex-treated group increased significantly at 36, 48 and 72 hrs after LPS administration when compared with the ALI group ($P < 0.01$). A significant difference in the SP-D content between the Dex-treated and the control group was noted only at 72 hrs after LPS administration ($P < 0.05$). **Conclusions** The SP-D content in lung tissue was reduced following ALI in young rats at the early stage. Early administration of Dex can significantly increase the pulmonary SP-D content.
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Key words: Lipopolysaccharide; Acute lung injury; Pulmonary surfactant protein D; Dexamethasone; Rats

地塞米松对脂多糖诱导的急性肺损伤幼鼠肺表面活性物质蛋白-D 的影响

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[**摘　要**] **目的**　肺表面活性物质蛋白 D(SP-D)被认为是急性肺损伤(ALI)和急性呼吸窘迫综合征(ARDS)有价值的生物指标。该研究旨在探讨幼鼠 ALI 时及地塞米松干预后肺组织 SP-D 的变化。**方法**　144 只 SD 大鼠被随机分为正常对照组、肺损伤组和地塞米松治疗组。腹腔内注射脂多糖(LPS,4 mg/kg)建立急性肺损伤模型,正常对照组注射等量生理盐水, 治疗组于注射 LPS 1 小时后注射地塞米松(5 mg/kg)。LPS 注射后 6,12,24,36,48,72 h 每组各处死 8 只大鼠。用 Western blot 方法测定肺组织 SP-D 的相对含量。**结果**　与正常对照组相比,ALI 组在注射 LPS 后 36,48,72 h SP-D 含量明显下降($P < 0.01$),在 48 hrs 达最低点(0.92 ± 0.11 vs 3.27 ± 0.52)。地塞米松治疗组于注射 LPS 后 36,48,72 h SP-D 含量明显高于 ALI 组 ($P < 0.01$), 6,12,24,36 和 48 h 与对照组相比差异无显著性, 72 h 差异有显著性($P < 0.05$)。**结论**　急性肺损伤早期幼鼠肺组织 SP-D 含量降低。早期应用地塞米松能使 ALI 肺组织下降的 SP-D 明显上升。
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[**关　键　词**]　脂多糖; 急性肺损伤; 肺表面活性蛋白 D; 地塞米松; 大鼠

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Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are common potential respiratory diseases in children. They result in a case fatality rate of 40%-70% [1-3]. ALI is the early stage of ARDS [1,4]. Pulmonary surfactant protein D (SP-D) is synthesized in alveolar type II (AT II) cells and is secreted into alveoli and conducting airways. It not only significantly contributes to surfactant homeostasis and pulmonary immunity[5,6], but is also involved in a range of immune functions including viral neutralization, clearance of bacteria, fungi and apoptotic and necrotic cells, down-regulation of allergic reaction and resolution of inflammation. SP-D is regarded as a valuable biomarker in ALI [7]. SP-D levels increased in bronchiolar alveolar lavage fluid (BALF) in response to intratracheal lipopolysaccharide(LPS)[8]. The increased levels of plasma SP-D in the early course of ALI/ARDS were associated with a greater risk of death[9]. Wang et al [10] reported that early use of dexamethasone (Dex) can increase SP-D levels in the tracheal fluid in premature infants with RDS. So far, the alterations of SP-D levels in lung tissue of rats with LPS-induced ALI following Dex treatment have not been reported in China. The purpose of this study was to explore the changes of SP-D in lung tissue in the early stage of ALI and the effects of Dex on lung SP-D levels.

Materials and methods

Materials

LPS was isolated from *E. coli* (O_{55}: B_5, Sigma Chemical Co.). Goat anti-rat SP-D polyclonal antibody and donkey anti-goat antibody were provided by Santa Cruz, Biotechnology, Inc.. Nitrocellulose membranes were provided by Millipore Corporation. 3 MM filter papers were obtained from Whatman, Clifton, NJ.

Subjects and ALI model

One hundred and forty-four pathogen-free 21-day-old Sprague-Dawley rats weighing 44-61 g, provided by the Animal Department of China Medical University with the permission of ethic committee, were randomly assigned into control, ALI and Dex-treated groups. Rats in the ALI group were intraperitoneally injected with 4 mg/kg LPS[7] in order to induce ALI, while the control rats were injected with normal saline instead. Dex was administered (5 mg/kg) 1 hour after LPS injection in the Dex-treated group. All rats were anesthetized with 10% chloral hydrate (4 mL/kg) and 8 rats were randomly chosen and sacrificed by incising the abdominal aorta at each time interval of 6, 12, 24, 36, 48 and 72 hours after LPS injection. Diarrhea, cyanosis and dyspnea occurred in the ALI group but the control rats had no such symptoms. The situation in the Dex-treated group was better than that in the ALI group. Dyspnea was not as serious as the ALI group at 36, 48 and 72 hours. The body weights of the survived rats in the ALI group were reduced compared with those of the control and the Dex-treated groups.

Left lung homogenate

The left lung was placed into a tube and stored in liquid nitrogen. While analysis, the tissues were placed into a homogenizing buffer (50 mM Tris · HCl, pH 7.5, containing 1 mM EDTA, 2 mM phenylmethylsulfonyl fluoride, and 2.5 mM N-ethylmaleimide) at a defined ratio of 1 g of lung tissue to 9 mL of homogenizing buffer. The lung tissues were homogenized on ice with a Polytron (Brinkman Instruments, Westbury, NY). The lung homogenate was sonicated on ice (20 s, 5 times), and spun at 300 g for 5 minutes to sediment tissue debris.

Total protein assay

Protein concentrations in lung homogenate were measured by Lowry's method in order to adjust the samples' concentrations identically before electrophoresis.

Western blot analysis

Western blot was employed for detecting the SP-D content in lung tissue [11,12]. The 20 μL of samples from each group were separated by 8% SDS-polyacrylamide gel electrophoresis (120 voltage, 1.5 hrs). Gels were blotted onto nitrocellulose membranes between two sheets of 3 MM filter paper, under constant 50 voltage 2 hours, with stirring at 4°C for 3 hours. The membranes were blocked for 2 hours, and then washed three times for 10 minutes. They were incubated in goat anti-rat SP-D 1∶500 for 1 hour, washed three times and then detected with donkey anti-goat serum 1∶2 000 for 1 hour. Finally they were scanned and photographied. Pictures of SP-D were *t* quantified by using the FlourChem Digital Imaging System V 2.0 (Alpha Innotech Corporation). The statistical data derived from integrated density values (IDVs) were computed with SPSS 11.5 software and were denoted in one in millionth.

Statistical analysis

Data are presented as $\bar{x} \pm s$. Statistical analysis was performed with ANOVA by SPSS 11.5 software. Statistical differences between groups were analyzed by unpaired *t* test. Statistical significance was accepted at the $P < 0.05$ level (two-tailed).

Results

SP-D levels in lung tissue in LPS-induced ALI

The SP-D levels in the ALI group had no significant differences at 6, 12 and 24 hours after LPS injection, and decreased significantly at 36, 48 and 72 hours compared with the control group. The lowest SP-D level was found at 48 hours in the ALI group (Figure 1 and Table 1).

Alterations of SP-D levels after Dex administration

SP-D levels in the Dex-treated group at 36, 48 and 72 hours increased significantly although there were no significant differences within 24 hours after LPS infection compared with the ALI group. The Dex-treated group had similar SP-D levels to the control group at 6, 12, 24, 36 and 48 hours after LPS injection. A significant difference in the SP-D levels between the ALI and the control group was noted only at 72 hours after LPS administration ($P<0.05$). See Figure 1 and Table 1.

Table 1 SP-D levels of lung tissue ($n=8$)

Group	6 h	12 h	24 h	36 h	48 h	72 h
Control	3.30 ± 0.44	3.64 ± 0.78	3.28 ± 0.50	3.54 ± 0.83	3.27 ± 0.52	3.52 ± 0.71
ALI	3.69 ± 0.64	3.68 ± 0.73	2.90 ± 0.83	1.69 ± 0.69[a]	0.92 ± 0.11[a]	1.20 ± 0.66[a]
Dex	3.65 ± 0.80	3.50 ± 0.76	3.48 ± 0.73	3.67 ± 0.84[b]	3.24 ± 0.69[b]	2.59 ± 0.80[b,c]

a Compared with the control group, $P<0.01$; b Compared with the ALI group, $P<0.01$; c Compared with the control group, $P<0.05$.

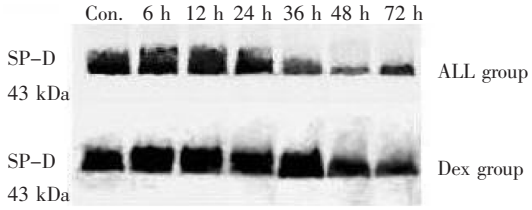

Figure 1 SP-D levels of lung tissue

Discussion

SP-D, the first line defense against pathogens, binds to LPS isolated from a variety of gram-negative bacteria[9, 13]. It is identified as major *E. coli* binding protein [11]. LPS, the core domain of *E. coli*, has been identified as a major ligand for SP-D of rats or humans [13, 14]. SP-D also binds to macrophages and neutrophils and promotes phagocytosis. It is chemotactic for alveolar macrophages, neutrophils, and monocytes and acts as a rapid scavenger molecule for clearance of potentially proinflammatory bacterial components such as LPS. It also acts as a soluble opsonin promoting rapid removal of pathogens and other noxious agents from the airways. The mice with SP-D deficiency showed an exaggerated inflammatory response after infectious challenge [15].

There were no significant alterations of SP-D levels within 24 hours after LPS injection in ALI rats compared with the control group. That meant the synthesis and storage of SP-D in AT II cells could meet the consumption during the first stage of interaction between SP-D and LPS as well as other factors. So this may be termed as the compensated stage.

SP-D synthesis is greatly dominated by the expression of SP-D mRNA. The down-regulation of SP-D mRNA led to insufficiency of SP-D production due to des-compensations[12]. With the development of ALI, the interactions of SP-D against LPS together with chemotaxis, phagocytosis, oxidative-burst and killing by neutrophils caused the great over-consumption of SP-D [12]. The SP-D levels dropped to the nadir at the late stage of ALI (48 hours after LPS injection). The necrosis and apoptosis of AT II cells also accelerated the reduction of SP-D. Thereby the des-compensated stage of ALI occurred.

Dex plays an important role in the elevation of SP-D levels in RDS [10, 16]. This study showed that Dex treatment increased significantly the SP-D levels at 36, 48 and 72 hrs after LPS injection. Dex exerts a variety of important anti-inflammatory actions. The balance between inflammatory and anti-inflammatory mediators was recovered by the application of Dex due to the inhibition to IL-1, IL-6, IL-8, TNF-α and granulocyte macrophage colony stimulating factor (GM-CSF) which caused lung tissue injury. The stability of lysosome was enhanced by the administration of Dex so that the permeability of capillaries was reduced. This attenuated the degradation of AT II cells caused by lysosomal enzymes and cytokines and the diffusion disorder caused by the pulmonary interstitial edema and hyaline mem-

brane formation. It was discovered that the content of Fas/FasL apoptosis-related genes was increased in BALF in the acute stage of ALI. The expression of TNF-α, IL-1β, IL-6 and iNOS mRNA was also elevated. It was also proved by the animal experiment that the expression of Fas/FASL was earlier than apoptosis of AT II cells in the recovery stage of ALI [17, 18]. Wen [19] found that the epithelial apoptosis induced by Fas antibody and INF-γ could be inhibited by Dex. The apoptosis of AT II cells was also suppressed by Dex [20]. The synthesis and secretion of SP-D by AT II cells was accelerated by the application of Dex [21]. Dex plays important roles in the anti-inflammation, stabilization to the cell membrane and promotion for the synthesis and secretion of SP-D.

This study suggested that SP-D levels decreased in lung tissue in the early stage of ALI. Early administration of Dex can significantly increase SP-D levels in the lung tissue of rats with ALI.

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