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Effects of dexamethasone on the ultrastructure of alveolar type II cells in young rats with lipopolysaccharide-induced acute lung injury

SHU Lin-Hua, WEI Ke-Lun, XUE Xin-Dong, HAN Xiao-Hua, SHANG Yun-Xiao, CAI Xu-Xu, LIU Chun-Feng, LI Jiu-Jun, WANG Li-Jie

Department of Pediatrics, Shenjing Hospital, China Medical University, Shenyang 110004, *China*

Abstract: Objective Alveolar type II (AT II) cells play a crucial role in the maintenance of pulmonary surfactant homeostasis and pulmonary immunity. The effects of dexamethasone (Dex) on the ultrastructure of AT II cells after acute lung injury remain unknown. This study focused on the ultrastructural changes caused by acute lung injury and on the effects of Dex administration on these ultrastructural changes in young rats. **Methods** Seventy-two 21-day-old Sprague-Dawley rats were randomly divided into control, acute lung injury and Dex-treated groups. Rats in the lung injury group were intraperitoneally injected with 4 mg/kg lipopolysaccharide (LPS) in order to induce acute lung injury, while the control rats were injected with the same amount of normal saline (NS). The Dex-treated group was injected first with LPS followed 1hr later by Dex (5 mg/kg) injection. Eight rats in each group were sacrificed 24, 48 and 72 hrs after LPS or NS injection. Lung samples were obtained from the lower parts of left lungs and fixed with 2.5% glutaraldehyde for transmission electron microscope examination. **Results** Microvilli of AT II cells disappeared and the number of lamellar bodies (LBs) increased in the lung injury group 24 hrs after LPS injection. The ring-like arrangement of LBs around nuclei was present until 48 hrs after LPS injection. By 48 hrs after LPS injection, giant LBs with vacuole-like abnormalities appeared. The shape of nuclei became irregular and the border of the nuclei became blurred. By 72 hrs after LPS injection, the number of LBs was obviously reduced; nucleoli disappeared; and karyolysis occurred in some of the nuclei. In contrast, in the Dex-treated group, LBs crowded on one side of AT II cells and exocytosis appeared on the same side by 24 hrs after LPS injection. By 48 hrs, the number of LBs was reduced. The number of mitochondria increased, and some of them became swollen and enlarged. However, by 72 hrs, the number of LBs increased and the ring-like arrangement of LBs around the nucleus again appeared. **Conclusions** Ultrastructural changes of AT II cells following lung injury induced by LPS were time-dependent in young rats. Dex may ameliorate AT II cell injury and promote functional restoration of AT II cells in LPS-induced acute lung injury. [**Chin J Contemp Pediatr, 2007, 9 (6): 521 – 525**]

Key words: Lipopolysaccharide; Acute lung injury; Alveolar type II cell; Lamellar bodies; Dexamethasone; Ultrastructure; Rats

地塞米松对脂多糖诱导的急性肺损伤幼鼠肺泡Ⅱ型上皮细胞超微结构的影响

舒林华，魏克伦，薛辛东，韩晓华，尚云晓，蔡栩栩，刘春峰，李玖军，王丽杰　中国医科大学附属盛京医院儿科，110004

[**摘　要**] **目的**　肺泡Ⅱ型上皮细胞对维持肺表面活性物质的动态平衡和肺免疫功能具有极其重要的意义。地塞米松对急性肺损伤肺泡Ⅱ型上皮细胞超微结构的影响目前仍不清楚。该研究探讨急性肺损伤时及应用地塞米松干预后肺泡Ⅱ型上皮细胞超微结构的动态变化。**方法**　72 只 21 d SD 幼鼠随机分为对照组、急性肺损伤组和地塞米松治疗组。肺损伤组的幼鼠腹腔注射脂多糖(LPS)(4 mg/kg)以建立急性肺损伤模型。对照组注射等量生理盐水。地塞米松治疗组在注射 LPS 1 h 后腹腔注射地塞米松(5 mg/kg)。每组随机选取 8 只幼鼠分别于注射 LPS 或生理盐水后 24、48、72 h 处死。取左肺下 1 mm^3 的肺组织固定于 2.5% 戊二醛中待透射电镜检查。**结果**　注射 LPS 24 h 后，肺损伤组大鼠 AT II 细胞微绒毛消失，板层小体数量增加。24 及 48 h 板层小体呈指环状绕核排列。48 h 出现巨大空泡变性样的板层小体。细胞核形态不规则，部分核边界不清。72 h 板层小体数目明显减少，核仁从细胞核消失，一些细胞核出现核溶解。注射 LPS 后 24 h 地塞米松治疗组板层小体聚集在 AT II 细胞内同侧，并于该侧发生“胞吐”现象。48 h 线粒体肿胀、聚集，脊断裂，板层小体数目减少。72 h 板层小体数目增多，并重新呈指环状绕核排列。**结论**　LPS 诱导的急性肺损伤肺泡Ⅱ型上皮细胞超微结构的一系列变化呈时间依赖性。

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[Biography] SHU Lin-Hua, Male, MD., Prelector, Specializing in acute lung injury (Email: shulinhua@126.com).

地塞米松能减轻急性肺损伤中肺泡Ⅱ型上皮细胞损伤和促进肺泡Ⅱ型上皮细胞的功能恢复。

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Acute lung injury and acute respiratory distress syndrome (ARDS) are common potential diseases in children, with a case fatality rate of 62.9%[1]. It is regarded that the nature of acute lung injury/ARDS comes from the extensive damage of pulmonary endothelial cells and pulmonary epithelial cells due to over inflammatory reactions. Acute lung injury is the early stage of ARDS[2,3]. Pulmonary surfactant which plays an important role in reducing alveolar surface tension and keeping gas-fluid balance in alveoli is synthesized and secreted by AT II cells. AT II cells contribute significantly to surfactant homeostasis and pulmonary immunity. The functions of AT II cells are dominated by the integrity of AT II cells' structures.

Early alterations of AT II cells including lamellar bodies (LBs) in response to endotoxin were studied by Fehrenbach[4] in 1998. The comparison of ultrastructural alterations of AT II cells between neonatal and adult rats with acute lung injury induced by LPS were reported in 2005[5]. The temporal ultrastructural changes of AT II cells in young rats with acute lung injury induced by LPS were presented by Shu[6] in 2007. However, little information is available regarding the alterations of AT II cells in acute lung injury after dexamethason (Dex) treatment. Dex not only inhibits the apoptosis of pulmonary epithelium induced by Fas antibody and INF-γ[7], but also increases the content of pulmonary surfactant protein D (SP-D)[8]. This study focused on the ultrastructural changes of AT II cells caused by acute lung injury and on the effects of Dex administration on these ultrastructural changes in young rats.

Materials and methods

Materials

LPS was isolated from *E. coli* (O_{55}: B_5, Sigma Chemical Co.). 2% Osmic acid (O_sO_4) was provided by England; Araldite (Epon812), by Japan; LKB ultromicrotome, by Sweden; transmission electron microscope (JEM 100CX-Ⅱ), by Japan.

Subjects and acute lung injury model

Seventy-two pathogen-free 21-day-old Sprague-Dawley rats, provided by the Experimental Animal Department of China Medical University with the permission of the ethics committee, were randomly divided into control, acute lung injury and Dex-treated groups. Rats in the acute lung injury group were intraperitoneally injected with 4 mg/kg LPS[9] in order to induce acute lung injury, while the control rats were injected with the same amount of normal saline (NS). The Dex-treated group was injected first with LPS followed 1 hour later by Dex (5 mg/kg) injection[8,10,11,12]. Eight rats in each group were sacrificed 24, 48 and 72 hours after LPS or NS injection.

Sample preparation

Lung samples (1 mm^3 in size) were obtained from the lower part of left lungs and fixed twice with 2.5% glutaraldehyde. The pellets were rinsed repeatedly in PBS. Before being embedded in araldite, the samples were post-fixed with 2% Osmic acid in PBS for 1 hour and then dehydrated through a graded series of alchol. Ultrathin sections were prepared by ultramicrotome and stained with uranylacetate and lead citrate. Ultrastructural changes of AT II cells were observed under the JEM 100CX-II transmission electron microscope.

Results

Ultrastructure of AT II cells in the control group

Nucleus of AT II cell and its border were clear and the chromatins in the nucleus were homogeneous. LBs in the cytoplasm were presented with round shape and normal density. The cell membrane was continuous and integrity. The microvilli on the surface of cells were clearly seen (Figure 1). in one AT II cell. The ring-like arrangement of LBs

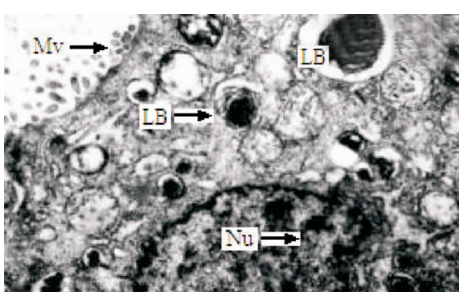

Figure 1 Ultrastructure of AT II cells in the normal lung (×10 000). Microvilli were clearly seen. Round and normal density LBs were well distributed in the cytoplasm. Nuclear membrane was clear and integrity. Chromatin in the nucleus was homogenous. LB: lamellar body. Nu: nucleus. Mv: microvilli.

Ultrastructural changes of AT II cells in the acute lung injury group

The microvilli on the surface of AT II cells disappeared in the early stage of acute lung injury. A series of pathological changes of LBs in the cytoplasm occurred after acute lung injury. By 24 hours after LPS injection, LBs increased in number and in size, but decreased in electronic density. There were two nuclei around nuclei was present until 48 hours after LPS injection (Figure 2A). By 48 hours after LPS injection, the electronic density of LBs was progressively reduced and emptying of LBs was enhanced. LBs obviously showed vacuole-like abnormalities. Giant LBs as one of typical manifestations of acute lung injury [4] were present. The inclusions in the LBs were nearly emptied (Figure 3A). By 72 hours after LPS injection, the number of LBs was obviously reduced. The remnants of scattered and ruptured LBs were seen in the cytoplasm (Figure 4A).

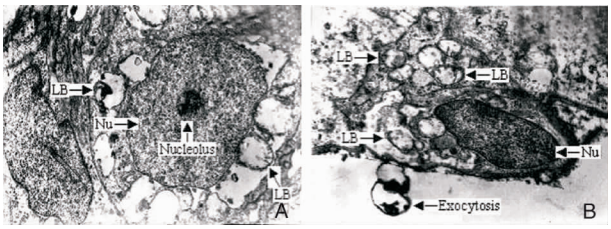

Figure 2 Comparison of the ultrastructure of AT II cells between the acute lung injury (A) and the Dex-treated groups (B) at 24 hrs of LPS injection (×7 200). **A**: The ring-like arrangement of LBs around the nucleus appeared. The border of the nucleus was integrity. The chromatins were homogeneously disseminated in the nucleus. Clear nucleolus was seen. **B**: LBs crowed on one side of the cell and exocytosis was presented at the same side. The nucleus was shrunk into ellipse shape. Inclusions in the nucleus were condensed homogenously.

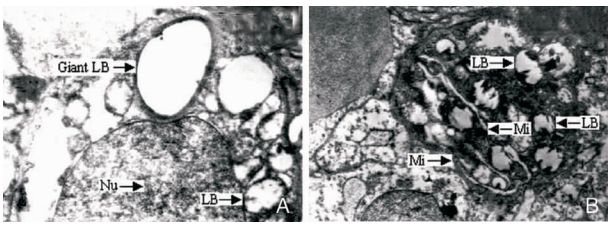

Figure 3 Comparison of the ultrastructure of AT II cells between the acute lung injury (A) and the Dex-treated groups (B) at 48 hrs of LPS injection (× 10 000). **A**: Giant LBs with vacuole-like deformity appeared. **B**: Mitochondria increased and crowded together. Some of them became swollen and enlarged. Mi: mitochondia.

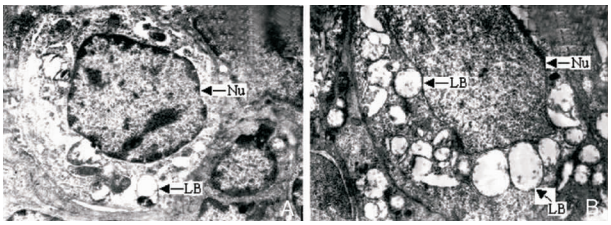

Figure 4 Comparison of the ultrastructure of AT II cells between the acute lung injury (A) and the Dex-treated groups (B) at 72 hrs of LPS injection (×7 200). **A**: The nucleus became smaller and irregular. The chromatin in the nucleus was misty. The number of LBs was significantly reduced. The residues of LBs in the cytoplasm were scattered around the shrunk nucleus. **B**: The nucleus became enlarged. The ring-like arrangement of LBs was presented with increased number of LBs. The density of LBs enhanced.

The nuclei of AT II cells showed progressive changes after LPS injection. At 24 hours after LPS injection they were round and full. The chromatins were concentrated into homogeneous granules and disseminated in the nucleus. Nucleoli can be noted in each nucleus (Figure 2A). By 48 hours after LPS injection, the shape of nuclei became irregular and the border of the nuclei became blurred. Two nucleoli were displayed in a nucleus. The chromatins were no longer homogeneous and clear and became concentrated and condensed. The nucleus became smaller. By 72 hours after LPS injection, the nucleus was shrunk and depressed. The border of the nucleus was stiff and the border of the nuclei was not complete or clear. The volume of nucleus became smallest. Nucleoli disappeared and karyolysis occurred in some of the nuclei. The chromatins were unclear or chromatinolysis appeared (Figure 4A).

Ultrastructural changes of AT II cells in the Dex-treated group

The ultrastructural changes of AT II cells in the Dex-treated were different from those in the untreated acute lung injury group. At 24 hours of LPS injection (23 hours of Dex administration), the secretion of AT II cells accelerated and LBs were moved to one side of the cell for exocytosis[13, 14]. The nucleus was shrunk into ellipse shape. Inclusions in the nucleus were condensed homogenously (Figure 2B). By 48 hours after LPS injection, the number of LBs was reduced and the density of LBs was attenuated. LBs showed different sizes and irregular shapes. Mitochondria increased and crowded together. Some of them became swollen and enlarged. Mitochondria cristae were broken. The shape of nucleus was irregular and the border of the nucleus was blurred (Figure 3B). However, by 72 hours after LPS injection, the number of LBs increased and the ring-like arrangement of LBs around the nucleus again appeared. The volume of nueleus was enlarged and the karyoplasms were homogenous and clear (Figure 4B).

Discussion

AT II cells which synthesize and secrete pulmonary surfactants are the most important component of pulmonary epithelium. The normal structures of AT II cells and the homeostasis of pulmonary surfactant secretion and metabolism are necessary for pulmonary physiological activities[15].

Tesfaigzi and his colleagues[16] reported that the apoptosis of AT II cells induced by LPS increased greatly in the early stage of acute lung injury. The induction of AT II cells apoptosis by LPS does not require TNF-α [17]. Injured AT I cell caused by LPS cannot regenerate by themselves. The recovery of AT I cells depends on the transformation from AT II cells. AT II cell injury induced by LPS is the key trigger for the development and recovery of acute lung injury.

The microvilli of AT II cells disappeared 24 hours after LPS injection [4]. The compensation of AT II cells was triggered by LPS in order to meet the consumption and metabolism of pulmonary surfactants. The number of LBs increased, presenting with ring-like arrangement around the nucleus of AT II cells. LBs increased in size, but decreased in electronic density compared with normal controls. Accelerated karyokinesis showed activated proliferation of AT II cells. The decompensation of AT II cells occurred at 48 hours after LPS injection when the characteristic alterations of LBs occurred. The number of LBs was significantly reduced compared with that at 24 hours. LBs were in different sizes. The appearance of giant LBs[4] with vacuole-like abnormalities and the lowest electronic density which was considered as one of the prominent features in acute lung injury indicated an extreme over-compensation of AT II cells. Pulmonary surfactant protein-A secreted from LBs was significantly exhausted so that the density of LBs was attenuated. The ring-like arrangement of LBs around the nucleus which was considered as the second main feature in acute lung injury showed that self-regulation and functional restoration of AT II cells when an imbalance between inflammation and anti-inflammation appeared. The energy for this process was probably provided by mitochondria. These changes of LBs were paralleled with the lowest level of SP-D in acute lung injury [9]. It could be deduced that many organelles were involved in the inflammatory and anti-inflammatory reactions against LPS and that they were motivated to the over-maximum exertion. It might be resulted from apoptosis[16, 17] and toxication of AT II cells caused by LPS and the exhaustion of pulmonary surfactants. All these pathophysiological and micromorphological changes of AT II cells led to serious clinical manifestations even death in acute lung injury. By 72 hours after LPS injection, LBs rupture appeared due to the over-dilation of LBs and over-consumption of pulmonary surfactant proteins. The ring-like arrangement of LBs around the nucleus vanished. The number of LBs decreased greatly and the remnants of LBs were scattered in the cytoplasm.

It was discovered that Dex can inhibit apoptosis of pulmonary epithelium induced by Fas-antibody and IFN-γ[7]. Dex can inhibit the combination between antigen and antibody as well as the interaction between inflammatory mediators and cytokines, and interfere with alexin activation induced by LPS. Dex also can stabilize the membranes of cells and lysosome so that the epithelial tissue can be protected. In this research exocytosis from AT II cells proved that the synthesis and secretion of pulmonary surfactants were activated and accelerated 24 hours after Dex use. A great amount of energy was supplied by mitochondria for pulmonary surfactant synthesis and LBs arrangement so that severe mitochondria damage occurred at 48 hours of Dex use. The over-compensation of mitochondria led to swelling and rupture of mitochondria. LBs were rearrayed like a ring around the nucleus with the energy from mitochondria 72 hours after Dex use. These showed that Dex ameliorated pulmonary injury and promoted pulmonary epithelium recovery and pulmonary functional restoration. This study also found that the improvement of clinical symptoms (dyspnea, diarrhea and cyanosis) of the rats in the Dex-treated group were paralleled with the improvement of AT II cells.

It can be concluded that a series of ultrastructural changes of AT II cells following acute lung injury induced by LPS were time-dependent in young rats. Dex may ameliorate AT II cell injury and promote functional restoration of AT II cells in LPS-induced acute lung injury.

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