· Original Article in English ·

Levels of surfactant protein A and TNF-α in bronchoalveolar lavage fluid of newborns with pulmonary hemorrhage

Xu-Xu CAI, Jian MAO, Ying XIN, Yu-Kun HAN

Department of Pediatrics, Second Affiliated Hospital, China Medical University, Shenyang 110004, *China*

Abstract: Objective Recent studies have suggested that acute lung injury (ALI) is an important cause of pulmonary hemorrhage of the newborn (PHN) and that surfactant protein A (SP-A) and tumor necrosis factor-α (TNF-α) are involved in the development of ALI. This study examined the levels of SPA and TNF-α in bronchoalveolar lavage fluid (BALF) and their relationship in newborns with pulmonary hemorrhage(PH). **Methods** Twenty newborn infants with PH and 15 sick neonates but without PH (Control group) were enrolled in this study. According to the prognosis, the PH group was subdivided into Survival group ($n = 14$) and Death group ($n = 6$). The Western-dot blot analysis and enzyme-linked immunoadsorbent assay (ELISA) were used to detect the levels of SP-A and TNF-α in BALF and serum TNF-α level. **Results** The SP-A levels in BALF in the survival PH patients in the acute stage and in the death PH cases were significantly lower than those of the Control group. The death PH patients showed a much lower SP-A level in BALF than the survival ones in the acute stage. Whilst the recovery stage of PH, the SP-A level in BALF in the survival patients increased significantly and remained the similar level as the Control group. The TNF-α levels in both serum and BALF in the survival PH patients in the acute stage and in the death PH cases were significantly higher than those in the Control group. The death PH cases showed a higher level of TNF-α in serum and BALF than PH survival cases in the acute stage. The increased extent of TNF-α level in BALF was obviously greater than of that in serum. In the recovery stage, the level of TNF-α in BALF was significantly reduced and almost returned to the level of the Control group in the survival PH cases compared with that in the acute stage. There was a negative correlation between the BALF SP-A and TNF-α levels in newborns with PH ($r = -0.635, P = 0.003$). **Conclusions** SP-A and TNF-α may be involved in the process of lung injury in PHN. Monitoring the levels of SP-A and TNF-α is useful for the early diagnosis and treatment and the evaluation of the outcome of PHN. [**Chin J Contemp Pediatr, 2005, 7(3):198 - 201**]

Key words: Pulmonary surfactant-associated protein A; Tumor necrosis factor-alpha; Bronchoalveolar lavage fluid; Pulmonary hemorrhage; Newborn

新生儿肺出血支气管肺泡灌洗液 SP-A 及 TNF-α 的变化及其相关性研究

蔡栩栩,毛健,辛颖,韩玉昆 中国医科大学附属第二医院儿内科,辽宁 沈阳 110004

[**摘 要**] **目的** 近年研究发现急性肺损伤(ALI)是致新生儿肺出血的重要原因。大量研究表明肺表面活性蛋白 A(SP-A)和肿瘤坏死因子-α(TNF-α) 参与了 ALI 的损伤过程,但有关两者在肺出血新生儿支气管肺泡灌洗液 (BALF)中的变化及关系鲜有报道。该研究旨在探讨 SP-A 和 TNF-α 在新生儿肺出血发生中的作用,两者间的关系及对预后的影响。**方法** 采用斑点免疫印迹法和酶联免疫吸附法分别测定对照组($n = 15$), 肺出血存活组急性期($n = 14$)、恢复期($n = 14$)和肺出血死亡组($n = 6$)新生儿 BALF 中 SP-A,TNF-α 和血清 TNF-α 水平。**结果** 存活组和死亡组新生儿肺出血急性期 BALF 中 SP-A 含量 (38.50 ± 7.62,29.43 ± 6.57)较对照组(44.88 ± 7.48)显著降低($P = 0.024, P = 0$),且死亡组 SP-A 明显低于存活组急性期($P = 0.015$),存活组肺出血恢复期 SP-A 水平(45.16 ± 7.25)明显升高,接近对照组($P > 0.05$);而肺出血存活组和死亡组 BALF 中 TNF-α 含量 (208.54 ± 64.69 ng/L, 319.16 ± 46.79 ng/L)较对照组(96.40 ± 37.82 ng/L)显著增加($P = 0.011, P = 0$), 死亡组比存活组急性期增加更明显($P = 0$),且 BALF 中 TNF-α 的变化较血清中更明显,存活组恢复期 BALF 中 TNF-α 水平(112.06 ± 35.22 ng/L)明显下降,接近对照组($P > 0.05$);肺出血患儿 BALF 中 SP-A 水平的下降与 TNF-α 的增高呈负相关($r = -0.635, P = 0.003$)。**结论** SP-A 和 TNF-α 参与了新生儿肺出血的肺损伤过程,为从 SP-A 及细胞因子角度进一步认识新生儿肺出血的发病机制提供了实验依据,为新生儿肺出血的早期防治及预后

[Received] October 9, 2004; [Revised] March 4, 2005

[Biography] Xu-Xu CAI (1963 -), Female, MD, Associate Professor, Specializing in pediatric respiratory disorders (Email: caixuxu2001@sohu.com).

判断提供了一种新的方法。 [中国当代儿科杂志,2005, 7(3):198 - 201]
[关 键 词] 肺表面活性物质相关蛋白 A; 肿瘤坏死因子 α; 支气管肺泡灌洗液;肺出血;新生儿
[中图分类号] R722.15 [文献标识码] A [文章编号] 1008 - 8830(2005)03 - 0198 - 04

Pulmonary hemorrhage of the newborn (PHN) is a common fatal disease in neonatal intensive care units and its mortality rate reaches as high as 40%. Its mechanism is not very clear[1]. Pathological and the clinical studies have indicated that PHN is a clinical syndrome caused by various diseases. Previous studies by the authors have suggested that acute lung injury (ALI) is an underlying cause of PHN[2,3]. It is known that the reduction and disfunction of pulmonary surfactant (PS) induced by various factors is an important cause that leads to lung injury and severe respiratory disfunction[4]. Tumor necrosis factor-α(TNF-α) is the key factor that causes the release of many kinds of proinflammatory cytokine. However, the research on the changes of SP-A and TNF-α levels in PHN is limited. This study was designed to investigate the changes of SP-A and TNF-α in BALF and serum in neonatal infants with pulmonary hemorrhage (PH) and to explore their roles in early diagnosis and treatment and prediction of outcome of PHN.

Subjects and methods

Subjects

Twenty newborn infants with PH (PH group) and 15 sick neonates but without PH admitted to the Second Affiliated Hospital of China Medical University between October 2000 and June 2001 were enrolled in this study. PHN was diagnosed according to the criteria revised in national neonatal conference[5]. The PH group consisted of 20 neonates with proven PH induced by severe neonatal asphyxia ($n = 7$), meconium or amnionic fluid aspiration syndrome ($n = 5$), septemia ($n = 7$), neonatal pneumonia ($n = 2$) and organophosphate poisoning ($n = 1$). The Control group consisted of 5 cases of neonatal asphyxia, 3 meconium or amnionic fluid aspiration syndrome, 2 septemia, and 5 neonatal pneumonia. According to the prognosis, the PHN group was subdivided into Survival group ($n = 14$) and Death group ($n = 6$). There was no statistical difference between the PHN and the Control groups in gestational age, postnatal age and weight (Table 1).

Bronchoalveolar lavage (BAL)

BAL was performed at the time of clinically indicated tracheal suctioning as described[6]. The informed parental consent was obtained before the procedure started. Briefly, the infant was placed in the supine position with the head turned to the left to direct the catheter into the right lower lobe. An FG 5 catheter was gently inserted in a segmental bronchus until resistance was felt, and two aliquots of 1 mL/kg saline were instilled. With the catheter in situ, after two or three ventilator breaths, the instilled fluid was aspirated back with a suction pressure of 5-7 kPa. The appeal course was repeated 3 times. The collected BALF was centrifuged at 500 g at 4°C for 10 minutes. The supernatant was stored at -70°C until assayed.

Table 1 Population characteristics

Group	n	Gestational age		Postnatal age		Weight (g)	
		<37w	≥37w	Mean	Range	Mean	Range
Control	15	7	8	6 d	0.5 h-17 d	3 089	1 900-4 200
PHN	20	6	14	4 d	0.5 h-22 d	2 890	1 770-4 400

Measurement of SP-A level

The level of SP-A in BALF was determined using Western-dot blot. Image analysis was carried by MAIS300 picture analytical system. There was no standard preparation of SP-A, so, only its relative value was calculated.

Detection of TNF-α level

The level of TNF-α in serum and BALF was determined using enzyme linked immunosorbent assay (ELISA) according to the reagent protocol.

Statistical analysis

All data were processed with SPSS10.0 for windows. Results were expressed as mean ± SEM ($\bar{x} \pm s$). Comparisons for variables were performed by the Dunnett t-test and the One-way ANOVA. The correlation analysis was performed by calculating the Pearson correlation coefficient.

Results

The SP-A level in BALF

The SP-A levels in BALF in the survival PH patients in the acute stage and in the death PH cases were significantly lower than those of the Control group. The death PH patients showed a much lower SP-A level than the survival ones in the acute stage. During the recovery stage of PH, the SP-A level in the survival patients increased significantly and remained the similar level as the Control group (Table 2).

Table 2 Levels of SP-A and TNF-α ($\bar{x} \pm s$)

Group	n	SP-A in BALF (gray scale)	TNF-α in BALF (ng/L)	TNF-α in serum (ng/L)
Control	15	44.88 ± 7.48	96.40 ± 37.82	67.85 ± 21.42
PH	20	36.28 ± 7.72[a]	241.73 ± 78.39[a]	100.64 ± 28.93[a]
PH survival (acute stage)	14	38.50 ± 7.62[a]	208.54 ± 64.69[b]	91.82 ± 26.36[b]
PH survival (recovery stage)	14	45.16 ± 7.25	112.06 ± 35.22[d]	79.42 ± 24.89
PH death	6	29.43 ± 6.57[d]	319.16 ± 46.79[b,d]	121.22 ± 25.46[b, c]

a vs Control group $P < 0.05$, b vs Control group $P < 0.01$, c vs Survival group (acute stage) $P < 0.05$, d vs Survival group (acute stage) $P < 0.01$

The TNF-α level in serum

As shown in Table 2, the serum TNF-α levels in the survival PH patients in the acute stage and in the death PH cases were significantly higher than those in the Control group. Of the PH group, the level of TNF-α in the death cases was significantly higher than that in survival ones in the acute stage. In the recovery stage, the survival PH patients did not show a significantly decreased level of TNF-α compared with those in the acute stage.

The TNF-α level in BALF

As shown in Table 2, the TNF-α levels in BALF in the survival PH patients in the acute stage and in the death PH cases increased significantly when compared with those in the Control group . The death PH cases showed a much higher level of TNF-α than the PH survival cases in the acute stage. The increased extent of TNF-α level in BALF was obviously greater than of that in serum. In the recovery stage of PH, the TNF-α level in BALF in survival cases was significantly reduced compared with that in the acute stage, and almost returned to the level of the Control group.

The correlation between levels of SP-A and TNF-α in BALF

The Pearson correlation analysis demonstrated a negative correlation between levels of SP-A and TNF in BALF from neonates with PH, with the correlation coefficient of - 0.635 and $P = 0.003$.

Discussion

PS is a lipoprotein substance, which is composed of lipids (90%) and SP (10%). It can reduce surface tension in the lung alveolus. Although phospholipids are critical for the generation of low surface tensions, SP is fundamental in the regulation of surfactant function and metabolism. SP is divided into the hydrophilic surfactant proteins (SP-A and SP-D) and the hydrophobic surfactant proteins (SP-B and SP-C). SP-A is the predominant phospholipid-associated glycoprotein in pulmonary surfactant and is specific to the lung. SP-A is required for formation of tubular myelin, an intermediate structural form of secreted surfactant and participates in the formation of surface active film. Moreover, this protein has an important role in lung immune defense and modulation of the inflammatory response to infection and other stimuli [7]. The decrease in SP-A level has been implicated in the pathogenesis of acute respiratory distress syndrome (ARDS). Balamugesh et al[8] reported that the SP-A levels in the bronchial aspirates in patients with ARDS at the start of mechanical ventilation were lower but increased after 24 hours and 48 hours of mechanical ventilation. In the present study, it was found that the SP-A levels in BALF from neonates with PH in the acute stage were obviously reduced, and returned to the Control group's level in the recovery stage. The SP-A level in the PH death cases was lower than that in the survival patients. These results indicate that the decrease of SP-A level in BALF may be one of important causes leading to lung injury in PHN. Thus, monitoring SP-A changes in BALF may be useful in the early diagnosis and treatment and evaluation of the outcome of PHN.

TNF-α is one of the important proinflammatory cytokines which plays a key role in host defense and in the acute inflammatory response related to tissue injury. TNF-α has fierce toxicity to lung. It stimulates neutrophilic granulocyte adherence to endothelial cell and induces apoptosis, activates neutrophilic granulocyte to release elastase, collagenase, myeloperoxidase, active oxygen and other inflammatory medium, and increases pulmonary alveolar-capillary membrane permeability. Previous animal experiment by the authors has shown that TNF-α plays an important role in initiating the inflammatory response in pulmonary hemorrhage of neonatal rats induced by lipopolysaccharide[9]. Many clinical studies have demonstrated that TNF-α, interleukin-1β and interferon-α in BALF and plasma were markedly increased in patients at risk for ARDS and developing ARDS after trauma, sepsis, or shock[10,11].

However, the relationship between SP and inflammatory cytokine in lung injury has not been identified. In the present study it was discovered that the levels of TNF- α in BALF and serum from neonates with PH were significantly increased, and that the TNF-α in BALF was higher than in serum. TNF- α levels in both BALF and serum in PH death patients were significantly higher than in PH survival cases. In the recovery stage of PH, the TNF-α level was decreased in survival cases. Moreover, there was a close negative correlation between the levels of SP-A and TNF-α in BALF in neonates with PH. These results indicate that TNF-α may be involved in the development of lung injury in PHN and may be an important cause that leads to SP-A reduction.

A previous study has shown that the SP-A expression could be regulated by cytokines. For example, interferon-γ stimulates the secretion of SP-A, but TNF-α and phorbol ester suppress the expression of SP-A[12]. Some research has indicated that TNF- alpha inhibits the SP-A gene expression by suppressing transcription[13]. On the other hand, it is reported that SP-A not only stimulates but also inhibits the proinflammatory activity of pulmonary macrophages. The expression of cytokines and chemokine mRNA (TNF-α, IL-6, MCP-1, MIP-α and MIP-2) increased in the lungs of infected SP-A-/-mice and the concentrations of proinflammatory cytokines, such as TNF-α, IL-6, and IL-1β, also increased in BALF of SP-A -/-mice. Harrod[14] reported that co-administration of purified hSP-A with virus may inhibit pulmonary inflammation in SP-A -/- mice. Mcintosh[15] also found that SP-A could reduce TNF-α activity in medium from isolated LPS-stimulated macrophages[12]. At present, a role of SP-A in the regulation of cytokines is not very clear. It is supposed that SP-A may activate a kind of signal transduction pathway by combining with SP-A acceptor at immune cell surface and finally activate nuclear factor-kappa B (NF-kappa B), which is essential for the transcription of many cytokine genes. Therefore, it is needed to further investigate whether SP-A regulates cytokines production by activating or suppressing NF-kappa B activity.

Therefore, replacement treatment of SP-A will be helpful for not only improving the function of PS, but also reducing the inflammatory reaction and enhancing the lung defense function. Obviously, the identification of the relationship between SP and the inflammatory reaction is useful in the prevention and management of PHN and lung injury.

[References]

[1] Chen K-Z, Wang W. Pulmonary hemorrhage model of neonatal rats and its clinical significance (in Chinese) [J]. Chin J Contemp Pediatr, 2002, 4(5): 357-360.

[2] Du Y, Li J, Li S-Q, Li Q-H. Analysis of clinical related factors in 25 cases pulmonary hemorrhage of the newborn (in Chinese) [J]. Pediatr Emergen Med, 1998,5 (4):178-179.

[3] Cai X-X, Du Y, Han X-H, Shang Y-X Han Y-K. The pathological findings and inflammatory responses in the lung tissue of neonatal rats following lipopolysaccharide treatment (in Chinese) [J]. Chin J Pediatr, 2003,41(8):617-618.

[4] Lewis JF, Jobe AH. Surfactant and the adult respiratory distress syndrome[J]. Am Rev Respir Dis, 1993, 147(1): 218-233.

[5] The Subspecialty Group of Neonatology, Pediatric Society, Chinese Medical Association, Editorial Board of Chinese Journal of Pediatrics. Diagnostic criteria and management for pulmonary hemorrhage of the newborn (in Chinese) [J]. Chin J Pediatr, 2001,39 (4):248.

[6] Kotecha S. Bronchoalveolar lavage of newborn infants [J]. Pediatr Pulmonol Suppl, 1999; 18: 122-124.

[7] Whitsett JA, Weaver TE. Hydrophobic surfactant proteins in lung function and disease [J]. N Engl J Med, 2002, 347(26): 2141-2148.

[8] Balamugesh T, Kaur S, Majumdar S, Behera D. Surfactant protein-A levels in patients with acute respiratory distress syndrome [J]. Indian J Med Res, 2003, 117: 129-133.

[9] Cai X-X, Wu W-B, Du Y, Liu C-F, Zhang H, Lu Y, et al. Effects of tumor necrosis factor-αand IκBα on pulmonary hemorrhage of neonatal rats induced by lipopolysaccharide (in Chinese) [J]. Chin J Contemp Pediatr, 2003,5(4):301-305.

[10] Suter PM, Suter S, Girardin E, Roux-Lombard P, Grau GE, Dayer JM. High bronchoalveolar levels of tumor necrosis factor and its inhibitors, interleukin-1, interferon, and elastase, in patients with adult respiratory distress syndrome after trauma, shock, or sepsis [J]. Am Rev Respir Dis, 1992, 145(5):1016-1022.

[11] Carter AB, Monick MM, Hunninghake GW. Lipopolysaccharide-induced NF-kappaB activation and cytokine release in human alveolar macrophages is PKC-independent and TK- and PC-PLC-dependent [J]. Am J Respir Cell Mol Biol, 1998, 18(3): 384-391.

[12] Pryhuber GS, Khalak R, Zhao Q. Regulation of surfactant proteins A and B by TNF-alpha and phorbol ester independent of NF-kappa B [J]. Am J Physiol, 1998, 274(2 Pt 1): L289-L295.

[13] Whitsett JA, Clark JC, Wispe JR, Pryhuber GS. Effects of TNF-alpha and phorbol ester on human surfactant protein and MnSOD gene transcription in vitro [J]. Am J Physiol, 1992, 262(6 Pt 1): L688-L693.

[14] Harrod KS, Trapnell BC, Otake K, Korfhagen TR, Whitsett JA. SP-A enhances viral clearance and inhibits inflammation after pulmonary adenoviral infection [J]. Am J Physiol, 1999, 277(3 Pt 1): L580-L588.

[15] McIntosh JC, Mervin-Blake S, Conner E, Wright JR. Surfactant protein A protects growing cells and reduces TNF-alpha activity from LPS-stimulated macrophages [J]. Am J Physiol, 1996, 271 (2 Pt 1): L310-L319.

(Edited by Xia WANG)