SURFACTANT PROTEIN B EXPRESSION IN BRONCHOALVEOLAR LAVAGE FLUID OF FULL-TERM NEONATES WITH RESPIRATORY DISTRESS SYNDROME

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SUMMARY – The aim was to investigate the surfactant protein B (SP-B) expression in the bronchoalveolar lavage fluid (BALF) of full-term neonates with respiratory distress syndrome (RDS). The enzyme-linked immunosorbent assay was performed to assess SP-B expression in BALF of 60 full-term neonates with RDS and 23 healthy neonates and correlation of SP-B level with RDS classification according to chest x-ray findings and PaO_2/FiO_2 before mechanical ventilation in neonates with RDS. The SP-B level was significantly lower in the RDS group (17.63±6.80 ng/mL) than in healthy neonates (103.95±6.38 ng/mL) (P<0.001). The SP-B level correlated positively with PaO_2/FiO_2 before mechanical ventilation (r=0.838, P<0.001). Moreover, the lower the SP-B level, the more severe was the RDS as determined by chest x-ray (P<0.001). In conclusion, full-term neonates with RDS had reduced SP-B in BALF, which was related to the severity of RDS, suggesting that SP-B supplement may be an effective strategy in the treatment of RDS in full-term neonates.

Key words: *Respiratory distress syndrome, newborn; Pulmonary surfactant-associated protein B; Pulmonary surfactants*

Introduction

Neonatal respiratory distress syndrome (NRDS) is a major disease causing respiratory failure in neonates and also a major cause of death in neonates, preterm neonates in particular. The pathogenesis of NRDS is related to the lack of pulmonary surfactants (PS), which may increase the alveolar surface tension resulting in alveolar collapse. Surfactant protein B (SP-B) is a key component of PS and may promote the spread and adherence of PS on the gas-liquid interface and plays an important role in the formation and stabilization of surface-active phospholipid monolayer[1]. In recent years, full-term neonates increasingly develop clinical manifestations of NRDS and pulmonary findings on chest x-ray. In the present prospective study, enzyme linked immunosorbent assay (ELISA) was employed to detect the SP-B expression in the bronchoalveolar lavage fluid (BALF) of full-term neonates with NRDS.

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Materials and Methods

Inclusion criteria

Full-term neonates, gestational age 37-42 weeks and birth weight 2500 g to 4000 g, were included. NRDS was diagnosed according to the following criteria: (1) moaning, bruising, progressive dyspnea and respiratory failure found within several hours after birth and auscultation of the lungs revealing moist rales and reduced breath sounds; (2) oxygen supplement *via* mask or nasal tube failed to rectify hypoxemia and mechanical ventilation had to be performed; (3) chest x-ray findings supporting the

diagnosis of NRDS: grade I: bilateral transmittance reduced and small mesh-like particle shadow present; grade II: grade I findings and air bronchogram; grade III: grade II findings and blurred heart and diaphragm contours; grade IV: white lung; (4) blood gas analysis before mechanical ventilation: PaO_2 <50 mm Hg, PaO_2/FiO_2 <200 mm Hg, and patient meeting the diagnostic criteria for respiratory failure; (5) cardiogenic pulmonary edema excluded by heart ultrasonography, electrocardiography and clinical manifestations; and (6) patient meeting the diagnostic criteria for NRDS in Practical Neonatology edited by Shao *et al.*[2]. In control group, healthy neonates had no manifestation of primary lung diseases or abnormalities on chest x-ray.

Exclusion criteria

Pulmonary hemorrhage, disseminated intravascular coagulation (DIC), systemic bleeding disorders, AB0/Rh hemolytic disease, anemia and cyanotic heart disease were excluded.

General information

A total of 83 full-term neonates with NRDS were recruited from the Neonatal Intensive Care Unit, Affiliated Bayi Children's Hospital, Beijing Military General Hospital from April 2010 to October 2011. Control group included 23 healthy neonates that received intubation during resuscitation for suffocation or due to suspected inhalation of meconium/amniotic fluid, and free from any chest x-ray abnormalities or dyspnea. There were no marked differences in birth weight, gender, gestational age, maternal age and pattern of delivery between the two groups (P>0.05) (Table 1). An informed consent was obtained from their parents and the study was approved by the Ethics Committee of the Beijing Military General Hospital. Blood culture, sputum culture, BALF culture, routine blood test, detection of C-reactive protein, blood biochemical testing, detection of neonatal hematolysis, screening for congenital metabolic diseases, echocardiography and chest x-ray were performed according to the disease status.

Methods

ELISA was performed to detect serum and BALF level of SP-B.

1) BALF: At 30 min after the diagnosis of NRDS, BALF was collected before endotracheal administration of surfactant. In control group, BALF was collected during resuscitation by intubation for suffocation or inhalation of meconium/amniotic fluid. Collection of BALF was done in a bronchoscope independent manner. At 15 min before collection, oxygen was administered at FiO_2 of 1.0 and sedation was also performed. Normal saline at 37 °C was administered *via* the intratracheal tube (0.5 mL/kg). Thereafter, pressed supplement of oxygen was done *via* a resuscitation bag 2-3 times, followed by turning the body over and patting the back. A suction tube was inserted *via* the intratracheal tube and then withdrawn when resistance was felt. Suction was done at the negative pressure of <6 kPa. The suction tube was withdrawn slowly during the suction. These procedures were done within 30 s and repeated three times. The mean volume of BALF was 2.5 mL (recovery rate 55%). BALF was placed on ice and used for further assay. BALF was filtered through single-layer gauze to remove mucilage. The filtrate was centrifuged at 4 °C for 30 min at 200xg. The supernatant was collected and stored at -70 °C until use.

2) Serum: Irritation was avoided throughout the

Table 1. General data of neonates with neonatal respiratory distress syndrome (NRDS group) and without NRDS (control group)

Group	n	Gender		Gestational age (wks)	Birth weight (g)	Maternal age (yrs)	Pattern of delivery	
		M	F	$\bar{X}$±s	$\bar{X}$±s	$\bar{X}$±s	Eutocia	Cesarean section
NRDS	60	33	27	38.0±0.7	2945.3±193.3	27.2±3.6	26	34
Control	23	11	12	38.2±0.8	2969.2±247.3	27.1±3.0	13	10
χ^2 or t	-	0.344		1.148	0.465	0.115	1.161	
P	-	0.558		0.255	0.643	0.908	0.281	

procedure. Tubes without coking and endotoxin were used to collect blood, which was centrifuged at 4 °C for 20 min at 1000xg. Serum was collected and stored at -80 °C.

3) ELISA: The assay was done in a bland manner. The serum and BALF level of SP-B was detected by ELISA according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed with the SPSS version 13.0. Quantitative data (such as gestational age, birth weight, maternal age, serum and BALF level of SP-B) were compared with independent t test. Comparisons of proportion of male/female and pattern of delivery were done with chi square test. One-way analysis of variance was done to evaluate the correlation of NRDS grades as determined by chest x-ray findings with serum and BALF level of SP-B; bivariate correlation analysis was performed to evaluate the correlation of BALF level of SP-B with PaO_2/FiO_2 before mechanical ventilation.

Results

Serum and BALF level of SP-B in the two groups

The BALF level of SP-B in NRDS group was significantly lower than that in control group (17.63±6.80 *vs.* 103.95±6.38 ng/mL, t=52.637, $P<0.001$). However, there was no significant difference in serum SP-B level between the two groups (18.93±5.50 *vs.* 18.68±5.26 ng/mL, t=0.186, $P=0.853$).

Correlation of BALF and serum level of SP-B with NRDS grades as determined by chest x-ray findings

In NRDS group, the lower BALF SP-B level was related to a more severe NRDS, but serum SP-B level was not associated with NRDS grades (Table 2).

Correlation of BALF and serum level of SP-B with PaO_2/FiO_2 before mechanical ventilation

In NRDS group, the BALF SP-B level (17.63±6.80 ng/mL) correlated positively with PaO_2/FiO_2 (114.1±5.4 mm Hg) before mechanical ventilation (r=0.838, $P<0.001$).

SP-B level between NRDS death and survivors

In the six neonates died from NRDS, the BALF SP-B level (7.73±0.72 ng/mL) was significantly lower than that in 54 survivors (18.73±6.25 mg/mL) (t=12.220, $P<0.001$). Three of these six patients were finally diagnosed with a genetic defect[3].

Discussion

Results of the present study showed that BALF SP-B level in NRDS neonates was significantly lower than that in healthy controls and correlated positively with PaO_2/FiO_2 before mechanical ventilation. Moreover, the lower the BALF SP-B level, the more severe was the NRDS graded by chest x-ray findings, suggesting an association between BALF SP-B level and NRDS severity. In this study, the influence of pulmonary hemorrhage, DIC, systemic bleeding disorders, AB0/Rh hemolytic disease and anemia on BALF and serum, as well as the influence of cyanotic heart disease on PaO_2/FiO_2 was excluded.

SP-B is a hydrophobic protein composed of a homodimer with molecular weight of 18 kD. SP-B is mainly synthesized in alveolar type II cells and is a key surfactant protein of PS because the SP-B knockout neonatal rats or neonates with homozygous SP-B defect will die of respiratory distress soon after birth[4]. Human SP-B gene is mapped to 2p12-p11.2 and contains 10 introns and 11 exons. The 11th exon is not translated. The exons 6 and 7 encode mature SP-B peptide. The functions of SP-B include the following[1]: (1) it can promote the adherence and spreading of surfactant phospholipids on the gas-liquid interface; (2) it can crosstalk with phospholipid head group to in-

Table 2. Serum and bronchoalveolar lavage fluid (BALF) level of surfactant protein B (SP-B) and neonatal respiratory distress syndrome (NRDS) grades determined by chest x-ray findings (ng/mL)

Group	Grade I (n=12)	Grade II (n=16)	Grade III (n=12)	Grade IV (n=20)	F	*P*
BALF SP-B	24.70±7.94	20.16±4.80	15.78±2.62	12.09±4.16	17.059	<0.001
Serum SP-B	20.82±4.65	18.67±5.59	20.65±4.81	16.96±5.94	1.798	0.158

crease the lateral stability of phospholipid monolayer; (3) it is a key factor determining the formation of lamellar bodies; (4) it may form tubular myelin with SP-A, phospholipids and calcium; (5) it may promote the uptake of phospholipid vesicles by alveolar type II epithelial cells; (6) it can bind to phospholipid bilayer and thus has membrane-lytic characteristics, which may facilitate the organization and assembling of surfactant phospholipid membrane in the lamellar bodies[5]; and (7) it is also involved in immune regulation and play important roles in the airway defense and stabilization[6].

The reduced BALF SP-B level in NRDS neonates cannot be simply explained by the leakage of surfactant proteins into circulation because our findings showed that serum SP-B level was comparable between the control and NRDS neonates and there was no relationship between the severity of NRDS and serum SP-B level. This also suggests that SP-B is mainly expressed in the lung. The reasons for reduced SP-B in the lung of NRDS neonates are as follows: (1) the plasma proteins and phospholipase A_2 leaking into the alveolar space and the inflammatory mediators and oxygen free radicals in the lung cause destruction or inactivation of PS[7]; and (2) damage to the alveolar type II cells may reduce the uptake and synthesis of PS.

Correlation between SP-B in the lung of neonates and NRDS: generally, NRDS in full-term neonates is different from that in preterm infants. In full-term neonates, the insufficiency of PS is not attributed to the immaturity of the lung. Thus, the pathogenesis of NRDS in full-term neonates is more likely related to the changes in chemical components of PS, which then reduce the surfactant function of PS resulting in NRDS. In the present study, full-term neonates were recruited. In NRDS group, the BALF SP-B level was markedly lower than that in healthy controls, suggesting the reduced SP-B in the lung of NRDS neonates, which reduced the surfactant function of PS resulting in NRDS. Greene *et al*.[8] report that the BALF SP-B level in neonates at a high risk of RDS was markedly lower than that in healthy controls, and the BALF SP-B level in RDS patients was significantly reduced when compared with those at a high risk of RDS. In addition, the SP-B expression is at a low level during the course of RDS. The reduced SP-B expression in RDS neonates significantly influences the alveolar stability, ventilation/perfusion ratio and ventilation function. Some investigators have attempted to treat RDS with SP-C, achieving poor efficacy, but SP-B is more effective for RDS than SP-C. This also indirectly indicates the correlation between SP-B and RDS[9,10].

Correlation of SP-B expression in the lung of neonates with the severity of NRDS: in the present study, the correlation of BALF SP-B level with PaO_2/FiO_2 before mechanical ventilation and grades of NRDS as determined by chest x-ray findings was evaluated to assess the relationship between SP-B and severity of NRDS. Results showed the lower the SP-B expression in the lung of neonates, the lower was the PaO_2/FiO_2 before mechanical ventilation and the higher was the grade of NRDS as determined by chest x-ray findings (more severe NRDS).

In addition, our results showed that the BALF SP-B level in six patients died of NRDS was significantly lower than that in survivors. Three of these six patients died because their parents refused further treatment. The remaining three patients were repeatedly treated with PS and mechanical ventilation but eventually died and were finally diagnosed with genetic SP-B defect by genetic analysis[3]. This suggests that the neonates with genetic SP-B defect usually do not respond to routine treatment, except for lung transplantation[11], and that the genetic and metabolic diseases of surface-active substances are involved in the pathogenesis of NRDS.

Taken together, NRDS neonates have reduced SP-B expression in the lung, which is related to the severity of NRDS. Treatment with exogenous SP-B may be an effective strategy in the treatment of NRDS. However, supplement of SP-B is ineffective for neonates with genetic SP-B defect, which requires gene therapy.

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Sažetak

IZRAŽENOST SURFAKTANTNOG PROTEINA B U BRONHOALVEOLARNOM ISPIRKU TERMINSKE NOVOROĐENČADI SA SINDROMOM RESPIRACIJSKOG DISTRESA

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Cilj studije bio je ispitati izraženost surfaktantnog proteina B (SP-B) u bronhoalveolarnom ispirku (BALF) terminske novorođenčadi sa sindromom respiracijskog distresa (SRD). Izraženost SP-B određena je testom ELISA u BALF 60 terminske novorođenčadi sa SRD i 23 zdrave novorođenčadi. Utvrđena je korelacija razine SP-B s klasifikacijom SRD prema rendgenskoj snimci prsišta i vrijednosti PaO_2/FiO_2 prije mehaničke ventilacije u novorođenčadi sa SRD. U skupini novorođenčadi sa SRD razina SP-B bila je značajno niža (17,63±6,80 ng/mL) od one u zdrave novorođenčadi (103,95±6,38 ng/mL) (P<0,001). Utvrđena je pozitivna korelacija razine SP-B i PaO_2/FiO_2 prije mehaničke ventilacije (r=0,838, P<0,001). Štoviše, što je bila niža razina SP-B, to je teži bio SRD procijenjen prema rendgenskoj snimci prsišta (P<0,001). Zaključuje se da terminska novorođenčad sa SRD ima sniženu razinu SP-B u BALF i to je povezano s težinom SRD. Ovi nalazi ukazuju na to da bi dodatak SP-B mogla biti učinkovita strategija u liječenju SRD kod terminske novorođenčadi.

Ključne riječi: *Sindrom respiracijskog distresa, novorođenče; Surfaktantni protein SP-B; Plućni surfaktanti*