A NOVEL METHOD FOR ALVEOLAR SURFACTANT ASSESMENT OF CLINICAL SAMPLES IN NORM AND PATHOLOGY

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ABSTRACT

The aim of the present study was to estimate the physiology condition of lung surfactant by biophysical analysis of different clinical samples in norm and pathology. The analyzed clinical samples included gastric aspirates (GA) from prematurely born and full term infants, tracheal aspirates (TA) from unventilated and ventilated lung during surgery in patients with nonsmall cells lung cancer (NSCLC) and broncho-alveolar lavage samples from a patient with pulmonary alveolar proteinosis, taken after each stage of the applied whole lung lavage procedure (WWL). By using the novel method of Axisymmetric Drop Shape Analysis, the equilibrium and dynamic surface characteristics (maximal and minimal surface tension during 10 cycles of compression-decompression) were determined. Our results showed that AS surface behavior differed in norm and pathology. The analyzed pathologies led to significant differences in the surface tension characteristics. The present study could find application into the clinical practice for fast surfactant maturity diagnostics in prematurely born children regarding lifesaving therapy with exogenous surfactants administration. It also showed that lung cancer, hypoxia and inhalation anesthesia affect the biophysical properties of AS which leads to changes in its surface behavior. In addition, it would be of great interest for the effective implementation of the procedure of whole lung lavage in the clinical practice.

Key words: *alveolar surfactant, surface tension, clinical samples*

Introduction

Alveolar surfactant (AS) is a complex lipoprotein mixture that covers the lung alveoli at the air-liquid interface. Its main function *in vivo* is to reduce the surface tension (γ, mN/m) during exhalation thus preventing the alveolar collapse. The insufficiency of AS in lung as well as deviation from its optimal biochemical composition results in respiratory activity impairment [5; 7; 8] One of the most severe respiratory dysfunctions is the neonatal respiratory distress syndrome (NRDS) [13]. The extreme immaturity including NRDS is a leading cause of the neonatal morbidity and mortality. So far the neonatal lung maturity was diagnosed by tests using amniotic fluid, tracheal aspirate, nasopharyngeal aspirate, etc. Some of these methods are traumatic and invasive, others can provide samples with insufficient quantity for their analyzes. Therefore, it is necessary to look for new diagnostic methods for assessment of surfactant maturity at birth that would be less invasive and painless and that would provide sufficient sample quantity.

Another extremely severe condition with high frequency and lethal outcome is the nonsmall cells lung cancer (NSCLC). This condition usually requires surgical intervention. During the lung resection the operated lung is under hypoxia and the ventilated lung is exposed to volatile anesthetics [3]. In some conditions alveolar epithelial cells are exposed to low oxygen concentrations and although they can adapt to hypoxia, there are alterations in cellular function that can impact clinical outcomes. Volatile anesthetics have been shown to change surfactant phospholipid biosynthesis.

Pulmonary alveolar proteinosis (PAP) is an extremely rare disorder, occurring worldwide with an estimated prevalence of 0.1 per 100,000 individuals. The onset of clinical disease is

atypical, with a subacute indolent course that often delays the diagnosis by months to years [1]. PAP is a diffuse pulmonary disease characterized by the accumulation of periodic acid-Schiff-positive lipoproteinaceous material, primarily phospholipid surfactant and specific surfactant apoproteins in the distal air spaces, which results in impaired gas transfer [1]. Whole lung lavage (WLL), introduced by Jose Ramirez-Rivera in the late 1960s, is still the gold-standard therapy [6]. Indeed, this technique has been much improved over the years, thus enhancing effective removal of material from the alveoli. Warmed normal saline solution in 1-liter aliquots (total volume up to 20 liters) is instilled into the lung, chest physiotherapy is performed and the proteinaceous effluent is drained. The sequence of events is repeated until the effluent becomes clear [2, 10].

The clinical therapy for respiratory dysfunctions currently includes the application of various native or synthetic exogenous surfactant preparations which substitute human alveolar surfactant. Analysis of the composition and the properties of the AS are crucial for the assessment of lung maturity and optimal function and justify the need of surfactant therapy application.

The aim of the present study was to estimate the functionality of lung surfactant by biophysical *in vitro* analysis of different clinical samples in norm and pathology. With this regard we investigated the properties of: (1) GA from 74 babies: 15 with NRDS, 6 prematurely born and 53 normally born and healthy; (2) TA from 15 patients with NSCLC and (3) 10 whole lung lavage samples, collected from a male patient during 15 WLL cycles.

Materials and Methods

Gastric aspirates: The gastric aspirates were collected in the first minutes after birth in sterile tubes via a nasogastral tube and were stored in -20ºC. The quantity of each sample (between 2 and 5 ml) was enough for biochemical and biophysical analyzes described below.

Tracheal aspirates: The tracheal aspirates were collected from patients with NSCLC. Surgery was performed under general anesthesia and a double lumen tube intubation. Bronchoalveolar lavage (BAL) was also performed with 20 ml 0.9% NaCl from the operated (nonventilated) lung after intubation and before reventilation, and from ventilated lung after intubation and at the end of anestesia. Anesthesia was conducted by sevoflorane, fentanyl and atracurium bromidum.

Broncho-alveolar lavage samples: The whole lung lavage samples were collected from a male patient during 15 WLL cycles for both lungs. Every cycle was made with 1 liter 0.9% NaCl saline (10 ml/kg). The procedure was performed first on the right lung and seven days later on the left lung. The analyzed samples were gathered as follows: sample 1: mean sample after lavage with 1l 0.9 % NaCl saline; sample 2: taken and analyzed after 4x1l 0.9 % NaCl saline was added to sample 1; sample 3: new lavage cycle with 4x1l 0.9 % NaCl saline for treated lung; sample 4: new lavage cycle with 4x1l 0.9 % NaCl saline for lung; sample 5: new lavage cycle with 3x1l 0.9 % NaCl saline for the lung.

Biophysical measurements of surface characteristics of clinical samples: The pending drop method is a novel approach for analysis of the surface behavior of small amounts (50µl) of the tested clinical samples. Tensiometer KSV CAM 101 (KSV Instruments Ltd., Finland) was used. The determination of surface tension was performed using the Axisymmetric Drop Shape Analysis (ADSA). Fifteen minutes were allowed after the symmetric drop of sample formation for adsorption of surface-active molecules at the air-water interface and reaching an equilibrium value of the surface tension (γ_{eq}, mN/m). After recording γ_{eq} the drop was subjected to 10-fold compression and decompression (from 100% to 20%) by specialized apparatus that imitate the inspiration/exhalation of the lung alveoli during respiration. Thus, the following surface parameters were detected: maximal surface tension at 100% drop surface (γ_{max}, mN/m) and minimal surface tension at 20% drop surface (γ_{min}, mN/m).

Results and Discussion

In order to assess the lung maturity at different respiratory disorders: NRDS, NSCLC, and PAP, biophysical analyzes of clinical samples were performed. The surface characteristics of the clinical samples were determined by the pending drop method. The adventage of this method is the short time for measurment of the AS surface characteristics and the small quantity required for the analysis. We measured the following surface parameters: equilibrium, maximal and minimal surface tension.

The GA studied were distributed into two groups: GA taken from healthy full term infants (53 samples, I group); GA of premature infants with NRDS (15 samples, II group). As shown on Fig. 1, the mean values of the equilibrium surface tension do not differ significantly between the two groups while the dynamic surface characteristics of GA, especially the minimal surface tension (γ_{min}) show much higher statistically significant difference. The mean γ_{min} value of GA from the healthy term infants was 11.5 mN/m, while in the group of premature infants with NRDS the average γ_{min} was significantly higher – 20.6 mN/m (Fig. 1). The minimal surface tension was proved to be a sensible and informative parameter for evaluation of fetal and neonatal lung maturity [9, 4]. It is well known that in conditions of compression *in vivo*, the AS can reach very low values of γ_{min}, less than 5 mN/m [12] which is of important significance for prevention of alveolar collapse at the end of exhalation. Our results were expected since AS consists mainly of phospholipids and specific surfactant proteins that provide normal alveolar function [8]. It is important to notice that optimal PL and protein concentration at the alveoalr surface is achieved after 36 week of gestation [13]

Our results suggest that the novel application of the pending drop method can be succesfully used in clinical practice for fast and reliable assesment of AS maturity and the need for exogenous surfactant therapy in newborn children.

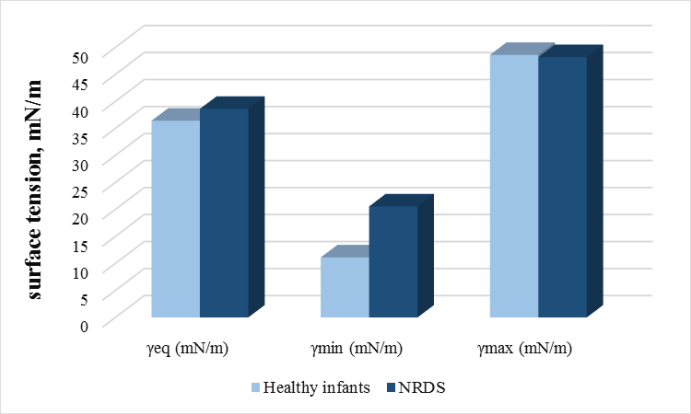

Figure 1. Surface tension values (γ_{eq},γ_{min} and γ_{max}) in gastric aspirates from healthy full term infants and premature infants with NRDS.

The next group of clinical samples were taken from 65 NSCLC patients: study group (n=36) and control group (n=29). The patients in the study group had more comorbidities compared to the patients in the control group. In the study group we determined a deterioration in the mean γ_{eq} in patients with malignant disease compared to the control group (45.27 vs. 24 mN/m, Fig. 2). During compression and decompression of monolayers from pulmonary surfactant no statistically significant differences were determined before and after anesthesia and before and after hypoxia.

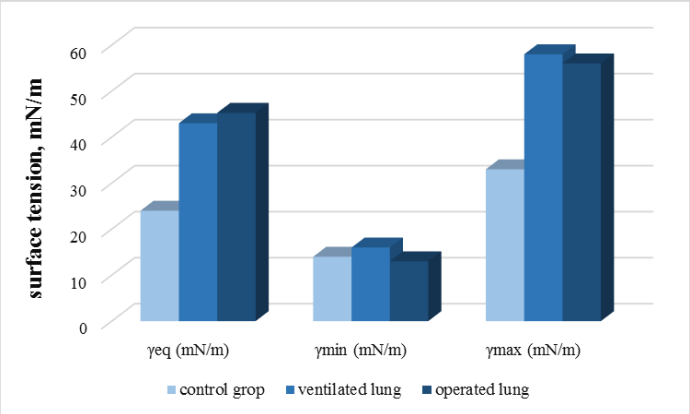

Figure 2. Surface tension values (γ_{eq},γ_{min} and γ_{max}) in traheal aspirates from NSCLC patients.

Our results suggest that hypoxia and inhalation anesthesia affects the surface properties of pulmonary surfactant with regard to equilibrium surface tension. Most probably, the effects observed was a result of an increase in serum proteins content in samples, which have a potent inhibitory effect on lipids spreading at the alveolar surface [11].

The third group of clinical samples used in the present study involved broncho-alveolar lavage liquid samples collected by WLL procedure from a patient with PAP. The impaired respiratory function with PAP is caused by inactivation of the alveolar surfactant due to the increased amount of proteins, most of which is the plasma albumin. It is known that in case of respiratory distress syndrome the albumin concentration of the alveolar liquid reaches up to 25-100 mg/ml. It is considered that as a result of its high speed of adsorption and concentration albumin (like other plasma proteins) covers the alveoli faster compared to the AS, thus forming a protein film that makes alveolar surface unreachable for the surfactant [5, 7, 10].

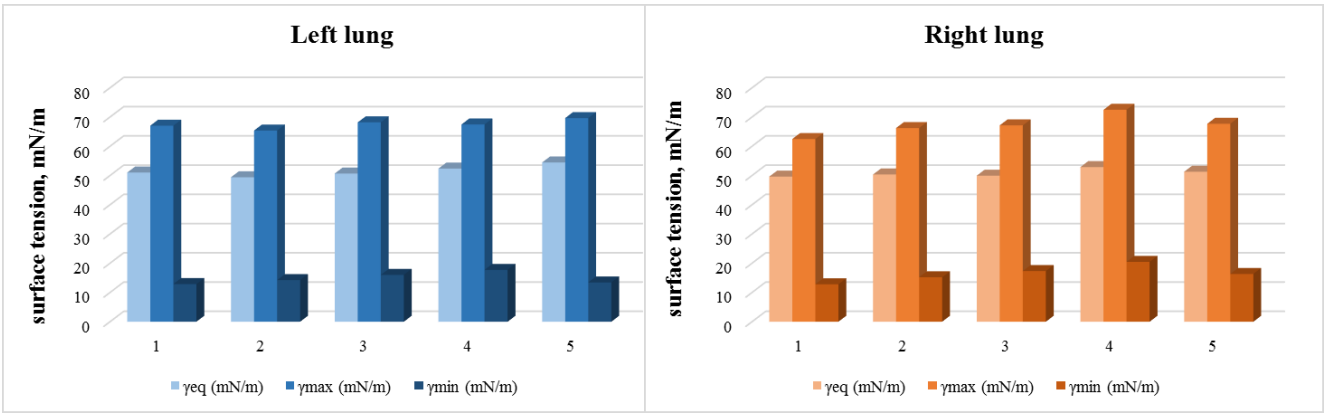

Figure 3. Surface tension values (γ_{eq},γ_{min} and γ_{max}) in broncho-alveolar lavage from patient with PAP.

Our results showed a tendency of increased γ_{eq} values after the initial saline infusion in the left lung (Fig. 3). This corresponded to the lower PL content in the samples (data not shown).This effect was less significant in the deteriorated right lung. It is known that normal function of alveolar surfactant equals optimal PL/protein values and lower surface tension [8, 13]. In the studied case of PAP this ratio is abnormal, especially for the right side of the lung. These results were also confirmed by image diagnostics showing deterioration of the condition of the right lung compared to the left (data not shown).

The mean values of the dynamic surface characteristics (γ_{max} and γ_{min}) confirm in less extend the deterioration of the right lung showing an increase in maximal and minimal surface tension values compared to these parameters of the left lung.

Conclusion

Our results showed that AS surface behavior differed in the clinical samples from patients with different respiratory dysfunctions leading to alteration of normal alveolar functioning.

The pending drop method used for a first time for assessment of clinical samples could find application into the clinical practice for fast surfactant maturity diagnostics in prematurely born children regarding lifesaving therapy with exogenous surfactants administration. It also showed that lung cancer, hypoxia and inhalation anesthesia affect the biochemical and biophysical properties of AS which leads to changes in surface behavior. In addition, novel approach of pending drop method will be of great interest for the effective implementation of the procedure of whole lung lavage in the clinical practice.

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