Pulmonary surfactant – structure, metabolism and the role in RDS and ARDS treatment

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Abstract
Pulmonary surfactant is a complex mixture of lipids and proteins that, by modulating surface tension during breathing, prevents alveolar collapse at exhalation and overdistention at inspiration. Surfactant is synthesized by type II pneumocytes, assembled in lamellar bodies and secreted at the air-liquid interface of the alveolus. This surface-active material is re-uptaken by alveolar type II cells and eliminated by macrophages. Deficiency or dysfunction of pulmonary surfactant contributes to several respiratory pathologies, such as among other respiratory distress syndrome (RDS) in newborns and acute respiratory distress syndrome (ARDS) in children and adults. Surfactant replacement therapy in the treatment and prophylaxis of RDS substantially reduces mortality and morbidity in preterm infants. However, ARDS treatment trials involving adult patients have not brought about good results. The aim of the paper is to present structure, function and metabolism of pulmonary surfactant as well as an overview of publications on the use of exogenous surfactants in RDS and ARDS treatment.

Key words: surfactant, RDS, ARDS

Introduction
Pulmonary surfactant is a mixture of lipids and proteins synthesized by the alveolar type II epithelial cells (pneumocytes). It acts within peripheral part of lower airways, where it is playing an important role in the lung environment homeostasis [1-3]. Its lipid component mainly consists of bipolar phospholipids and it is responsible for lowering surface tension of the thin aqueous layer lining the respiratory epithelium of lungs [1, 4]. The protein constituent is composed of 4 proteins bearing their names according to the sequence of their discovery, namely: SP-A, SP-B, SP-C and SP-D (surfactant protein A, B, C, D). These proteins play a key role in surfactant metabolism regulation, leading to a complex and dynamic cycle of surfactant material at the air-liquid interface of alveoli [2-4]. Moreover, pulmonary surfactant proteins take part in local defence mechanisms and immunomodulation within the terminal portion of lungs [5-7].

The main function of surfactant is to stabilize gases in the alveoli and to increase the lung compliance [8]. Its deficiency causes respiratory distress syndrome (RDS) in premature neonates. The multiform defect of surfactant function also leads to the development of acute respiratory distress syndrome (ARDS) in both infants and adults [9-11]. Several attempts to find a cause of morphological changes accompanying RDS and ARDS and understanding of their development have encouraged clinicians to use exogenous surfactants in treatment. According to a number of publications, surfactant replacement therapy in newborns with respiratory disorders gave beneficial therapeutic results [12-14]. However, ARDS treatment trials involving adult patients revealed that exogenous surfactant may improve oxygenation but did not improve mortality. Future studies may potentially discover an approach to use exogenous surfactants in ARDS [9, 15, 16].

The structure and metabolism of surfactant
The alveolar-capillary barrier (so-called air-blood barrier) is the place in which main gas exchange in the lung takes place. These are unusually delicate structures made by the cytoplasm of alveolar type I cells and the capillary endothelium separated by the common basement membrane (fig. 1) [7, 17]. Pulmonary surfactant seems to be essential for the proper functioning of air-blood barriers. This lipid-protein complex constitutes a layer which separates the gas phase from the aqueous layer covering the alveoli (hypophasė) and reducing surface tension almost to zero. This protects the alveoli from collapse during expiration as well as prevents them from overdistention during inspiration [1-3]. Surfactant mainly consists of lipids (90 per cent of its mass). The major fraction is composed by phosphatidylcholine with saturated and unsaturated fatty acids. The most frequent substituents among the unsaturated fatty acids are
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palmitic and oleic acids [1, 3, 18]. The other components include phosphatidylglycerol, phosphatidylethanolamine, sphingomyeline, neutral lipids and glycolipids [11].

Surfactant proteins are divided into 2 groups: large and water-soluble SP-A and SP-D proteins and small, hydrophobic SP-B and SP-C proteins. SP-A and SP-D proteins belong to the calcium-dependent type of lectins [5, 19]. They are characterized by ability to bind to bacteria, viruses and other pathogens as well as to activate alveolar macrophages. Both mechanisms are of great importance to defense mechanisms of the lung [5, 6, 11]. The basic function of SP-B and SP-C proteins is to increase the stability of phospholipidic surface film [4].

Surfactant proteins and lipids are synthesized by the alveolar type II cells, which are also known as granular pneumocytes. A certain amount of surfactant (except SP-C) is also produced in Clara cells [1, 19]. Phospholipids are synthesized from the precursors like glycerol, fatty acids and choline in the smooth endoplasmic reticulum of aforementioned cells. Then they are moved to be converted in the Golgi apparatus in which lamellar bodies, serving as storages, are finally formed [1, 3]. The lipid synthesis ensues relatively quickly (approx. 1-2 hrs). The production and processing of surfactant proteins does not basically differ from the general pattern of protein production [1]. Glucocorticoids, prolactin, estrogenses, thyroid hormones and catecholamine exert stimulating influence on the surfactant constituents synthesis whereas fetal hyperinsulinism (caused usually by maternal diabetes), hypoxia and acidosis delay its production. Such conditions result in the frequent development of RDS in neonates [1]. Lamellar bodies are surrounded by a membrane round or oval special organelles where the surfactant material is assembled in series of densely packed bilayers as seen by electron microscopy [4, 20]. These structures begin to arise in the alveolar type II cells in the human fetus around 17-24 weeks of gestation – a long time before the alveoli are formed and lung are prepared for breathing [18].

Surfactant secretion occurs by means of exocytosis, during which the membrane limiting lamellar bodies undergoes a fusion with the apical plasma membrane of the type II pneumocytes. The process involves microtubules and other elements of the cytoskeleton (actin filaments among others) [1, 19]. The surfactant released from alveolar type II cells undergoes a number of morphological and functional changes ranging from transformation into tubular myelin to conversion into a lipid monolayer at the air-liquid interface (fig. 2). It is worth to notice that the above mentioned changes are mainly dependent on the interaction between lipids and proteins [4, 19].

Tubular myelin formation process needs the presence of calcium ions, phospholipids and SP-A, SP-B proteins [4, 15]. The research by Walski and co-workers showed that posttranslational blocking of protein synthesis with puromycine leads to irregularities in lamellar bodies as well as it contributes to the underdevelopment of myelin-like structures. At a longer period of puromycine treatment any tubular myelin structures were observed [21, 22].

Tubular myelin is the immediate precursor of a monomolecular surfactant form, which as an ultrathin film lines the surface of the alveolus [20]. The main constituent of the monolayer is dipalmitoylphosphatidylcholine (DPPC), which is a bipolar lipid (it has a hydrophilic ‘head’ and a lipophilic ‘tail’). A drop of this substance ‘spills around’ on the surface of water due to the polar group attraction by water molecules [17]. DPPC determi-
nes the stability of the surfactant monolayer irrespective of the changeable pressure parameters. SP-B and SP-C proteins seem to be essential to the transformation of tubular myelin into its monomolecular form [4, 23]. An electron microscope examination indicated that hydrophobic proteins show an ability to bound, aggregate and cut the phospholipid complexes. SP-A protein can play a role in monolayer formation as well, however it is much less effective [20].

Surfactant secretion may be stimulated or inhibited by physicochemical factors and by stimulating corresponding receptors. For instance, hyperventilation, temperature growth or calcium ions concentration have been proposed as possible inducers of surfactant secretion. A similar effect may be produced by stimulating cholinergic, beta-adrenergic, purine and vasopressin receptors [1, 18, 24]. Atelectasis cause a reduction of surfactant in atelecatic areas due to exhaustion abilities on its synthesis [1]. Also, its secretion seems to be inhibited by SP-A reabsorption in the alveolus [25].

Pulmonary surfactant is in a state of continuous change. There is a dynamical balance between the monomolecular film which lines the alveoli and the part of surfactant in the type II pneumocytes [1]. Surfactant is constantly removed from the alveoli surface through reuptake by the type II pneumocytes, where it can be recycled or degraded and reused to synthesize new amount of surfactant. Yet another phenomenon is its clearance by alveolar macrophages [5]. Clara cells do not engage in recirculation [6]. It is highly probable that lipid disintegration may also occur in the monolayer of surfactant [1].

The whole process of surfactant lipids exchange lasts from 5 to 10 hours. The rate of recirculation depends on fraction and a morphological form of surfactant. The uptake of extracellular surfactant is stimulated by SP-A, SP-B, SP-C proteins and phosphatidylglycerol. The amount of recycled surfactant components is dependent on the lung aeration (after birth 90 percent of surfactant undergoes recirculation) [1].

SP-A protein is essential for regulating the recycling of surfactant. According to its C-type lectin activity SP-A can bind to the carbohydrate groups on the type II pneumocytes. It facilitates the uptake of phospholipids by both type II pneumocytes and alveolar macrophages and protects surfactant lipids from degradation in type II cells [5, 25]. It is worth mentioning that SP-A is uptaken by the alveolar type II cells and incorporated into lamellar bodies independently [1, 25].

A small amount of surfactant is permanently removed from the lung through airways and circulatory system. The clearance may take place in alveolar macrophages and is connected with their migration from lung tissue. Moreover, surfactant may be removed as a result of ciliary movement, which causes the part of material to be moved into trachea and then into the esophagus [1, 26].

The use of surfactant in RDS and ARDS treatment

Exogenous surfactant treatment was introduced into clinical practice by Fujiwara and co-workers, who administered it to neonates suffering from breathing disturbances [12]. Since then, a number of published clinical reports have proven the effectiveness of surfactant replacement therapy [9, 13] The use of surfactants combined with mechanical ventilation has made it possible for premature babies weighting merely 750 g to survive [1]. There were ahead published research involved a range of different preparations, including so-called synthetic surfactants as well as semi-natural preparations. Synthetic surfactants being chemically-synthesized substances contained a mixture of lipids, which were supposed to approximately match the basic composition of alveolar surfactant. Semi-natural preparations were derived from animals and contained not only lipids but also some naturally-found proteins [3, 10]. At present, production of natural surfactants preparation from amniotic fluid collected during cesarean sections in full-term pregnancy, have become a history. Although they contain all the essential phospholipids and proteins, for single therapeutic dose it is needed up to 1.6 l of amniotic fluid [10, 14, 27].

Over the last years various methods for surfactant administration into trachea have been developed [3]. The most frequent one is to deliver surfactant in a bolus through the endotracheal intubation during artificial ventilation or soon after the respirator has been removed. In case the ventilator is not used simultaneously, an Ambu bag has to be used to assure an even distribution of surfactant [3, 28]. It seems essential to adjust surfactant administration to a given breathing cycle phase so as to avoid reverse flows into the trachea [1]. Usually, one dose of surfactant is recommended to be administered. However, if the patient is kept under controlled respiration in addition to being given oxygen, it may be necessary to administer another dose after 12 hours. The OSIRIS Collaborative Group studies have indicated that there is no need to continue surfactant administration to infants who show RDS symptoms after two standard doses have been applied [13].

At the beginning of the “surfactant era” the preparation was given only to neonates with RDS confirmed by both chest radiography and biochemical tests for the
maturity of lung tissue. At present surfactant is more and more often used prophylactically in neonates at risk of severe RDS, it means born before 28 and after 28 week of gestation with a confirmed immaturity of lungs [28]. As prophylactic treatment we describe instillation of the drug within 5 to 30 minutes after birth, very often in the delivery room. The infant must be intubated and stabilized so that surfactant can be administered. As it was published earlier neonates with a 5-minute Apgar score under 3 are not suitable for preventive treatment [28]. Clinical trials have shown that prophylactic surfactant therapy brings a number of benefits. For instance there is a decrease in the requirement for ventilatory support in the first days of life as well as a reduction in the incidence of air leak syndrome [13, 28]. However, intratracheal surfactant administration to newborns induces a number of technical problems. There were described of administering preparation to not a fully-stabilized patient, without radiographic verification of endotracheal tube placement. Another disadvantage of the method is an impossibility to verify acid-base equilibrium before administration [28]. Amniotic puncture are routinely carried out for estimation of fetal lung maturity so the procedure seems to be safe for the mother and her baby. The trials to administer surfactant prenatally directly into amniotic fluid as a new RDS preventive treatment are made. The results from animal and observational human studies have seemed promising, however there is no current evidence from randomized controlled trials to guide the use of intraamniotic instillation of surfactant for women at risk of preterm birth [3, 29, 30].

The optimistic results of surfactant treatment in neonates have encouraged clinicians to employ a similar therapy for children and adults with ARDS. ARDS is a respiratory failure caused by various factors that directly and indirectly damage the lung (table 1). Inflammatory process in lungs causes pulmonary surfactant dysfunction, which finally leads to alveolar flooding and atelectasis [31, 32].

Trials involving the use of synthetic surfactant (Exosurf) in ARDS, conducted by Anzueto and co-workers, did not produce expected results [33]. In the studied group, neither mortality rate decrease nor peripheral blood oxygenation improvement were found. The reason for failure of such therapy may have been the use of protein-free surfactant. The presence of proteins, as it was mentioned above, is essential for proper surfactant spreading on the alveoli surface. Undoubtedly, the method of surfactant delivery used by Anzueto and co-workers was meaningful for results of therapy [1, 33]. The exogenous preparation was administered in the form of aerosol and as researches estimated less than 5% of the dose reached the lungs [33]. The effects produced in Walmarth’ trials show that better results may be achieved by instilling surfactant with a bronchoscope directly into segmental bronchi [34].

Meta-analysis conducted by Davidson and co-workers reveal that exogenous surfactant may improve oxygenation but did not improve mortality of ARDS patients. One potential explanation for the lack of effectiveness of surfactant therapy is that ARDS often develops in association with many serious medical disorders, which dramatically influences the prognosis [16]. The most frequent cause of mortality among patients with ARDS is not the respiratory failure itself, but a primary disease. It seems that surfactant replacement therapy may play an important role in treatment of ARDS caused by factors that directly damage the lung [16, 32, 35].

Besides, we should take into consideration that the pathophysiology of ARDS is complex, involving a variety of insult leading to neutrophil infiltration, pulmonary fibrosis and increased alveolocapillary permeability [36]. Several in vitro-studies show that neutrophil elastase can damage surfactant proteins and impaired the function of surfactant [37]. This finding has important implication for surfactant therapy in ARDS. Advanced cases might require bronchoscopic focal lavage to remove plasma proteins and inflammatory mediators prior to surfactant instillation to areas of the great needs [11, 36].

Another important issue is proper dosage of exogenous preparation. In ARDS patients, as mentioned above, the triggering of pro-inflammatory cascades leads to a markedly reduction of surfactant activity by different mechanism, for example degradation of lipids and proteins by lipases and proteases. Supplementation with

### Table 1. Disorders associated with the development of the acute respiratory distress syndrome (ARDS) (modification from [31])

<table>
<thead>
<tr>
<th>Extrapulmonary causes of ARDS</th>
<th>Pulmonary causes of ARDS</th>
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<tbody>
<tr>
<td>Sepsis</td>
<td>Pneumonia (bacterial, viral, by fungi, parasitical)</td>
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<tr>
<td>Disseminated intravascular coagulation</td>
<td>Aspiration of gastric content</td>
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<tr>
<td>Severe trauma with shock</td>
<td>Fat emboli</td>
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<td>Multiple transfusion</td>
<td>Lung contusion</td>
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<tr>
<td>Acute pancreatitis</td>
<td>Near-drowning</td>
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<tr>
<td>Drug overdose</td>
<td>Inhalational injury</td>
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<tr>
<td>Malaria</td>
<td>(toxic gases)</td>
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<tr>
<td>Severe burns</td>
<td>Reperfusion pulmonary edema</td>
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<tr>
<td>Intracranial hypertension</td>
<td>Mountain sickness</td>
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<tr>
<td>Extracoporeal circulation</td>
<td>Thorax irradiation</td>
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exogenous surfactant in ARDS requires enough amount of preparation to reduce inactivation [9]. On the other hand, the trials on animals by Wąsowicz and co-workers indicated that an excessive amount of surfactant per surface unit may lead to air-blood barrier damage and activation of the inflammatory process [17]. In addition numerous of studies demonstrated that synthetic preparations induce more adverse effects in lungs compared with semi-natural surfactant preparations [17, 23].

Recent intensive clinical research has led to the development of new surfactants, which can enhance the therapeutic potential of treatment patients with ARDS. These new generation of preparations are made of synthetic peptides that mimic the function of hydrophobic proteins SP-B and SP-C combined to phospholipids [9, 38]. The results of two randomized control trials with use of recombinant SP-B analog called KL4 seem promising [39]. Moreover phospholipase-resistant phospholipid analogues have been designed, synthesized, and tested as potential components in new preparation [9]. It is likely that proteins containing synthetic surfactants with highly reproducible composition, produced in large amount at a low cost, will become available in the near future [9, 38].

Conclusion
The major function of surfactant is to lower surface tension at the air-liquid interface in alveoli and terminal conducting airways, which prevents alveolar collapse at exhalation and overdistention at inspiration.

Administration of surfactant to newborns with RDS has been established as appropriate preventive and treatment therapy. However ARDS treatment trials involving adult patients have not brought about expected results. New preparation, dosage and methods of administration are among the issue that may improve the efficiency of surfactant treatment. New well planned laboratory studies as well as clinical trials are expected.

References
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