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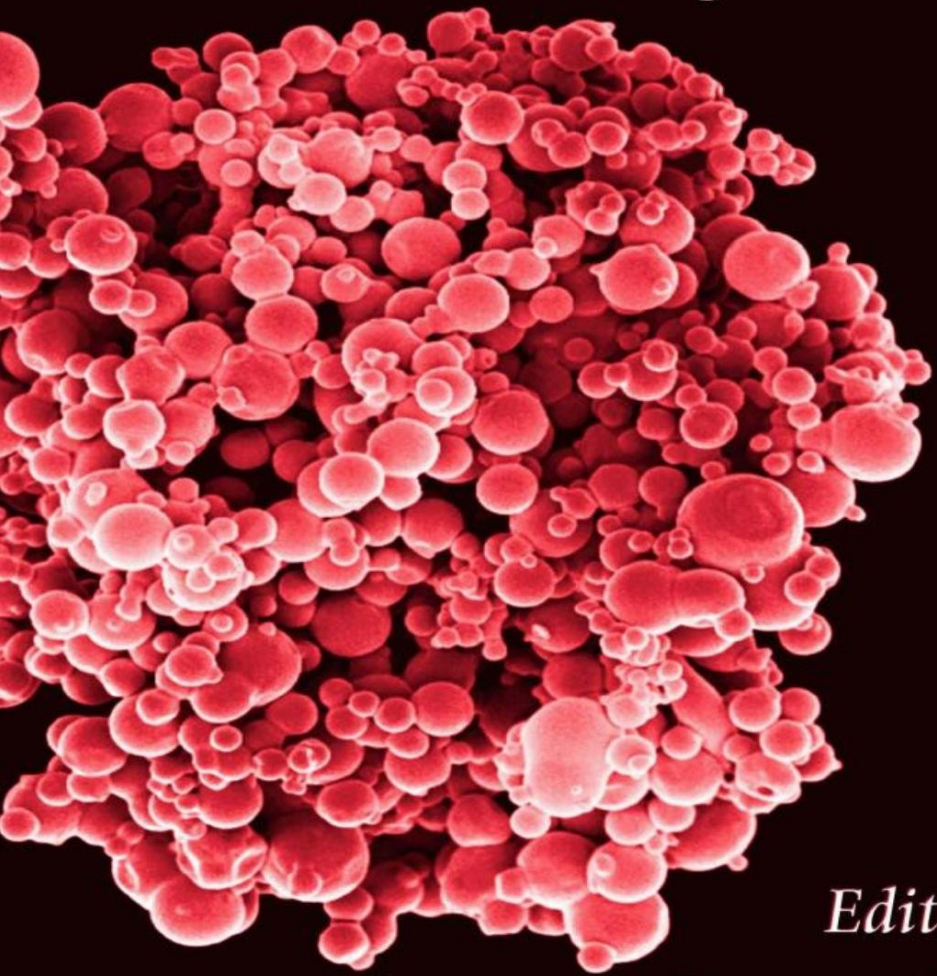

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Lung Surfactants: Correlation Between Biophysical Characteristics, Composition, and Therapeutic Efficacy

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INTRODUCTION

Lung surfactant is a lipoprotein complex covering the alveolar epithelial surface of the lungs (1). It was discovered about 50 years ago when the pathogenesis of respiratory failure, which some premature newborns suffered from immediately after birth, was being investigated. In 1959, Avery and Mead (2) first found out that bronchoalveolar lavage (BAL) fluid of newborns with the disease of hyaline membrane, which is now known as respiratory distress syndrome in infants (IRDS), lowered surface tension less than BAL of healthy newborns.

Lung surfactant is synthesized in type II pneumocytes, stored in the lamellar bodies (LBs), and secreted to the alveolar space (3). It reduces the surface tension at the air–water interface from 72 mN/m to 20 to 25 mN/m and makes alveolar ventilation and gas exchange possible preventing alveoli from collapsing, i.e., it ensures respiratory mechanics. Surfactant also prevents pulmonary edema formation and provides host defense properties in the lung.

Abnormalities of pulmonary surfactant system have been described in IRDS (2), acute lung injury (ALI), and acute respiratory distress syndrome (ARDS) (4–6), pneumonia (7–10), cystic fibrosis (11,12), idiopathic pulmonary

fibrosis (13,14), atelectasis (15), radiation injury (16), asthma (17–23), chronic obstructive pulmonary diseases (COPD) (24), sarcoidosis (25), tuberculosis (26,27), and others (24). The surfactant system undergoes both qualitative and quantitative alterations. In ARDS, the main biochemical abnormalities comprise an 80% fall in the total phospholipids (PLs), decrease in comparative content of dipalmitoyl phosphatidylcholine (DPPC), phosphatidylglycerol (PG) and other lipid fractions, and loss of surfactant-associated proteins (5). Surfactant function is also inhibited by leaked plasma proteins, oxygen radicals, and proteases in the alveolar compartment.

In 1980, Fujiwara et al. (28) first demonstrated high therapeutic efficiency of PL extract from bovine lung with the addition of palmitic acid (PA) and DPPC in IRDS. Surfactant therapy of IRDS is considered to be one of the major advances in neonatology in our time. About 10 preparations of lung surfactant have been developed and applied for IRDS treatment. This success induced the attempts of application of exogenous surfactants in the treatment of ALI/ARDS and other lung diseases. However, clinical trials in ARDS have had rather conflicting results (29). Parallel with efficient usage of surfactants (30,31), some studies did not result in any improvement in either oxygenation or survival (32). Among the reasons for the failure can be different etiology of ARDS (33), late surfactant administration (31), wrong dose (33), mode of delivery (32,34,35), difference in the surfactants themselves, and mistakes in planning and conducting of clinical trials (36).

In this article, we have made an attempt to analyze the experience in the clinical application of exogenous lung surfactants and discuss some conditions of their usage whose observing or neglecting can lead to success or failure of the treatment. We have tried to answer the questions of what is an ideal formulation of pulmonary surfactant and what is the mode of its application for the treatment of different lung diseases.

BASIC BIOPHYSICAL AND BIOCHEMICAL PROPERTIES OF THE LUNG SURFACTANT SYSTEM

Composition of Lung Surfactant

The composition of the surfactant may vary with such factors as species, age, lung compartment, disease states, diet, method of isolation, and so on (37). Surfactant isolated from lung BAL of healthy mammals consists of about 90% lipids and 10% proteins. Ten percent to twenty percent of the lipids are neutral and the remaining 80% to 90% is PL. About 80% of PL is phosphatidylcholine (PC), about 50% to 60% of PC is DPPC, and about 10% of PL is PG. There are also small quantities of phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), and sphingomyelin (SM) (1,37–39).

About a half of protein fraction of surfactant is composed of four surfactant-associated proteins: SP-A, SP-B, SP-C (40), and SP-D (41).

Whereas SP-B and SP-C are extremely hydrophobic low-molecular-weight proteins, SP-A and SP-D are hydrophilic high-molecular-weight proteins from the protein family of collectins. SP-A represents 4% of surfactant and SP-B and SP-C each make up less than 1% (37).

The lipid and protein components of the surfactant are assembled and packaged in type II cells as LB, which are then secreted into the airspace and form tubular myelin, the direct precursor to the surfactant film at the air–liquid interface. LB and tubular myelin are dense forms of alveolar surfactant. The less dense and smaller aggregates of surfactant are formed during respiratory motion. They are taken up by type II cells or by macrophages, which results in a consistent ratio between functionally active large surfactant aggregates and dysfunctional small aggregates in normal lung.

Functions of Lung Surfactant System

Initially, surfactant was thought to be a key player only in the biophysical behavior of the lung. It is known that during the cycle of inspiration and expiration, fast and repeated alteration of alveolar surface size and, correspondingly, the area of surfactant cover occur. The surface tension of water which covers glicocalex of alveolar cells is 72 mN/m. Surfactant adsorption on alveolar surface decreases the surface tension to 23 mN/m, which facilitates the work of breath and provides respiratory mechanics (42).

Experimental data in vitro (42) and in vivo (43–45) shows that the surface tension at compression (expiration) falls to about 0 mN/m at the water–air interface (42). However, both we and other investigators have been confused by the lack of physical sense in this finding (46). We think that the following statements can explain surface phenomenon in inspiration/expiration cycle more profoundly. The quantity of surface-active molecules in water phase of alveoli is much more than necessary for monolayer formation on the air–water interface. Therefore, the molecule adsorption on the surface is maximum, and the surface tension coincides with one on the PL–air interface and is about 25 mN/m (42,47,48). Furthermore, many experimental data show that the surfactant film on the air–water interface may consist (probably partly) of not one but three layers (42,49,50).

The high concentration of surfactant molecules on the interface means that when the surface area decreases, they come tightly to each other; and on reaching the tightest packing, repulsive force will result in exertion in the film, which will compensate the force compressing the surface. In rheology, it is named concatenation of viscosity and elasticity. The force that compresses the surface is surface tension on air–water interface in alveolar (25 mN/m after adsorption). At pressure reduction (expiration), this force tries to reduce the surface. Finally, elastic stress will balance the surface tension force, and the resulting "force" will be equal to zero. This is the resulting surface force, which is measured as surface tension. Surfactant surface

tension cannot be less than 25 mN/m, (PL surface tension on air–water interface), while the resulting surface "force" can fall to zero. Because surfactant film is not solid, its molecules are squeezed out of the surface of the water phase. Surfactant bilayer located under the monolayer may prevent molecule squeezing out and increase the stability of the film.

When the surface area is the least at expiration, and surfactant film is in the condition of its maximum compression, the force of elastic tension is practically completely balanced by surface tension force and resulting "force" is equal to zero. Therefore, there are no reasons for the following reduction of alveolar surface and its collapse. The available data on surface forces in surfactant films on air–water interface can be explained by this concept.

Although stabilizing the lungs is undoubtedly the major physiological function of surfactant, there is evidence that surfactant system may also serve other functions: it affects the permeability of the alveolar–capillary barrier to soluble compounds (51) and contributes to innate and adaptive immunity of the lung. Surfactant proteins act as a first-line defense against invading microorganisms and viruses (51–53). Moreover, they possess binding capacity for aeroallergens, highlighting the possible role of the pulmonary surfactant system in allergic diseases such as asthma (54,55).

Every component of surfactant complex plays its own role in polyfunctional surfactant activities. The key element in all pulmonary surfactants, DPPC, is considered to be the most important component with respect to its biophysical function (56). Anionic PL, especially PG, are responsible for modulating the properties of surfactant interfacial films, improving their stability during compression, and facilitating the adsorption and refining of PL on the air–lipid interface. PG can stimulate uptake of liposomal PC by type II cells (57). PA interacts with DPPC and/or SP-B to increase the movement of surfactant from the subphase and to stabilize the surfactant complex at the air–water interface (58–61). Cholesterol may play an important role in the lateral phase organization of surfactant structures (62).

Of particular interest are the specific surfactant-associated proteins that control the normal lifecycle of endogenous surfactant. SP-B and SP-C are mainly important for the biophysical properties of surfactant. SP-A and SP-D contribute essentially to host defense, which is realized in two ways: interaction with potentially injurious agents and alteration of the behavior of immune cells (63). SP-A and SP-D bind various microorganisms (64,65), lipids, and other exogenous substances. They stimulate alveolar macrophages (AM) (5,65–68) and influence the behavior of mast cells, dendric cells, and lymphocytes (69). SP-A inhibits the maturation of dendric cells, whereas SP-D enhances the ability of the cells to take up and present antigen, thereby enhancing adaptive immunity. SP-D may reduce the number of apoptotic cells (70,71). Transgenic models (SP-A null mice and SP-D null mice) demonstrates the importance of these proteins in the setting of bacterial and virus pneumonia (72). SP-A and SP-D have differential roles in modulating

the inflammatory response to noninfectious lung injury (73). The overall effect of SP-D might be anti-inflammatory, whereas SP-A can contribute to both pro- and anti-inflammatory activity.

SP-B and SP-C play an important role in lung mechanics. Genetic deactivation of the SP-B gene induces irreversible and lethal respiratory failure both at birth (74,75) and in adults (76) due to incapability to maintain an opened respiratory surface. However, the controversial role of SP-B in monolayer refining and formation of a DPPC enriched layer is being discussed. It is thought now that SP-B brings lateral stability to the DPPC-rich monolayer of PL by both electrostatic and hydrophobic interactions (77). The analysis of the structure of lipid films at the nanoscopic level suggests that SP-B and SP-C alter the structure of surfactant films to optimize film rheological behavior under the dynamic conditions imposed by the lungs (78,79). Besides SP-A, SP-B is necessary for the formation of tubular mielin from secreted LB material. SP-B plays a role in host defense of the lung together with SP-A (80–82). SP-C, the smallest pulmonary surfactant-associated polypeptide, can have several functions: it contributes to the formation and dynamics of surfactant films at the air–liquid interface (83,84), prevention of surfactant inactivation by serum proteins, modulation of surfactant PL turnover, and binding to bacterial lipopolysaccharides (LPS).

ABNORMALITIES OF LUNG SURFACTANT IN DIFFERENT PATHOLOGIES

Infant Respiratory Distress Syndrome

The surfactant deficiency in IRDS results in direct biophysical consequences, i.e., high abnormalities in the mechanical properties of the respiratory system (84). There is evidence that variation in the level of surfactant-associated proteins expression or genetic variation in their genes is associated with IRDS (85) and congenital pulmonary alveolar proteinosis (86,87).

Acute Respiratory Distress Syndrome

ARDS described in 1967 by Ashbaugh et al. (4) can develop after the action of both direct injurious factors such as pneumonia, aspirated toxic agents, gastric contents, and others (direct ARDS), and as a result from inflammatory processes due to numerous systematic disorders such as sepsis, multitrauma, multiple blood transfusions, and others (indirect ARDS). It is associated with biochemical and biophysical abnormalities in the surfactant. In ARDS, marked increase in alveolar surface tension is observed. It resulted from a lack of surface-active compounds, changes in PL, fatty acid, neutral lipid, and surfactant-associated proteins; loss of the surface-active large surfactant aggregate fraction; inhibition of surfactant functions by leaked plasma proteins, inflammatory mediators, oxygen radicals, and

proteases in the alveolar compartment; incorporation of surfactant PL and proteins into polymerizing fibrin (4,6,39,88,89).

The studies of the PL composition of bronchoalveolar lavage fluid (BALF) samples from patients with ARDS discovered the overall reduction of PL content; significant change in the distribution of PL classes including a marked decrease in PG, increase in the portion of the minor components (PE, PS, PI, and SM), and reduction of PC; significant decrease (to about 80% of control values) of the portion of PA, and the increase of the portion of unsaturated fatty acids in PL; nearly twice reduction of DPPC (6,88–90). In ARDS, a significant decline of SP-A, SP-B, and SP-C but not of SP-D was demonstrated (6,88,90,91). SP-A and SP-B levels remained decreased at least within 14 days after ARDS beginning (91).

Surfactant disturbance also involves some abnormalities at the higher levels of its structural organization. In model lung injury and ARDS (6,92,93), an increase of smaller surfactant aggregates occurs. It is paralleled by a loss of SP-B and surface activity. The increase in air–blood barrier permeability in ARDS causes plasma protein leakage into alveolar space. Among them, albumin (94,95), hemoglobin (96), and particularly fibrinogen or fibrin monomers (95–99) have strong surfactant-inhibitory properties. The presence of SP-B and SP-C in physiologic quantities reduces the sensitivity of surfactant to fibrinogen inhibition (99,100). The process of fibrinogen polymerization in surfactant presence results in loss of surfactant PL from the soluble phase due to their binding to fibrin strands, which is accompanied by the complete loss of surface activity in these areas (101,102). The surface activity can be largely restored by adding fibrinolytic agents (103,104).

Other mechanisms leading to surfactant dysfunction include nitration of some surfactant-associated proteins (particularly SP-A), degradation of surfactant lipid components due to increased phospholipase activity, and direct oxidation of surfactant (92).

The abnormalities of lung surfactant in ARDS cause dramatic pathophysiologic changes: alteration in lung mechanics, alveolar instability, atelectasis, and the decrease of lung compliance, which results in impairment of gas exchange (105), and a decreased resistance to secondary lung infection. Although the exact contribution of individual surfactant component to the alveolar host defense system is not completely clear, the marked decrease in SP-A content (6,89–91) and the evidence of degradation of SP-A in vivo in the lungs of ARDS patients (106) suggest a loss of opsonizing capacity to pathogens (90,107).

Very few data are available on the influence of surfactant treatment on biochemical and biophysical parameters of surfactant in ARDS (35,108). BALs were performed three hours prior to, and 15 to 18 hours and 72 hours after, surfactant administration to the patients and healthy volunteers (35). Surfactant treatment resulted in a marked increase in the lavagable PL, but predominance of the alveolar surfactant-inhibitory proteins was still

encountered. Essential or even complete normalization of the PL profile, large surfactant aggregates fraction, SP-B and SP-C (but not SP-A) content, and the fatty acid composition of the PC was noted. So, surfactant administration in severe ARDS causes restoration of surfactant properties.

Asthma

Accumulating data indicate that airway obstruction, which is thought to be caused by smooth muscle constriction, mucosal edema, and secretion of fluid into the airway lumen, may partly be due to a dysfunction of pulmonary surfactant (54,55,109,110). Surfactant obtained from BAL and sputum of patients with asthma has decreased surface activity and changes in composition (17). It has been shown in animal models of asthma that though the change in the amount of surfactant is little, it may be in a less functional form (111). Cheng et al. (112) demonstrated that, in a guinea-pig model of chronic asthma, the surfactant pool size and content of large surfactant aggregates was decreased.

Pneumonia

The surfactant in BAL fluid from patients with pneumonia has reduced PC and PG content, and alterations in fatty acid composition. These changes are qualitatively similar to those registered in patients with ARDS. The amount of SP-A is also decreased and the surfactant surface tension lowering function is disturbed, partly due to the alterations in lipid components (6). As found in other conditions, where hydrophilic surfactant protein content is diminished, host defense functions may be impaired.

Tuberculosis

In experimental tuberculosis model (26), it was shown that the neutral lipids increase in BAL, whereas the total PL decreases. The enhancement of the permeability of endothelia and alveolar cell membranes results in intracellular edema and liquid leakage into alveolar space. Metabolic processes in type II cells and, therefore, the synthesis and recycling of new surfactant are disturbed, resulting in its deficiency. The functions of AM are also impaired: incomplete phagocytosis results in *Mycobacterium tuberculosis* persisting in AM. Antituberculosis drugs usually stop inflammation development in tuberculosis animal model, but long application of these drugs, for example, the combination of isoniazid, rifampicin, and ethambutol, causes disturbances of biosynthetic processes in type II cells (26).

Surfactant abnormalities often result in very severe consequences, even death. So the attempts to stop this process by means of surfactant administration seem to be quite promising and a logical way for the treatment of these pathologies.

EXOGENOUS LUNG SURFACTANTS AND METHODS OF OBTAINING THEM

Available preparations of lung surfactant can be divided into two types: the preparations made of synthetic compounds and the preparations of natural origin (Table 1).

Synthetic Preparations of Lung Surfactant

The design of synthetic preparations is based on the studies of the functions of different surfactant components with following construction of the

Table 1 The Preparation of Lung Surfactant

Chemical name	Trade name	Source	Specific proteins
Synthetic surfactants			
Pumactant	ALEC		None
Colfosceril	Exosurf		None
KL4, sinapultide, lucinactant	Surfaxin		Synthetic peptide KL4
rSP-C, lusupultide	Venticute		Recombinant SP-C
Natural surfactants			
Nonmodified surfactants			
SF-RI1	Alveofact	Lavaged bovine lung	SP-B, SP-C
Surfactant-BL	Surfactant-BL	Minced bovine lung	SP-B, SP-C
Calfactant	Infasurf	Lavaged bovine lung	SP-B, SP-C
Modified surfactants			
Surfactant TA	Surfacten	Minced bovine lung	SP-B, SP-C
Beractant	Survanta	Minced bovine lung	SP-B, SP-C
Poractant alfa	Curosurf	Minced porcine lung	SP-B, SP-C
HL-10	Surfactant HL-10	Minced porcine lung	SP-B, SP-C
CLSE	BLES	Lavaged bovine lung	SP-B, SP-C
Human surfactant			
Amniotic fluid derived	Amniotic fluid derived	Amniotic fluid	SP-A, SP-B, SP-C
Surfactant-HL	Surfactant-HL	Amniotic fluid	SP-B, SP-C

Abbreviations: CLSE, calf lung surfactant extract; BLES, bovine lipid extract surfactant; SP, surfactant-associated proteins.

preparations from the substitutes that can be obtained easier, cheaper, or safer. The ability of surfactants to decrease surface tension and increase oxygen concentration in blood was thought to be its most important function. Four synthetic preparations are known: Exosurf, ALEC, Surfaxin, and Venticute.

Exosurf (Glaxo-Wellcome, Inc., Research Triangle Park, North Carolina, U.S.A.) is a protein-free preparation devised by J Clements. It is composed of 85% DPPC, 9% hexadecanol, and 6% tylaxopol, in the form of powder. DPPC serves biophysical functions of surfactant, whereas hexadecanol imitate the functions of surfactant proteins, PG, and other lipids to some degree (37). Hexadecanol facilitates secondary spreading and sorption of DPPC on liquid surface. Tylaxopol is a strong detergent, that contributes to DPPC dispersion. The preparation is delivered at a dose of 67.5 mg/kg body weight. Now, Exosurf marketing is very limited.

ALEC (Pumactant, Britannia Pharmaceutical, Redhill, Surray, U.K.) is a protein-free surfactant composed of DPPC and PG in weight ratio 7:3 (113). It was usually used as a suspension in physiological solution, in two to four doses, 100 mg in 1 to 1.2 mL each.

Surfaxin (KL4, Discovery Laboratories, Doylestown, Pennsylvania, U.S.A.) is a suspension in 0.9% NaCl containing DPPC and palmitoyl-oleoyl-PG in the ratio of 3:1, 15% of PA and 3% of synthetic SP-B-like peptide, Sinapultide. The latter is amphiphathic helix of repeated subunits of one lysine and four leucines (114). The manufacturing method is the following. First, the peptide in the mixture of chloroform/methanol (1:1) is added to the mixture of DPPC and PG (1:10), heated up to 43°C, and dried either in N_2 current or under vacuum. Dried sediment is then resuspended in water at 43°C, added NaCl up to 0.9%, and incubated during one hour. The mixture can be exposed to several cycles of freezing and thawing.

Venticute (Byk Gulden, Kinslum; Atlanta Pharma, Konstanz, Germany) contains 1.8% of rSP-C, 63% of DPPC, 28% of palmitoyl-oleoyl PG, 4.5% of PA, and 2.5% of $CaCl_2$ after suspension in 0.9% of NaCl. rSP-C is a sequence of 34 amino acids and differs from human SP-C by amino acid substitutes. Phenylalanine in four and five positions of amino acid sequence of native protein substitutes for cysteine, and isoleucine substitutes for methionine. These substitutes are made to intensify the interaction between rSP-C and PL, stabilize the film at the air–water interface, and finally prevent molecular aggregation (115).

Surfactant Preparations of Natural Origin

The preparations of natural origin can be divided into two subgroups: modified natural surfactants (Surfacten, Survanta, Curosurf, and Surfactant-HL-10) and nonmodified natural surfactants [Alveofact, Infasurf, bovine lipid extract surfactant (BLES), Surfactant-BL, Surfactant-HL, and human surfactant from amniotic fluid]. They are obtained from bovine and

porcine lungs or from human amniotic fluid and contain surfactant-associated proteins and all classes of PL.

Modified Natural Surfactant Formulations

Surfacten (Surfactant TA, Tokyo Tanabe, Japan) is the first commercial preparation of lung surfactant developed by Fujiwara et al. in 1980 (28). To obtain Surfacten, the cow lungs are minced and extracted by organic solvents. Ballast proteins, neutral lipids, and nonlipid admixture are removed. Then the product is modified by adding DPPC, PA, and triglycerides. The final freeze-dried product contains 48% DPPC, 16% unsaturated PC, SM, 4% triglycerides, 8% fatty acids, 7% cholesterol, and about 1% SP-B and SP-C. Surfacten is administered as a sonicated emulsion at a dose of 100 mg/kg body weight, in concentration of 25 mg/mL of PL. Electron microscopy of pellets of Surfacten demonstrates heterogeneity of forms comprising lamellae, vesicles of different sizes, and amorphous substance resembling protein (116). Surfacten is marketed in Japan and Southeast Asia.

Survanta (Beractant, Abbot Ltd., Ross Laboratories, Columbus, Ohio, U.S.A.) is modified Surfacten. It is a natural bovine lung extract comprising PL, neutral lipids, fatty acids, and SP-B and SP-C with the adding of DPPC, PA, and tripalmitin for improving tension-lowering properties and standardizing the finished product. Unlike Surfacten, Survanta is produced as a frozen suspension. The preparation contains 25 mg/mL PL (including 11.0–15.5 mg/mL DPPC), 0.5–1.75 mg/mL triglycerides, 1.4–3.5 mg/mL free-fatty acids, no cholesterol, and less than 1% proteins. Electron microscopy shows that the preparation consists of about 55% crystals and 45% lamellar-vesicular forms. It is administered at a dose of 4 mL/kg (37).

Curosurf (Poractant alfa, Chiesi Farmaceutichi S.P.A., Parma, Italy) is a surfactant from porcine lungs. Its production consists of several stages: water–salt extraction of minced porcine lung, centrifugation, chloroform–methanol extraction, and liquid–gel column chromatography on Lipidex-5000. The fraction of polar lipids is resolved in chloroform and filtered consecutively through filters of 0.45 and 0.2 μm. After the removal of organic solvent, the sediment is suspended in 0.9% NaCl with sodium bicarbonate (pH 6.2) and sonicated at 50 W, 40 kHz. Curosurf contains about 99% polar lipids (30–35% DPPC) and about 1% SP-B and SP-C in the ratio of 1:2. Neutral lipids and cholesterol are removed (117), that is why it is considered to be modified natural surfactant. The finished product is 1.5 or 3 mL emulsion with PL concentration of 80 mg/mL. Ninety percent of Curosurf emulsion is the particles with the size less than 5 μm. It is used at a dose of 120 to 200 mg/kg (118).

Nonmodified Natural Lung Surfactants

The surfactants from human amniotic fluid—one developed in the United States (California) (119) and Surfactant-HL (Biosurf, Russia)

(120,121)—are the closest to the pulmonary surfactant in situ. The former surfactant contains surfactant PL and SP-A, SP-B and SP-C, whereas Surfactant-HL contains PL and SP-B and SP-C. Although these preparations were efficient in clinical trials, they are not produced because of the difficulty with obtaining raw material.

Alveofact (SF-RI 1, Thomae GmbH, Biberach/Riss, Germany) is a chloroform–methanol extract of BAL bovine lung. It comprises 88% PL, 4% cholesterol, 8% other lipids, and 1% SP-B and SP-C. It contains relatively higher amount of SP-B (37).

Infasurf (Calfactant, Forrest Labs, St. Louis, Missouri, U.S.A.) is a chloroform–methanol extract of neonatal calf lung lavage. It comprises 35 mg/mL PL, 55% to 70% of which is saturated PC, SP-B, and SP-C (122).

BLES (BLES Biochemicals, Inc., London; Ontario, Canada) is isolated by organic extraction of bovine lung lavage. It comprises 63% saturated PC, 32% other PL, 2% SP-B and SP-C, and no neutral lipids (37,123).

Surfactant-BL (Biosurf, St. Petersburg, Russia) is isolated from bovine lung. Its manufacturing consists of the following stages. Lung tissue is homogenized to pieces with the side sizes not more than 5 mm in the stream of 0.9% NaCl. Debris and cells are removed by centrifugation. Supernatant is frozen at -20°C and thawed at $+4^{\circ}$C to increase the size of surfactant aggregates, which allows raising the output of intermediate material. The suspension is centrifuged, at $10{,}000 \times g$, for 30 minutes, at $+4^{\circ}$C. The precipitate is resuspended in water and extracted by chloroform–methanol mixture (124). The lower phase of two-phase system is collected, organic solvents are removed by rotary evaporation, the dry residuum is resuspended in water, and lyophilized. The preparation comprises 75% to 80% PL, 5% to 6% neutral lipids, 9% to 11% free cholesterol and its ethers, 1.8% to 2.5% SP-B and SP-C, and 3% to 4% nonidentified components. It should be mentioned that it contains all classes of PL of natural surfactant: PC, 62% to 70% of all PL (66% of PC is DPPC); lysolecithine, 1.1%; SM, 9.7%; PE, 13%; PI + PS, 6.8%; PG + diphosphatidyl glycerine, 5%; and nonidentified lipids containing phosphorus, 1.6%. The group of neutral lipids (5–6%) comprises triglycerides (4.5–5.5% of PL), diglycerides (1%), and free-fatty acids (the quantity is not estimated). Electron microscopy of the emulsion of Surfactant-BL shows that the preparation consists of aggregates of 1.6 to 1.8 μm, which in their turn are formed by 0.2 to 0.5 μm vesicles and does not contain crystal structures. We think that the presence of the aggregates shows the nativity of the preparation because they derive from self-assembly (121). The preparation is permitted for newborns and adults. The dose of Surfactant-BL is 75 mg/kg body weight for newborns and 6 mg/kg every 12 hours for adults with ARDS. Two to three administrations are usually enough for the course. Surfactant-BL is marketed in Russia.

The presented data show that available commercial preparations of lung surfactants vary a lot in their composition and properties.

Methods of Obtaining Lung Surfactants

The properties of surfactant preparations very much depends on the approaches applied for their obtaining. Synthetic surfactants are produced by mixing PL, usually from soy (DPPC, palmitoyl-oleoyl PG), with long-chain spirits (hexadecanol) and emulsifiers (tylaxopol), and in some cases synthetic peptide KL-4 (Surfaxin) or rSP-C (Venticute). To obtain Surfaxin, a peptide is added to PL in the mixture of chloroform–methanol with following removal of organic solvents in the current of rare gases, repeated emulsification in NaCl, and heating at 43°C. Several cycles of freezing–thawing or sonication are used. Such techniques are widely used in liposome technology for better peptide building into PL membrane and producing more homogenous preparations. However, electron microscopy shows that the preparations with narrow spectrum of saturated PL have crystal structure in emulsion, which might cause poor interaction with alveolar epithelium.

The methods of obtaining modified and nonmodified natural surfactants differ from each other in several ways. First, different raw materials are used: BAL or minced lung. Alveofact and Infasurf are extracted from BAL, which results in less lung tissue components in preparations (37). For production of other surfactants, either water–salt extraction of minced lung with following precipitation by ultracentrifugation of crude unpurified surfactant with subsequent extraction by organic solvent mixture (Curosurf) or extraction of minced lung by the mixtures of the same solvents (Surfacten, Survanta, Surfactant-HL 10) is used. Sometimes, neutral lipids and cholesterol are removed from lipid extract by precipitation with acetone, in other cases by means of liquid–gel column chromatography on Lipidex-5000 (Curosurf).

The design of many surfactants used to be aimed at making the substance, which could only lower surface tension with maximum efficiency. That is why the components, which deteriorated this parameter (neutral lipids, cholesterol and its ethers, ballast proteins and SP-A and SP-D), are removed from finished products. This can also result in the loss of many native surfactant components (plasmalogen and other minor PL, nonidentified substances), which are very important for improving surface properties (125,126). To compensate the loss, DPPC, PA, and tripalmitin are added to Surfacten and Survanta. The content of surfactant minor lipids that contribute to biophysical properties of the preparations was studied in three commercial surfactants: Alveofact, Curosurf, and Survanta (126). Lipid compositions had strong differences. Survanta had the highest portion of unsaturated PL and the lowest portion of acid-containing PL. The highest plasmalogen and acid-containing PL concentrations were found in Curosurf. Different lipid compositions could explain some of the differences in surface viscosity. PL pattern and minor surfactant lipids are important for biophysical activity. The removed components may also be

responsible for innate local immunity of lungs, host defense properties, and increase of mucociliary clearance.

Marked differences among surfactants were observed in vitro in the presence of possible surfactant inhibitors (127). Inactivation effect of fibrinogen, albumin, and hemoglobin was studied with various surfactants. Curosurf and Survanta were inhibited by all three proteins, whereas BLES and Alveofact demonstrated low sensitivity (128).

The characteristics of surfactant preparations listed above lead to very different results of their clinical usage. The next part of the article is devoted to the clinical results of surfactant replacement therapy.

THERAPEUTICAL EFFICACY OF DIFFERENT LUNG SURFACTANTS

Bonĉuk-Dayanikli et al. (37) described the requirements for ideal therapeutic surfactant, which include the attributes of any ideal preparation and characteristics specific for surfactants: mimic effect of pulmonary surfactant in vitro, nonimmunogenicity, ability to improve gas exchange, lung mechanics and functional residual capacity, resistance to inactivation, optimal distribution characteristics, known clearance mechanisms, and minimal toxicity. Furthermore, the preparation must possess such properties of lung surfactant in situ as host defense ability and innate immunity (53).

Infant Respiratory Distress Syndrome

Although none of the surfactants meets all these requirements, the efficient application of surfactant replacement therapy for IRDS was started in 1980 (28). Not all the newborns with IRDS respond positively to surfactant administration, which can be explained by different degree of prematurity and infection constituent. Considerable experience in IRDS treatment and some clinical studies showed that synthetic protein-free Exosurf is less efficient than Curosurf and Survanta (129,130). The wide application of surfactants for IRDS treatment allowed reducing mortality rate significantly. It has been shown that newborns treated with surfactant have much less respiratory problems later compared to the newborns without surfactant treatment. Now surfactants are being used more and more in other lung pathologies in newborns such as meconium aspiration, innate pneumonia, and so on.

Acute Lung Injury and Acute Respiratory Distress Syndrome

The pathophysiology of ALI/ARDS is much more complex, that is why the development of optimal treatment strategies is a challenge. ARDS is caused by secondary surfactant deficiency. The first attempt of surfactant application for ARDS treatment was made in 1987 (131). Since then, rather controversial data have been obtained (Table 2). In spite of the introduction

of some modern techniques for ARDS treatment such as "safe" conventional mechanical ventilation (CMV), usage of the concept of "open lung" (132), and so on, the mortality rate due to ALI and ARDS is still very high, and according to consolidated data on 10 European countries it was 53% in 2003. So, the development of new approaches for ALI and ARDS treatment is well-justified.

Table 2 shows that the majority of clinical studies registered positive alterations in oxygenation and lung compliance, though significant reduction of mortality rate was achieved only with the application of natural surfactants (27,30,133–138). Randomized clinical trials (RCT) of different surfactants in accordance with evidence-based medicine (EBM) requirements

Table 2 Surfactant Application in Acute Lung Injury/Acute Respiratory Distress Syndrome

Surfactant trade name	Number of patients	Mode of administration and dose	Result	References
Exosurf	725	Aerosolized surfactant 5 mg/kg for five days	No effect	(32)
Survanta	59	50–100 mg/kg, via an endotracheal tube	Mortality reduction from 43–18.8%	(108)
Infasurf	21	2.8 g/m^2, via an endotracheal tube	Mortality reduction	(133)
Alveofact	27	200–500 mg/kg, via bronchoscope	Mortality rate 44% compared to calculated rate of 74%	(134)
Venticute	448	200–400 mg/kg up to four intratracheal instillations	No effect	(135)
Surfactant-HL-10	35	200 mg/kg intratracheal instillations	Mortality reduction	(29)
Surfaxin	22	50–60 mg/kg, via bronchoscope (lavage) 400 mL of emulsion	Significant decrease in mortality	(29)
Surfactant-BL	183	10–12 mg/kg, via bronchoscope	Reduction of mortality rate to 15% (direct lung injury), and 25% to 30% (indirect lung injury)	(27,136)

resulted in the negative third phases of RCT (29,63,135). The only exception is Surfaxin and Venticute, whose clinical trials are in process at the moment (29,63).

The contradictions of the results of the efficiency of surfactant therapy in ALI/ARDS, and negation of the prospects of surfactant application due to some unsuccessful attempts (29,63,135), have induced us to analyze possible reasons for failure. They can be the following:

- Late administration of surfactant preparations
- Incorrect therapeutic dose and methods of preparation administration
- The injustice of EBM principle usage in patients in critical conditions
- Great variety in surfactant compositions

Timing for Surfactant Administration

Surfactant therapy is usually started very late, within first 48 to 72 hours of CMV or even later (29,134,135,138). High efficiency of early surfactant administration compared to late administration was first demonstrated for the treatment of the children and adults with ALI and ARDS (31,136,139). Multicenter case-uncontrolled clinical trials of Surfactant-BL were carried out in 58 patients with ALI and ARDS who met the requirements of American-Europe consensus conference (AECC) of 1994 (140). The patients had oxygenation index [arterial oxygen tension (PaO_2)/inspiratory oxygen fraction (FiO_2) ratio] equal to 119.4 ± 5.7 mmHg, and lung injury score (LIS) 3.04 ± 0.25 before surfactant administration. The analysis of treatment results allows dividing the patients into two groups: those who responded to surfactant positively (81.03%) and those who did not respond to surfactant administration (18.87%). In the first group, 24 hours after administration, PaO_2/FiO_2 ratio increased by 78.4% and LIS decreased by 57.9%. After 6.4 ± 1.2 days, 70.7% of the patients of the first group were weaned from CMV, the mortality rate was 14.9%, whereas the mortality in the second group was 90%. The main difference in therapeutic modes was the period of time between the moment of PaO_2/FiO_2 ratio drop less than 200 mmHg and surfactant administration: it was 18.7 ± 2.72 hours in the first group and 31.9 ± 5.6 hours in the second group (31,136).

Searching Therapeutic Dose and Method of Surfactant Administration

The question of a dose seems to be very difficult. Some investigators (35,138) believe that high doses of the preparation are necessary for successful treatment of ARDS. This approach is based both on clinical experience and data about inhibiting effect of leaking plasma proteins. We think that the calculation of leaking inhibitors quantity was based on wrong assumptions (6,35). Protein quantity was measured in pooled BAL samples. The increase in permeability of alveolar–capillary barrier in ARDS does not mean that it

is permeable completely: the leakage of the proteins has a definite speed. After the first lavage, the protein concentration in the following lavage sample will be very small, and protein concentration gradient between capillary and airspaces is created, which causes the following protein leakage. So the total protein measured in pooled lavage fractions does not reflect the true situation in alveolar space. To test this assumption, we assessed albumin, fibrinogen, and total protein content separately in five successive 40-mL portions of lavage fluids. The period of time between the lavages should be minimal, and usually it was 30 to 60 seconds. The sharp increases in protein content in BALF are turned out to be followed by deep falls with protein concentration equal to zero in some patients (Fig. 1). So we think that the protein content in airspaces was significantly overestimated

The fact that healthy adult has 3 to 15 mg/kg of surfactant also supports the application of the less dose of preparation. Nevertheless, the therapeutic dose for the majority of surfactants is very high, and in some cases reaches 200 to 800 mg/kg per course (30,134,135,138). Such high and variable doses can be explained only by the diversity of surfactants. Surfactant-BL is the only surfactant whose therapeutic dose, 6 to 12 mg/kg for ALI/ARDS (136,141), is close to the surfactant content in vivo (121).

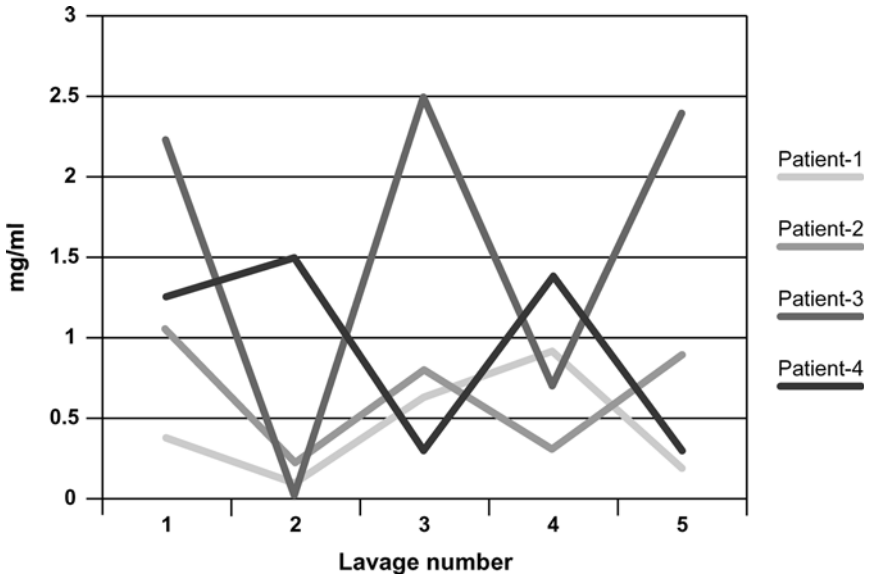

Figure 1 Protein content in separate bronchoalveolar lavage fluid fractions of patients with acute lung injury/acute respiratory distress syndrome. Total protein content separately in five successive 40-mL portions of lavage fluids was determined. The period of time between the lavages was 30 to 60 seconds. The sharp increases in protein content in bronchoalveolar lavage fluid are turned out to be followed by deep falls with protein concentration equal to zero in some patients.

Experimental data prove that only 4.5% of surfactant reaches alveolar surface at aerosol way of administration (32). Clinical trials demonstrated that aerosol way is less efficient in newborns (142) and inefficient in adults (32). Larger volumes are better for particle distribution among different parts of lungs, but at the same time the preparations hardly reach injured lung areas (143). Now the most efficient way of administration is considered to be endobronchial administration of preparation into every lung segment.

The Injustice of EBM Principle Usage in Patients in Critical Conditions

The correctness of observing the principles of EBM in patients in critical condition is questionable (36,144–146). Not long ago, mortality rate in ARDS reached 60% to 70%. Such conditions are considered to be fatal, and RCT of the preparations, whose efficiencies has been proved in experiments or has physiological ground, are not justified (146). The interpretation of the results of RCT is complicated very much by wide heterogeneity of the patients with ARDS who must not be enrolled in the same groups according to the etiology and severity of the disease. For example, additional analysis of the negative results of RCT of Venticute for ALI/ARDS treatment showed that it significantly reduced mortality in direct lung injury (29,63,135). Surfactant-BL was proved to be more efficient in direct lung injury compared to systemic lesion (29,147–150). Carrying out clinical studies of the surfactants in homogenous groups of patients gave more promising results and allowed recommending the treatment of patients of certain etiology of ARDS (147,148,150,151).

Another very important thing, which is not taken into account by EBM at planning RCT, is the number of patients treated in one particular intensive care unit (ICU). The desire to minimize the period of the third phase of RCT, which should involve a large number of patients, causes the distribution of the patients among many hospitals. For example, the third phase of RCT of Venticute (135) enrolled 448 patients treated in 109 hospitals. So, on average, four patients (two treated with surfactant and two control patients) were in each hospital, which makes data comparison incorrect. The procedure of surfactant treatment is quite complex. Differences in basic therapy and respiratory support methods as well as very small experience in surfactant application can affect the result.

Different Surfactant Preparations Have Different Therapeutic Effect

The efficiency of surfactant therapy depends on the composition of a chosen preparation (152). Higher efficiency of Survanta and Curosurf for IRDS treatment compared to Exosurf (153) and Exosurf inefficiency for ARDS treatment (32,154) prove that natural surfactants give better responses than protein-free synthetic surfactants. Phase II of clinical study

of recombinant SP-C (Venticute) in patients with ARDS showed marked improvements in the oxygenation index, ventilator-free days, and the percentage of successfully weaned patients. However, mortality rate in this group was 29% compared to 33% in the control. Patients delivered up to 200 mg/kg of total PL in four doses (154).

The application of modified natural surfactants Curosurf (137) and Survanta (30,108) demonstrated gas exchange responses (30,108,137) and, in case of Survanta, a trend toward reduced mortality. BALF analysis revealed partially improved surfactant functions (108).

The most promising are the results of clinical application of natural nonmodified surfactants: Alveofact (35,138) and Surfactant-BL (27,31,136, 147,148,151). Uncontrolled multicenter study showed that bronchoscopic application of a high dose of Alveofact in patients with severe ARDS and septic shock is both feasible and safe, resulting in pronounced improvement in gas exchange and far-reaching, though incomplete, restoration of the severely changed biochemical and biophysical surfactant properties (35,138). A total of 15 patients survived the 28-day study period (mortality rate 44.4%, compared to a calculated risk of death for the given acute physiology, age, and chronic health evaluation (APACHE) II scores of 74.0%) (138). Another controlled, randomized, open-label study of the efficiency of Infasurf in 42 children with ARDS demonstrated a rapid improvement in oxygenation, reduced duration of CMV, and an earlier discharge from the pediatric ICU in the surfactant treatment group (133,136).

Multicenter uncontrolled clinical trials of Surfactant-BL have been carried out in the patients with ALI and ARDS of different etiology such as sepsis, multiple trauma, multiple transfusion, aspiration of gastric content, thromboembolism of lung artery, severe pneumonia, thermochemical burns of respiratory tracts, and postbypass lung injury (Table 3). Surfactant administration at a dose of 6 to 12 mg/kg per course reduced significantly the duration of CMV and 28-day mortality rate (from 60% to 23.2%). The mortality rate in the patients who responded to surfactant administration was 15%. Seven patients with severe burns of respiratory tracks treated by Surfactant-BL survived compared to 1 survivor of 15 patients in the control group (150).

Several ways of improving surfactants are under study (152). The investigators have been developing some substitutes for natural surfactant components: first, either synthetic or recombinant surfactant proteins or their analogues to generate proteins that are free of animal contaminants; second, PL analogues that may improve surface activity of surfactant and be resistant to phospholipase and, third, the substances to prevent surfactant inactivation, for example, such nonionic polymers as dextran or polyethylene glycol (155).

We think that only surfactant preparations with complex and similar to native surfactant in situ composition and structure can have high therapeutic effect. These preparations can not only improve biophysical

Table 3 Surfactant-BL Application in Homogenous Groups of Patients with ALI Acute Respiratory Distress Syndrome

ALI/acute respiratory distress syndrome etiology	Type of lung injury	Number of patients	28-day survival
Aspiration of gastric content	Direct	18	17 (94%)
Severe pneumonia	Direct	26	22 (85%)
Respiratory tract burns	Direct	11	10 (91%)
Complication after pulmonectomy in tuberculosis, ALI	Indirect	26	24 (92%)
Sepsis	Indirect	28	17 (61%)
Massive hemotransfusion	Indirect	16	10 (68%)
Postbypass lung injury	Indirect	36	25 (69%)
Severe multiple trauma	Indirect	22	15 (68%)

Abbreviation: ALI, acute lung injury.

parameters of injured lung but also serve as a substrate and stimulator for own endogenous surfactant synthesis (156), involve uninjured lung parenchyma areas in respiration, contribute to lung parenchyma immunity, lung defense system, and removal of toxic compounds from alveolar space with sputum.

Surfactant Therapy of Other Lung Disease

The finding of abnormalities of pulmonary surfactant system in practically all lung pathologies encourages the attempts of surfactant treatment of others than IRDS and ARDS lung disease. The experience in this field is not very wide. Surfactant preparations have been used for the treatment of pneumonia (27,157), atelectasis (158), asthma (159–161), and tuberculosis (27,162–164).

Limited experience with selective bronchial instillation of surfactant in a patient with pneumonia has suggested the possibility of benefit (157). Surfactant-BL was used in more than 60 children (from 9 months to 14 years old) with acute bronchopneumonia complicated by stable atelectasis. The treatment resulted in significant reduction of the number of fibrobronchoscopies, an increase in complete and partial atelectasis solvability (158).

Clinical use of surfactant in asthma is currently under investigation. A study in which 12 asthmatic children received aerosolized bovine surfactant indicated that there were no changes in lung functions (159). In another clinical investigation, 11 adult asthmatic patients with stable airway obstruction were given aerosolized surfactant six hours after an asthma attack (160). All patients showed an improvement in pulmonary function. The investigation of the effect of a porcine natural surfactant on inflammatory changes in patients with mild asthma following segmental allergen challenge

(165) showed that allergen-induced inflammatory response was increased by surfactant pretreatment compared to placebo. It is unknown whether this pulmonary action is restricted to one specific preparation or true for various formulations. The researchers conclude that surfactant treatment of patients with asthma may require specifically designed preparations (23,54,110).

There is limited information on the value of surfactant treatment of patients with COPD. In a single study of the effect of surfactant PL in COPD, patients with chronic bronchitis who received aerosolized PL three times daily for two weeks had a modest dose-related improvement in mucociliary transport and airflow compared to that in patients who received saline (166). The Discovery Labs has been developing an aerosolized surfactant solid form to treat hospitalized COPD patients. Work with aerosolized surfactant has demonstrated improved pulmonary function in such patients.

The surfactant application in lung tuberculosis is quite efficient (63,162–164). Surfactant-BL was used in complex therapy for 52 patients with lung tuberculosis (162–164). All the patients discharged bacteria with sputum and had multidrug resistance. Small doses of surfactant (25 mg a day, 0.3 mg/kg) were administered according to a specially designed scheme during two months together with four to five antituberculosis preparations. Six to eight weeks after the beginning of the treatment, 85.7% of patients demonstrated conversion of sputum to negative (vs. 65% in the control group); two to four months later, 94% of patients had infiltrate resolutions (vs. 67% in the control group), and 83% of patients had reduction or close of cavities (vs. 47% in the control group).

CONCLUSION

Although there are still a lot of questions regarding feasibility, efficiency, and methods of surfactant therapy for the diseases others than IRDS, the future of surfactant preparations seems to be quite promising. The application of surfactant preparations in patients with direct lung injury is more efficient than in the patients with indirect lung injury. Surfactant application can be fearlessly recommended for the patients with aspiration of gastric content, severe burns of respiratory tracts, severe pneumonia, lung contusion, and others. In any case, the analysis of the efficiency of surfactant therapy should be carried out in homogenous groups of patients as the definition of ARDS is too broad and includes the variety of patients with different and extremely complex pathophysiologies. Some patients may be more responsive to exogenous surfactant than others.

The therapeutic efficiency of surfactant formulations varies a lot. It is necessary to emphasize that the closer preparation composition and structure are to the characteristics of the surfactant in situ, the better results it has. The preparation must be administered as early after the onset of ALI/ARDS as possible, preferably within the first 24 hours. Later administration

often leads to failure. The dose varies essentially depending on the chosen surfactant preparation. The application of surfactants for some subacute pulmonary diseases and tuberculosis requires working out the treatment regimens different from ones in ALI/ARDS.

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