

Surfactant in Airway Disease.

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Abstract. Beta₂-adrenergic agonists cause a release of pulmonary surfactant into lung airways. The surfactant phospholipids maintain patency of conducting airways, but this function is inhibited by plasma proteins entering an inflamed airway. The physical behavior of the surfactant can be studied with the Pulsating Bubble Surfactometer (PBS) and the Capillary Surfactometer (CS). Calf Lung Surfactant Extract (CLSE) was found to be inhibited by plasma proteins and by a lowering of temperature. BALB/c mice inhaling ozone or infected with Respiratory Syncytial Virus (RSV) developed severe breathing difficulties and malfunctioning surfactant, mainly as a result of proteins invading the airways. Patients with asthma were challenged with allergens in an area of one lung. Lavage fluid from such an area had a surfactant that functioned poorly (i.e., inability to maintain airway openness) compared with lavage fluid from the other lung or from lungs of healthy volunteers. When proteins in lavage fluid were removed, surfactant performance clearly improved. Eosinophils, so prominent in asthmatics, synthesize the enzyme lysophospholipase which, together with the enzyme PLA₂ catalyses hydrolysis of surfactant's main component phosphatidylcholine. Such hydrolysis incapacitates surfactant's ability to maintain airway patency. Treatment of asthma with beta₂-adrenergic agonists and with steroids will have a valuable effect on the surfactant system. It will cause a release of fresh surfactant into terminal airways. Surfactant can also be nebulized and inhaled, which has been shown to be an effective treatment.

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Introduction. All over the world asthma is treated with beta₂-adrenergic agonists. They cause smooth muscles to relax. They also cause pulmonary surfactant to be released from alveolar cells type II, a widely overlooked effect, since the importance of airway

surfactant in asthma is not yet appreciated. Surfactant maintains openness of alveoli and terminal conducting airways and thereby promotes normal blood gases and low airway resistance. Excellent reviews on surfactant's ability to maintain low airway resistance and its vital importance for asthma patients, have been published.^{1,2} The present review includes a description of surfactant's physical properties.

The importance of pulmonary surfactant was early recognized by neonatologists. Airway liquid of term infants contains surfactant, synthesized in alveolar cells type II. It consists mainly of phospholipids; dipalmitoyl-phosphatidylcholine (DPPC) dominating.³ The phospholipid molecules are amphipathic, i.e., at one end, where the fatty acids are located, they are hydrophobic, whereas the polar heads are hydrophilic. Air-liquid interfaces of expanded lungs constitute ideal locations for phospholipids. The fatty acids can avoid water by staying in air while the polar heads remain in water. A film of these molecules, at least monomolecular, will spontaneously form at the lungs' air-liquid interfaces.

The law of Laplace as it applies to the **spherical** surface of an alveolus

$$\Delta P = 2\gamma/R,$$

shows how the pressure difference, ΔP , at the air-liquid interface is dependent on γ , surface tension of alveolar moisture, and alveolar radius, R . Since the alveolus communicates with ambient air, ΔP is approximately equal to the negative pressure surrounding the alveolus. If γ does not change during breathing, the law shows that during expiration ΔP would increase, as R decreases. Consequently, small alveoli might not be surrounded by the suction they require to maintain expansion; they collapse. However, breathing **does** change γ . During expiration, surfactant phospholipids are forced to come closer, causing γ to diminish, and if diminishing more than R , ΔP will not increase; alveoli will not collapse.

Plasma proteins are also amphipathic, but their hydrophobicity is weak; explaining why they are water soluble and evenly distributed in the airway moisture. Due to their weak hydrophobicity they are not strongly attached to the air-liquid interfaces and during expiration they will probably be squeezed out from the interface film. They prevent phospholipids from coming as close to each other as they normally do. For that reason surface tension is not lowered the normal way, possibly causing alveolar collapse.

The Pulsating Bubble Surfactometer (PBS) (US patent 4,800,750.

Manufactured by General Transco, Seminole, Florida). The PBS^{4,5} is intended for an evaluation of alveolar pulmonary surfactant. Its function is based on Laplace's law. In a small chamber, containing the liquid to be evaluated, a bubble is created. It communicates with ambient air and simulates an alveolus during breathing. Bubble radius, R , is shifting from 0.4 to 0.55 mm. By measuring pressure around the bubble, ΔP is obtained and when correlated with R , γ can be calculated and recorded.

The law of Laplace as it applies to the **cylindrically** shaped conducting airway,

$$\Delta P = \gamma/R,$$

shows how the pressure difference, ΔP , between moisture lining the airway and air in its center is dependent on the surface tension of airway moisture, γ , and R , the terminal airway radius. During expiration, when R is diminishing, phospholipids are forced to come closer, causing surface tension, γ , to be lowered. If surface tension of the airway moisture, γ , is reduced more than R , ΔP will diminish and liquid will not be sucked into

the narrowest part of the airway (Fig.1). However, if the phospholipids are too few, absolutely or in relation to proteins, surface tension will not be adequately lowered. ΔP will then increase, indicating that in the narrowest airways pressure of liquid will be reduced; resulting in moisture being attracted from wider airways, perhaps causing airway blockage. This occurs during expiration which in a patient with asthma is offering the greatest resistance.

According to what has now been written surfactant helps to maintain airway patency, as seen in Fig. 2, ⁶ where the lungs from two rabbit neonates are shown. The rabbits were littermates, delivered by hysterotomy on day 27, when surfactant synthesis is inadequate. A tube was introduced into each trachea, so that pressure in the lungs' airways simultaneously and repeatedly could be raised to 35 cm of water. Before the lungs were expanded in this way a surfactant preparation, obtained from adult rabbits, was instilled into the tracheal tube of the lungs to the right. When pressure had been lowered to zero a clear difference was seen. The lungs to the right had evenly expanded alveoli, and conducting airways were open. A few alveoli in the lungs to the left were expanded, but they were over expanded, and there was no air in conducting airways. Thus, the surfactant malfunction, in this experiment resulting from deficiency, showed pathology similar to that of fatal asthma: conducting airways not open, air trapping, over expanded alveoli.

Pulmonary surfactant maintains airway patency. An airway inflammation, characteristic of asthma, ^{7,8} will allow plasma proteins to leak into the airway lumen and since they are known to inhibit the surfactant function, ⁹ it was felt that an instrument evaluating surfactant's ability to maintain airway patency, with or without inhibiting contaminants, would be valuable.

The Capillary Surfactometer (CS). (US patents 4,970,892 and 6,814, 936). Manufacturer: Calmia Medical, Toronto, Canada. The CS uses a glass capillary, modeling a terminal conducting airway ⁵. In a short section the capillary is very narrow; its inner diameter being 0.24 to 0.25 mm, similar to the width of a human terminal airway.¹⁰ If during breathing moisture were to accumulate and block any part of the airway it would be in the narrowest section. Thus, a small volume (0.5 μ L) of broncho-alveolar lavage fluid (BALF) is deposited in the capillary's narrowest section. Airflow through the capillary, 0.3 mL/min, raises pressure until the sample is extruded and spreads in a wider capillary section. Pressure instantaneously drops to zero. The question is: will the capillary remain patent or will liquid return? If the capillary remains open pressure stays at zero, but if liquid returns, airflow meets substantial hindrance. Considering that the sample volume required for the CS is only 0.5 μ L it might in the future be possible to directly examine the moisture from a terminal airway (Suggested by Dr. Sorkness, University of Wisconsin). It could be sucked into a fine polyethylene catheter (PE10), sliding through a wider tubing, introduced via a bronchoscope. The need for concentration of BALF surfactant would then be avoided.

Following initial extrusion of the sample, pressure is recorded for two minutes. With well functioning surfactant, the liquid will not return to the narrow section; pressure remains at zero. If liquid returns, pressure is raised, once or repeatedly, indicating impaired surfactant function. A microprocessor calculates how many percent of the two-

minute period the capillary remained open. The printed report would be “Open 100%” if function was optimal, or “Open 0%” if maximally poor.

The capillary, in shape and width, is imitating a terminal conducting airway. However, its material is glass, so the question could be raised: will surfactant function similarly in lung airways? To answer that question terminal conducting airways of rats were studied.^{11, 12} It was found that when there was a surfactant deficiency the airway would close repeatedly, as the glass capillary did. However, when surfactant was instilled into the terminal airway, there was a clear trend for the airway to remain open.

The CS was used for a study of Calf Lung Surfactant Extract (CLSE), (commercially available as Infasurf, ONY Inc., Buffalo, NY). CLSE (2 mg/mL) without proteins maintained patency 100%, but that ability was hampered when plasma proteins were added. Fibrinogen was particularly inhibiting and when added at a concentration of 0.07 mg/mL had the same inhibiting effect as albumin, 7 mg/mL.

The surfactant inhibiting effect of the weakest plasma inhibitor, albumin, was tested at a final concentration of 10 mg/mL when added to surfactant, 2mg/mL.¹³ The assays were carried out at temperatures from 42° to 25° C. It was noted that a lowering of temperature to 34° C inhibited some of the surfactant ability to maintain patency, and when temperature was lowered to 32° C the loss of this function was devastating. We wondered if this might explain why patients with mild asthma, and most likely a moderate airway inflammation, will sometimes develop severe breathing difficulties when physically exercising in cold air.

Conditions causing surfactant malfunction. An airway inflammation, characteristic of asthma,^{7, 8} may be caused by certain pollutants, viruses, or allergic reactions. Will it lead to a lowering of surfactant concentration, or might there be other factors having a negative effect on surfactant performance? The inflammation will cause an oozing of plasma proteins into the airway.⁷ Those proteins, particularly fibrinogen, inhibit surfactant function.⁹ The CS will specifically show if inflammatory proteins harm surfactant’s ability to maintain airway patency.

We theorized that any airway inflammation might be a reason for a surfactant inhibition, since it will induce a leakage of plasma proteins into the airway lumen. We tested this theory by investigating how breathing and surfactant function of mice was affected when the animals had been breathing ozone or when they were infected with Respiratory Syncytial Virus (RSV).

BALB/c mice exposed to ozone.^{14, 15} The animals were exposed to 1 or 2 parts per million (ppm) for two to eight hours. After 24 hours they were examined with whole body plethysmography. Breathing was significantly affected after only two hours. Rate of breathing diminished as did the pressure change caused by each breath. Possibly these changes were due to a significant worsening of surfactant function. For a study of that possibility, the mice were killed and BALF was obtained. To increase the surfactant concentration of the BALF the cell free fluid was centrifuged at 40,000xG and 4° C for one hour. A pellet of large aggregate surfactant was obtained. It was re-suspended after removal of supernatant, constituting 80% of the BALF.

The ability of BALF surfactant to maintain capillary patency was successively lost the longer the animals had been exposed to ozone. At the same time protein

concentration gradually increased which we assumed had caused the surfactant malfunction. This assumption was supported by the result of a “washing” procedure, aiming at removal of water soluble proteins. The principle was to add a large volume of saline solution to the concentrated surfactant, vortex and once again centrifuge for one hour at 40,000xG. As before, the surfactant formed a pellet and following removal of supernatant, equal in volume to the previously added saline solution, the pellet was re-suspended and evaluated with the CS. It was found that following this “washing” procedure the surfactant had an almost perfect ability to maintain patency.

Mice infected with RSV.^{16, 17} BALB/c mice were nasally inoculated with RSV, in a high or a low dose. Six days later their breathing was clearly affected by both doses, but more by the higher. They were killed so BALF could be obtained. The technique was identical to the one used for the ozone experiments. The surfactant functioned poorly, and was washed as in the ozone experiments. The function improved, but surfactant from infected mice never approached the values of non-infected animals. This suggested that a surfactant inhibition caused by invading proteins might not have been the only reason for the deteriorating surfactant function. There might also have been time for hydrolysis of phospholipids, or perhaps of the surfactant associated protein, SP-B.

BALF from patients with asthma following an allergen challenge. Two studies were carried out in collaboration with departments where segmental allergen challenge of asthmatics was routine.^{18, 19} Asthmatics were examined with fiber-optic bronchoscopy. A segment of one lung was challenged with allergen. The other lung served as control. The bronchoscope was reintroduced after 24¹⁸ or 48¹⁹ hours so the two segments could be lavaged. Non-asthmatic volunteers were similarly examined. The BALF surfactant was concentrated as in the ozone and RSV studies. With the CS it was found that BALF from challenged segments of asthmatic patients showed a serious surfactant malfunction compared with BALF from the patients’ control lungs or healthy volunteers. A washing procedure greatly improved the function. We thus felt that protein leakage was the dominating reason for the malfunction. Only a segment of one lung had been challenged. Had large areas of both lungs been challenged, causing surfactant malfunction, dyspnea and low FEV₁ most likely would have developed.

Eosinophilia and surfactant dysfunction. Airway inflammation is characteristic of asthma as is eosinophilia. Recent studies explain how eosinophils may affect pulmonary surfactant.^{20, 21} In patients with asthma phospholipase A₂ (PLA₂) is excessively released. It catalyzes hydrolysis of the main surfactant component, phosphatidylcholine (PC).^{22, 23} The hydrolysis, yielding free fatty acids and lysophosphatidylcholine (LPC), does not seriously affect surface activity.²⁴ However, the LPC serves as substrate for another hydrolytic process, catalyzed by lysophospholipase (LPLase), which is synthesized by eosinophils.

Thin Layer Chromatography (TLC) was used to demonstrate the catalytic effect of the two enzymes. CLSE (2mg/mL) was incubated in two groups of four test tubes. Tube one contained CLSE only. Tube two CLSE plus PLA₂. Tube three CLSE and LPLase, and tube four contained CLSE and both enzymes. Incubation lasted fifteen minutes in one group, four hours in the other. After these periods lipid distribution was studied with TLC and surface activity with a CS (Fig.3). After four hours the PC

concentration was drastically lowered when both enzymes had been present. The CS then showed that surfactant's ability to maintain airway patency was debilitated. It appeared that PC hydrolysis was greatly accelerated only when LPC continuously was being removed with the hydrolysis catalyzed by LPLase.

Therapeutic aspects. A most important treatment of asthma is with steroids. Since 1972 this prophylaxis has successfully been used to accelerate surfactant synthesis of premature fetuses.²⁵

Beta₂-adrenergic agonists are used to treat asthma and COPD. They cause fresh surfactant to be released into alveoli,^{26, 27} and from there it is extruded into terminal conducting airways. This scenario would enhance the quantity and quality of the airway surfactant, probably causing a noticeable clinical improvement.

Surfactant can also be inhaled as a nebulized spray,²⁸⁻³⁰ a promising treatment, which has been shown to improve asthma symptoms, but there are unsolved questions. What is the composition of the most effective artificial surfactant? Considering the hydrophobicity of the phospholipids they are not easily nebulized. Is a surfactant preparation and a method for its supply accepted as asthma treatment? Are there limitations to the use of inhaled surfactant?

In the future, it might be possible to obtain BALF from asthma patients for an evaluation of their surfactant status. If surfactant inhibiting proteins are found, could enzymes be inhaled that will catalyze hydrolysis of those proteins without damaging airway epithelium? Does the BALF supernatant contain enzymes that hydrolyze surfactant components, possibly the combination of PLA₂ and LPLase? If so, could an enzyme be developed that will catalyze hydrolysis of LPLase? It should be supplied as a nebulized spray.

Conventional treatment of asthma will have a beneficial effect on the surfactant system. If in the future asthma research is also specifically focused on that system treatment is likely to improve even further.

Word count: 2504

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Figure legends

Fig.1. A. If surfactant phospholipids are not present, or are deficient, pressure of moisture in the narrow airway section ($R_1 < R_2$) will be less than what it is in the wide section. This will cause moisture to move from the wide to the narrow airway, which eventually might become blocked.

B. If surfactant phospholipids **are** present (blue symbols) in an adequate number they will be pressed together at end expiration. That lowers surface tension and will prevent liquid from moving into narrow airway sections.

C. If protein molecules are present in the surfactant film (red symbols) they will be squeezed out of the film during expiration and be dissolved in the liquid under the film. The number of phospholipids at the surface will not be adequate and surface tension will not be lowered as it normally would be, and liquid will move from wide to narrow sections of terminal airways.

Fig. 2. Lungs have been expanded when surfactant was deficient (left) and when it had been boosted from an exterior source (right). Experiment fully explained in text. From reference #6. Reproduced with permission of the Editor of *J Appl Physiol*.

Fig. 3. TLC showing the hydrolysis that occurs after enzymes from eosinophils have catalyzed hydrolysis of the surfactant phosphatidylcholine

(PC). When the two enzymes, PLA₂ and LPLase were both catalyzing the hydrolysis for 4 hours, PC concentration clearly diminished and the surfactant ability to maintain airway patency was poor. From reference # 21. Reproduced with permission of the Editor of J Allergy Clin Immunol.