Chapter 1

Pulmonary Surfactant and Lung Transplantation

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Abstract

Already in 1965 Waldhausen reported that surfactant from lung transplants had an impaired capacity to form a stable monolayer of phospholipids at an air-water interface, or, in short, that surfactant had an impaired function (1). This conclusion was underlined 10 years later by Benfield (2). However, due to the use of minced lung extracts (containing both alveolar and intracellular surfactant as well as other membrane fractions) for measurement of surfactant function and due to poor study design, no reliable conclusions could be drawn from these early studies. Now, 30 years after the initial study, new studies, investigating pulmonary surfactant obtained by bronchoalveolar lavage, have provided evidence that lung transplantation indeed alters the components of pulmonary surfactant in such a way that the function of surfactant deteriorates. In this article we review the effects that lung transplantation has on pulmonary surfactant and the relation between surfactant alterations and lung transplant function. Possible effects of rejection or immunosuppressive therapy on pulmonary surfactant will not be treated. We will introduce normal pulmonary surfactant and how it is affected by acute lung injury. In the injury caused by lung transplantation we recognize, the effects of three phases with different effects on pulmonary surfactant: donor management, storage of lungs, and reperfusion. Finally, we review the perspectives for surfactant treatment in lung transplantation.

Pulmonary surfactant in the normal lung

Biophysical function

Pulmonary surfactant reduces surface tension at the air/fluid interface of fluid that lines the alveolar surface. This reduction of surface tension is achieved by forming a stable monolayer of surfactant phospholipids (see Figure 2). In this way surfactant stabilizes the alveoli at end-expiration which prevents atelectasis and alveolar edema and secures an optimal surface area for gas exchange (3).

Surfactant composition

Pulmonary surfactant consists of a mixture of phospholipids and at least four surfactant proteins, SP-A, SP-B, SP-C, and SP-D (4). The main surfactant phospholipid class phosphatidylcholine (PC), in particular its saturated form, dipalmitoylphosphatidylcholine (DPPC), has the unique property to form a stable monolayer (Figure 2). To maintain the monolayer of DPPC during the
respiratory cycle the presence of other surfactant lipids and proteins are required. The hydrophobic surfactant proteins SP-B and SP-C enhance the transport of lipids into the monolayer and are therefore essential for the regulation of the surface tension in the alveoli during the respiratory cycle (4, 5). The hydrophobic SP-A may have a protective and regulatory role in this process. SP-A, and the related protein SP-D, are also important in innate lung defense (6).

Surfactant metabolism and subtypes

During the respiratory cycle surfactant components are lost from the monolayer that must be replenished (7). This dynamic process of renewing and clearance is driven by the type II pneumocytes which synthesize and secrete surfactant, take up inactive remnant surfactant particles, and recycle surfactant phospholipids and proteins (3, 4, 7, 8) (Figure 2). The phospholipids, SP-B and SP-C are secreted together in lamellar bodies (9). SP-A is probably secreted independently by type II cells (10) and Clara cells (11). In the alveolar lining fluid lamellar bodies transform into tubular myelin (see figure 1), for which SP-A needs to be incorporated (7, 12). Tubular myelin can be considered to be the extra cellular reservoir of surfactant which protects surfactant from inactivation and from which the monolayer is formed. Because of their relatively heavy weight, the lamellar bodies and tubular myelin are called heavy subtype surfactant. Inactive remnant surfactant particles consist of smaller and lighter unilamellar vesicles and
Surfactant function in a normal alveolus

Figure 2. This figure is a schematic representation of the function of pulmonary surfactant in a normal alveolus. The function of surfactant is to reduce the surface tension at the air-fluid interface of the fluid that lines the alveolar surface. For its function surfactant consists of a mixture of phospholipids and three surfactant proteins, SP-A, SP-B, SP-C, all produced by the type II pneumocyte. Crucial is that dipalmytoylphosphatidylcholine (DPPC), the disaturated form of phosphatidylcholine (PC), forms a stable monolayer that reduces the surface tension. Efficient absorbance of DPPC occurs via the transformation route from the lamellar body structure into tubular myelin. These two morphological forms of surfactant are called the heavy subtype surfactant and are surface active. Used phospholipids form small vesicles with reduced amounts of surfactant proteins, also called light subtype surfactant. The small vesicles are taken up by the type II pneumocytes and recycled.
Surfactant changes caused by lung transplantation

Figure 3. After transplantation the composition of surfactant is altered in such a way that its function is impaired. The changes include lower percentages of heavy subtype surfactant and of PC relative to total phospholipids, and a reduced amount of SP-A. The altered surfactant is more prone to inactivation by serum proteins that leak from the blood vessels into the alveolus. Both the surfactant alterations and the leaked serum proteins make the monolayer unstable and incapable to reduce the surface tension adequately. The remaining high surface tension causes alveolar collapse and aggravates the pulmonary edema.

therefore are called light subtype (7, 12). The light subtype surfactant contains reduced amounts of surfactant proteins rendering it as surface inactive (13). Remnant surfactant is taken up by the type II pneumocytes for recycling or, to a lesser extent (5-10 % of total ), cleared by alveolar macrophages(14)

Regulation of surfactant metabolism
Multiple substances and factors regulate the synthesis and secretion of surfactant. We will focus mainly on the factors that determine extracellular surfactant metabolism. In the alveolar lining fluid SP-A may regulate secretion and re-uptake of surfactant by the type II cell and the formation of tubular myelin. In addition, SP-A has been reported to have a synergistic effect on the SP-B-mediated adsorption of lipids at the air-fluid interface. SP-B is proposed to regulate the transformation route from tubular myelin via the monolayer into unilamellar vesicles. On its turn the action of SP-B is influenced by an enzyme called convertase (a serine protease). The action of transformation depends on an ongoing increase and decrease of the alveolar surface area during respiration (15, 16). The exact physiological relavance of convertase and conversion of heavy to light surfactant subtypes remains to be established. Besides respiration other factors such as parasympathetic and sympathetic innervation of the lung influence pulmonary surfactant metabolism. These two factors will be discussed later in this article in relation to the procedures affecting donor lungs for transplantation.

Impairment of the biophysical function of surfactant in acute lung injury

The biophysical function of surfactant can be impaired by acute lung injury in several ways (17). First, the adsorption of DPPC is directly inhibited by serum proteins that have leaked through the alveo-capillary barrier. The leaked serum proteins directly compete with the DPPC for a place at the air-fluid interface thereby making the DPPC monolayer unstable at expiration (18). Second, due to changes in surfactant phospholipids, such as the decrease of PC, DPPC and phosphatidylglycerol glycerol and the increase of lysophosphatidylcholine after lung injury the monolayer might become unstable (18). Finally decreased amounts of SP-A, often found after acute lung injury, will result in a less organized surfactant structure that is more prone to inactivation (19, 20). In vitro experiments have shown that especially the combination of a decreased SP-A and an increased amount of serum proteins is detrimental for the biophysical function of surfactant (21). It is likely that SP-A protects surfactant from inactivation by serum proteins. Support for this notion comes from experiments in vitro (21) and, recently, also from studies in vivo (22, 23).

Pulmonary surfactant in lung transplantation
Donor management

Already in the donor the surfactant system can be affected by lung injury. Although surfactant has not been investigated specifically in lung donors it has been investigated in similar pathophysiological conditions. Events that influence pulmonary surfactant are brain injury and brain death, mechanical ventilation, and disruption of the vagal nerve. All these events are obligatory in the lung transplantation procedure.

Brain injury and brain death

To our knowledge effects of brain injury or brain death on composition of surfactant have not been investigated. However, it is generally known that brain injury or brain death can cause alveolar edema (24). Experimentally, mechanically applied head injury causing brain death in monkeys caused massive leakage of serum proteins resulting in inhibition of the biophysical function of surfactant in parallel with lung dysfunction (25). Sympatholytic drugs reduced the amount of serum protein leakage (25), which is most likely beneficial for the function of surfactant.

Mechanical ventilation

The effects of mechanical ventilation on surfactant secretion or alveolar amounts of surfactant depend on the duration and the mode of ventilation. A single inflation increases the amount of alveolar surfactant. A longer duration of mechanical ventilation of in situ or excised lungs was found to increase the secretion of surfactant leading to higher alveolar amounts of phospholipids (26-30). In one study it was suggested that ventilation did not increase secretion of surfactant but modified the metabolism, inducing synthesis de novo and decreasing recycling of alveolar surfactant (31). In more detail others showed that ventilation increases the amount of heavy subtype surfactant in excised lungs (27). However, ventilation of in situ lungs caused an increased conversion of heavy to light subtype resulting in low amounts of heavy subtype (32). The alveolar conversion of heavy subtype to light increased when greater tidal volumes were used (32). Recently, Novick et al indeed showed that large tidal volume ventilation of donor lungs in dogs reduced the fraction of heavy subtype surfactant (33). In parallel the ventilation caused an increased alveolar amount of serum proteins. As shown by others, mechanical ventilation, particularly when large tidal volumes without PEEP is used, results in protein leakage (34). Overall the decreased fraction of heavy subtype surfactant and the increased leakage of serum protein after mechanical ventilation will impair the
biophysical function of surfactant from donor lungs. A few large breaths, however, might be beneficial.

Disruption of vagal nerve

High cervical disruption of the vagal nerves does not affect the ability of surfactant secretion but it causes massive serum protein leakage into the lungs (35, 36). In this way, vagal disruption might further contribute to the inhibition of the biophysical function of surfactant as a consequence of serum protein leakage.

Thus, management of the lung transplant donor will effect the state of pulmonary surfactant in the donor lung. The major threat to pulmonary surfactant in the donor is leakage of serum proteins which will inhibit the biophysical function of surfactant at reperfusion. Ventilation with PEEP and small tidal volumes might be beneficial in preventing this serum protein leakage. In addition, a few large breaths before storage will cause a shift of surfactant from intracellular to alveolar. Such a high amount of surfactant inside the alveolus might be beneficial during reperfusion (37) as will be discussed in that section.

Lung storage

During storage, components of surfactant might change as a result of damage to lung cells. Ischemia, both warm and cold, impairs lung cell viability because toxic metabolic products are formed after a certain duration (38). To preserve lung cell viability the lung transplants are usually cooled to 10 - 4 °C by flushing the pulmonary artery with cold preservation solutions (38, 39). Clinically, a storage period up to 8 hours is considered to be save. Debate occurs whether the lung should be stored inflated or deflated and which preservation solution is to preferred (38-41)

Inflated or deflated lung storage

Pulmonary surfactant becomes polluted with cell membrane phospholipids after 2 hours of warm storage of lungs without use of preservation solutions as a result of cell death (42). However, during warm storage after the use of preservation solutions, inflation with room air preserves the ability of the type II pneumocyces to secrete surfactant (43). Active secretion can be provoked by lung ventilation or lung lavage (43, 44). Remarkably, this ability to secrete surfactant was absent when lungs were stored inflated with nitrogen or deflated (43). In line with these results the presence of oxygen during warm storage seems to be necessary to enable secretion of surfactant. Cooling of the
lungs below 13 °C totally blocks surfactant secretion (29, 44). In concert with the absent active secretion of surfactant during unventilated storage or cooled storage, the amount of surfactant, the relative fraction of heavy subtype, and the phospholipid composition of alveolar surfactant remained virtually unchanged during warm storage for up to 2 h (45) and during cold storage from 2 h up to 28 h (46, 47). In some studies, however, surfactant deteriorated quickly in stored rat lungs (41, 48). Immediately after preservation with Eurocollins solution (EC) the amount of DPPC decreased, which was further aggravated by storage (41). Very low fractions of heavy subtype surfactant were found after 4 and 8 h deflated or inflated stored lungs after preservation with EC (lower than 50%); the lowest fraction (about 10%) was found after 8 h deflated storage (41).

The effect of warm storage on SP-A has not been investigated; also the effect of cold storage on SP-A levels is unclear. It has been shown that the amount of SP-A in lungs inflated with fifty percent oxygen decreases to 50% of control values after 12-h cold storage (46) and to 75% after 20-h storage (49). Although not statistically significant deflated cold storage decreased SP-A to the same extend (49).

The in vitro biophysical function of surfactant remains normal after storage (inflated or deflated) presuming that there is no accumulation of (serum) proteins in the alveolar space (46, 49, 50). If the level of alveolar protein is increased during storage the in vitro biophysical function becomes impaired (41, 43, 48)

Preservation solutions

A direct comparison of preservation solutions in regard of effects upon pulmonary surfactant was only performed once (46). In that study the Belzer University of Wisconsin solution (UW) was compared with Eurocollins solution (EC) used for preservation of dog lungs. Both solutions preserved the amount and composition of surfactant; Eurocollins solution caused a slightly lower percentage PC and a somewhat higher amount of serum proteins in BALF (46). Indirect comparison of EC and Perfadex solution is possible by comparing two identical studies in rats performed by one research group (41, 50). This comparison reveals that Perfadex preserves the fraction heavy subtype during 16 h cold storage whereas EC fails to do so after 4 h cold storage.

In conclusion, the amount and composition of surfactant phospholipids and the function of surfactant in BALF can remain normal after significant periods of either warm storage (up to 2 h) or cold storage (up to about 24 h). However, likely depending on the method of storage, storage can have
deleterious effects on surfactant, especially after longterm storage of deflated lungs. The influence of different preservation solutions on pulmonary surfactant needs further evaluation. There is some indication, at least in rat lungs, that EC solution is deleterious for pulmonary surfactant.

**Lung reperfusion**

The degree of injury of the lung transplant clearly emerges after reperfusion (38). The effect of reperfusion upon pulmonary surfactant depends on the degree of lung injury in the donor, the length of warm or cold lung storage, the method of lung storage, or the used preservation solution. The gross changes in pulmonary surfactant after transplantation are depicted in Figure 3.

**Warm storage and reperfusion**

Reperfusion after one hour warm storage caused a significant loss of lamellar bodies from type II cells (51), and a decreased alveolar percentage PC with a relative increase of non-surfactant phospholipids, an impaired biophysical function, and increased amounts of serum proteins (45). The impaired function was attributed to the combination of the altered surfactant composition and the disturbance of the monolayer by serum proteins. An increase from 1 to 2 h warm ischemia impaired the surfactant function further (45).

**Cold storage and reperfusion**

In lungs reperfused after short cold storage periods, up to 4 h, the pulmonary surfactant was virtually not affected. After 2 h cold inflated storage (EC as preservation solution) and a 6 h reperfusion period no changes in the proportion heavy subtype, the percentage PC, the amount of SP-A and the biophysical function of surfactant were found in dog lung transplants (46) (table). In parallel, the lung transplant function remained at its preoperative level (46). After 4 h cold inflated storage (EC solution) and a 24 h reperfusion period a small (3 %) decrease in percentage DPPC of total PC was found in parallel with an insignificant decrease in PO\(_2\) of the transplant compared to preoperative values (47). In rat lung transplants, Hausen and colleagues (41) found that after 4 h cold inflated storage (EC
Pulmonary surfactant and lung transplantation

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Data are mean ± std error of the mean
# = minimum surface tension measured in isolated heavy subtype surfactant
‡ = data are estimated means

solution) and 2 h reperfusion the fraction heavy subtype remained higher than after 4h deflated storage (table). This difference however was not caused by reperfusion but was already present after EC preservation and storage (see Inflated or deflated lung storage). The transplant function remained good in both groups (41).

Prolonged cold storage periods for more 4 h do cause significant changes in pulmonary surfactant and, sometimes, impairment of lung transplant
function. If the storage period of dog lungs was prolonged from 2 h to 12 h, then the fraction of heavy subtype decreased with more than 25 percent, together with a slight decrease in percentage PC (only EC solution) and PG (EC and UW solution) and a significant increase in percentage SM (EC and UW solution) (46). The amount of SP-A, already lowered after storage, remained at the same low level after subsequent reperfusion (46) (table). Due to the decrease in heavy subtype surfactant also the biophysical function of surfactant was impaired. In parallel, the function of these lung transplants deteriorated (46). Further prolongation of lung storage to 38 h in a following study by the same group decreased heavy subtype with 40% and deteriorated the lung transplant function (52) (table). In the study of Hausen in rats (41), prolongation of a 4 h storage period of fully inflated lungs to 8 h, caused a significant decrease in heavy subtype (table). This decrease was not paralleled by an impaired surfactant function in vitro; the lung transplant function, however, significantly worsened (41). Pulmonary surfactant of deflated stored lungs was not available because of complete failure of the transplant upon reperfusion (41). In another rat study, 6 or 20 h cold deflated lung storage (saline as preservation fluid) and a 1 h reperfusion period reduced heavy subtype surfactant by 50%, induced a small decrease in the percentage of PC and a more than 90% decrease in the amount of SP-A (53) (table). In concert, the biophysical function of surfactant (serum protein-free) was significantly impaired in the 20 h stored lungs. The lung transplant function was deteriorated after both the 6 h and 20 h storage period (53). Similarly, sixteen hours inflated storage of rat lungs with Perfadex® followed by 2 h reperfusion caused a more than 80% of heavy subtype surfactant and a deterioration of lung transplant function. Prostacyclin, infused intravenously into the donor and added to the Perfadex® solution, improved lung transplant function but had no effect on the lowered fraction of heavy subtype surfactant (50). The effects of transplantation injury on surfactant were shown to persist for at least one week after transplantation in rats (54). Specifically, 20 h storage was associated with a low amounts of surfactant phospholipids and an impaired lung transplant function.

Importantly, the impact of the described changes in composition of pulmonary surfactant on its function and the function of the lung transplant greatly depends on the amount of serum protein leakage. Massive leakage of serum proteins will strongly inhibit the surfactant function, as has been described in ARDS (17). Leakage of serum proteins as part of the ischemia-reperfusion injury was observed in most studies (45, 46, 53, 55) and impairs the surfactant function and the function of the lung transplant (45).
It can be concluded that pulmonary surfactant is most severely affected as the lung donor is ventilated with high tidal volumes and by ischemia-reperfusion injury after a significant warm or cold ischemic period. Alterations in composition of surfactant after reperfusion are comparable for warm (< 2h) storage and long term cold storage, for inflated or deflated storage, and among different preservation solutions. However, there is some indication that inflated storage might be beneficial for pulmonary surfactant upon reperfusion. The surfactant alterations may result from a disturbed alveolar surfactant metabolism (altered intracellular synthesis or increased conversion from heavy to light surfactant subtype) or a direct breakdown of surfactant components (17). The loss of intracellular lamellar bodies after reperfusion (51) supports the hypothesis of an impaired synthesis of surfactant. Importantly, the changes in surfactant are paralleled by an increased leakage of serum proteins that further inhibit surfactant function and that may contribute to the conversion of heavy to light surfactant subtypes (56).

Surfactant treatment in lung transplantation

The rationale for surfactant treatment during the transplantation procedure is to improve the insufficient biophysical function of surfactant and thereby the lung transplant function. The efficacy of surfactant treatment in lung transplantation might be expected to be dependent on similar factors as in other acute lung injury models, including early timing of surfactant treatment, the severity of the injury at the time of treatment, the mode of surfactant delivery, and the amount and kind of surfactant used. In lung transplantation studies surfactant treatment was usually given before reperfusion with a high dose of modified natural surfactant, containing phospholipids and SP-B and SP-C but no SP-A. In one study surfactant treatment was started at a very early stage in the lung donor with the goal to prevent lung injury caused by ventilation.

Effects on alveolar surfactant

As might be expected treatment with supra-normal amounts of exogenous surfactant phospholipids (minimally 50 mg/kg BW per lung) in the donor lung or in the lung transplant just before reperfusion increased the amount of surfactant phospholipids, increased the fraction of heavy subtype and altered the composition of phospholipids in BALF obtained 1 h to 6 h after reperfusion of the transplant (53, 55). Additionally, surfactant treatment
preserved the amount of endogenous SP-A (53, 57) These improvements normalized the biophysical function of surfactant when measured in vitro, even in transplants with severe reperfusion injury after 20-h storage (53). In addition surfactant instillation (after 1 h warm storage) prevented the loss of lamellar bodies from the type II pneumocytes, indicative for an effect on the surfactant metabolism (51). Another parameter, indicating uptake of the exogenous surfactant, is the degree of association of the given surfactant with the lung transplant tissue. This measure was introduced to interpret the results of a combined surfactant treatment of the lung donor and of the lung transplant just before reperfusion after implantation into the recipient. The degree of association of surfactant with lung tissue proved to be higher after the combined treatment than after separate donor or recipient treatment (52). Very early surfactant nebulization, starting before lung donors were ventilated for 8 h with high tidal volumes causing lung injury, preserved the heavy subtype surfactant and prevented protein leakage and neutrophil accumulation. This preservation of surfactant components was still apparent after 17 h cold inflated storage and 6 h reperfusion (33). In another study surfactant instillation before reperfusion prevented an decrease of the amount of surfactant phospholipids 1 week after transplantation after severe transplantation injury caused by 20h ischemia (54).

**Effects on lung transplant function**

The effect of surfactant treatment on lung transplant function varied among studies. In the first study surfactant treatment failed to improve transplant function in dogs; exogenous surfactant was given into the lung donor before cold inflated storage (35-h) and into the donor lung in the recipient just before reperfusion (58). In another study surfactant treatment before reperfusion improved lung transplant function in 3 out of 8 treated dogs (38 h cold storage) (55). The improvement of transplant function in the three dogs started after 3 h of reperfusion. In a following study, again the efficacy of surfactant treatment was improved by nebulizing an extra dose in the lung donor followed by an instilled dose just before reperfusion. This strategy resulted in a normal gas exchange and compliance immediately after reperfusion whereas instillation just before reperfusion had no effect (52). In rats prolongation of the storage period decreased the effect of surfactant instillation just before reperfusion: after 6 h cold deflated storage the gas exchange and dynamic compliance were significantly better improved than after 20 h storage (53). Lung function of 20 h stored lungs assessed 1 week after transplantation was however improved by instillation before reperfusion.
although the immediate function was not improved (54). In a later study using inflated instead of deflated storage for 20 h, instillation of surfactant before reperfusion could not improve the immediate lung transplant function, possibly because the inflated storage had resulted in a relatively good transplant function in parallel with mild changes in surfactant components (57). Suppletion of surfactant with SP-A, as an attempt to counteract serum protein inactivation of surfactant, was not more effective than surfactant alone. Very early surfactant treatment of the lung donor preserved donor lung function and, after reperfusion, lung transplant function at pretransplantation levels (33). One of the explanations for the preservative effect of the early surfactant treatment was that the surfactant mitigated ventilator induced lung injury.

Clinically, a successful rescue treatment with surfactant is reported after a single lung transplantation. Surfactant nebulization was initiated several hours after reperfusion as treatment of reperfusion injury of the transplant. The diagnosis reperfusion injury was based on an impaired gas exchange, a decreased dynamic lung compliance and edema (clinically and on chest X-rays) requiring a higher FiO$_2$ and PEEP. The effect of surfactant treatment was an increase of the dynamic compliance after 30 minutes. After several hours the FiO$_2$ and PEEP could be lowered. The patient was extubated the following day (59).

These studies show that surfactant treatment is a potential option to improve the function of lung transplants immediately after reperfusion. However, the best delivery strategy needs to be developed. Early surfactant treatment of lung donors was most successful. The high association of exogenous surfactant given to the donor as part of a combined treatment of donor and recipient might indicate that in the donor surfactant is incorporated in the still normally functioning type II pneumocyte. The excellent transplant function after the combined treatment might indicate that both the alveolar as well as the intracellular pool need to be supplemented to optimize transplant function. Another way to explain the excellent lung transplant function after the combined treatment is that nebulization of surfactant in the donor enhances the spreading of the instilled surfactant in the recipient since an adequate surfactant layer promotes spreading (60). Surfactant treatment of an inflated lung before reperfusion, now necessarily as bolus instillation, might be improved when more efficient nebulization methods are available because there is some indication that nebulized surfactant spreads better than instilled surfactant.
Conclusions

In this review we have focused on the biophysical function of surfactant, which is important for the immediate lung transplant function after reperfusion. Other functions of surfactant, such as its role in innate lung defense and immunemodulating properties, might be important for long term transplant function. For the peri-operative period the following general conclusions can be drawn.

Careful donor management, especially ventilation with small tidal volumes, is important to preserve pulmonary surfactant and lung transplant function after implantation. Storage (within certain time limits) does not necessarily affect the amount, phospholipid composition and function of pulmonary surfactant. In contrast, reperfusion can deteriorate the pulmonary surfactant after transplantation depending on the duration and method of storage. Inflation during prolonged cold storage seems to preserve both surfactant function and subsequent lung transplant function after reperfusion. There is some indication that the kind of preservation solution used during storage affects the outcome on pulmonary surfactant. Early surfactant treatment, preferentially before the start of transplant injury, improves the function of the lung transplant. Nebulization of surfactant was effective in the lung donor. It remains to be investigated whether instillation or nebulization of surfactant is to be preferred after storage or after reperfusion.

Acknowledgements
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Chapter 1b

Aims of this thesis
Aims of this thesis

The first aim of this thesis is to elucidate whether lung storage and lung transplantation are harmful to the pulmonary surfactant system. The second aim is to investigate the effects of treatment by intrabronchial instillation of surfactant on composition and function of pulmonary surfactant, and the effects on lung transplant function.

We first investigate the composition and function of pulmonary surfactant after cold storage of rat lungs (chapter 2A). Pulmonary surfactant is obtained by bronchoalveolar lavage of the left or right lung. Next, composition and function of pulmonary surfactant in relation to lung transplant function is investigated at 1 day after transplantation (chapter 2B).

In the next part of the study, we treat lung transplants by instillation of a modified surfactant into the bronchus just before reperfusion to improve the quality of pulmonary surfactant and the function of the lung transplant. We analyze the immediate function of the transplants during 1 hour after reperfusion (chapter 3A) and at 1 week after transplantation (chapter 3B). It is shown that the effect of the surfactant instillation on the transplant function is correlated to the changes in surfactant components. Based on the findings of these two studies, we enriched surfactant with surfactant-associated protein-A (SP-A) to improve the efficacy of surfactant instillation (chapter 3C).

Finally, we investigate in a pilot study to what extent the pulmonary surfactant changes in patients after lung transplantation. In this study pulmonary surfactant is sampled by aspiration of tracheal fluid during the first 24 h after sequential double lung transplantation (chapter 4A).