

REVIEW ARTICLE

## Surfactant alterations and treatment of lung transplant ischemia–reperfusion injury

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### Abstract

This review addresses surfactant alterations and treatment in lung transplant ischemia–reperfusion injury. Lung ischemia–reperfusion injury damages the endogenous surfactant system as a result of the production of reactive oxygen species, proteolytic enzymes and (phospho)lipases. Surfactant is composed of phospholipids and proteins and its main function is to reduce the surface tension inside the alveolus. Impairment of surfactant will cause atelectasis, influx of serum proteins, pulmonary edema, decreased lung compliance and impaired gas exchange. Surfactant therapy restores the quantity and composition of surfactant and reduces the inhibitory effect of serum proteins; other effects are that it serves as an antioxidant and anti-inflammatory agent. Pretreatment may be more beneficial than treatment after the development of lung ischemia–reperfusion injury. However, the cost of surfactant must be weighed against the clinical outcome.

**Key words:** Ischemia–reperfusion, lung pathology, lung transplantation, surfactant

### Lung transplantation

Lung transplantation is nowadays a well-accepted treatment option for patients with end-stage pulmonary diseases. Although improvements in lung preservation, pre- and postoperative care and surgical techniques have been made, the outcome of human lung transplantation remains limited (1,2). Development of primary acute graft failure (PAGF) is the main cause of early morbidity and mortality after lung transplantation, resulting in a 1-year survival rate of  $\approx 80\%$  (1,2). Moreover, long-term prognosis is also limited (5-year survival  $< 50\%$ ) due to the development of bronchiolitis obliterans syndrome (BOS) (1). Lung ischemia–reperfusion injury (LIRI) is thought to contribute significantly to both PAGF and BOS (1,3,4).

### PAGF

PAGF, which symptomatically resembles the acute respiratory distress syndrome (ARDS), usually develops within 72 h after transplantation (5).

Symptoms of PAGF consist of non-cardiogenic pulmonary edema, increased pulmonary artery pressure, decreased lung compliance and impaired gas exchange (5–9). Histological analysis of PAGF lungs shows diffuse alveolar damage with micro-atelectasis. Although  $\approx 97\%$  of the recipients show some degree of reperfusion edema on chest X-ray, severe LIRI occurs in 15–30% of lung transplant recipients (6).

Experimental studies of LIRI (7–10) have shown that abnormalities and depletion of pulmonary surfactant (surfactant) contribute significantly to the symptoms seen in PAGF of the lung. Furthermore, surfactant obtained by means of bronchoalveolar lavage of human lung transplant recipients demonstrated surfactant dysfunction up to 7 years after transplantation, thereby contributing to lung malfunction (11).

Pulmonary surfactant is essential for normal breathing, as it diminishes the surface tension at the air–fluid interface inside the alveolus (12). Herewith, the alveolus is kept open at the

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end of expiration and fluid homeostasis is preserved (12).

**BOS**

Although severe LIRI contributes considerably to the development of PAGF, the impact of LIRI on long-term mortality is still the subject of debate. The major obstacle to long-term survival is the development of post-lung transplant BOS, which is associated with chronic transplant dysfunction (1,13). BOS affects ≈50% of patients who survive for >3 months after transplantation (13). The pathogenesis of BOS is not completely understood, but appears to involve a “response to injury” type of pattern, where multiple injuries may finally result in BOS (Fig. 1). Both donor characteristics and transplant procedure complications may result in early lung injury. Reperfusion injury may worsen early lung injury, whereafter rejection and alloantigen-independent factors (pneumonia, cytomegalovirus) can act as subsequent injuries and increase the risk of BOS (2,3,13,14).

**LIRI**

LIRI predominantly occurs with lung transplantation. However, LIRI symptoms have also been described after cardio-pulmonary bypass, isolated lung perfusion and pulmonary sleeve resection.

As severe LIRI plays a significant role in the development of PAGF after lung transplantation and as LIRI is an early participant in the multiple-hit theory of BOS, treatment of LIRI may decrease early morbidity and mortality after LIRI, but may also prevent or delay the onset of BOS, thereby influencing the late morbidity and mortality of lung transplantation.

*Pathophysiology*

Ischemia can be defined as a blood (oxygen) deficiency in the lung caused by constriction or obstruction of its blood vessels. Reperfusion occurs when the blood flow to the organ is restored. During the transplantation period, the lung is stored hypothermically to reduce the rate of biochemical reactions, which results in decreased degradation of important cellular components. Nevertheless, adenosine triphosphate (ATP) is depleted during ischemia, which ultimately causes inactivation of ATP-dependent membrane pumps, accumulation of intracellular calcium, the formation of eicosanoids and reactive oxygen species (ROS), inflammation and cell death (Fig. 2) (2,15).

*Inactive ATP-dependent membrane pumps and intracellular calcium accumulation*

Under normal conditions, the action of the Na<sup>+</sup>/K<sup>+</sup>-ATP synthase (ATPase) pump sets up a

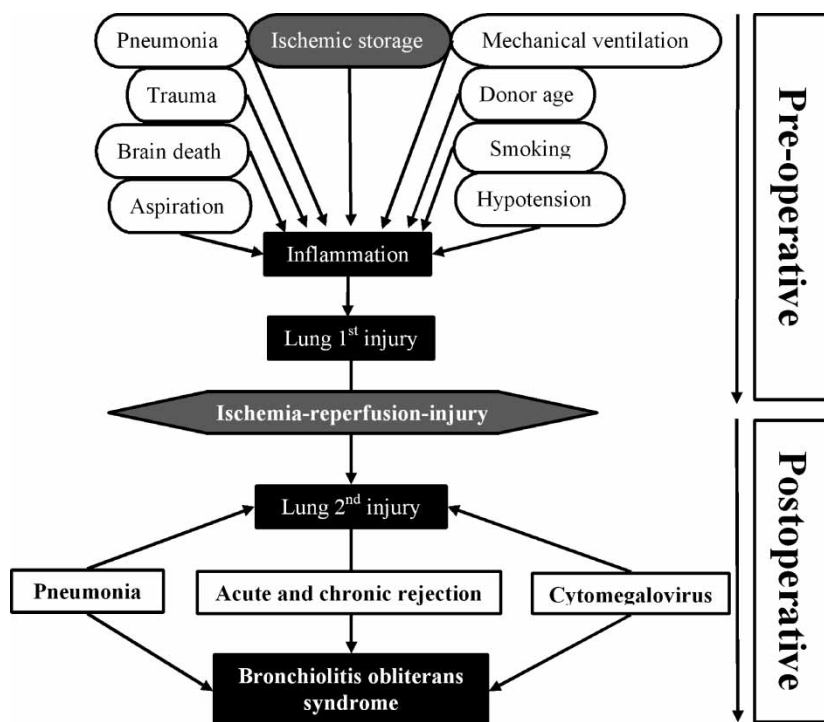


Fig. 1. Lung transplantation: a multiple-hit theory for the development of BOS.

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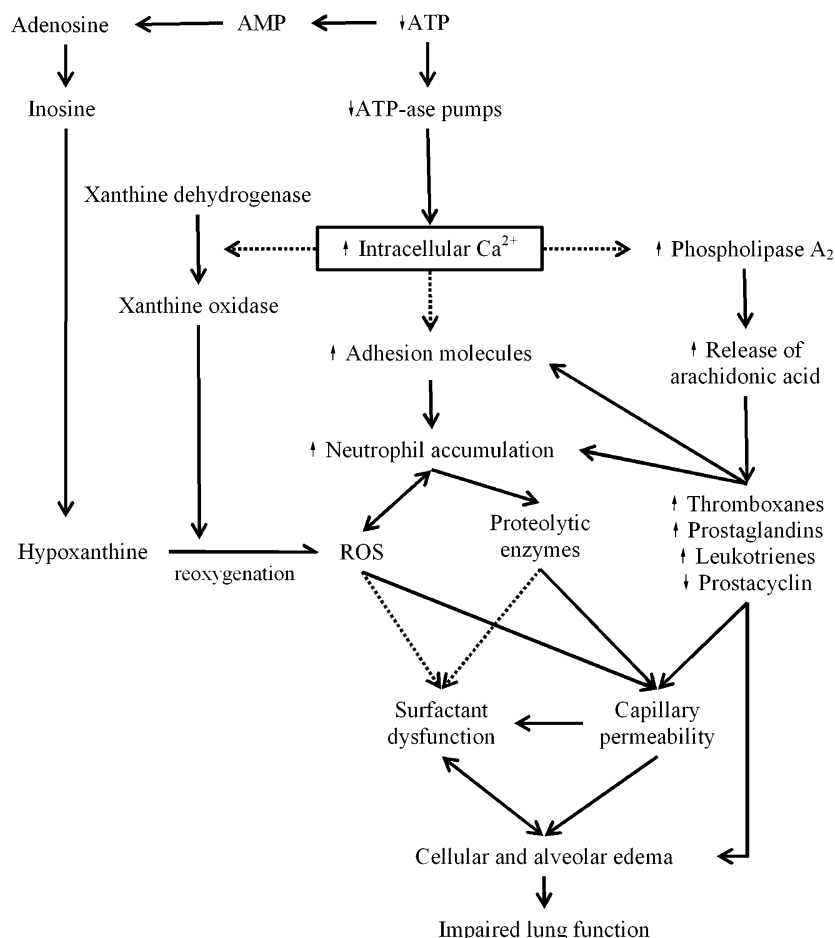


Fig. 2. Pathophysiology of LIRI. See text for details. AMP = adenosine monophosphate.

gradient of high extracellular  $\text{Na}^+$  relative to intracellular levels, which in turn drives the  $\text{Na}^+/\text{Ca}^{2+}$ -exchanger, so that  $\text{Ca}^{2+}$  is pumped out of the cell. During ATP depletion, the  $\text{Na}^+/\text{K}^+$ -ATPase pump becomes inactivated, leading to an increase in intracellular  $\text{Na}^+$ . As a result, the  $\text{Na}^+/\text{Ca}^{2+}$  pump will not function, causing  $\text{Ca}^{2+}$  to accumulate inside the cell. Other mechanisms contributing to high intracellular  $\text{Ca}^{2+}$  levels are an inactive plasmalemmal ATP-dependent  $\text{Ca}^{2+}$  pump, liberation of stored cytoplasmic  $\text{Ca}^{2+}$  due to acidosis and a decreased uptake by the sarcoplasmic/endoplasmic reticulum (2,15).

Cytosol-elevated  $\text{Ca}^{2+}$  activates phospholipase  $\text{A}_2$ , which results in the induction of arachidonic acid. Arachidonic acid is normally incorporated in the cell membrane and functions as a precursor for the production of eicosanoids, consisting of thromboxanes, leukotrienes, prostacyclin and prostaglandins. Whereas thromboxanes are predominantly produced by platelets, leukotrienes are formed by leukocytes, prostacyclin by endothelial cells and prostaglandins by smooth muscle cells. The effects

of eicosanoids are various but include vasoconstriction, activation of adhesion molecules, an increase in capillary permeability and the accumulation and extravasation of neutrophils (2,15).

Finally, increased intracellular  $\text{Ca}^{2+}$  causes transformation of xanthine dehydrogenase into xanthine oxidase, thereby facilitating the production of ROS, as described in the next section (2,15).

#### Production of ROS

In the aerobic setting, ATP is converted to urea and xanthine by the effect of xanthine dehydrogenase. However, due to the formation of xanthine oxidase in LIRI, hypoxanthine is broken down into ROS at the moment of reoxygenation. A second system for generating ROS is the reduced nicotinamide adenine dinucleotide phosphate oxidase system, which is predominantly present on the membrane surfaces of monocytes, macrophages, neutrophils and endothelial cells and catalyzes the reduction of oxygen to superoxide and hydrogen peroxide. The superoxide anion,

hydrogen peroxide and hydroxyl radical, which are all part of the ROS family, are very unstable and damage cellular membranes by lipid peroxidation (2,15).

### Inflammation

LIRI causes release of proinflammatory cytokines by macrophages. Consequently, neutrophils and lymphocytes are recruited into the lung. Because of the expression of adhesion molecules on endothelium and leukocytes, leukocytes roll, adhere to and extravasate into the lung tissue. Macrophages and neutrophils contribute to cellular damage by the production of ROS and several other mediators, such as proteolytic enzymes, lysozyme and lactoferrin (2,15).

### Studying LIRI

Several experimental models can be used to study treatment modalities for LIRI and its pathophysiology. Although the best model is obviously a lung transplantation model, this is a very time-consuming procedure, especially in small animals, and is technically difficult, often with high mortality rates. Therefore, an in situ lung clamp model, in which the bronchus, pulmonary veins and artery of (usually) the left lung are clamped to induce LIRI, has been developed. The clamping time generally ranges from 60 to 150 min (10,16,17). Although this model is technically much easier than the aforementioned transplantation model, it still has some disadvantages. Firstly, only warm ischemia can be studied as the lung is kept in situ. Furthermore, no preservation solutions are used and mortality may still be high because the animals are (preferably) ventilated after reperfusion for as short a period as possible due to the confounding effects of ventilation on LIRI (18).

Although the clamp model does not totally resemble the events of real lung transplantation, the symptoms of LIRI found in this model approximate those seen with PAGF. The macroscopic result of severe LIRI (120 min) is demonstrated in Fig. 3. Figure 4 shows the microscopic effect of 120 min of warm ischemia, 24 h after reperfusion, as compared to the right (non-ischemic) lung. LIRI results in intra-alveolar and septal edema, inflammation, atelectasis and intra-alveolar hemorrhage.

### Surfactant

Surfactant is responsible for decreasing the surface tension between air and the alveolo-capillary membrane. It thereby prevents the alveoli of the lung

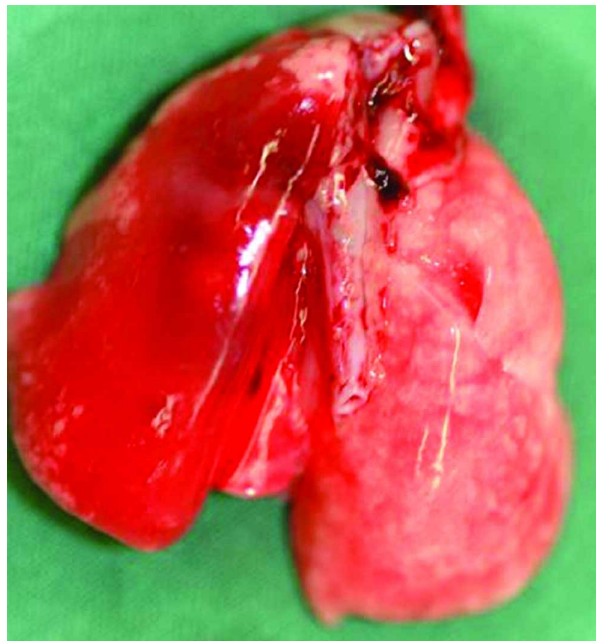


Fig. 3. Dorsal macroscopic view of rat lungs which underwent 120 min of left lung warm ischemia and 180 min of ventilation after reperfusion. The right lung did not suffer LIRI.

from collapsing at the end of expiration, facilitating inflation of the lung with minimal effort (12). In addition, lowering the surface tension is important for fluid homeostasis in the lung. Furthermore, surfactant protects the lung against microorganisms and serves as a functional barrier in the alveolus, so that the transfer of molecules across the alveolo-capillary membrane is limited. Finally, surfactant is presumed to have immune downregulating effects (12,19).

Surfactant is composed of lipids (90%) [mainly dipalmitoyl-phosphatidylcholine (DPPC)] and surfactant-associated proteins (SPs) (10%). The proteins can be divided into two groups: the hydrophilic (SP-A and -D) and hydrophobic (SP-B and -C) proteins (12,19).

The surface tension-lowering capacity of surfactant is predominantly due to DPPC. SP-B and -C have been demonstrated to enhance lipid insertion into the monolayer at the air-liquid interface. In this way they protect the surface film from being contaminated by non-surfactant proteins, which degrade surfactant (12,19).

As a result of their ability to recognize a broad spectrum of pathogens, SP-A and -D are believed to be molecules of the innate immune system. Several studies have shown that SP-A and -D interact with a number of viruses, bacteria, fungi and allergens. SP-A has also been suggested to play an important role in phospholipid secretion and recycling, the formation of tubular myelin and the blocking of surfactant inhibition by serum proteins (12,19).

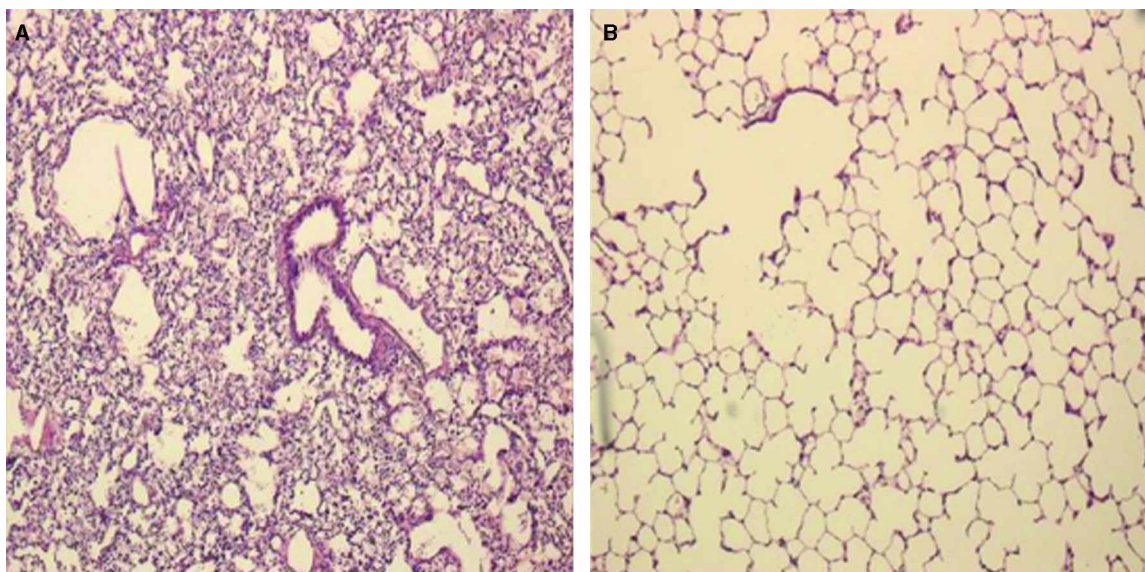


Fig. 4. Microscopic views of (A) a left and (B) a right rat lung which underwent 120 min of left lung warm ischemia. Histological assessment was made 24 h after reperfusion.

Surfactant can be divided by ultra centrifugation into two subfractions, which differ in terms of their morphological appearance and density. The heavy subtype or large aggregate (LA) subform of surfactant is highly surface-active, contains a high amount of SPs and is comprised of tubular myelin, lamellar bodies and large vesicles. The light subtype or small aggregate (SA) subform has a poor surface tension-lowering capacity and consists of small vesicles. When a lung is damaged, conversion of LAs to SAs occurs, resulting in an increased SA:LA ratio (7,10,12,19).

Production and secretion of surfactant is done by alveolar type II (ATII) cells. ATII cells and alveolar macrophages are important for the recycling of surfactant lipids, which is essential for maintaining homeostasis of the endogenous surfactant pool (19–21).

### Surfactant damage

Figure 5 summarizes the pathways by which LIRI exerts a damaging effect on surfactant.

#### *Surfactant-associated proteins*

As mentioned before, SPs play an important role in maintaining the quality of the surfactant lining of the alveolar epithelium. SPs are damaged due to the formation of proteases and ROS, resulting in impairment of surfactant recycling (SP-A), the ability to block surfactant inhibition by serum proteins (SP-A) and lipid insertion (SP-A–C). Decreased levels or inactivation of SPs can result in a diminished quantity of phospholipids, but also

in a changed composition of surfactant, thereby impairing surfactant-lowering properties (22).

A decrease in SP-A–C was measurable after prolonged ischemic storage without reperfusion and it decreased further after the start of reperfusion (22). Moreover, in lung transplant recipients, the level of SP-A was found to be decreased >1 year after transplantation (23). It was also shown (24) that the level of SP-A decreased with increasing severity of LIRI, suggesting that preservation of SP-A is essential for improvement after LIRI.

Some studies (7,8) have reported surfactant dysfunction without an alteration in the overall amount of phospholipid, but with changes in surfactant composition, which could be the result of impaired SPs. Both decreases in DPPC and phosphatidylglycerol and an increase in sphingomyelin have been described (7,8,22,25).

#### *Plasma proteins*

The presence of plasma proteins in the alveoli after LIRI has been described in many studies (7,8,22,24,26–35). Both warm and cold ischemic intervals have resulted in increased levels of plasma protein 1–24 h after reperfusion (7,8,24,26–29). Owing to ROS, proteolytic enzymes and phospholipases, endogenous surfactant and the endothelial and epithelial membranes are damaged (2,15). This results in leakage of serum proteins into the alveolus and of surfactant components into the bloodstream. As surfactant is rate-limiting for the transfer of proteins across the alveolo-capillary membrane and is either inactivated or lost due to the increased endothelial permeability after LIRI, a further influx

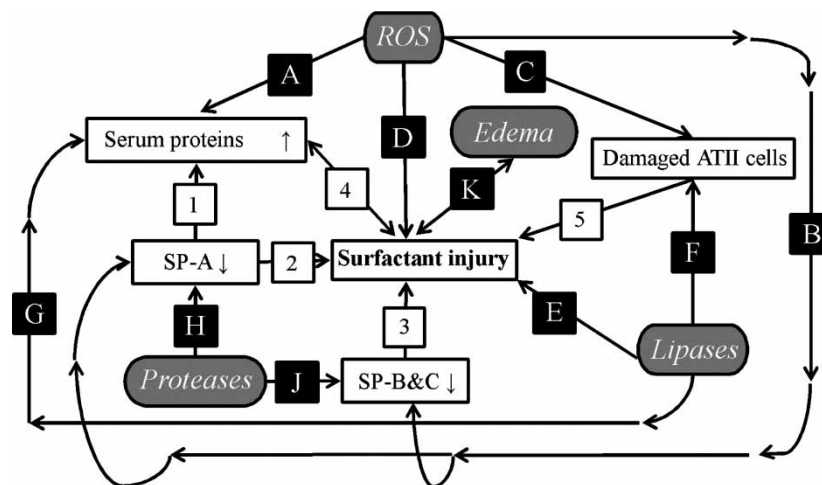


Fig. 5. ROS directly damage (A) the alveolo-capillary membrane, thereby facilitating the influx of serum proteins into the alveolus, (B) SP, (C) ATII cells and (D) the LA surfactant subform. Phospholipases cause degradation of (E) surfactant phospholipids inside the alveolus, (F) the membranes of ATII cells and (G) capillary endothelium, resulting in the influx of serum proteins. Proteinases break down (H) SP-A and (J) SP-B and -C. (K) Edema results in dilution of the surfactant phospholipids inside the alveolus, which results in further formation of edema. A decrease in SP-A leads to (1) less inhibition of serum proteins and (2) decreased phospholipid secretion, recycling and formation of tubular myelin. (3) Degradation of SP-B and -C causes less phospholipids to be inserted into the phospholipid monolayer lining the alveolar epithelium. (4) Once serum proteins have infiltrated the alveolus, they compete for a place at the air-liquid interface, thereby dose-dependently inhibiting surfactant function. Furthermore, once the phospholipid monolayer is damaged, the molecule transfer-limiting function of surfactant is also impaired, resulting in further influx of serum proteins, so that a vicious circle has developed. (5) ATII cells, which are important in the production, recycling and secretion of surfactant phospholipids are damaged, so that less LA is secreted and a smaller amount of SA is being recycled. Owing to these factors, a decrease in LA and an increase in SA has been noticed after LIRI.

of proteins is facilitated. Because proteins, once accumulated in the alveolus, then dose-dependently inhibit surfactant, this results in a self-triggering mechanism of surfactant inactivation (36). Under normal conditions, SP-A is partly able to counteract the inactivating effects of serum proteins (37). However, after LIRI, a decrease in SP-A was found in human lung transplant recipients and in animal models of LIRI (23,24).

### Surfactant therapy

Because damaged surfactant contributes to symptoms seen with PAGF, surfactant replacement therapy has been investigated using experimental ARDS and LIRI models (9,22,24,26–31,38,39). The effect of surfactant therapy has also been investigated in some case studies.

#### Clinical (case) studies

In 1995, Struber et al. (40) reported on a 26-year-old female who underwent right lung transplantation and developed severe LIRI 5 h after transplantation. She was subsequently treated with an intrapulmonary nebulized synthetic surfactant. Shortly afterwards, lung compliance, PaO<sub>2</sub> and tidal volume increased. Moreover, 24 h after therapy, the edematous infiltrate of the transplanted lung observed on chest X-ray film was

resolved. Another study (41) in 6 lung transplant patients also suggested improvement in LIRI due to surfactant replacement. However, in 1 of the 6 recipients, surfactant therapy failed, which could be attributed to the application approach or to the type of surfactant used.

#### Experimental studies

Although surfactant damage can be reduced by changing the preservation solution from Euro-Col-lins to low-potassium dextran solution, and by flushing the graft in a retrograde instead of an antegrade fashion, there is still a rationale for using surfactant replacement therapy in the case of severe LIRI (31–35,42). It was demonstrated (26,28) that surfactant administration improved lung compliance and PaO<sub>2</sub> and prevented an increase in the SA:LA ratio.

Most studies in which the effect of surfactant replacement therapy has been investigated have only addressed the first hours after reperfusion. Longer-term studies of LIRI and surfactant treatment are scarce. In this regard, Erasmus et al. (38) demonstrated that surfactant treatment just before reperfusion enhanced recovery from LIRI 1 week postoperatively. We confirmed (10) that LIRI resulted in an increased SA:LA ratio and in impaired PaO<sub>2</sub> and lung compliance throughout the first week after reperfusion. However, even months after re-

perfusion, diffuse alveolar damage and decreased lung compliance were still visible (NP van der Kaaij, unpublished observations, 2005). Surfactant pretreatment completely normalized these parameters from Day 3 to Day 90 after reperfusion (10).

#### Pre- or post-treatment

Some studies (28,43–45) have suggested that treatment with exogenous surfactant before the onset of ischemia is more beneficial compared to treatment at or after reperfusion. This can be explained as the result of an enlarged surfactant pool before the lung sustains LIRI, which prevents deterioration of the entire endogenous surfactant pool as a result of LIRI. This can be illustrated by the fact that the normal endogenous surfactant pool is  $\approx 10\text{--}15$  mg lipid/kg, and that the amount of surfactant used for treatment is in the range 50–400 mg lipid/kg (9,44). It was shown that the size of the surfactant pool is inversely proportional to the duration of ischemia because of the remaining activity of phospholipases during ischemia, so that enlargement of the pool before ischemia reduces the risk of total inactivation of the surfactant pool.

Pretreatment also has the advantage of preserving the endogenous SPs, although some (natural) surfactants may contain SP-B and -C. Also, surfactant given to the donor results in a more homogeneous distribution as compared to treatment after reperfusion, when alveolar damage has already occurred (Fig. 6) (9). In the latter case, intratracheally instilled surfactant will predominantly accumulate in open areas of the lung instead of atelectatic areas, where it is most needed.

Although surfactant pretreatment seems more beneficial than treatment after LIRI, the clinical use of pretreatment in human lung transplantation is inhibited by the cost of the material. Therefore, if surfactant pretreatment is to be considered in the clinical setting, the cost of surfactant must be weighed against the possible improved outcome and shorter hospitalization period.

### The pathways of surfactant therapy for LIRI

The rationale behind surfactant replacement therapy is to ameliorate the damage caused by ROS, to preserve the levels of DPPC and SPs and to decrease the inhibitory effects of serum proteins.

#### Anti-inflammatory and antioxidant function

Surfactant has been shown to inhibit cytokine release from activated monocytes and macrophages (46,47); the modulation of lymphocytes has also been sug-

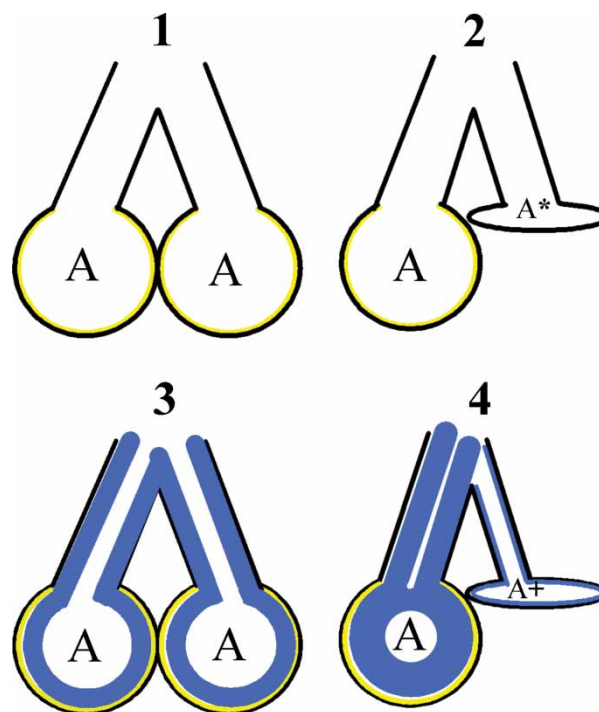


Fig. 6. The hypothesis that pretreatment of the lung is more beneficial than treatment after development of LIRI. (1) Normal alveoli (A) of the lung, in which the normal endogenous surfactant system (yellow) keeps the alveoli open at the end of expiration. (2) Lung alveoli after the development of LIRI: the endogenous surfactant system is inactivated, resulting in collapse of the alveoli of the lung (A\*). (3) Surfactant pretreatment results in an homogeneous distribution of the exogenous surfactant (blue), so that all alveoli of the lung are optimally protected against forthcoming injury. (4) Surfactant treatment after LIRI: exogenous surfactant will predominantly accumulate in the open areas of the lung, whereas it will not arrive in those areas of the lung where it is most needed (A+), resulting in an inhomogeneous distribution of the exogenous surfactant.

gested (10). Furthermore, surfactant is known to have antioxidant capacities, resulting in reduced ROS injury (48). Surfactant treatment can thus ameliorate the effects of inflammatory cells, so that endothelial and ATII cell injury is decreased, normalizing capillary permeability and surfactant recycling. It was shown (23) that the decreased function of ATII cells after LIRI is prevented by surfactant therapy.

#### Restoration or preservation of surfactant composition

Surfactant therapy mainly consists of administration of the LA subform (DPPC), which is the active surface tension-lowering form of surfactant, so that the level of LA surfactant in the alveolus is restored, which has a major impact on lung function. Surfactant pretreatment preserves the level of LA before the lung sustains ischemia and reperfusion, thereby decreasing lung injury instead of treating it (10).

Furthermore, restoring or preserving the levels of SPs contributes to normal phospholipid recycling and secretion (22). It was demonstrated (24) that SP-A-enriched surfactant was able to improve lung function after prolonged ischemia, whereas this was not possible to the same extent with SP-A-deficient surfactant. Also, a decrease in the LA subform was found, indicating an increased recycling capacity of the SP-A-enriched surfactant compared with SP-A-deficient surfactant (24).

#### *Decreasing the inhibitory effects of serum proteins*

When the quantity of surfactant is low, its composition has changed or capillary permeability has developed, serum proteins leak into the alveolus, where they further interfere with surfactant (49). Surfactant post-treatment may interrupt this vicious circle by restoring the quantity and composition of surfactant phospholipids (26,36,50,51).

Pretreatment with surfactant decreases capillary permeability and preserves the composition and quantity of surfactant phospholipid and the level of SPs. Preservation of SP-A is important, due to its inhibiting effect on serum proteins. Cockshutt et al. (37) showed reversed inhibition of serum proteins when SP-A was administered.

#### **Conclusions**

In an experimental setting, surfactant therapy for severe LIRI has proven to be effective. However, in human lung transplantation it has not yet become standard treatment for PAGF. Further clinical studies should investigate whether surfactant (pre)treatment is a realistic option in the field of human lung transplantation.

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