Ischemia-reperfusion Injury of the Lung: Role of Surfactant

N. P. Van der Kaaij, A. J. J. C. Bogers, and B. Lachmann

▪ Introduction: Lung Transplantation and Lung Ischemia-reperfusion Injury

Lung transplantation is an accepted treatment for patients with end-stage pulmonary disease. Despite refinement in lung preservation and improvement in surgical techniques and peri-operative care, lung ischemia-reperfusion injury remains a significant cause of early morbidity and mortality after lung transplantation [1]. Clinical symptoms will usually occur within the first 72 hours after lung transplantation and consist of non-cardiogenic lung edema, increased pulmonary artery pressure and resistance, decreased lung compliance and hypoxemia [2–7]. Microscopy shows diffuse alveolar damage with micro-atelectases. Although approximately 97% of the recipients show some degree of reperfusion edema on chest X-ray, severe lung ischemia-reperfusion injury occurs in 15–30% of lung transplant recipients [8]. The most severe form of lung ischemia-reperfusion injury is primary acute graft failure which clinically resembles the acute respiratory distress syndrome (ARDS); both conditions lead to increased utilization of intensive care resources, extended hospital stay and mortality [9–11].

The acute phase of ARDS can either resolve quickly or result in death. Already 4–7 days after onset of the clinical symptoms, the acute phase can develop into a 'chronic' fibroproliferative state, which is characterized by hyperplasia of alveolar type II (AT II) cells, infiltration of activated fibroblasts, collagen deposition, and remodeling of lung architecture [12]. However, this fibroproliferative state was thus far not described after lung ischemia-reperfusion injury. We now confirm in an animal model that also after severe lung ischemia-reperfusion injury, impaired oxygenation capacity of the lung, decreased lung compliance, and a fibroproliferative pre-stage are observed months after reperfusion (van der Kaaij, et al., unpublished data).

Lung ischemia-reperfusion injury is the main cause for early morbidity and mortality after lung transplantation. Nevertheless, one-year survival after lung transplantation is about 75%. The long-term prognosis also remains limited, with a 5-year survival of less than 50% [1]. The major obstacle to long-term survival is the development of post lung transplant bronchiolitis obliterans syndrome, which is associated with chronic transplant dysfunction. This affects about 50% of patients who survive beyond 3 months after transplantation [13]. The exact etiology of the bronchiolitis obliterans syndrome is not fully understood but its pathogenesis appears to involve a 'response to injury' type of pattern, where multiple periods of injury may result in this syndrome (Fig. 1). Both donor characteristics (age,
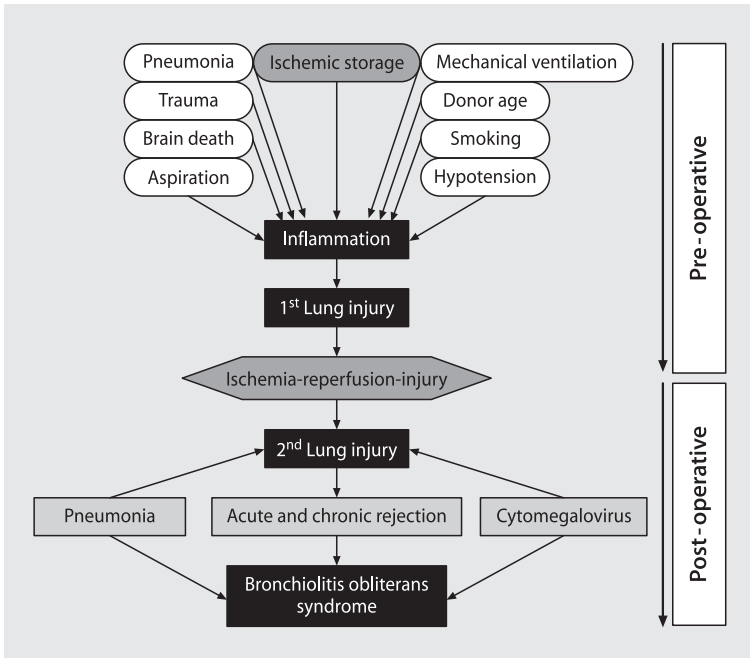
Fig. 1. Lung transplantation: A multiple hit theory for the development of bronchiolitis obliterans syndrome, which is associated with chronic transplant dysfunction of the lung

smoking years, cause of death) and complications during the donor procedure (ischemic time, hypotension, mechanical ventilation, aspiration) may result in early lung injury. Lung ischemia-reperfusion injury can aggravate this early lung injury, whereafter rejection and alloantigen independent factors (pneumonia, cytomegalovirus infection) can act as subsequent 'injuries' and increase the risk of bronchiolitis obliterans syndrome [10, 14, 15]. Because lung ischemia-reperfusion injury is an early contributor to lung injury, intervention at this stage may prove to decrease early morbidity and mortality after lung ischemia-reperfusion injury, but may also prevent or delay the onset of the bronchiolitis obliterans syndrome, thereby also influencing late morbidity and mortality after lung transplantation.

Several studies have highlighted the possibility that alterations in pulmonary surfactant (*surf*ace *act*ive *age*nt) are present shortly after lung ischemia-reperfusion injury, contributing to early morbidity and mortality [2–4, 16–19]. Furthermore, in bronchoalveolar lavage (BAL) fluid from human lung transplant recipients, surfactant dysfunction was still visible up to seven years after transplantation [20]. The changes in pulmonary surfactant after lung transplantation resemble the changes seen in ARDS and contribute to a great extent to lung dysfunction [11]. Clinical trials studying the effect of exogenous surfactant on ARDS appear promising, providing a rationale to study the possibilities of surfactant treatment for lung ischemia-reperfusion injury.

▌ Surfactant

To facilitate normal breathing with minimal effort, pulmonary surfactant lowers the surface tension at the alveolo-capillary membrane. In addition, lowering of the surface tension is important for the fluid homeostasis across the alveolo-capillary membrane. Furthermore, surfactant serves as a functional barrier in the alveolus, so that the transfer of molecules across the alveolo-capillary membrane is limited. Finally, surfactant protects the lung against microorganisms [10, 21, 22].

Surfactant is composed of lipids (90%), of which dipalmitoyl-phosphatidylcholine (DPPC) is the most surface tension lowering lipid, and surfactant associated proteins (SP) (10%). The proteins of pulmonary surfactant can be divided into two groups: the hydrophilic proteins SP-A and SP-D, and the hydrophobic proteins SP-B and SP-C. SP-B, as well as SP-C, have been demonstrated to enhance lipid insertion into the monolayer at the air/liquid interface. In this way they maintain a low surface tension, thereby protecting the surface film from being contaminated by non-surfactant proteins, which can result in inactivation or degradation of the surfactant film. SP-A and SP-D are believed to be molecules of the innate immune system through their ability to recognize a broad spectrum of pathogens. Several studies have shown that SP-A and SP-D interact with a number of viruses, bacteria and fungi, and with inhaled glycoconjugate allergens, such as pollen grains and mite allergens [10]. Furthermore, SP-A has been suggested to play an important role in phospholipid secretion and recycling, formation of tubular myelin and blocking surfactant inhibition by serum proteins [21, 22].

Surfactant can be divided by ultra centrifugation into two subfractions, which differ in morphological appearance and density. The heavy subtype or large aggregate subform of surfactant is highly surface active, contains a high amount of SP and is made up of tubular myelin, lamellar bodies and large vesicles. The light subtype or small aggregate subform has a poor surface lowering capacity and consists of small vesicles [22].

Production and secretion of surfactant is done by the AT II cells. Both AT II cells and alveolar macrophages are important for recycling of surfactant lipids, which is essential for maintaining homeostasis of the endogenous surfactant pool [22, 23].

▌ Fluid Homeostasis in the Lung

As discussed previously, surfactant is essential for maintaining normal fluid homeostasis in the lung and preventing pulmonary edema. Fig. 2 presents a diagram of fluid balance across the lung. The normal plasma oncotic pressure of 37 cmH_2O is opposed by the capillary hydrostatic pressure of 15 cmH_2O, the oncotic pressure of interstitial fluid proteins of 18 cmH_2O and by the surface tension conditioned suction pressure of 4 cmH_2O. In general, alveolar flooding will not occur when the surfactant system is properly functioning. However, when the surface tension rises above a critical level, alveolar flooding will occur, leading to influx of proteins into the alveolar space which results in further inactivation of surfactant [10, 24].
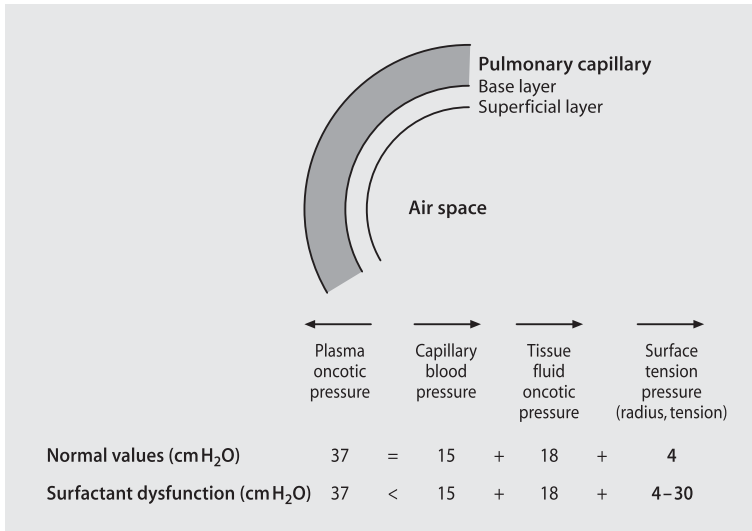
Fig. 2. Simplified diagram showing the factors influencing fluid balance in the lung (from [11] with permission)

▌ Lung Ischemia-reperfusion Injury: Pathophysiology

Lung ischemia-reperfusion injury, which occurs to a certain extent during lung transplantation, can damage the surfactant system. After the lung has been removed from the donor, the organ is hypothermically stored to reduce the rate of biochemical reactions, which results in a decreased degradation of important cellular components. Nevertheless, adenosine triphosphate (ATP) is depleted during ischemia, which ultimately causes inactivation of ATP-dependent membrane pumps, an increase in intracellular calcium, inflammation, the formation of reactive oxygen species (ROS), and cell death [10, 25] (Fig. 3).

Lung Ischemia-reperfusion Injury: Inactive ATP-dependent Membrane Pumps and Intracellular Calcium Accumulation

Under normal conditions, the action of the Na^+/K^+-ATPase pump sets up a gradient of high extracellular Na^+ relative to intracellular levels, which in turn drives the Na^+/Ca^{2+}-exchanger, so that Ca^{2+} is pumped out of the cell. During ATP depletion, the Na^+/K^+-ATPase pump becomes inactivated, leading to an increase in intracellular Na^+. As a result, the Na^+/Ca^{2+} pump will not function, causing Ca^{2+} to accumulate inside the cell. When ischemia is prolonged, the ionic balance may be so upset that the Na^+/Ca^{2+}-pump activity is reversed, resulting in import of Ca^{2+} in exchange for Na^+, thereby exacerbating calcium overload. Other mechanisms contributing to high intracellular Ca^{2+} levels are an inactive plasmalemmal ATP-dependent Ca^{2+}-pump, important to move Ca^{2+} out of the cell, liberation of stored cytoplasmic calcium due to the acidosis, and a decreased uptake by the sarcoplasmic/endoplasmic reticulum [10, 25].

Cytosol elevated Ca^{2+} activates phospholipase A_2, which results in the induction of arachidonic acid. Arachidonic acid is normally incorporated in the cell mem-
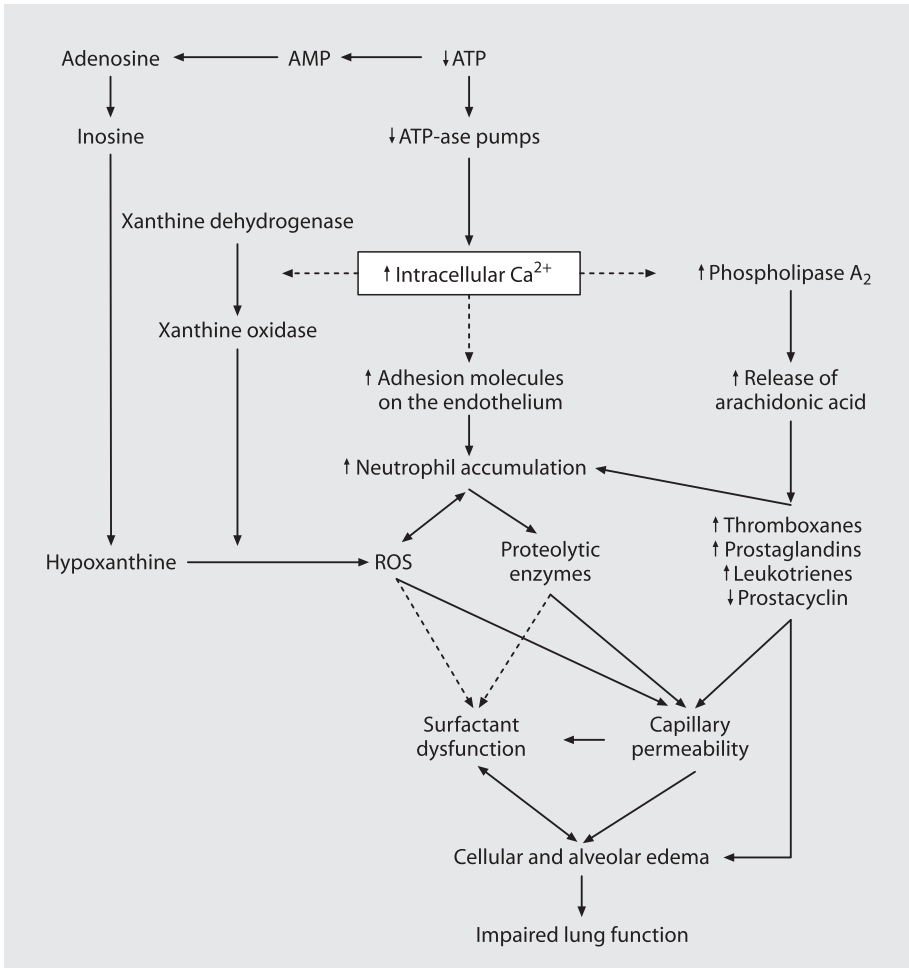
Fig. 3. Pathophysiology of lung ischemia reperfusion injury. See text for explanation. ATP: adenosine triphosphate; AMP: adenosine monophosphate; ROS: reactive oxygen species

brane and functions as a precursor for the production of eicosanoids, consisting of thromboxanes, leukotrienes, prostacyclin and prostaglandins. The effects of the eicosanoids due to tissue injury are various [10, 25].

While thromboxanes are predominantly produced by platelets, leukotrienes are formed by leukocytes (mostly neutrophils), prostacyclin by endothelial cells and prostaglandins by smooth muscle cells. Thromboxane A_2 has a potent vasoconstriction action, induces leukotriene production by neutrophils, and activates neutrophil adhesion receptors to facilitate interaction with the endothelium, which also expresses adhesion molecules due to the elevated Ca^{2+}. Leukotrienes too cause vasoconstriction, but may as well increase vascular permeability, and enhance neutrophil accumulation, adhesion and extravasation through the endothelium. Prostacyclin plays an important role in vascular function because it inhibits platelet adhe-

sion to the vascular endothelium and is a strong vasodilator. Damaged endothelial cells do not produce PGI_2, thereby making the vessel more susceptible to thrombosis and vasospasm. Prostaglandins have both a vasoconstrictor and vasodilator function. Next to leukotrienes, prostaglandins can also make the vascular endothelium more 'leaky' thereby promoting edema formation during inflammation [10, 25]. Furthermore, increased intracellular Ca^{2+} causes transformation of xanthine dehydrogenase into xanthine oxidase, thereby facilitating the production of ROS, as described in the next section [10, 25].

Lung Ischemia-reperfusion Injury: 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 lung ischemia-reperfusion injury, hypoxanthine is broken down into ROS. A second system to generate ROS is by the NADPH 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 ROS, are very unstable and damage cell membranes by lipid peroxidation [10, 25].

Lung Ischemia-reperfusion Injury: Inflammation

After lung ischemia-reperfusion injury, pro-inflammatory cytokines (like interleukin [IL]-8, IL-10, IL-12, IL-18, tumor necrosis factor [TNF]-α, and interferon [IFN]γ) are released by macrophages due to activation during ischemia. Consequently, neutrophils and lymphocytes are recruited into the lung. Because of expression of adhesion molecules to both endothelium (E-selectin, P-selectin, intercellular-adhesion-molecule-1) and leukocytes (L-selectin, β-integrins), leukocytes roll (selectins), adhere (β-integrins, intercellular-adhesion-molecule-1), 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 (gelatinases, collagenases and elastases), lysozyme, and lactoferrin [10, 25].

To summarize these different pathways: the increase in intracellular Ca^{2+} and Na^+ and the formation of ROS, eicosanoids, proteolytic enzymes and (phospho)lipases damage the lipid membrane of the cell, causing increased cellular permeability, the formation of cellular edema and eventually cell death. Finally all these pathways lead to a disturbed surfactant system [10, 25].

◼ Experimental Models of Lung Ischemia-reperfusion Injury

To study the complex pathophysiology of lung ischemia-reperfusion injury and to investigate surfactant treatment possibilities, an animal model is often used. Three major types of animal models have been reported: an isolated *ex vivo*, perfused lung system, a whole lung transplantation model, and an *in situ* warm ischemia model of the lung.

The isolated, perfused lung system is a model whereby the organ is taken out of the animal, hypothermically stored for a certain period of ischemia and subsequently reperfused by the use of a Langendorff system. Although this model has

advantages (e.g., the use of lungs of knock-out mice), the most important disadvantage clearly is the *ex vivo* situation, disturbing normal physiological interactions. Furthermore, only short follow-up periods after reperfusion (hours) can be established [26].

Animal whole lung transplantation has the advantage that it best reflects human transplantation and allows the investigation of the effect of cold ischemic storage, the use of storage solutions, and the study of allo-antigen settings. However, disadvantages are that, especially in small animals, it is a time-consuming and technically difficult procedure with often high mortality rates, limiting the study of longer follow-up periods after transplantation [3, 4, 18, 19, 27]. Also, larger animals, like pigs and dogs have been used to avert the possible high mortality and the technical difficulties [6, 28–30]. Nevertheless, the limitations of this model are the difficulties in activating and blocking specific pathways (due to costs and receptor specificity) and the unavailability of genetically modified animals.

To overcome some of the disadvantages in transplantation models, an *in situ* warm ischemia model of the lung in small rodents has been developed, in which the ischemia is induced by clamping the pulmonary artery, veins and bronchus of (usually) the left lung. Clamping time generally ranges from 60 to 120 minutes (van der Kaaij, et al., unpublished data) [31, 32]. After declamping, reperfusion occurs. Although this *in vivo* model is technically much easier than the aforementioned transplantation models, there are some disadvantages. Firstly, warm ischemia is used, accompanied by a high metabolic rate. However, the use of short periods of warm ischemia is accepted as an accelerated model of ischemia-reperfusion injury of the lung [33]. Additionally, because of often long warm ischemic periods, severe ischemia-reperfusion injury is induced resulting in still high mortality. As a result, most studies still investigate short time periods (max. 6 hours) after the start of reperfusion. Only a few studies report long reperfusion times of about 1 week [27, 32]; however, in these studies mortality was still very high after extubation of the animals. We have recently developed a model of lung ischemia-reperfusion injury in which (by adjusting the anesthetic protocol and ventilator strategy) we are able to study intervals up to months after reperfusion, with acceptable mortality (van der Kaaij, et al., unpublished data).

▌ Surfactant Dysfunction and Lung Ischemia-reperfusion Injury

Using these experimental models, several research groups have gained valuable information on how specific parts of the surfactant system are affected by lung ischemia-reperfusion injury [2, 3, 16, 17, 34] (Fig. 4).

Surfactant Dysfunction: Alveolar Proteins

The presence of alveolar proteins after lung ischemia-reperfusion injury has been described in many studies [2–5, 16, 19, 28, 29, 35–40]. Both different warm (1–2 h [3, 36], van der Kaaij, et al., unpublished data) and cold (2–20 h [2, 4, 5, 19, 35]) ischemic intervals have resulted in increased levels of alveolar protein between 1 and 24 hours after reperfusion. Due to ROS, proteolytic enzymes and phospholipases, endogenous surfactant and the endothelial and epithelial membrane are damaged. This results in leakage of proteins into the alveolus and surfactant com-
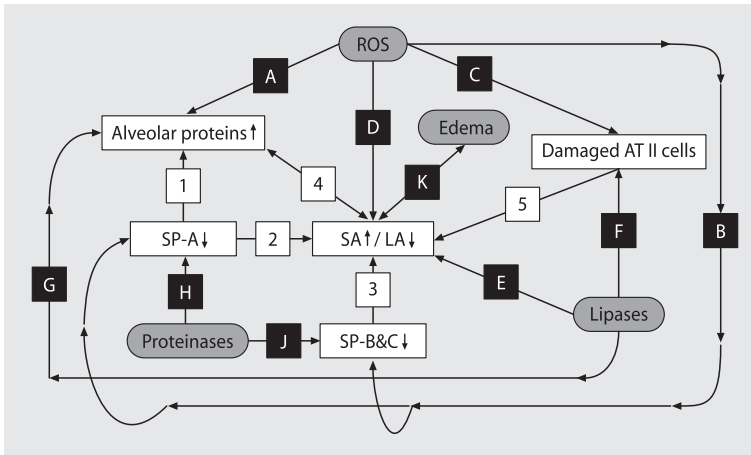
Fig. 4. *Reactive oxygen species* (ROS) directly damage **[A]** the alveolo-capillary membrane, thereby facilitating the influx of serum proteins into the alveolus, **[B]** the surfactant-associated proteins (SP), **[C]** the alveolar type II (AT II) cells, and **[D]** the large aggregate (LA) surfactant subform. *Phospholipases* cause degradation of **[E]** the 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]** SP-B&C degradation 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]** Finally AT II cells, 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. Due to these factors, a decrease in LA and an increase in SA has been noticed after lung ischemia-reperfusion injury

ponents into the bloodstream. Since 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 lung ischemia-reperfusion injury, a further influx 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 [24]. Under normal conditions, SP-A is able to counteract the inactivating effects of serum proteins on surfactant [41]. However, after lung ischemia-reperfusion injury, a decrease in SP-A was found in human lung transplant recipients and animal models of lung ischemia-reperfusion injury [19, 42].

Surfactant Dysfunction: Inactivation of Surfactant Associated Proteins

A decrease in SP-A was already visible after prolonged ischemic storage without reperfusion and decreased further after the start of reperfusion [2, 4, 19]. Moreover, in lung transplant recipients, the level of SP-A is still low more than one year after transplantation [42]. SP-A can be degraded by ROS and proteolytic enzymes. It was

also shown that the levels of SP-A decreased with ascending severity of lung ischemia-reperfusion injury, suggesting that preservation or amelioration of SP-A is essential for improvement after lung ischemia-reperfusion injury [19]. Besides the protein inhibiting function of SP-A, SP-A also plays a central role in phospholipid secretion and recycling, contributing to a decrease in surface activity of surfactant.

SP-B and SP-C have also been found to be decreased after lung ischemia-reperfusion injury [28]. Decreased levels or inactivation of SP-B and SP-C can result in a diminished quantity of phospholipids, but also in a changed composition of the surfactant on the surface of the alveolar epithelium, thereby impairing the surfactant lowering properties [28].

Studies have reported surfactant dysfunction without a change in the overall amount of phospholipids [2, 3]. Both a decrease in DPPC and phosphatidyl-glycerol and an increase in sphingomyelin have been described [2, 3, 17, 28]. DPPC, the saturated form of phosphatidylcholine, is the most important phospholipid known to reduce the minimum surface tension. Although Klepetko et al. demonstrated a correlation between an impaired oxygenation capacity of the lung and a decrease in DPPC (signifying the possible importance of this phospholipid in normal lung function), Veldhuizen et al. found no relationship between the changes in surfactant composition and surface lowering properties of surfactant, illustrating the complex pathophysiology of surfactant dysfunction [2, 17].

▋ Experimental Results of Surfactant Treatment in Lung Ischemia-reperfusion Injury

Several authors have examined the effect of the lung preservation protocol and surfactant replacement therapy on surfactant function in experimental models of cold and warm lung ischemia-reperfusion injury [4–6, 16, 19, 27–29, 35–40, 43, 44]. By changing the lung preservation solution from Euro-Collins to low potassium dextran solution, and by flushing the graft retrograde instead of antegrade, the damaging effects of lung ischemia-reperfusion injury on pulmonary surfactant could be ameliorated [29, 37–40, 44]. Furthermore, it was demonstrated that when, in the case of replacement therapy, surfactant was administered just before, at or after reperfusion, it improved lung compliance and PaO_2 and prevented an increase in the small aggregate/light aggregate ratio directly after lung ischemia-reperfusion injury [4, 35]. However, other studies have shown that treatment with exogenous surfactant before the onset of ischemia is more beneficial when compared to treatment at reperfusion [30, 35, 45, 46]. This can be explained by less complement activation, diminished membrane damage, and an enlarged surfactant phospholipid pool after donor treatment, thereby preventing deterioration of the entire endogenous surfactant pool [6, 46]. Also, surfactant given to the donor may result in a more homogeneous distribution in the lung as compared to treatment after reperfusion, when alveolar damage has already occurred [6].

Most studies investigating the effect of surfactant replacement therapy have only addressed the first hours (2–6) after reperfusion. Studies on the longer term effects of lung ischemia-reperfusion injury and surfactant treatment are scarce. In this regard, Erasmus and colleagues demonstrated that surfactant treatment just before reperfusion enhanced recovery from lung ischemia-reperfusion injury at one week postoperatively [27]. We confirm that lung ischemia-reperfusion injury resulted in

the conversion of surfactant into less active surfactant, and impaired PaO_2 and lung compliance throughout the first week after reperfusion. However, even months after reperfusion, diffuse alveolar damage and decreased lung compliance were visible. Surfactant treatment before the induction of warm ischemia completely normalized these parameters from 3 to 90 days after reperfusion (van der Kaaij et al., unpublished data).

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

Surfactant Therapy: Decreasing the Inhibitory Effects of Serum Proteins

When the quantity of surfactant is low or its composition is changed, serum proteins (like albumin, fibrin, fibrinogen, C-reactive protein [CRP], and hemoglobin) leak into the alveolus [47]. This protein leakage can be ameliorated by surfactant therapy [4, 24, 26, 48] (van der Kaaij et al., unpublished data). However, some studies failed to show a decrease in leakage of serum proteins into the alveolus, which can probably be explained by the different treatment strategies [6, 35, 48]. We hypothesize that surfactant administration to the donor may be more beneficial in inhibiting serum protein leakage than treatment at the time of reperfusion. However, once alveolar proteins accumulate in the alveolus, the lung can resist against surfactant inactivation by the interference of SP-A. Cockshutt et al. showed *in vitro* a reversed inhibition of serum proteins on the surface lowering function of surfactant when SP-A was administered [41].

Surfactant Therapy: Preservation of Surfactant Composition

With surfactant treatment the surface tension in the lung remains low, thereby maintaining the ventilation and perfusion of the lung, resulting in optimal oxygenation [6]. As mentioned earlier, an increase in the small/large aggregate ratio occurs due to lung ischemia-reperfusion injury. Since most of the exogenous surfactant administered to lung is in the large aggregate subform, a larger pool of surface-active phospholipids (DPPC) is created, so that the rise in small/large aggregate ratio is prevented [6, 28]. Thus, the instilled exogenous surfactant protects the endogenous surfactant pool against damage. This is illustrated by the fact that the normal endogenous surfactant pool is about 10–15 mg lipid per kilogram, and that the amount of surfactant used for treatment is in the range of 50–400 mg lipid per kilogram [6].

Furthermore the preservation of SP-A, SP-B, and SP-C can result in normal phospholipid recycling and secretion [28]. As already mentioned, exogenous surfactant treatment preserves the endogenous surfactant, resulting in normal endogenous SP-A, SP-B, and SP-C. Moreover, it was shown 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 within one hour after reperfusion [19]. Also, a decrease of the large aggregate subform was found indicating an increased recycling capacity of the SP-A enriched surfactant compared with SP-A deficient surfactant [19].

Surfactant Therapy: Anti-inflammatory and Antioxidant Function

Surfactant has also been shown to inhibit cytokine release from activated mono-cytes and macrophages [49, 50]; the modulation of lymphocytes has also been suggested. Furthermore, surfactant is known to have antioxidant capacities, resulting in reduced ROS damage at the level of the alveolus [51]. Surfactant treatment can thus ameliorate the accumulation and adherence of inflammatory cells, so that endothelial and AT II injury is prevented, normalizing cell permeability and surfactant recycling. Semik and colleagues showed that the decreased function of AT II cells after lung ischemia-reperfusion injury is prevented by surfactant treatment [42].

▌ Clinical Studies of Surfactant Treatment after Lung Transplantation

Some investigators have used surfactant to treat lung transplant recipients who developed severe lung ischemia-reperfusion injury after transplantation [52–54]. In a case report by Strüber and colleagues in 1995, a 26-year old woman who underwent right lung transplantation and developed severe reperfusion injury 5 hours after transplantation, was treated with intrapulmonary nebulized synthetic surfactant [53]. Shortly after surfactant therapy, lung compliance, PaO_2, and tidal volume increased. Moreover, 24 hours after therapy, the edematous infiltrate of the transplanted lung on chest X-ray film was resolved. Another study in six lung transplant patients also suggested improvement in lung ischemia-reperfusion injury due to surfactant replacement. However, in 1 of the 6 recipients, surfactant therapy failed to improve dynamic lung compliance, which could be attributed to the application approach or the type of surfactant used (synthetic versus natural surfactant) [52].

   Although the use of surfactant in human lung transplantation seems promising, no prospective randomized clinical trial has so far been set up to treat severe lung ischemia-reperfusion injury. Also, a clinical trial should investigate possible additional effects of donor pretreatment as compared to treatment after reperfusion.

▌ Conclusion

In this chapter we have discussed the effects of lung ischemia-reperfusion injury on the surfactant system. Lung ischemia-reperfusion injury damages the endogenous surfactant system by the production of ROS, proteolytic enzymes, and (phospho)lipases. Surfactant is composed of phospholipids and associated proteins and its main function is to reduce the surface tension inside the alveolus, allowing normal breathing. Impairment of the surfactant system will increase surface tension (leading to instability and collapse of alveoli), atelectasis formation, influx of serum proteins into the alveolus, pulmonary edema, decreased lung compliance, and impaired gas exchange.

   The use of surfactant replacement therapy (either before or after lung ischemia-reperfusion injury) ameliorates lung ischemia-reperfusion injury. Surfactant therapy restores the activity of the endogenous surfactant pool and reduces the inhibitory effect of serum proteins; possible other effects are that it serves as an anti-oxidant and an anti-inflammatory agent. Although human data on the use of surfactant in

lung transplant patients are scarce, the positive results in experimental models and a few patient reports suggest that (pre)treatment with surfactant in lung transplantation patients could improve outcome. Future studies should further investigate the effect of surfactant on lung ischemia-reperfusion injury markers on both short and long term.

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