Surfactant pretreatment decreases long-term damage after ischemia-reperfusion injury of the lung

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Abstract

Objective: Lung ischemia-reperfusion injury (LIRI) is a risk factor for primary acute graft failure following lung transplantation. LIRI hereby contributes to morbidity and mortality after lung transplantation. We have previously shown that surfactant pretreatment ameliorates LIRI up to 1 week after reperfusion. However, the impact of surfactant pretreatment on long-term outcome following LIRI is unknown. Therefore, the objective of this study was to investigate the effect of surfactant pretreatment on long-term outcome following LIRI. Methods: Male Sprague-Dawley rats (n = 63) were randomized to receive intratracheally administered porcine surfactant (400 mg/kg) or no pretreatment. One hour thereafter, animals underwent 120 min of warm ischemia by clamping the bronchus, pulmonary artery and vein of the left lung. A third group was sham-operated; a fourth group served as unoperated controls. Animals were killed on day 30 or 90 after surgery. Arterial oxygenation and lung compliance were determined. Broncho-alveolar lavage fluid (BALf) was collected to assess surfactant function and alveolar protein. Leukocyte infiltration was determined by flow cytometry in BALf, lung tissue and thoracic lymph nodes. Lungs of three animals per group were used for histological assessment. Results: Lung compliance was lower on day 30 and day 90 after LIRI than in sham-operated controls (day 30 Vmax 6.1 ± 2.1 vs 12.6 ± 1.3, day 90 6.9 ± 3.0 vs 12.1 ± 1.6; Cmax day 30 0.49 ± 0.17 vs 1.08 ± 0.21, day 90 0.67 ± 0.31 vs 1.11 ± 0.17). Furthermore, the number of CD45RA+-lymphocytes in left lung tissue was decreased on day 90 compared to unoperated animals (230.633 vs 867.454). The number of macrophages elevated in left BALf on day 90. HE slides of LIRI animals were scored as fibroproliferative with moderate atelectasis. Surfactant pretreatment improved lung compliance (Vmax day 30 11.7 ± 1.8, day 90 11.1 ± 1.2; Cmax day 30 1.04 ± 0.23, day 90 1.16 ± 0.21) and normalized the number of CD45RA+-lymphocytes (769.555 ± 421.016) in left lung tissue. Furthermore lung architecture on HE slides was on return to normal. However, more CD54+-lymphocytes were on day 30 (754.788 ± 97.269 vs 430.409 ± 109.909) and more macrophages on day 90 (2.144.000 ± 630.633 vs 867.454 ± 383.220) were measured in pretreated lung tissue compared to LIRI animals. Conclusions: Severe LIRI caused extensive pulmonary injury up to 90 days postoperatively. Surfactant pretreatment normalized pulmonary function, but resulted in an increased number of CD54+-cells and macrophages in lung tissue.

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1. Introduction

Primary acute graft failure (PAGF) is the main cause for early morbidity and mortality after lung transplantation, resulting in a 1-year survival rate of less than 80% [1]. Lung ischemia-reperfusion injury (LIRI) is thought to significantly contribute to the development of PAGF, which symptomatically resembles the acute respiratory distress syndrome (ARDS) [2,3]. The symptoms of PAGF occur within the first 72 h after transplantation and consist of non-cardiogenic pulmonary edema, increased pulmonary artery pressure, decreased lung compliance and impaired gas exchange [4-8]. The clinical expression of LIRI ranges from mild hypoxemia and mild
pulmonary edema on chest X-ray to PAGF, which is the most severe form of injury. Experimental and clinical studies on LIRI have shown that abnormalities and depletion of pulmonary surfactant contribute to the symptoms of LIRI [4–8].

In broncho-alveolar lavage fluid (BALf) of human lung transplant recipients, surfactant dysfunction was demonstrated up to 7 years after transplantation [9]. Pulmonary surfactant is essential for normal respiration, since it diminishes the surface tension at the air-fluid interface inside the alveolus. This keeps the alveolus open at the end of expiration and preserves fluid homeostasis. [3]. To control the disturbed intra-alveolar fluid homeostasis after LIRI, surfactant replacement therapy has been investigated using experimental models demonstrating that surfactant is capable of restoring lung function within hours after administration [3,4,7,10–14]. Recently, Struber et al. showed in a prospective clinical trial that surfactant pretreatment resulted in decreased capillary permeability, improved surfactant function, and a higher forced expiratory volume in 1 s after the induction of ischemia improved lung function and lung recovery up to 1 week in an experimental warm LIRI model [4]. However, it remains unknown what the effect of surfactant pretreatment is on long-term severe LIRI.

Furthermore, the major impediment to long-term survival after lung transplantation is development of chronic transplant dysfunction, referred to in the lung as bronchiolitis obliterans syndrome (BOS) [16]. Although still subject to debate, LIRI is suggested to contribute to BOS development by priming the immune system [17,18]. Since it is presumed that surfactant has an immune down regulating effect, surfactant therapy may mitigate the inflammatory response after LIRI [19]. Therefore, the goal of this study was to investigate the effect of surfactant pretreatment on long-term outcome after LIRI, as assessed by lung function, histology and leukocyte infiltration.

2. Materials and methods

2.1. Experimental design

The experimental protocol was approved by the Animal Experiments Committee under the national Experiments on Animals Act and complied with the 1986 directive 86/609/EC of the Council of Europe. Male Sprague-Dawley rats (n = 63, Harlan, Horst, The Netherlands), weighing 286 ± 31 g, were randomized into four groups: surfactant pretreated LIRI (n = 18), untreated LIRI (n = 18), sham-operated (n = 18), and unoperated controls (n = 9). Measurements were made 30 or 90 days after the operation.

2.2. Surfactant pretreatment

Exogenous porcine surfactant (HL-10, Leo Pharmaceutical Products, Ballerup, Denmark and Halas Pharma, Oldenburg, Germany), dissolved in 50 mg/ml of saline, was administered intratracheally in three gifts (total dose 400 mg/kg bodyweight) within 1 h before operation, as described previously [4].

2.3. Experimental model

The animal model has been described in detail before [4]. In brief, whereas unoperated rats underwent no intervention, a left dorsolateral thoracotomy in the fourth intercostal space was done in sham-operated, untreated LIRI and pretreated LIRI animals. The untreated and pretreated LIRI groups underwent 120 min of warm lung ischemia by clamping the bronchus, pulmonary artery and pulmonary veins of the left lung in inflated state, using one microvascular clamp. After 120 min ischemia, the clamp was removed and the lung was recruited by a stepwise increase of PIP and PEEP (maximum respectively 50 and 18 cm H2O) until the lung was visually expanded. Throughout the whole experiment, body temperature was kept within normal range by the use of a heating pad. All animals recovered with additional oxygen during the first 12 h.

2.4. Blood gas values

At the end of the experiment, animals were ventilated for 5 min (12 cm H2O PIP, 2 cm H2O PEEP, frequency 30 breaths/min and a FiO2 of 1.00). Blood gas values were recorded in 0.3 ml heparinized blood taken from the carotid artery (ABL555 gas analyzer, Radiometer, Copenhagen, Denmark). Ultimately, animals were euthanized by an overdose of pentobarbital (200 mg/kg), administered intravenously.

2.5. Static lung compliance

A static pressure–volume curve (PVC) of the left lung was obtained by clamping the contralateral hilum and recorded using conventional techniques [4]. Maximal compliance of expiration (Cmax) was determined as the steepest part of the lung deflation curve. Furthermore, maximal lung volume (Vmax), corrected for body weight, was recorded at a pressure of 35 cm H2O.

2.6. Broncho-alveolar lavage, lung tissue and thoracic lymph nodes

BALf was obtained separately for each lung by clamping the contralateral hilum, as described previously [4]. Left lung tissue and thoracic lymph nodes (TLN) were dissected and mashed through a filter. The suspensions were centrifuged to pellet the cells, whereafter red blood cells were lysed and cells were washed. Cells were counted, using a Bürker-Turk cell counter (Erma, Tokyo, Japan).

2.7. Surfactant and protein analysis

Supernatant of BALf was centrifuged at 4 °C for 15 min at 40,000 × g to separate surface-active surfactant pellet (large aggregate (LA)) from a non-surface active supernatant fraction (small aggregate (SA)). Concentration of LA and SA was determined by phospholipid extraction, followed by phosphorus analysis [4]. The supernatant was also used to determine alveolar protein concentration by using the
Bradford protein assay (Bio-Rad, Munich, Germany) with bovine serum albumin as standard.

2.8. Flowcytometry

Cells of BALf, lung tissue and TLN were incubated with 2% normal rat serum (NRS) in murine FACS buffer for 15 min to prevent non-specific binding of the primary antibody to Fc-receptors. Hereafter, cells were washed, centrifuged and surface-stained for 30 min with the following primary mouse anti rat antibodies: biotin conjugated CD5 (OX191), phycoerythrin (PE) labeled CD8 (OX82), fluorescein-isothiocyanate (FITC) labeled CD4 (OX331), and CD45RA-PE (OX33). Primary staining of CD5-biotin was revealed by secondary staining with streptavidin PE-Cy-5 (phycoerythrin-cychrome) (STAR891). Antibodies were obtained commercially from Serotec1 (Kidlington, UK) and PharMingen2 (BD, Franklin Lakes, New Jersey, USA).

Cellular differentiation was calculated based on morphology, autofluorescence and specific antibody staining. Cells were identified as follows: lymphocytes low FSC, low SSC, non autofluorescent and expressing either CD45RA+ (B-lymphocytes), CD5+CD4+ (T-helper lymphocytes) or CD5+CD8+ (T-cytotoxic lymphocytes); macrophages as high SSC and FSC and autofluorescent.[4]

Data were acquired on a FACS Calibur flowcytometer (BD, Franklin Lakes, New Jersey, USA) and were analyzed using Cell Quest (BD, Franklin Lakes, New Jersey, USA) and FlowJo software (Tree Star, Ashland, Oregon, USA).

2.9. Histology

Histological assessment was performed in three animals per group. The heart and lungs were excised en bloc, whereafter the lungs were fixated at a pressure of 10 cm H2O in 4% paraformaldehyde for 24 h, and embedded in paraffin wax. Sections were cut and stained with hematoxylin and eosin (H&E). A histopathologist ( MDB), blinded for the treatment, performed histological examination on the following parameters: intra-alveolar and septal edema, hyaline membrane formation, inflammation (classified as histiocytic, lymphocytic, granulocytic, or mixed), fibrosis, atelectasis, intra-alveolar hemorrhage, and overall classification. Each parameter was scored as (0) absent, (1) mild/scattered, (2) moderate/occasional, or (3) severe/frequent. Sections were overall classified as (N) normal, if no abnormalities were seen, (E) exsudative, if pulmonary edema and/or hyaline membranes were present, (F) fibroproliferative, if activated fibroblasts and/or proliferating alveolar type II cells were found, and (R) resolving, if injury was on return to normal. Slides were scored on a Leica DC500 camera (Leica Microsystems AG, Wetzlar, Germany).

2.10. Statistical analysis

The results in text, tables and figures are presented as mean ± standard deviation (SD). Data was analyzed using SPSS version 11.1 statistical software (SPSS Inc., Chicago, Illinois, USA). If the distribution within a group was normal, as assessed by the Kolmogorov—Smirnov test, and if the condition of equal variances was met by the Levene’s test, differences between groups were tested for significance by one-way ANOVA. If the overall level of the ANOVA was significant, intergroup comparisons were made by the Bonferroni post hoc test. In the case of unequal variances or an abnormal distribution, a non-parametric Kruskal—Wallis test was performed, followed by Mann—Whitney U tests for intergroup comparisons. A p value <0.05 was considered to be significant.

3. Results

3.1. Survival

The mortality in the surfactant pretreated LIRI and the untreated LIRI group was 11% (2 of 18 in both groups). All sham-operated animals survived the experimental period.

3.2. PaO2/FiO2 and PaCO2

The PaO2/FiO2 ratio of LIRI animals on day 30 was slightly but significantly lower than in sham-operated controls (505 ± 12 mmHg vs 572 ± 12 mmHg, p = 0.006). This difference had disappeared on day 90. Surfactant pretreatment resulted in a significantly better PaO2/FiO2 ratio as compared to untreated LIRI animals on day 30 (559 ± 11 mmHg vs 505 ± 12 mmHg, p = 0.003). All groups had normal PaCO2 values 30 and 90 days postoperatively (data not shown).

3.3. Static lung compliance

After sham-operation, left lung compliance was completely normal (Fig. 1). However, LIRI resulted in significantly decreased Cmax and Vmax on day 30 and 90 postoperatively as compared to sham-operated (Cmax day 30 p = 0.008; Vmax day 30 p = 0.002) and unoperated controls (Cmax day 30 p = 0.000, day 90 p = 0.017; Vmax max days 30 and 90 p = 0.000). After surfactant pretreatment, Cmax and Vmax were comparable to values found in sham-operated and untreated animals (Cmax day 30 p = 0.000, day 90 p = 0.001 vs untreated LIRI; Vmax day 30 p = 0.001, day 90 p = 0.003 vs untreated LIRI).

3.4. Surfactant and alveolar protein

No differences were found in the amount of alveolar protein between the groups on both time points. However, 30 days after LIRI a decrease in the level of LA was found in the left lung of both untreated LIRI (p = 0.008 vs unoperated) and surfactant pretreated animals (p = 0.008 vs unoperated), but not in the sham group (Fig. 2). Interestingly, the level of LA had further decreased on day 90 in the ischemic left (p = 0.008 vs unoperated, p = 0.004 vs sham-operated, p = 0.008 vs untreated LIRI), but also in the non-ischemic right lung of surfactant pretreated LIRI animals (p = 0.017 vs unoperated, p = 0.052 vs sham-operated, p = 0.008 vs untreated LIRI). Also, the level of SA found in the left and right lung on day 90 was significantly higher than in unoperated (p = 0.029), and untreated LIRI controls (left p = 0.030; right p = 0.019).
3.5. Lymphocytes in BALf and lung tissue

Although no significant differences in the number of CD45RA+ and CD5+CD4+-cells were measured in BALf between groups on both time points (Fig. 3A and C), LIRI resulted in infiltration of CD5+CD8+-cells in BALf on day 30 (Fig. 3B, p = 0.032 vs sham-operated).

After surfactant pretreatment, more CD5+CD4+-cells were measured in lung tissue on day 30 as compared to unoperated (p = 0.046), sham-operated (p = 0.038) and untreated LIRI animals (p = 0.046) (Fig. 4A). The number of CD45RA+ cells in lung tissue on day 90 was significantly higher than in untreated LIRI controls (Fig. 4C, p = 0.032). On day 90 the number of CD5+CD4+-cells in the surfactant pretreated group had returned to baseline values (Fig. 4A).

3.6. Lymphocytes in thoracic lymph nodes

No significant differences between untreated LIRI animals and controls were found in CD5+CD8+, CD5+CD4+ and CD45RA+-cells (Fig. 5A—C).

However, the lymphocyte population in TLN of surfactant pretreated animals showed elevated numbers of CD5+CD4+ and CD5+CD8+-cells on day 30 as compared to unoperated controls with numbers returning to normal on day 90 (Fig. 5A and B; p = 0.005 CD5+CD4+, p = 0.014 CD5+CD8+).

3.7. Macrophages in BALf and lung tissue

While sham-operation had no effect on the number of macrophages found in BALf of the left lung, LIRI resulted in an increased number of macrophages 90 days postoperatively as compared to unoperated (p = 0.004) and sham-operated controls (p = 0.03) (Fig. 3D).

In the surfactant pretreated group, significantly more macrophages were found in BALf (Fig. 3D; p = 0.032 vs sham-operated, p = 0.05 vs untreated LIRI) and lung tissue (Fig. 4D; p = 0.001 vs unoperated, p = 0.000 vs sham-operated) on day 30. Although the number of macrophages in lung tissue was...
still elevated on day 90 ($p = 0.013$ vs unoperated, $p = 0.014$ vs sham-operated, $p = 0.005$ vs untreated LIRI), it had returned to normal in the BALf on day 90 (Fig. 3D; $p = 0.016$ vs untreated LIRI).

Fig. 3. Mean number of inflammatory cells ± SD in BALf of the left lung of unoperated, sham-operated, untreated LIRI, and surfactant pretreated groups on days 30 and 90 postoperatively. Shown are: (A) CD5⁺CD4⁺-cells (helper T-lymphocytes), (B) CD5⁺CD8⁺-cells (cytotoxic T-lymphocytes), (C) CD45RA⁺-cells (B-lymphocytes), and (D) macrophages. BALf: broncho-alveolar lavage fluid; LIRI: lung ischemia-reperfusion injury; SD: standard deviation; Su: surfactant pretreatment; U: $p < 0.05$ vs unoperated animals; S$: p < 0.05$ vs sham-operated animals on day x; L$: p < 0.05$ vs LIRI animals on day x.

Fig. 4. Mean number of inflammatory cells ± SD in left lung tissue of unoperated, sham-operated, untreated LIRI, and surfactant pretreated groups on days 30 and 90 postoperatively. Shown are: (A) CD5⁺CD4⁺-cells (helper T-lymphocytes), (B) CD5⁺CD8⁺-cells (cytotoxic T-lymphocytes), (C) CD45RA⁺-cells (B-lymphocytes), and (D) macrophages. LIRI: lung ischemia-reperfusion injury; SD: standard deviation; Su: surfactant pretreatment; U: $p < 0.05$ vs unoperated animals; S$: p < 0.05$ vs sham-operated animals on day x; L$: p < 0.05$ vs LIRI animals on day x.
3.8. Histology

No histological abnormalities were seen on H&E slides of sham-operated animals on days 30 and 90 (Fig. 6, Tables 1 and 2). LIRI resulted in atelectasis, fibrosis and moderate inflammation, consisting mainly of histiocytes. The overall classification of LIRI animals on days 30 and 90 was fibroproliferative in five out of six animals. Although surfactant pretreatment resulted in mild atelectasis and prevented fibrosis, mild inflammation with predominantly histiocytes and lymphocytes was still noticed. However, the overall classification was scored as resolving, since injury was on return to normal. H&E: hematoxylin and eosin staining; LIRI: lung ischemia-reperfusion injury; Su: surfactant pretreatment.

4. Discussion

LIRI is suggested to be a major risk factor for the development of PAGF following lung transplantation, thereby contributing profoundly to early morbidity and mortality after lung transplantation. The clinical course of PAGF symptomatically resembles the acute phase of ARDS and consists of symptoms like hypoxemia, decreased lung compliance, increased pulmonary artery pressure, and development of non-cardiogenic pulmonary edema [20,21]. The acute phase of PAGF can either resolve quickly or result in death. If patients survive the acute phase of PAGF, a ‘chronic’ fibroproliferative state, characterized by hyperplasia of alveolar type II cells, infiltration of macrophages and activated fibroblasts, collagen deposition and pulmonary remodeling, may develop within 4—7 days after onset of the first clinical symptoms [20,21]. In an earlier report, we have demonstrated that 120 min warm ischemia resulted in our model in symptoms that resembled the symptoms seen in the acute phase of PAGF, suggesting that LIRI is a major risk factor for PAGF in the absence of other influencing factors, such as alloimmunity [4]. The 11% early mortality found in the present study was due to the development of severe pulmonary edema shortly after reperfusion. The 89% of the
untreated LIRI animals that survived the acute phase developed chronic abnormalities, as demonstrated by impaired lung compliance up to 90 days after reperfusion and fibroproliferative changes on H&E slides. Although we did not confirm collagen deposition with histology, a fibroproliferative state with tissue remodeling can further be illustrated by the increased levels of macrophages and CD5+CD8+-lymphocytes in BALf of untreated LIRI animals, which both play an important role in lung fibrosis. In addition, the decreased numbers of CD45RA+-lymphocytes found in lung tissue of untreated LIRI animals on day 90 also suggests the occurrence of tissue remodeling after LIRI. Although extensive injury was found in the left lung of untreated LIRI animals, only mild hypoxemia was demonstrated on day 30. This discrepancy may be explained by the fact that PaO2 was dependent on both lungs, and that the loss of left lung function may be compensated by the right lung.

While LIRI is suggested to be a major risk factor for the development of PAGF, it is also thought to be one of the factors contributing to BOS, which affects 50% of patients surviving beyond 3 months after transplantation [2,16–18]. The exact etiology of BOS is not fully understood, but its pathogenesis appears to involve a ‘response to injury’ type of pattern. BOS probably develops as a result of multiple periods of injury, like brain death, LIRI, rejection and infection [2,16,18]. Although BOS is restricted to the pathology after lung transplantation, and not used in relation to ischemic injury per se, we clearly show that severe LIRI caused a progressive deterioration of the lung. Because LIRI is one of the first injuries the lung sustains in clinical lung transplantation, tackling LIRI is an important strategy to improve outcome following lung transplantation.

One approach to ameliorate LIRI is to treat the lung with surfactant. In both experimental models and in clinical lung transplantation, it has been shown that surfactant therapy, administered after the development of severe LIRI, is capable of restoring lung function within hours after administration [3,4,7,10–13,22]. However, treatment with surfactant before the onset of ischemia has been suggested to be more beneficial than treatment after reperfusion, because an enlarged surfactant phospholipid pool inside the alveolus is already present before the onset of injury [14]. Surfactant pretreatment also results in a more homogeneous distribution in the lung as compared to treatment after reperfusion, when alveolar damage has already occurred and surfactant will accumulate in the already open areas of the lung instead of the atelectatic areas, where it is most needed. Previous data from our group demonstrated that
surfactant treatment before the induction of ischemia resulted in improved lung function in the first week after LIRI [4]. In the present study we demonstrate that surfactant pretreatment normalized lung compliance up to 3 months after reperfusion, restored the number of CD45RA+ lymphocytes in lung tissue to the level observed in unoperated animals, and decreased lung damage on H&E slides. However, more macrophages were found, accompanied by a CD5+CD4+-lymphocyte infiltrate in both lung tissue and TLN on day 30, which had disappeared on day 90.

Since reduction of the inflammatory reaction in the context of ischemia-reperfusion has proven to be successful in amelioration of injury, infiltration of leukocytes after LIRI should be considered as unwanted [2,23,24]. From the literature, it is known that surfactant is capable of down regulating the activation of macrophages and dendritic cells in vitro, and to decrease the production of cytokines [19]. The finding that surfactant pretreatment resulted in increased numbers of CD5+CD4+ lymphocytes could be explained by the fact that amelioration of LIRI by surfactant may have unmasked a different inflammatory reaction, or, that the material itself elicited an inflammatory reaction. Another possibility is that surfactant induced a regulatory T-cells response, which warrants further analysis of this subset of cells using CD25 or FOXP3. In our previous study we also observed increased numbers of macrophages after surfactant pretreatment in the BALF up to day 7 after reperfusion [4]. The fact that they are still present up to day 30 in the BALF may be explained by the surfactant recycling capacity of macrophages [4].

Although our study clearly demonstrates a long-term beneficial effect of surfactant pretreatment on lung function and pulmonary architecture following severe LIRI, this study has some limitations. Shortcomings of our experimental model are that warm instead of cold ischemia is used, and that it is impossible to easily administer pulmoplegia. However, this experimental model is an accepted and useful model to study the effect of LIRI in small rodents with acceptable mortality. Furthermore, it has been demonstrated that there are no major differences between short periods of warm and longer periods of cold ischemia [25].

In conclusion, the present study demonstrates that severe LIRI causes extensive pulmonary injury up to 90 days after reperfusion, which can be ameliorated by surfactant pretreatment 1 h before LIRI. Surfactant therapy may thus be a promising strategy to prevent short-term lung injury after ischemia and reperfusion of the lung, and to prevent a chronic fibroproliferative state.

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References


Appendix A. Conference discussion

Dr H. Reichenspurner (Hamburg, Germany): Do you have any clinical experience? I mean, do you do that in the Netherlands pretreating the donors now?

Dr van der Kaaij: Well, not yet, but hopefully we will be able to do so in the future. However, first we are performing an experimental study to investigate whether a lower dose of surfactant pretreatment is more beneficial than a high dose of surfactant at the time of reperfusion. At the moment we do not use surfactant in a clinical setting.

Dr M. Strueber (Hannover, Germany): Of course, I’d like to thank you that you brought this topic up again, but my comment is a little bit frustrating that we are not able to get this into the clinical application.

And the points are, first, we learned that to put the surfactant into a donor is a real problem. I think Heidi Goerler had made most of this experience being there with lots of small vials of surfactant and slowing down the retrieval procedure to put it in with a bronchoscope. That was one point.

And the other is we learned that with a lower dose, we are not achieving what we want to achieve so we’re ending up that we need a high dose. Probably we need pretreatment before flush perfusion of the lung, and we probably need additional treatment at the time of transplant.

So it comes to a question of how much does it cost, and can we afford it in this transplant? This is the frustrating part of it.

Dr van der Kaaij: That’s a very important comment. A major problem of surfactant treatment is the cost of the material itself. However, just as any other new drug, the cost may decrease in the future if it gets produced in large quantities. Regarding your first comment, since I am only into experimental work on this subject, it’s hard to comment on the clinical use of surfactant. I have not treated patients with surfactant, so I cannot comment on how difficult it is to administer the surfactant. In the experimental setting, it’s actually quite easy because you only have to administer a very small volume.

Dr Reichenspurner: It’s certainly an important question of how to finance the clinical application. We have a similar discussion about heart preservation at the moment, and the problem is: where does the financing come from? Is it from the health insurance companies? They say, if it is not the recipient, we are not paying.

And so that’s an important question which at some stage one needs to answer.

Dr van der Kaaij: Indeed, this is a very important question. Although, I don’t know what the cost of ECMO is, it definitely will be very expensive to treat all donor lungs with surfactant. Considering the price of surfactant at this moment, this is not yet a clinical option.

Dr Reichenspurner: Okay. Thank you very much for bringing this up.