Improvements in lung compliance after pulmonary transplantation: correlation with interleukin 8 expression☆

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Abstract

Objective: Previous studies have suggested reductions in lung reperfusion injury when initial reperfusion is undertaken with the addition of pharmacological modulators. We investigated three pharmacological agents in a porcine model of left single lung transplantation to determine the effect on lung compliance and its relationship with the expression of the cytokine, interleukin-8 (IL-8).

Methods: Donor lungs were preserved with modified Euro-Collins for a mean ischaemic time of 18.6 h. Pulmonary venous oxygenation, lung compliance and IL-8 expression were assessed over a 12-h period. Group A (n = 5) was a control group with no interventions added, Group B was reperfused with the addition of intravenous inositol hexakisphosphate (InSP6) (0.02 mg/kg per min), Group C received the nitric oxide donor, 3-morpholinosydnonimine (SIN-1) (0.02 mg/kg per min) and Group D received intravenous Pentoxifylline (2 mg/kg per h). All interventions were administered at a pulmonary artery pressure of 20 mmHg.

Results: Group D yielded the best oxygenation (P = 0.0041) while Groups B and C were similar. All were superior to Group A (P < 0.001). Lung compliance was significantly improved in Groups B, C and D compared to group A. In Group D, the greatest improvements in lung compliance were observed (P < 0.0001). Similar observations were seen with regard to pulmonary vascular resistance. IL-8 expression was delayed until after 30 min of reperfusion in Group D, but was evident after 10 min in all the other groups. This correlates with the compliance and oxygenation data. Conclusions: The addition of InSP6 or SIN-1 at reperfusion significantly attenuates reperfusion injury compared with controls and improves lung compliance. The unique comparison with Pentoxifylline afforded by this study indicates that at the doses studied Pentoxifylline appears to be superior, correlating with a greater inhibition of IL-8 expression.

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1. Introduction

Despite advances in lung preservation technology, early graft dysfunction remains a significant problem. Lung graft ischaemia–reperfusion injury is complex, involving the pulmonary sequestration of activated neutrophils and the release of toxic granular enzymes and oxygen free radicals.

Several pharmacological interventions are thought to modulate reperfusion injury but have seldom been directly compared in a single standardized model. Pentoxifylline administration has been successful in ameliorating reperfusion injury after skeletal muscle ischaemia and in experimental lung and liver transplantation. It acts through a variety of mechanisms including inhibition of leukocyte-endothelial interactions and oxygen free radical scavenging [1–4]. There may also be inhibitory effects on cytokines.

Inositol polyanions typified by inositol hexakisphosphate (InSP6) is a phytic acid derivative. Its structure enables it to inhibit leukocyte-endothelial interactions by blocking selectin mediated adhesion [5]. Its unique comparison with Pentoxifylline afforded by this study indicates that at the doses studied Pentoxifylline appears to be superior, correlating with a greater inhibition of IL-8 expression.

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benefits of these interventions on lung compliance and sought to determine a relationship with the pattern of expression of the important cytokine, interleukin-8 (IL-8) which is thought to be one of the pivotal mediators in the pathophysiology of reperfusion injury.

2. Materials and methods

Twenty female Landrace pigs (mean weight of 45.4 kg) were divided randomly into four groups (n = 5 in each). A similar number were size-and weight-matched and acted as donor animals. All grafts were reperfused at a mean pulmonary artery pressure of 20 mmHg.

Group A was a control group with no intervention administered. Group B was reperfused with the addition of InSP6 (0.02 mg/kg per h). Group C was reperfused using SIN-1 at a dose of 0.02 mg/kg per h while Group D was reperfused with the addition of intravenous Pentoxifylline (20 mg/kg loading dose, then 2 mg/kg per h). All interventions were administered to the recipient animal only and commenced 5 min prior to reperfusion of the graft.

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Academy of Science and published by the National Institutes of Health (NIH publication 85-23, revised 1985). All conditions associated with the United Kingdom Animals (Scientific Procedures) Act, 1986 were also met.

2.1. Donor operation

Our model of single lung transplantation has been described previously [8]. In summary, animals were anaesthetized with initial intramuscular premedication using Diazemuls (2 mg/kg) and Ketamine (15 mg/kg). Subsequent anaesthesia was induced with Propofol (20 mg/kg) and maintained with Isoflurane and intravenous Alfentanil. Animals were intubated with an endotracheal tube (outside diameter 9.5 mm) and ventilated at a tidal volume of 15 ml/kg at an inspired oxygen concentration of 100%.

Heart–lung blocks were retrieved in a standard manner [9] and lungs preserved by flush perfusion with 60 ml/kg of modified Euro-Collins via the main pulmonary artery.

After separation of the left lung from the block, a pulmonary artery pressure monitoring line (Cavafix Certo 18G, Braun, Melsungen, Germany) was inserted through a purse-string suture into the distal left pulmonary artery. A pulmonary venous sampling line (Flocare, Nutricia, Madrid, Spain) was similarly placed through the left atrial cuff directed into a distal pulmonary vein, allowing for later sampling of venous blood form the graft without mixing from the contralateral native lung after transplantation and reperfusion. Lungs were stored inflated at a temperature of 4 °C for a mean ischaemic time of 18.6 h.

2.2. Recipient operation

Recipient animals were pre-medicated with intramuscular Azaperone (8 mg/kg) and Diazemuls (2 mg/kg). After induction with intravenous Propofol, animals were maintained on intravenous Pentobarbitone (30 mg/kg per h) and Alfentanyl. Venous and arterial pressure monitoring lines were inserted as in donor animals.

Two endotracheal tubes were inserted via a tracheostomy. A 9.5-mm outside diameter tube was placed into the trachea to ventilate both lungs initially and subsequently the native lung alone. The second 6.5-mm tube was advanced through the left bronchial Anastomosis after its completion to ventilate the graft lung independently. Each endotracheal tube was connected to a separate ventilator to permit individual lung ventilation following the transplant, with a tidal volume of 15 ml/kg at 12 breaths per minute for each lung.

A left thoracotomy was performed followed by left pneumonectomy. Implantation of the donor lung proceeded in an established fashion constructing anastomoses of the left atrium, bronchus and left pulmonary artery in order. The contralateral pulmonary artery was encircled by a tape and snugger such that the left pulmonary artery pressure could be manipulated. A pressure monitoring line was placed in the recipient left atrium and a dedicated sampling line was inserted into the proximal left pulmonary artery.

Pulmonary artery flow was measured via a 10-mm Transonic A-Series flow probe (Linton Instruments, Norfolk, UK) placed around the left pulmonary artery distal to the anastomotic line. A similar 12-mm probe was placed around the descending aorta to provide a guide to the cardiac output. Both flow probes were connected to a dual channel HT207 Medical volume flow meter (Transonic Systems Inc., Ithaca, NY, USA).

All of the above pressure and flow data sources were routed through a CED 1401 32 channel digital to analogue converter (Cambridge Electronic Design Ltd., Camb, UK) and acquired on a Gateway 2000 PC running Microsoft Windows 95 and Spike 2 (Version 4.0) data acquisition software (Cambridge Electronic Design). Data were collected continually over the 12-hour post-operative period and stored on hard disk for subsequent analysis.

As the pulmonary artery pressure at reperfusion may influence the subsequent graft function, this was kept at 20 mmHg by constricting or releasing the tourniquet on the contralateral pulmonary artery as required. The same principle was applied to the other groups to ensure that reperfusion pressure was constant in all cases. In all animals, the pulmonary venous oxygen partial pressure (mmHg) was obtained from pulmonary venous sampling line samples analysed immediately on a blood gas analyser (Nova Biomedical Stat Profile 5, Waltham, USA). Pulmonary vascular resistance (mmHg/l per min) was calculated from the formula: (Mean pulmonary artery pressure
saccharide (LPS) (100 £g/ml) from porcine vascular endothelium activated with lipopoly-
water to achieve 3 mg/ml. The concentration of RNA specimens, as determined by the
ratio after spectrophotometry of greater than 1.9. The proteins having a 260/280 nm (RNA/protein) absorbance
7 solution. The final preparation was free of DNA and
with 75% ethanol and was then dissolved in 1 mM EDTA, pH
4. Cell monolayers were used as the substrate for RNA extraction at 6 h after exposure.
RNA was then transcribed to analogous cDNA to be used
as a template for the PCR, which amplified the porcine IL-8 coding segments of the cDNA, analogous to porcine messenger RNA for IL-8. PCR was performed using a Perkin Elmer Geneamp RNA PCR Core Kit (N808-01K3)
(Perkin Elmer Applied Biosystems Division, Foster City,
CA, USA). After 20–40 cycles the amplified sequence was
detected by electrophoresis.
Gels were run using 1% agarose. cDNA samples (20 µl)
were added to 3 µl of Bromophenol blue/Ficoll dye after
submerging the gel in 1% TBE solution in a Bio-Rad tank
and placed in each lane. Lambda DNA HindIII marker (250
units per µl) (Sigma) was used in the leftmost lane, with the
IL-8 positive control adjacent. The tank was then connected
to the power supply (80 V, 45 mA) for 1.5 h.
The gel was stained in ethidium bromide for 30 min,
before analysis in a Bio-Rad Gel Doc 1000 analyser cabinet
using Molecular Analyst v1.4 software (Bio-Rad Labs,
Molecular Bioscience Group, Hercules, CA, USA).

To ensure that RNA strands were intact, porcine actin
primers were used in a PCR experiment. All lung biopsy
specimens demonstrated the presence of actin.

Data from groups of animals were compared using
Scheffe’s analysis of variance, with P < 0.05 indicating
statistical significance at a power of 90%.

3. Results

The pulmonary venous oxygen partial pressure in all of
the intervention groups (as assessed by the summary measure
of area under the curve) were significantly higher than in
controls animals which were subjected to similar pressure
control at the time of reperfusion (P < 0.001) (Fig. 1).
There was no statistically significant difference between
SIN1 and InSP6 (P = 0.99), but Group D (reperfused with
Pentoxifylline) was superior to both (P < 0.0001). No
difference in partial pressure of carbon dioxide was noted in
either treatment group compared with controls (P > 0.05).

Regarding the lung compliance measurements, we noted a
decline in compliance with time in the control Group A, as we
might expect as reperfusion injury progresses. We observed
significantly higher lung compliance in animals in Groups B
and C compared to control low pressure reperfused lungs
(P = 0.012 and P = 0.004, respectively), although they too
showed a decline in compliance with time. However in
Group D, lung compliance was significantly higher than in
any other group (P < 0.0001) and was sustained throughout
the time course of the experiment (Fig. 2).

Using the area under the curve for total pulmonary
vascular resistance (TPVR) of the left lung, the TPVR
was lower in all drug intervention groups compared with
controls though least impressive with InSP6-treated animals
(P = 0.036). Pentoxifylline and SIN 1 were more effective
in reducing TPVR compared with controls (P = 0.0011 and

Fig. 1. Pulmonary venous PO2 (mmHg) with time after administration of
pharmacological interventions. Results are shown as the mean value for
animals in each intervention group (n = 5; Group A, control; Group B,
inositol polyanions; Group C, SIN-1; Group D, Pentoxifylline). Error bars
indicate the 95% confidence interval. Note superior function obtained with
Pentoxifylline-treated lungs.
P0.0001, respectively) but they were statistically similar when compared with each other ($P = 0.12$) (see Fig. 3).

Left atrial pressure and right ventricular pressures were no different in the control group animals compared with any other group ($P > 0.05$).

Repeated sampling did not significantly affect the haematocrit of animals in any group over the time course of the experiment.

Regarding the qualitative assay of IL-8, PCR analyses of 35 specimens of normal right porcine lungs did not show any detectable IL-8 expression. Similarly, 35 biopsies from lungs prior to reperfusion, i.e. after 18 h of cold ischaemic preservation, also did not show any basal IL-8 activity.

Control lungs showed the faint presence of IL-8 at 2 min with obvious expression at 10 min and 30 min. Lungs reperfused at low pressure with the addition of SIN 1 or InSP6 altered the pattern of IL-8 expression after reperfusion such that the early observation of IL-8 at 2 min was absent but expression at 10 and 30 min was still apparent. However, in all five lung specimens from lungs reperfused with the addition of Pentoxifylline, IL-8 was only just seen at 30 min and not prior to this as was the case in the other intervention groups (Table 1 and Figs. 4–6).

4. Discussion

Reperfusion injury is a major problem following pulmonary transplantation, a procedure indicated in many forms of end stage lung disease. The pathophysiology is complex, but involves the sequestration of neutrophils in the lung graft within minutes of reperfusion with subsequent release of damaging enzymes and oxygen free radicals. Cytokines play an important role in the regulation of interactions between circulating leucocytes and pulmonary endothelium and parenchyma during reperfusion injury. Interleukin 8 (IL-8), released principally from alveolar macrophages, is one of the important contributors to the complex events occurring at reperfusion and a key chemotactic factor for neutrophils [10,11]. It dramatically enhances neutrophil transmigration through pulmonary endothelium and epithelium [12] and as well as PMNL chemoattraction and activation, it accentuates IL-1, IL-2, IL-4 and IL-6 production with ensuing tumour necrosis factor (TNF)-α formation. Indeed studies on lung reperfusion injury in a rabbit model have suggested that neutrophil infiltration and the destruction of pulmonary architecture could be modulated by a monoclonal antibody against IL-8 [11]. A number of potential interventions have been investigated in an attempt to modulate pulmonary reperfusion injury in clinical practice to improve graft function, reduce patient morbidity, mortality and perhaps permit the use of longer organ ischaemic times.

We investigated the expression of IL-8 in response to a variety of interventions in a porcine model to determine the role of IL-8, its pattern of expression, its modulation by various interventions and its relationship to oxygenation and lung compliance.

The IL-8 assay technique used in this study allowed the isolation of RNA from snap frozen lung biopsy specimens with subsequent IL-8 expression measurement with the reverse transcriptase PCR using porcine IL-8 primers. The method was effective in isolating pure (260 nm/280 nm absorbance ratio of 1.5–2.0) and intact RNA strands from the biopsy specimens.

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Table 1

Results of PCR analysis for porcine IL-8

$^a$ The presence or absence of IL-8 expression with each intervention is indicated. Lung biopsies were taken at the time intervals shown.
Although not quantitative, the results indicate the pattern of IL-8 expression in the lung biopsy specimens with time and uniquely in the presence of different interventions. Perioperative immunosuppression and surgery alone may influence cytokine levels but in a comparative study such as this these factors should be equivalent across the intervention groups.

Preserved but unreperfused lungs and normal samples of contralateral lung did not show appreciable expression of IL-8. No lungs in any group expressed IL-8 at 12 h post-reperfusion.

It has been postulated that in brain-stem-dead donors, the accompanying release of stress hormones such as epinephrine may increase IL-8 production but this was not observed in our study. It is known that brain-stem death influences cardiac graft function in experimental models and the fact that the donor animals in our experiments were cerebrally intact may be a disadvantage.

The results indicated that lungs reperfused normally express IL-8 within 2 min of reperfusion with maximal expression at 10–30 min. In contrast, lungs reperfused with SIN-1 or InSP6 show a different pattern of expression, with IL-8 expressed for the first time at 10 min and maximally at 30 min.

In contrast, Pentoxifylline attenuated IL-8 expression, with minimal expression only appearing 30 min after perfusion. This is a mechanism of Pentoxifylline action which has not been previously described and may be important in understanding how this agent influences neutrophil sequestration not only in the lung but in other tissues.

Such rapid expression of IL-8 by gene transcription is remarkable (within 2 min of reperfusion in control group animals) given the mRNA half life of 4.6 h in healthy volunteers [13]. Physicochemically it is a stable entity, resistant to proteolysis, suggesting a longer term role in mediating inflammatory responses. Imamura showed that in a model of lipopolysaccharide induced lung injury, TNF-α peaked within 30 min of injury, while IL-1 and IL-8 peaked at 2 h [14]. This indicates the respective roles of initiator and effector in this important group of cytokines. In alveolar macrophages from porcine lungs stimulated with lipopolysaccharide, IL-8 mRNA expression was detectable within 30 min, peaking at 3–6 h at a 30-fold higher level than basal unstimulated cells [15].

Numerous factors influence local synthesis and secretion of IL-8 and consequent neutrophil recruitment and activation. Defensins (non-enzymatic cationic polypeptides) released from neutrophils at the site of lung injury enhance IL-8 transcription to increase IL-8 mRNA 12-fold [16]. In human peripheral blood neutrophils, IL-8 mRNA increases
5-fold by 2 h after stimulation with lipopolysaccharide but levels reached baseline levels by 12 h after stimulation. In contrast, monocytes maintain high levels of IL-8 secretion over a 12-h period [17]. Although monocytes secrete 70 times more IL-8 than neutrophils, the abundance of the latter means that they are the principal source of IL-8. Thus neutrophil derived IL-8 may play an autocrine or paracrine role in the early stages of lung inflammation.

Oxygen free radicals, abundant during reperfusion injury, regulate gene expression of IL-8 and even low levels of reactive oxygen species may serve to initiate IL-8 production which then recruits neutrophils to sites of lung injury [18].

The timing of IL-8 transcription in lung ischaemia–reperfusion injury has not been studied in any great detail and no previous studies have looked at an earlier time frame or in pulmonary reperfusion injury specifically.

The observed pattern of IL-8 expression appeared to correlate with functional indices of lung graft preservation. Control lungs showed the worst oxygenation and a decline in lung compliance over the time course of the experiment. There was significant improvement in oxygenation and lung compliance in Groups B and C, in parallel with the later expression of IL-8 in these groups compared to controls. In Group D, treated with Pentoxifylline, lung compliance did not decline significantly with time and was superior throughout the experiment to all other intervention groups. Oxygenation was also best in this group and again correlates with the later expression of IL-8 in these grafts.

In a study such as this, however, it is possible that the reduction in IL-8 expression is instead a reflection of attenuated reperfusion injury itself. This presents us with a situation in which a pharmacological intervention may reduce IL-8 and thus modulate reperfusion injury. Conversely, however, interventions attenuating reperfusion injury may consequently modulate IL-8 expression to produce the same observation. Further studies are clearly warranted to clarify the sequence of events.

We believe that Pentoxifylline treatment immediately before lung graft reperfusion significantly attenuates reperfusion injury, improves oxygenation and halts the deterioration in lung compliance with time. Our experiments suggest that an important mechanism may be through the delayed expression of the important cytokine, IL-8. Further studies using a quantitative assay are required to clarify this potentially important mode of action of Pentoxifylline.

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References