CARDIAC AND PULMONARY REPLACEMENT

CONTROLLED REPERFUSION AND PENTOXIFYLLINE MODULATE REPERFUSION INJURY AFTER SINGLE LUNG TRANSPLANTATION

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Objective: Rodent models have suggested that initial low-pressure reperfusion of transplanted lungs reduces injury after ischemia. We investigated this phenomenon and the use of pentoxifylline in a porcine model of left single lung transplantation. **Methods:** Donor lungs were preserved with Euro-Collins solution for a mean ischemic time of 18.4 hours. Neutrophil trapping in the graft, pulmonary artery pressure, and gas exchange were assessed over a 12-hour period. Partial occlusion of the contralateral pulmonary artery allowed manipulation of the pulmonary artery pressure in the transplanted lung. Group A ($n = 5$) was perfused at a mean pulmonary artery pressure of 20 mm Hg, group B was reperfused at a mean pulmonary artery pressure of 45 mm Hg for 10 minutes before reducing the pressure to the same as group A, and group C was reperfused at a mean pressure of 20 mm Hg for 10 minutes, then increased to a mean of 45 mm Hg for the remainder of the experiment. Group D was reperfused as in group A with the addition of intravenous pentoxifylline. **Results:** Leukocyte sequestration was observed in the first 10 minutes after reperfusion in groups A, B, and C, with maximal sequestration at 2 minutes. Significantly more sequestration was observed in the first 6 minutes in group B than in groups A and C, which were similar. Pentoxifylline significantly reduced leukocyte sequestration. Pulmonary venous oxygen tension in the allograft lung was worst in group B. Groups A and C were similar, but group D was superior to all other groups ($p < 0.001$). **Conclusions:** Low-pressure reperfusion, even when limited to the first 10 minutes, modulates reperfusion injury possibly through a leukocyte-dependent mechanism. The addition of pentoxifylline in the recipient confers significant additional benefit. (J Thorac Cardiovasc Surg 1998;115:1335-41)

Despite advances in lung preservation technology, early graft dysfunction remains a significant problem, affecting up to 20% of lung transplant recipients worldwide.^{1,2}

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Lung graft ischemia-reperfusion injury is complex and multifactorial but is principally determined by the sequestration of activated neutrophils within the lung on reperfusion with subsequent release of injurious granular enzymes and oxygen free radicals.³

Controlled-pressure reperfusion of the graft has been suggested as a potential strategy for attenuating acute pulmonary dysfunction. This involves the reperfusion of a lung graft at 50% of physiologic pressure for 10 minutes before reperfusion at physiologic pressure.^{4,5} Bhabra and colleagues⁴ showed beneficial effects in an isolated rodent lung model, but its inherent limitations prompted us to investigate the phenomenon in more depth and to evaluate

Fig. 1. Neutrophil sequestration in the lung graft with time. The number of neutrophils exiting the lung is expressed as a percentage of the number of neutrophils entering the lung. A figure less than 100% therefore indicates neutrophil trapping in the graft, whereas points more than 100% show neutrophil release from the lung graft.

it in conjunction with pentoxifylline administration, which has been successful in ameliorating reperfusion injury after skeletal muscle ischemia and experimental lung and liver transplantation.⁶⁻⁸ Furthermore, although controlled-pressure reperfusion has been demonstrated at physiologic pressures, we wished to investigate whether the benefits remained when supranormal pressures were applied—conditions more common in clinical lung transplantation.

Methods

Twenty female Landrace pigs (mean weight 45.2 kg) were divided into four groups ($n = 5$ in each). A similar number were size and weight matched and acted as donor animals.

Group A animals ($n = 5$) were perfused at a mean pulmonary artery pressure (PAP) of 20 mm Hg throughout the 12-hour experiment; group B animals were reperused at a mean PAP of 45 mm Hg for 10 minutes before reducing the pressure to the same as group A for the remainder of the time course; and group C animals were reperused at a mean pressure of 20 mm Hg for 10 minutes, then increased to a mean of 45 mm Hg. Group D animals were reperused as in group A with the addition of intravenous pentoxifylline administered to the recipient

animal only (20 mg/kg loading dose, then 2 mg/kg per hour) beginning 5 minutes before reperfusion.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared 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.

Donor operation. Animals were anesthetized with initial intramuscular premedication with diazepam (2 mg/kg) and ketamine (15 mg/kg). Subsequent anesthesia 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%. Cannulas (Cavafix Certo 18G, Braun, Melsungen, Germany) were inserted into the external jugular vein and common carotid artery for monitoring of central venous pressure and arterial pressure, respectively.

After median sternotomy, the superior and inferior venae cavae, main pulmonary artery (PA), trachea, and ascending aorta were isolated. Animals were then given heparin (300 U/kg), 10 mg/kg methylprednisolone, and a cannula (model 145016, Polystan, Walgerholm, Denmark)

Fig. 2. MDA (oxygen free radical marker) with time after reperfusion.

was inserted through a purse-string suture in the main PA. Ligation of the cavae and venting of the right side of the heart by incision of the inferior vena cava was followed by crossclamping of the aorta. A 60 ml/kg dose of cold (4° C) modified Euro-Collins solution was infused through the cannula in the main PA, and the left side of the heart was vented by incision of the left atrial appendage.

After topical cooling with saline solution (1° C), the trachea was clamped at end-inspiration and the heart-lung block excised.

After subsequent separation of the left lung from the block, a PAP monitoring line (Cavafix Certo 18G, Braun, Melsungen, Germany) was inserted through a purse-string suture into the distal left PA. 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 from the graft without mixing from the contralateral native lung after transplantation and reperfusion.

Recipient operation. Recipient animals were premedicated with intramuscular azaperone (8 mg/kg) and diazepam (2 mg/kg). After induction of anesthesia with intravenous propofol, animals were maintained on intravenous pentobarbital (30 mg/kg per hour) and alfentanil. Venous and arterial pressure monitoring lines were inserted as in donor animals.

Two endotracheal tubes were inserted through 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 asynchronous ventilation after the transplant.

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 PA in order. The contralateral PA was encircled by a tape and snigger such that the left PAP 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 PA.

PA flow was acquired through a 10 mm Transonic A-Series flow probe (Linton Instruments, Norfolk, United Kingdom) placed around the left PA 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 flowmeter (Transonic Systems Inc., Ithaca, N.Y.).

All the above pressure and flow data sources were routed through a CED 1401 32-channel digital-to-analog converter (Cambridge Electronic Design Ltd., Cambs, United Kingdom) and acquired on a Gateway 2000 PC running Microsoft Windows '95 and Spike 2 (Version 4.0)

Fig. 3. Pulmonary venous oxygen partial pressure in the graft lung with time.

data acquisition software (Cambridge Electronic Design Ltd.). Data were stored continually over the 12-hour postoperative period on hard disk for subsequent analysis.

Pulmonary venous oxygen partial pressure was obtained from pulmonary venous sampling line samples analyzed immediately on a blood gas analyzer (Nova Biomedical Stat Profile 5, Waltham, Mass.).

Pulmonary vascular resistance (in millimeters of mercury per liter per minute) was calculated from the formula (Mean PAP [mm Hg] – Left atrial pressure [mm Hg])/Left PA flow [L/min].

Neutrophil trapping within the lung was calculated by expressing the neutrophil count of blood exiting the lung as a percentage of that entering the lung at each sampling interval.

Malonaldehyde (MDA), an important decomposition product of lipid peroxides, is an indirect measure of free radical activity.

A spectrophotometric assay using an LPO-586 method was used to quantify MDA in pulmonary venous blood. Condensation of one molecule of MDA with two molecules of a proprietary chromogenic agent produces a stable chromophore with maximal absorbance at 586 nm.

Five milliliter samples of whole blood were collected in 48 μ L of potassium ethylenediaminetetraacetic acid 0.17 mol/L. After centrifugation at 2500g for 10 minutes at 4°C, 200 μ L of the supernatant was collected in duplicate for use in the assay.

The sample was incubated for 40 minutes at 45°C with the chromogenic substance, then cooled on ice, and absorbance measured by spectrophotometer at 586 nm. The sensitivity of this method was determined as 0.5 μ mol/L. In samples of 200 μ L, the lower limit of measurable MDA was 2.5 μ mol/L. Reproducibility was determined by assays done over 10 days under identical experimental conditions. With standard concentrations from 0 to 20 μ mol/L, standard error of the mean was less than 5%.

Statistical methods. The summary measure of area under the curve was used to describe the behavior of individual animals.⁹ Groups of animals were then compared by use of Scheffe analysis of variance, with $p < 0.05$ indicating statistical significance at a power of 90%. We acknowledge the assistance of Dr. H. R. Matthews, Senior Lecturer in Medical Statistics at the University of Newcastle-upon-Tyne, for his advice regarding the statistical analysis of these data.

Results

Leukocyte sequestration in the lung graft was observed in the first 10 minutes after reperfusion (Fig. 1) in groups A, B, and C, with maximal sequestration occurring at 2 minutes. After a plateau phase lasting to 240 minutes after reperfusion, neutrophils were released from the lung. In group D

Fig. 4. Pulmonary vascular resistance of the transplanted lung with time.

the administration of pentoxifylline abolished this biphasic pattern of sequestration and subsequent release.

Analysis of variance showed that overall neutrophil trapping in the graft was significantly reduced in group D ($p = 0.029$) compared with the other groups. No differences in neutrophil sequestration were observed in the other groups overall. However, analysis of the first 30 minutes indicated significantly more leukocyte sequestration in group B, reperfused at high pressure, than in groups A and D ($p = 0.001$).

MDA levels were also significantly higher in lungs reperfused at high pressure ($p < 0.0001$), whereas those in groups A and C were similar ($p = 0.30$). Lungs in the pentoxifylline treatment group showed significantly lower free radical levels than those in the other groups ($p < 0.0001$) (Fig. 2).

Pulmonary venous oxygen tension in the allograft lung was worst in group B ($p < 0.001$). Groups A and C were similar ($p = 0.14$), but group D, treated with pentoxifylline, was superior to all other groups ($p < 0.0001$) (Fig. 3). Some deterioration in function

was noted 9 hours after reperfusion in lungs reperfused at low pressure for an initial period of 10 minutes only compared with low-pressure reperfusion throughout the 12-hour follow-up period.

Pulmonary vascular resistance was lowest in the pentoxifylline-treated group (Fig. 4). Groups A and C were statistically similar, but group B, reperfused at high pressure, initially showed the highest pulmonary vascular resistance and lowest graft flows over the 12-hour study period.

Discussion

The original work on controlled-pressure reperfusion in isolated rat lungs suggested benefits in terms of better oxygenation when lungs were reperfused at 50% of physiologic pressure for 10 minutes before reperfusion at physiologic pressure.^{4,5}

These studies, however, are limited by the inherent limitations of small animal models, the short perfusion time of just 60 minutes, and the presence of an extracorporeal circuit, which activates neutrophils and may influence pulmonary graft function.

Clearly, this strategy needed to be investigated in

a large animal model, enabling in vivo techniques of implantation, longer (12-hour) reperfusion times, and by allowing more substantive analysis to achieve some insight into the mechanism.

Our model of left single lung transplantation provides a stable and reproducible model, allowing fine control of ventilation and perfusion of the lung graft while the animal is supported by the contralateral lung irrespective of the function in the newly implanted graft. The rigorous separation of the lungs in terms of sample collection ensures that there is no mixing of blood from the contralateral side.

The original rat model described controlled reperfusion at physiologic pressure. This is rarely encountered during clinical lung transplantation, when pressures may approach systemic levels. It was important to establish, therefore, whether controlled-pressure reperfusion would still provide benefit under supraphysiologic conditions. The use of a high-pressure reperfusion group at an initial PAP of 45 mm Hg, with low-pressure groups being physiologic (20 mm Hg), indicated that this is indeed the case.

Our experiments have also determined that low-pressure reperfusion, even when limited to the first 10 minutes, modulates reperfusion injury. Our porcine model has established that the phenomenon can be reproduced in a large animal model and that the benefits of low-pressure reperfusion over high-pressure reperfusion persist for as long as 12 hours. Our data on reperfusion for a limited 10-minute period suggest that benefit is similar to those lungs reperfused at low pressure throughout, although by 9 hours after reperfusion the pulmonary venous oxygen tension began to decline. The long-term functional results of a limited period of perfusion at low pressure has yet to be determined.

The mechanism behind controlled-pressure reperfusion may involve the recovery of endothelial integrity, possibly through prostacyclin or nitric oxide pathways, or may modulate shear stresses known to influence adhesion molecule expression and cytokine release. Our data have indicated that less neutrophil trapping occurs under conditions of low-pressure reperfusion, even when limited to the first 10 minutes, with lower levels of oxygen free radical release. Some studies have suggested that controlled-pressure reperfusion acts through a purely mechanical mechanism by ameliorating the shedding of pulmonary vascular endothelium with rapid reperfusion. Our studies indicate that the active

involvement of neutrophils is also important in the process.

The addition of pentoxifylline in the recipient confers significant additional benefit. Pentoxifylline has many functions, in particular inhibiting the adherence of neutrophils to endothelium and preventing leukocyte degranulation. Pentoxifylline may also inhibit the important interleukin, interleukin-1, and tumor necrosis factor, which are critical in the pathogenesis of ischemia-reperfusion injury.^{10, 11}

Although pentoxifylline clearly improves and augments function in conjunction with low-pressure reperfusion by reducing neutrophil trapping in the lung and subsequent free radical release, any benefits attained through its combination with high-pressure reperfusion remain to be seen.

Our experiments administered pentoxifylline to recipient animals only and has been beneficial using this regimen in previous porcine models.¹² There has been some suggestion that pentoxifylline is effective when administered in flush solutions but not in the recipient alone.¹³ Our findings seem to support the former, although results are difficult to compare between different dose regimens and models.

These simple methods of controlled-pressure reperfusion and pentoxifylline administration can be instituted easily in the operating theater in human recipients. Pentoxifylline (already licensed for use in human beings with peripheral vascular disease) is an inexpensive and safe drug for use in lung transplant recipients. We now hope to begin a clinical trial of these techniques to determine whether the benefits observed in this porcine model are true in clinical practice, which may lead to considerable improvements in mortality and morbidity among lung transplant recipients, as well as shorter intensive care stays and costs.

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