CONTROLLED REPERFUSION AFTER LUNG ISCHEMIA: IMPLICATIONS FOR IMPROVED FUNCTION AFTER LUNG TRANSPLANTATION

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Objectives: Despite improvements in organ preservation, reperfusion injury remains a major source of morbidity and mortality after lung transplantation. This pilot study was designed to investigate the effects of controlled reperfusion after lung ischemia. **Methods:** Twenty adult pigs underwent 2 hours of warm lung ischemia by crossclamping the left bronchus and pulmonary artery. In five (group 1), the clamp was simply removed at the end of ischemia (uncontrolled reperfusion). The 15 other pigs underwent modified reperfusion using blood from the femoral artery to perfuse the lung through the pulmonary artery (pressure 40 to 50 mm Hg) for 10 minutes before removing the pulmonary artery clamp. In five (group 2), the blood was mixed with crystalloid, resulting in a substrate-enriched, hypocalcemic, hyperosmolar, alkaline solution. In five (group 3), the blood was circulated through a leukocyte-depleting filter, and the last five (group 4) underwent reperfusion with both a modified solution and white blood cell filter. Lung function was assessed 60 minutes after reperfusion, and biopsy specimens were taken. **Results:** Controlled reperfusion with both a white blood cell filter and modified solution (group 4) completely eliminated the reperfusion injury that occurred with uncontrolled reperfusion (group 1), resulting in complete preservation of compliance ($98\% \pm 1\%$ vs $77\% \pm 1\%$; $p < 0.001$, and arterial/alveolar ratio ($97\% \pm 2\%$ vs $27\% \pm 2\%$; $p < 0.001$); no increase in pulmonary vascular resistance ($106\% \pm 1\%$ vs $198\% \pm 1\%$; $p < 0.001$); lowered tissue edema ($82.1\% \pm 0.4\%$ vs $84.3\% \pm 0.2\%$; $p < 0.001$), and myeloperoxidase activity (0.18 ± 0.02 vs 0.35 ± 0.02 δ OD/min/mg protein; $p < 0.001$). In contrast, using either a white blood cell filter or modified solution separately improved but did not avoid the reperfusion injury, resulting in pulmonary function and tissue edema levels that were intermediate between group 1 (uncontrolled reperfusion) and group 4 (white blood cell filter and modified solution). **Conclusion:** After 2 hours of warm pulmonary ischemia, (1) a severe lung injury occurs after uncontrolled reperfusion, (2) controlled reperfusion with either a modified reperfusion solution or white blood cell filter limits, but does not avoid, a lung reperfusion injury, (3) reperfusion using both a modified perfusate and white blood cell filter results in complete preservation of pulmonary function. We therefore believe surgeons should control the perfusate after lung transplantation to improve postoperative pulmonary function. (J Thorac Cardiovasc Surg 1998;115:415-25)

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Since its resurgence in the late 1980s, lung transplantation has been accepted as the treatment of choice for end-stage pulmonary disease. Demand continues to outpace availability of donor lungs. As such, the number of lung transplantations performed worldwide has plateaued since 1993.¹ Compared with other solid organs, little clinical progress has been made in using less than perfect lungs and in prolonging ischemic time. At present, only 10% to 15% of multiorgan donors have lungs suitable for transplantation, and most institutions do not routinely accept ischemic time longer than 6 hours.¹ Despite these conservative procurement practices, transplanted lungs remain vulnerable to preoperative injury and severe graft dysfunction (reperfusion injury or reimplantation response) that occurs in 10% to 20% of cases.¹⁻⁵ Clinically, this syndrome results in progressive hypoxemia, decreased pulmonary compliance, pulmonary edema, increased pulmonary vascular resistance (PVR), and diffuse infiltrates on chest radiographs.¹⁻³ Histologically, these lungs show alveolar damage, sequestration of neutrophils, interstitial edema, and in severe cases, hemorrhagic infiltrates.⁶ Although not always fatal, this syndrome is associated with a prolonged intensive care unit stay and mechanical ventilation, which has been associated with higher incidence of airway healing complications.¹ This ischemia-reperfusion injury has also recently been found to predispose grafts to early rejection by up-regulation of class II major histocompatibility antigens.⁷ This up-regulation of inflammatory mediators during the immediate postoperative period has been shown to not only play a role in injury to the transplanted lung but also the deterioration often seen in native lung function in single lung transplants.^{8,9}

Understanding and controlling the ischemia-reperfusion injury seems to be one of the keys to increasing the available donor pool and improving short- and long-term outcomes in lung transplantation. Although it is clear that changes that occur during ischemia are the trigger, it has become increasingly evident that most of the injury occurs during the first few minutes of reperfusion.¹⁰⁻¹² Current clinical practices in lung transplantations are primarily aimed at decreasing damage during ischemia (i.e., single cold flush perfusion, topical cooling and inflated cold storage).^{1,2} Several experimental studies have, however, been done to try to minimize the reperfusion injury.^{4,13-17} Most have centered around adding substrates to the flush solution, such as oxygen-derived free radical scavengers,

agents that block neutrophil-endothelial interaction, or endothelial protective agents. Few of these additives have gained clinical acceptance. Much less attention has been paid to directly manipulating the onset of reperfusion, despite the documented effectiveness of this approach after myocardial ischemia.^{18,19}

The objectives of this pilot study were, therefore, to determine the effect of using controlled reperfusion in preventing a reperfusion injury after a period of lung ischemia.

Material and methods

Twenty healthy mature Duroc-Yorkshire pigs (25 to 35 kg) were sedated with intramuscular ketamine (20 mg/kg), anesthetized with intravenous pentobarbital (25 mg/kg), and paralyzed with intravenous pancuronium (0.3 mg/kg). Anesthesia was maintained by intermittent intravenous pentobarbital. Mechanical ventilation was achieved with a tracheostomy using a Servo 900-B volume-controlled ventilator (Siemens-Elema, Solna, Sweden). All animals received 1 gm of cefazolin intravenously before surgery, and 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 Institute of Health (NIH publication 96-03, revised 1996).

Heart rate was monitored continuously by electrocardiogram. The left femoral artery and vein were cannulated for continuous monitoring of arterial blood pressure, blood gas determinations, and intravenous infusions. Urine output was assessed with an indwelling urinary catheter. Intravenous furosemide (Lasix), 0.5 to 1 mg/kg, was given as indicated to maintain urine output greater than 1 mg/kg per hour. Body temperature was monitored using esophageal temperature probe (Mon-A-Therm 400H, Mallinckrodt, St. Louis, Mo.) and maintained at 36° to 38° C with a heating blanket. Periodic arterial blood samples were drawn and analyzed for blood gas, potassium, ionized calcium, and hemoglobin (Ciba-Corning, Blood Gas System 288, Medfield, Mass.). Electrolytes were kept within normal limits, and all animals had an initial hemoglobin value higher than 10.0 gm/dl.

After a median sternotomy, the pericardium and both pleural spaces were entered. A 7F balloon-tipped thermodilution catheter (Baxter Healthcare Corp., Deerfield, Ill.) was placed through the right internal jugular vein into the main pulmonary artery for determination of pressure and cardiac output. An 18-gauge catheter was placed in the left atrium for pressure measurements. The right and left hilar structures (pulmonary arteries, veins, and main bronchi) were dissected. Special attention was paid to skeletonizing the left bronchus for a length of approximately 1 cm, thereby obliterating the bronchial blood supply. Animals were then given 3 mg/kg heparin intravenously. The right femoral artery and left pulmonary artery were cannulated using an 8F DLP arterial cannula (DLP, Inc., Grand Rapids, Mich.). This allowed blood to be

Fig. 1. **A,** Our experimental model of controlled pulmonary reperfusion. Blood is taken from the femoral artery and combined with either a modified crystalloid solution using a BCD as a mixer, passed through a WBC filter before being returned to the pulmonary artery, or both. The pressure in the distal pulmonary artery is constantly measured and kept between 40 and 50 mm Hg. **B,** The modified reperfusate is infused into the left pulmonary artery distal to the clamp and is able to return to the pig through the pulmonary veins.

withdrawn from the femoral artery and, through a roller pump, passed through either a mixer/heater (BCD, Shiley Corp, Irvine, Calif.), a leukocyte-depleting filter (WBC filter) (Pall BC-1, Pall Corp, Glen Cove, N.Y.), or both, and returned to the pulmonary artery (Fig. 1). A catheter was also placed to allow constant monitoring of left pulmonary artery pressure during controlled reperfusion.

Study groups. The animals were divided into four groups, five in each. The left and right hilar structures were transiently clamped separately to obtain baseline pulmonary functional measurements (compliance, arterial/alveolar ratio [a/A ratio], and PVR) for each lung. Clamps were then placed on the left main-stem bronchus and left pulmonary artery. The left main-stem bronchus was clamped to ensure complete warm ischemia by eliminating all bronchial blood and lymphatic flow. Ventilation was adjusted for one-lung ventilation, and the animals were observed for 2 hours. At the end of 2 hours, the left lung was reperfused by one of four different methods.

Uncontrolled reperfusion

GROUP I (UNMODIFIED BLOOD). The clamps were simply removed from the pulmonary artery and bronchus, allowing unmodified blood to reperfuse the lung by restoring native pulmonary flow.

Controlled reperfusion

GROUP II (MODIFIED SOLUTION). The left lung was reperfused by taking blood from the femoral artery, mixing it in a 4:1 ratio with a modified crystalloid solution

Table I. Modified reperfusate solution

Solution additive	Volume added (ml)	Component modified	Concentration delivered*
MgCl ₂ (2 mEq/L)	24	Mg ⁺²	10-12 mEq/L
THAM (0.3 mol/L)	225	pH	pH 7.5-7.6
CPD	225	Ca ⁺²	0.2-0.3 mmol/L
Aspartate/glutamate	250	Substrate	13 mmol/L each
D ₅₀ W	40	Glucose	>400 mg/dl
D ₅ W	200	Osmolarity	380-400 mOsm
Nitroglycerin (5 mg/ml)	1	Vasodilator	1 µg/ml

Tham, Tromethamine; CPD, citrate-phosphate-dextrose; D₅₀W, 50% dextrose in water.

*When mixed in a 4:1 ratio with blood.

(Table I), and then pumping this solution into the left pulmonary artery distal to the clamp for 10 minutes (Fig. 1). The reperfusate was allowed to return to the pig by way of the pulmonary vein. Perfusion pressure was continuously monitored and kept between 40 and 50 mm Hg in the distal pulmonary artery. This resulted in blood flows between 50 and 70 ml/min. After 10 minutes, the controlled perfusion was stopped, and the pulmonary artery and bronchial clamps were removed, restoring native pulmonary circulation.

GROUP III (WBC FILTER). The left lung was reperfused by taking blood from the femoral artery, passing it through a WBC filter (Pall BC-1), and then pumping it into the left pulmonary artery distal to the clamp for 10 minutes (Fig. 1). The pressure was again continuously monitored and kept between 40 and 50 mm Hg in the distal pulmonary artery. After 10 minutes, the perfusion was stopped and the clamps removed, restoring native pulmonary blood flow.

GROUP IV (WBC FILTER AND MODIFIED SOLUTION). Perfusion was the same as in groups 2 and 3, but both a WBC filter and modified solution were used during the first 10 minutes of reperfusion. The clamps were then removed as above.

All animals were observed for 1 hour after reperfusion and functional measurements were repeated in the left and right lungs. Bilateral lung biopsy specimens were then obtained for tissue edema and myeloperoxidase activity, and the animals euthanized.

Pulmonary functional measurements. Pulmonary functional measurements were obtained for each lung by transiently clamping the contralateral bronchus and pulmonary artery so all blood flow and ventilation were directed through the lung being assessed. All measurements were obtained before ischemia (baseline) and 60 minutes after reperfusion. Postreperfusion measurements are expressed as a percentage of baseline to allow each pig to act as its own control.

ARTERIAL/ALVEOLAR RATIO. With ventilation unchanged between preischemic and postreperfusion settings, the a/A oxygen ratio for each lung was calculated using the following formula:

a/A tension ratio =

$$\frac{P_{O_2}}{F_{iO_2}(P_B - PH_2O) - PCO_2(1.25 - 0.25 F_{iO_2})}$$

where P_{O_2} is partial pressure of oxygen, F_{iO_2} is inspired percentage of oxygen, PCO_2 is partial pressure of carbon dioxide, P_B is barometric pressure, and PH_2O is partial pressure of water.

PVR. Cardiac output was measured using a Swan-Ganz catheter and a Edwards 9502 computer (Baxter Healthcare Corp., Edwards Division, Santa Ana, Calif.). Averages from three measurements were used. PVR was calculated using the following formula:

$$PVR (\text{dynes} \times \text{sec}^{-1} \times \text{cm}^{-5}) = \frac{PAP - LAP}{CO} \times 80$$

where PAP is mean pulmonary artery pressure, LAP is mean left arterial pressure, and CO is cardiac output.

LUNG COMPLIANCE. Peak airway pressure was measured using a tidal volume of 15 ml/kg without positive end-expiratory pressure. Static compliance was calculated as change in volume/change in pressure (ml/cm H_2O).

Tissue measurements

LUNG EDEMA. A wedge biopsy was taken from the inferior portion of the right and left upper lobes at the end of the experiment for tissue water and myeloperoxidase activity. A portion of each biopsy specimen was weighed and then dried to a constant weight at 80° to 85 °C. Results

are expressed as percentage tissue water using the following formula:

$$\% \text{Tissue water} = \frac{\text{Wet weight} - \text{Dry weight}}{\text{Wet weight}} \times 100$$

MYELOPEROXIDASE ACTIVITY. The other portion of each biopsy was immediately frozen and stored in liquid nitrogen. The frozen tissue was then crushed using a liquid nitrogen-cooled motor and pestle, and lyophilized (Savant Speed Vac Systems, Farmingdale, N.Y.). Quantitative myeloperoxidase activity was determined using the modified procedures of Okabayashi and associates.²⁰ Ten to 15 mg of dry lung tissue was homogenized in 1 ml of 0.5% hexadecyltrimethylammonium bromide (to release myeloperoxidase from the cells), 5 mmol/L EDTA, and 50 mmol/L potassium phosphate buffer (pH 6.2) with Kontes glass tissue grinder (Kontes Glass Co, Vineland, N.J.). The homogenate was centrifuged at 10,000g for 15 minutes at 4° C. The supernatant was assayed for total soluble protein by the method of Pierce Laboratories.²¹ Fifty microliters of supernatant was combined with 0.6 ml Hanks bovine serum albumin, 0.5 ml of 100 mmol/L potassium phosphate buffer (pH 6.2), 0.1 ml 0.05% H_2O_2 , and 0.1 ml of 1.25 mg/ml *o*-dianisidine. The optical density was measured at 460 nm (Beckman DU7400, Beckman Instruments, Inc., Fullerton, Calif.). Color development from 5 to 20 minutes is linear. Enzyme activity is expressed as the change in optical density units per minute per milligram of tissue protein ($\delta OD/\text{min}/\text{mg}$ protein).

WBC count and platelet counts. WBC and platelet counts were obtained from animals in which a WBC filter was used (Sysmex K-100, Baxter, Deerfield, Ill.). Differential counts were performed manually by a single technician. Leukocyte and platelet counts were obtained proximal and distal to the WBC filter during reperfusion and expressed as cells $\times 1000/\text{mm}^3$ per cubic millimeter.

Statistical analysis. Data were analyzed using JMP V2.0 (SAS Institute Inc., Cary, N.C.) on a Macintosh IIVX Computer (Apple Inc., Cupertino, Calif.). All data are expressed as mean $\pm$ standard error of the mean. Paired Student's *t* test and two-way analysis of variance with interaction (factorial analysis) were used for comparison of variables among experimental groups. If analysis of variance revealed a significant interaction, pair-wise tests of individual group means were contrasted by way of multiple comparisons (Tukey's test) using a level of significance of $p < 0.01$.

Results

No statistical difference was found in baseline (preischemic) measurements of compliance (14.8 ± 0.3 ml/cm H_2O), PVR (959 ± 14 dynes/sec/cm), or a/A ratio (0.78 ± 0.03) between any group. All animals remained stable throughout this experiment.

Pulmonary function. Results are summarized in Figs. 2 to 4 and Table II. All postoperative values are expressed as a percentage of baseline measurements to allow each pig to act as its own control. After 2 hours of ischemia, uncontrolled reperfusion

Fig. 2. Postreperfusion pulmonary compliance expressed as percent recovery compared with baseline values. Uncontrolled reperfusion with unmodified blood significantly lowered pulmonary compliance, indicating a reperfusion injury. Controlled reperfusion with either a modified solution or WBC filter partially blunted this injury but did not fully restore pulmonary compliance. Conversely, using both a WBC filter and modified solution avoided the reperfusion injury, resulting in complete recovery of pulmonary compliance. * $p < 0.01$ versus uncontrolled reperfusion. ** $p < 0.001$ versus all other groups.

Fig. 3. Postreperfusion PVR expressed as percent increase compared with baseline values. The PVR doubled when the lung was reperfused in an uncontrolled fashion using unmodified blood. Conversely, this increase was lessened by using controlled reperfusion with either a modified solution or WBC filter alone. However, the reperfusion injury was only completely avoided when both a WBC filter and modified solution were combined. Indeed, in this group, there was no increase in PVR compared with preischemic values. * $p < 0.001$ versus uncontrolled reperfusion. ** $p < 0.001$ versus all other groups.

(group 1) caused a significant pulmonary injury manifested by decreased compliance, elevated PVR, and decreased oxygenation (a/a ratio). In contrast, this injury was essentially avoided by controlling pulmonary reperfusion using a modified solution and WBC filter (group 4), resulting in complete preservation of compliance, PVR, and oxygenation

(a/a ratio). Use of only one of these modalities (modified solution, group 2; or WBC filter, group 3) resulted in intermediate preservation of pulmonary function, implying that the reperfusion injury was only partially modified.

In the nonischemic right lung, no significant change occurred in postoperative pulmonary func-

Fig. 4. Postreperfusion a/A oxygen ratio expressed as percent recovery of baseline values. Uncontrolled reperfusion using unmodified blood resulted in a marked decrease in a/A ratio, indicating a reperfusion injury. This injury was partially avoided by using either a WBC filter or modified solution alone and was completely avoided using controlled reperfusion with both a WBC filter and modified solution. * $p < 0.01$ versus uncontrolled reperfusion. ** $p < 0.01$ versus all other groups.

Table II. Left lung functional and tissue results

Reperfusion method	Compliance (%)	PVR (%)	a/A ratio (%)	Lung water (%)	Myeloperoxidase
Uncontrolled (Group 1)	77 ± 1*	198 ± 1	27 ± 2	84.3 ± 0.2	0.35 ± 0.02
Modified solution (Group 2)	86 ± 1*	153 ± 2*	51 ± 1*	83.4 ± 2*	0.21 ± 0.03*
WBC filter (group 3)	91 ± 1*	133 ± 1‡	77 ± 2‡	83.3 ± 2*	0.17 ± 0.01*
Solution and filter (group 4)	98 ± 1‡	106 ± 1‡	97 ± 2‡	82.1 ± 0.4‡	0.18 ± 0.02*
	F = 5.7, $p < 0.03$	F = 142, $p < 0.001$	F = 11.8, $p < 0.004$	F = 98, $p < 0.0001$	F = 9.7, $p < 0.007$

* $p < 0.01$ vs group 1 (uncontrolled reperfusion).

‡ $p < 0.01$ vs group 1 and group 2.

‡ $p < 0.01$ vs all groups.

tion (compliance, PVR, a/A ratio) in any group undergoing controlled reperfusion (groups 2 through 4). In contrast, when the left (ischemic) lung underwent uncontrolled reperfusion (group 1), slight deterioration of pulmonary function in the right (nonischemic) lung compared with baseline was noted (compliance $96\% \pm 1\%$, PVR $111\% \pm 2\%$, a/A ratio $95\% \pm 2\%$), suggesting it had sustained a mild pulmonary injury from metabolic products liberated during reperfusion of the ischemic left lung.

Biochemical analysis. Results are summarized in Table II and follow the same trends as functional results. The highest rise in lung edema and myeloperoxidase activity, once again, occurred with un-

controlled reperfusion of the ischemic left lung, implying the greatest injury. These changes were completely prevented by modifying the perfusate with a WBC filter and a modified solution (group 4) and partially altered if only one perfusate modality (modified solution group 2 or WBC filter group 3) was used.

In the nonischemic right lung, tissue edema ($83.2\% \pm 0.2\%$ vs $81.5\% \pm 0.1\%$, $p < 0.001$) and myeloperoxidase activity (0.33 ± 0.02 vs 0.19 ± 0.01 , $p = 0.003$) were significantly higher after uncontrolled reperfusion (group 1) compared with pigs receiving a controlled perfusate with a WBC filter and modified solution (group 4). This further implies that the contralateral lung is injured by metab-

olites released during uncontrolled reperfusion of the ischemic left lung.

WBC and platelet counts. The WBC (pre 17.5 ± 1.2 vs post 0.4 ± 0.1 WBCs $\times 1000/\text{mm}^3$; $p < 0.001$) and neutrophil (pre 12.2 ± 1.1 vs post 0.3 ± 0.1 neutrophils $\times 1000/\text{mm}^3$; $p < 0.001$) counts were significantly decreased by the WBC filter. Platelet counts also decreased but to a lesser extent (pre 437 ± 18 vs post 281 ± 19 platelets $\times 1000/\text{mm}^3$; $p < 0.001$).

Discussion

These data indicate that after 2 hours of warm pulmonary ischemia, a severe lung injury occurs with uncontrolled reperfusion, this injury is minimized but not avoided by using either a modified solution or WBC filtration, and that using both a modified solution and a WBC filter results in complete preservation of pulmonary function. These findings are almost identical to results with controlled reperfusion after myocardial ischemia and support the importance of applying these concepts after lung ischemia.^{18, 19}

Ischemia-reperfusion injury remains a significant problem in lung transplantation.¹⁻³ Recent studies have shown that injury to the endothelial cell may be the trigger that eventually leads to total organ failure, but the cause of this injury is multifactorial.^{11, 12, 22} Although the dysfunction begins during ischemia, it is significantly enhanced and accelerated by reperfusion.^{5, 19, 23} One reason is that the inevitable restoration of oxygen during reperfusion leads to formation and activation of a variety of humoral mediators of injury and inflammation.⁵ These humoral factors cause an initial endothelial dysfunction that leads to marked reduction in nitric oxide and prostaglandin release.⁵ That along with chemotactic factors (PAF, LTB₄, CSa) and up-regulation of adhesive molecules on the endothelial cell (ICAM-1, ECAM-1, GMP140) accentuates adherence of neutrophils to the endothelium and diapedesis into the organ.^{1, 5, 24} Activated neutrophils release a host of proinflammatory mediators that promote cell injury and amplify initial endothelial dysfunction.^{5, 23, 25} Several pharmacologic agents are known that can block the endothelial injury. Many of these have been studied, including adding different neutrophil adhesives molecule blocking monoclonal antibodies to the initial flushing solution or giving some of the deficient chemicals, such as, nitric oxide, during reperfusion.^{13, 26, 27}

Because controlling the conditions of reperfusion and the composition of the reperfusate have been shown to modify the reperfusion injury in myocardial tissue, this study investigates whether application of these principles can prevent the reperfusion injury after pulmonary ischemia. We used a clinically relevant (in vivo) model because most studies of lung reperfusion have been done using isolated preparations, making extrapolation of results from these studies to clinical practice difficult.

WBC filter. Use of an efficient WBC filter (Pall BC-1) removed over 95% of leukocytes in one passage in all pigs. However, in comparison with some other filters only approximately 60% of the platelets were removed. Breda and associates^{16, 17} were the first to show that leukocyte depletion during reperfusion decreased lung injury after ischemia. By use of a variety of experimental models, these observations have been confirmed after both short-term warm ischemia and 24 hours of cold storage.^{2, 6, 24, 28} Several other studies have used various neutrophil-modulating agents to demonstrate that WBCs play a major role in reperfusion injury of the lung.^{27, 28} Steimle and associates,²⁹ on the other hand, found that profound neutropenia did not fully protect an organ from an ischemia-reperfusion injury. Therefore the reperfusion injury appears to be complex, consisting of both neutrophil-independent and neutrophil-mediated events. Our results concur with that conclusion. We found that WBC depletion did not fully prevent this injury but significantly blunted the inflammatory cascade. Several studies performed using an isolated preparation have raised the question of whether WBC depletion for a longer period of time may be necessary for full benefits. This question is not addressed by our results, and further studies using an in vivo model need to be done to determine the optimal length of leukocyte-depleted reperfusion.

From a clinical standpoint, the two major concerns of the WBC filtrations have been the possible increased rate of infection, especially in the setting of immunosuppression, and the concomitant platelet depletion. However, with our method of reperfusion, the total body WBC and platelet counts are minimally affected because only approximately 20% to 30% of the pigs' total blood volume is filtered, and platelet elimination with this filter is only about 60%. Recent studies have also demonstrated that neutrophil depletion during surgery may actually decrease the infection rate and that neutrophil levels quickly return to normal levels once the filter

is removed.³⁰ It is therefore unlikely that this poses a problem.

Modified reperfusate solution. Despite compelling evidence that the biochemical composition, as well as cellular constituents of reperfusate solutions, significantly affect the amount of ischemia-reperfusion injury in the heart, kidney, and peripheral muscle, few attempts have been made to apply this knowledge to lung transplantation.^{18, 19} Several studies have been done adding cytoprotective, oxygen radical scavengers or neutrophilic inhibitors to the recipient's blood before reperfusion.^{6, 23} Serrick and associates,¹⁵ recognizing the importance of the initial reperfusion and the detrimental effects of uncontrolled blood reperfusion, studied the effects of a rinse solution before reperfusion. They found that using an extracellular solution with additives, including antioxidants, before reperfusion significantly increased viability of endothelial cells versus uncontrolled blood reperfusion. Others found that 10 minutes of hemodilute reperfusion after ischemia significantly decreased the lung injury.⁴

The composition of our modified reperfusion solution was based on principles established in myocardial tissue, with each component addressing one of the adverse effects of ischemia/reperfusion. To counteract acidosis, the solution was made alkalotic using the intracellular buffer THAM. Because calcium is one of the primary mediators of the reperfusion injury, calcium levels were decreased by adding citrate, and the solution was enriched with magnesium to prevent calcium influx. The substrates aspartate, glutamate, and glucose were used to replenish depleted energy stores. The solution was made hyperosmolar to limit edema formation, and nitroglycerin was added to dilate vascular endothelium that might be damaged from ischemia, thus improving perfusate distribution. Finally, it was mixed with blood in a 4:1 ratio to provide oxygen and endogenous buffers and oxygen radical scavengers. Although the solution therefore resembles our cardioplegic solution, it has been modified for the lung. It is obvious from our results that the composition of the initial perfusate, even without leukopenia, plays an integral part in preventing the reperfusion injury. This is very similar to the findings in other organs, especially the myocardium where modified reperfusates are used routinely in heart operations.¹⁸ No doubt exists that the composition of the perfusate used for controlled lung reperfusion is going to change. The effects of several additives, such as, exogenous oxygen radical scavengers, vaso-

dilators, calcium channel blockers, nitric oxide precursors, and other agents, need to be investigated. Also, the optimal amount of hemodilution and length of controlled reperfusion is unknown. These and other technical refinements in the method of controlled reperfusion will remain a fertile ground for enthusiastic investigators in the near future.

Pressure. Besides changing the reperfusate composition with leukocyte depletion and a modified solution, the conditions of reperfusion were controlled by fixing the perfusate pressure between 40 and 50 mm Hg. This may have helped modify the reperfusion injury. However, because only group 4 (WBC filter and modified solution) recovered complete function, controlling perfusion pressure does not by itself explain these results. Indeed, the magnitude of its effect cannot even be gauged from this study because variations in perfusion pressure were not investigated. Furthermore, simply removing the clamps and allowing uncontrolled reperfusion (group 1) resulted in similar mean pulmonary reperfusion pressures (30 to 50 mm Hg), and yet these animals sustained a severe pulmonary injury. Reduction of reperfusion injury by controlling reperfusion pressure is well recognized in the heart and lung.^{4, 18, 19} Bhabra and associates⁴ showed that controlling the pressure during the first 10 minutes of pulmonary reperfusion significantly improved graft function, independent of storage medium or ischemic time. They also found that this beneficial effect was not achieved with a shorter period of controlled reperfusion and that a longer period had no added effect. Why controlled pressure during reperfusion has a protective effect is not fully known. Hydrostatic pressure resulting in rapid edema, shear stress, and vasomotor dysfunction have been implicated. Whatever causes the injury, it seems that during the low-pressure period, the endothelium recovers sufficiently to subsequently tolerate full pressure perfusion. We chose a pressure between 40 and 50 mm Hg because it is what has been shown to be effective in the heart, and this was the pressure that resulted with uncontrolled reperfusion. However, because the normal pulmonary pressure is usually substantially lower, perhaps this pressure is too high and actually exacerbated the injury. Whether lower pressures would be even more beneficial must be balanced by the need to deliver a minimal amount of modified perfusate to the lung and allow for adequate distribution. The optimal perfusion pressure and the amount of its

importance in reducing this injury is, therefore, another area for investigation.

Finally, we found, as did Watanabe and associates,⁹ that the contralateral (nonischemic) right lung also sustained an injury after uncontrolled reperfusion of the ischemic left lung. This was evident by 5% to 10% worsening of right lung function and the increase in tissue edema and myeloperoxidase activity. Conversely, the injury to the contralateral (non-ischemic) right lung was almost completely prevented with controlled reperfusion (groups 2 through 4). This strongly suggests that metabolic by-products liberated as a result of the reperfusion injury play a significant role in the contralateral lung injury and that controlled reperfusion of the ischemic lung prevents this from happening.

In conclusion, our study strongly suggests that the ischemia-reperfusion injury can be significantly decreased by modifying the perfusate, controlling the perfusion pressure, and filtering white blood cells during the first 10 minutes of reperfusion. Because the existence of a reperfusion injury continues to plague surgeons after lung transplantation, this study suggests that reperfusion should always be controlled after pulmonary ischemia. Additional investigations are needed to verify these results after long-term cold storage and further refine our method. Because this study was done using a clinically relevant model, this technique can be used in human transplant recipients.

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Discussion

Mr. John H. Dark (*Newcastle Upon Tyne, United Kingdom*). What was the pressure of the reperfusion in the control group?

Dr. Halldorsson. We used a pressure between 40 and 50 mm Hg, which gave us a flow of approximately 50 ml/min.

Mr. Dark. In the uncontrolled group, the group who had no separate reperfusion, where you simply removed the clamp, did you measure the pressure in that group?

Dr. Halldorsson. Yes. After the clamps were removed, it was simply the pressure in the native pulmonary artery which had a mean of 30 to 50 mm Hg. The pressure was therefore similar to the group undergoing controlled reperfusion. Since we did not control the reperfusion, however, we are unsure how much flow was directed to the ischemic lung compared to the nonischemic lung.

Mr. Dark. So in addition to the solution, you are also addressing the aspect of pressure-controlled reperfusion?

Dr. Halldorsson. Correct. I did not emphasize that point, but we believe that controlling the pressure during the initial period of reperfusion is also important in preventing the reperfusion injury. In this study, we chose a pressure of 40 to 50 mm Hg for the controlled reperfusion based on what we found worked well in ischemic myocardium. Since the normal pulmonary artery pressure is usually lower than the myocardial perfusion pressure, it is possible that a lower pressure would improve results and this is currently an area of investigation. This must, however, be balanced with the knowledge that a lower perfusion pressure may not allow adequate distribution of the reperfusion.

Mr. Dark. A second question is how long do you think you should put the WBC filter in the circuit? We have used a very similar experimental model with a WBC filter of 30 minutes.

Dr. Halldorsson. That question remains unanswered. We chose 10 minutes on the basis of our experience in myocardial tissue and several reports after pulmonary ischemia. However, we previously found that longer periods of controlled reperfusion were beneficial in the myocardium, and so you may be right that the controlled

reperfusion should be delivered over a longer period of time. This is another current area of investigation.

Mr. Dark. Did you observe WBC sequestration even after removing the filter from the circuit?

Dr. Halldorsson. We have no way of knowing as we only examined the tissue for myeloperoxidase activity at the end of the experiment.

Mr. Dark. Thank you very much. I might add that we have repeated a very similar set of experiments with an orthotopic transplant model. You can duplicate all the benefits of control-pressure reperfusion and the WBC filter by giving pentoxifylline.

Dr. Halldorsson. Thank you for your comments, but we would disagree. We do not believe any one drug can completely prevent the reperfusion injury, as it is a complex injury that must be addressed by a multifaceted approach. Our method of using controlled reperfusion to prevent reperfusion damage is based on our extensive experimental and clinical experience after myocardial ischemia. In these studies, we investigated many facets of controlled reperfusion and found each principle to be important. Omitting any one of these principles resulted in decreased myocardial recovery. This study further supports this belief, as the best results were achieved only when all aspects of pulmonary reperfusion were controlled (group 4). Although several drugs such as pentoxifylline are known to block WBC function, and could be used instead of leukocyte depletion, this would only address one aspect of this multifaceted injury. In this study, we chose to use a WBC filter, since they have been proven clinically to be very effective without the potential complications of pharmacologic agents.

Dr. G. Alexander Patterson (*St. Louis, Mo.*). I would be interested in your speculation as to what you think the mechanism of this benefit is. At the recent meeting of the International Society for Heart and Lung Transplantation (ISHLT), the Toronto group presented a very nice experiment that showed a significant structural injury in lungs that were reperfused, uncontrolled, versus those that were reperfused more gradually. Perhaps all this is structural and does not have a lot to do with white cells. If I remember your slide correctly, myeloperoxidase assay was similar with or without the filter. This suggests that the filter really did not make any difference in terms of leukocyte sequestration.

Dr. Halldorsson. That's correct.

Dr. Patterson. Why do you think this works?

Dr. Halldorsson. The ischemic reperfusion injury is extremely complex, so it is unlikely that controlling a single variable will prevent all the detrimental effects of reperfusion. On the basis of this study, as well as our studies after myocardial ischemia, we believe that it is important to control *all* aspects of reperfusion. The transplant surgeon is in the unique position to counteract the potential reperfusion damage since both the condition of reperfusion and the composition of the reperfusion are under the surgeon's direct control. We controlled the composition of the reperfusion by using a leukodepleted modified solution, and the conditions of reperfusion by regulating the pressure and flow. Since ischemia and reperfusion damage cells by numerous pathways, we try to address each adverse effect independently. The reperfu-

sate solution is therefore alkalotic to counteract the acidosis that occurs with ischemia; since excess calcium can be detrimental, the solution is made hypocalcemic with citrate, and magnesium added to prevent calcium influx; we added the substrates aspartate, glutamate, and glucose to replenish depleted energy stores; to prevent edema, the solution is hyperosmolar; and lastly, nitroglycerin is added to improve distribution of the solution. White cells are depleted, as they are detrimental via numerous mechanisms. We also controlled the reperfusion pressure as ischemic cells are more susceptible to a high pressure injury.

We believe it is careful attention to all of these aspects of reperfusion that allowed complete return of pulmonary function. Each is important and exclusion of any will significantly increase reperfusion damage.

Dr. Larry R. Kaiser (*Philadelphia, Pa.*). In your control group, in your injury group, how long did you follow those animals, and was there any reversal in the injury?

Dr. Halldorsson. In this study, all measurements were done 1 hour after reperfusion. In another study, how-

ever, we did follow animals for 4 hours and found there was no improvement with uncontrolled reperfusion. In contrast, animals undergoing a controlled reperfusion showed a slight improvement in pulmonary function after 4 hours.

Dr. Nasser K. Altorki (*New York, N.Y.*). In the modified perfusion only, you showed some blunting of the reperfusion injury. Is that a benefit of improved rheology, or is it just dilution of the granulocytes?

Dr. Halldorsson. Our reperfusate solution is mixed 4 parts blood to 1 part crystalloid, so there is minimal WBC dilution or change in viscosity. It is therefore doubtful that this explains our findings. Furthermore, even in the absence of leukodepletion, modifying the conditions (pressure) and composition (solution) of the reperfusate reduced the reperfusion injury in both pulmonary and myocardial tissue. We therefore believe each principle of reperfusion is important, and only by completely controlling the period of reperfusion can optimal pulmonary myocardial function be obtained.