CARDIOTHORACIC TRANSPLANTATION

RAPID REPERFUSION CAUSES STRESS FAILURE IN ISCHEMIC RAT LUNGS

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Objective: Rapid reperfusion may be injurious to the ischemic lung. Our aim was to confirm that slow reperfusion improves postischemic pulmonary function and to elucidate the ultrastructural changes associated with slow versus rapid reperfusion. **Methods:** We used an ex vivo perfused rat lung transplant model to study the effect of slow versus rapid reperfusion on subsequent lung function and morphologic condition. Functional assessment was performed in (1) fresh lung, slowly reperfused; (2) fresh lung, rapidly reperfused; (3) ischemic lung (4 hours at 22°C), slowly reperfused; and (4) ischemic lung, rapidly reperfused. **Results:** In group 4, the shunt fraction ($P = .001$), airway pressure ($P = .001$), and wet/dry ratio ($P = .01$) were significantly higher than in groups 1 through 3. Light and electron microscopy of slowly reperfused ischemic lungs ($n = 4$) appeared normal. Rapidly reperfused ischemic lungs ($n = 4$) demonstrated massive alveolar edema, hemorrhage, and epithelial "blebbing" by light microscopy. Electron microscopy identified the blebbing as separation of the epithelial layer from an intact basement membrane by edema fluid. The epithelial layer was disrupted in numerous locations. Complete disruption of all layers of the blood-gas barrier was occasionally present. **Conclusion:** Rapid reperfusion of the ischemic lung is an important contributing factor to reperfusion lung injury resulting in mechanical stress failure of the alveolar/capillary barrier. Gradual reintroduction of blood flow to the ischemic lung improves oxygenation. (J Thorac Cardiovasc Surg 1998;116:932-42)

Reperfusion injury continues to be a significant problem after lung transplantation; however, little attention has been paid to the role of mechanical factors during the early reperfusion period. Specifically, how

quickly or slowly should we reintroduce blood flow to the lungs after re-implantation? The current clinical practice is to initiate the perfusion flow rate rapidly.¹ After completion of the bronchial and vascular anastomoses, the vascular clamps are removed, and pulmonary arterial flow is instantaneously restored. The rate of reperfusion is potentially an important issue because rapid reperfusion may cause shear stress injury and disruption of the pulmonary endothelium. There are experimental data in other organ systems, such as the heart, in support of the hypothesis that the rate of reperfusion may be an important factor in determining the severity of ischemia/reperfusion injury.^{2,3} There is now evidence that this is also the case for the ischemic lung.⁴ Thus there are a number of reasons to suspect that gradual restoration of blood flow over several minutes (slow reperfusion), rather than instantaneous full flow (rapid reperfusion), may prevent mechanical damage to the pulmonary microvasculature and improve postischemic pulmonary function. Our aim in the present study was

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Supported by the Ontario Thoracic Society and the Medical Research Council of Canada. Dr Pierre is a recipient of the Ortho/Biotech Research Fellowship from the International Society for Heart and Lung Transplantation. Dr Pierre and Dr DeCampos are recipients of fellowships from the Canadian Cystic Fibrosis Foundation.

Received for publication March 27, 1998; revisions requested June 11, 1998; revisions received July 9, 1998; accepted for publication July 15, 1998.

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0022-5223/98 \$5.00 + 0 12/1/93297

to confirm that slow reperfusion improves postischemic pulmonary function and to examine the ultrastructural changes associated with slow versus rapid reperfusion of the ischemic lung by the use of light, scanning, and transmission electron microscopy.

Materials and methods

Experimental design. Donor lungs were harvested from 20 adult male Wistar rats (300 to 400 g; Charles River Inc, St-Constant, Quebec). The donor lung operation was performed as previously described.⁵ Briefly, rats were anesthetized with 65 mg of sodium pentobarbital (MTC Pharmaceuticals, Cambridge, Ontario) by intraperitoneal injection. A tracheostomy was performed, and the animal was ventilated with room air at a rate of 70 breaths/min and a tidal volume of 3 mL. A midline laparotomy was performed, and the caudal vena cava was exposed. One thousand units of heparin was injected into the inferior vena cava and was followed by a median sternotomy. The thymus was removed to expose the pulmonary artery. Next the pulmonary artery was cannulated via the right ventricle with a 14-gauge angiocatheter (Becton Dickinson Vascular Access, Sandy, Utah) that was then secured in place with a silk tie around the transverse sinus. Positive end-expiratory pressure (PEEP), 3 cm H₂O, was added to the ventilator at this point. The heart-lung block was extracted, and the right hilum was ligated with a silk tie. The left lung was then inflated to 10-mm Hg pressure with room air. Only the left lung was used to reduce the amount of blood necessary to prime the ex vivo circuit.

Lungs were then randomly allocated into 4 groups (n = 5 per group), according to the ischemic interval (fresh or ischemic) and to the rate of lung reperfusion (slow or rapid):

- Group 1: Fresh lung, slowly reperused
- Group 2: Fresh lung, rapidly reperused
- Group 3: Ischemic lung, slowly reperused
- Group 4: Ischemic lung, rapidly reperused

Fresh lungs were reperused immediately in the isolated rat lung system after harvest and had an average ischemic time of only 20 minutes. Ischemic lungs were stored for 4 hours at 22°C before being reperused. This ischemic interval was chosen on the basis of previous studies of lung viability after ischemia that indicated that there should be a measurable worsening of lung function after 4 hours of 22°C ischemia.⁵ Storage at lower temperatures necessitates longer storage times to produce similar degrees of lung injury, and the model becomes unstable.

Reperfusion circuit. The lung reperfusion system used in this study has been described in detail elsewhere.⁶ Briefly, the perfusion circuit was primed for each experiment with 25 mL of fresh venous blood obtained from 2 heparinized (1000 IU/rat) and anesthetized rats. A double-head roller pump (Cole-Parmer Instrument Co, Chicago, Ill) was used. During reperfusion, the effluent of the study lung (a single left lung) was continuously deoxygenated by a second pair of lungs

(the “deoxygenator”), which were ventilated with a hypoxic gas mixture (Harvard ventilator model 683; Harvard Apparatus Co, Inc, South Natick, Mass) 4% oxygen, 8% carbon dioxide, tidal volume of 3.0 mL, at a rate of 70 beats/min, with 3-cm H₂O PEEP) to produce mixed venous blood for delivery back to the study lung. The study lung was ventilated with room air with a tidal volume of 1.5 mL, a rate of 40 beats/min, and 1-cm H₂O PEEP. The perfusion flow rate at any given time was identical for both the study lung and the deoxygenator. Sodium bicarbonate was added whenever necessary to maintain the blood pH between 7.3 and 7.5. The entire circuit was housed in a warmed (37°C) and humidified chamber. Forty rats in total were used as blood donors (2 rats per perfusion), 20 of which were also used as donors for the deoxygenator.

In groups 1 and 3, a slow reperfusion was carried out with a technique similar to that described by Deeb and associates.⁷ The flow was initiated at 0.4 mL/min and increased by 0.4 mL/min every minute until a flow rate of 4 mL/min was achieved; thereafter, the flow was kept constant. In groups 2 and 4 (rapid reperfusion), the flow was initiated at 4 mL/min and maintained constant throughout reperfusion. This perfusion flow rate corresponds to approximately 33% of the flow to the left lung of a 350-g rat under normal physiologic conditions.⁸

Physiologic assessment. The airway pressure and mean pulmonary artery pressure were continuously monitored (Uniflow; Baxter Healthcare Corp, Deerfield, Ill; 8A Multi-Channel Recorder; Hewlett-Packard Company, Andover, Mass). Blood gases were measured (Model 278 Blood Gas System; Ciba Corning Diagnostics Corp, Mass) with samples withdrawn from the venous reservoir below the deoxygenator lung and from the left atrial effluent of the study lung after 10, 30, 60, 90, and 120 minutes of reperfusion. Perfusion was discontinued if gross pulmonary edema was present as detected by fluid in the tracheal cannula of either lung. The intrapulmonary shunt fraction (Qs/Qt) was calculated by standard equations⁹:

$$Qs/Qt = (Cc - Ca)/(Cc - Cv) \times 100\%,$$

where Cc, Ca, and Cv are the oxygen contents of the pulmonary capillary blood, the arterial blood (study lung effluent), and the venous blood (deoxygenator lung effluent), respectively. The end-capillary PO₂ was calculated based on the FIO₂ and PaCO₂ and assuming a respiratory quotient R = 0.8. At the end of the 2-hour reperfusion or when massive pulmonary edema developed, the study lung was weighed and dried for 48 hours at 70°C; wet/dry weight ratios were calculated.

Ultrastructural assessment. To examine the ultrastructural correlates of rapid reperfusion lung injury in ischemic lungs, another 8 reperfusion were carried out (4 slowly reperused ischemic lungs and 4 rapidly reperused ischemic lungs). These lungs underwent 4 hours of ischemia at 22°C and were reperused in the ex vivo rat lung circuit described earlier. To specifically examine events related to the critical early period,

Fig 1. A, Intrapulmonary shunt fraction (shunt %) and (B) arterial PCO_2 during reperfusion in fresh lungs and in lungs subjected to 4 hours of 22°C ischemia (s fresh + slow; , fresh + rapid; j ischemia + slow; m ischemia + rapid). In fresh lungs and in slowly reperfused ischemic lungs, the shunt fraction remained within the normal range throughout reperfusion. In rapidly reperfused ischemic lungs, the shunt fraction was significantly higher than in the other 3 groups ($P = .001$), and the reperfusion was interrupted after 10 minutes because of massive pulmonary edema. Values are mean $\pm$ SD.

the blood perfusion was discontinued after 10 minutes. In addition, 4 normal/nonreperfused rat lungs were examined by electron microscopy. After reperfusion all lungs were inflated to 10-mm Hg pressure and maintained at that level during the fixation process. Saline-dextran (11.06 g/L NaCl, 350 mOsm; 3% T-70 dextran; and 1000 U heparin/100 mL) was infused via the pulmonary artery from a height of 15 cm until the outflow appeared clear of blood cells (approximately 25 mL). Then fixative (phosphate-buffered 2.5% glutaraldehyde

with 3% T-70 dextran; total osmolality 500 mOsm; pH adjusted to 7.4) was perfused for 10 minutes from a height of 15 cm. We chose this fixation process based on the work of West,¹⁰ Bachofen,¹¹ and their associates, who found this technique to be optimal for studying the alveolar barrier. After fixation random samples were taken from the mid-zone of the left lung and cut into smaller blocks (1 mm^3). The samples were postfixed in 1% osmium tetroxide. Dehydration was carried out in graded alcohols, followed by propylene oxide; the samples were then embedded in epon. One-micrometer-thick sections were stained with toluidine blue, and ultrathin sections were stained with uranyl acetate and lead citrate. Transmission electron microscopic examination was performed using a Philips 201 (N.V. Philips, Gloeilampenfabrieken, Eindhoven, Germany) transmission electron microscope. Tissue for scanning electron microscopic examination was postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, critical point dried, mounted on stubs, coated with a thin layer of gold, and examined under a JMS 820 (JOEL, Peabody, Mass) scanning electron microscope. The samples for light microscopy were fixed in 10% buffered formalin and embedded in paraffin; sections were stained with hematoxylin and eosin.

All animals received humane care in compliance with the Principles of Laboratory Animal Care formulated by the National Society for Medical Research, 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 No. 86-23 revised, 1985).

Statistical analysis. Two independent analyses of variance were carried out (1) to assess the effects of ischemia and reperfusion rate on pulmonary hemodynamics and gas exchange at a given reperfusion time (10, 30, 60, 90, and 120 minutes) and (2) to assess the effects of reperfusion time on hemodynamics and gas exchange in a given preservation group. Wherever the overall F test was significant, the Newman-Keuls multiple comparisons procedure was used to compare the different groups. An arbitrary significance level of $\alpha = 0.05$ was adopted. All data analysis was performed with the SAS statistical software package (SAS Inc, Cary, NC). The results are presented as mean $\pm$ standard deviation (SD).

Results

Using this ex vivo system, we were able to reperfuse isolated rat lungs and collect reliable gas exchange and hemodynamic data. Our success rate with this system was 100%. No reperfusion data were excluded.

Functional assessment. The mean values of venous pH, PO_2 , and PCO_2 from the deoxygenator lung for the 4 groups were stable throughout the reperfusion period. There were no statistically significant differences among groups in venous blood gas tensions or pH before or during reperfusion. In all 20 reperfusions, the addition of HCO_3^- to the blood was necessary to maintain the

pH within the physiologic range. The requirement of HCO_3^- during reperfusion was similar in the 4 groups (0.44 ± 0.12 mEq/perfusion; mean $\pm$ SD).

In groups 1, 2, and 3 all lung perfusions were carried out to completion (2 hours). In contrast, all lungs in group 4 (ischemia + rapid reperfusion) developed copious pulmonary edema during the first 10 minutes of reperfusion. The procedure was discontinued at this point in group 4. Fig 1 shows the study lung shunt fraction and PCO_2 during reperfusion. Values of PCO_2 in fresh lungs (groups 1 and 2) were in the range of 27 to 29 mm Hg, indicating that the lungs were slightly hyperventilated relative to the perfusion flow. There were no significant differences in PCO_2 between groups 1 to 4 at any time point during reperfusion. In fresh lungs (groups 1 and 2), the shunt fraction was minimal and remained stable throughout the perfusion period, indicating good oxygenation. The shunt fraction in group 3 (ischemia + slow reperfusion) was slightly greater than in fresh lungs, but the difference did not reach statistical significance. In contrast, lungs in group 4 (ischemia + rapid reperfusion) demonstrated a significantly higher shunt fraction after 10 minutes of perfusion, compared with those found in groups 1 to 3 ($P = .001$), indicating poor oxygenation.

Slow reperfusion resulted in a gradual stepwise increase in pulmonary artery pressure over the first 10 minutes (Fig 2, A) in groups 1 and 3. Nevertheless, there were no significant differences in pulmonary artery pressure at 10 minutes or over the 2-hour reperfusion period in groups 1 through 3. In contrast, in group 4 (ischemia + rapid reperfusion) the highest pulmonary artery pressures were observed within the first 10 minutes of reperfusion; however, this did not reach statistical significance.

In fresh lungs (groups 1 and 2), there was no significant difference in the mean airway pressure during reperfusion. The mean airway pressures in rapidly reperfused ischemic lungs (group 4) were significantly higher ($P = .001$) than in slowly reperfused ischemic lungs (group 3) or fresh lungs (groups 1 and 2; Fig 2, B)

In groups 1 through 3, the wet/dry weight ratios at the end of reperfusion were within the normal range and not statistically different (group 1, 6.3 ± 0.9 ; group 2, 6.0 ± 0.5 ; group 3, 6.9 ± 1.1). By contrast, a 2-fold increase in wet/dry weight was observed at the end of reperfusion in group 4 (12.0 ± 1.6 ; $P = .01$).

Light and ultrastructural assessment. Of the lungs used for morphologic assessment, the shunt fraction for slowly reperfused lungs at 10 minutes was significantly better than that of rapidly reperfused lungs at 10 minutes (slow = $8.8\% \pm 3.5\%$ versus rapid = $53.5\% \pm 8.2\%$; $P = .001$).

Fig 2. A, Mean pulmonary artery pressure (P_{pa}), and (B) mean airway pressure (P_{aw}) during reperfusion in fresh lungs and in lungs subjected to 4 hours of 22°C ischemia (s fresh + slow; r fresh + rapid; j ischemia + slow; m ischemia + rapid). In rapidly reperfused ischemic lungs the mean pulmonary artery pressure during the first 10 minutes of reperfusion was higher than in the other 3 groups but did not reach statistical significance. In rapidly reperfused ischemic lungs, the mean airway pressure during the first 10 minutes of reperfusion was higher than in the other 3 groups ($P = .001$). In group 4, the reperfusion was interrupted after 10 minutes because of massive pulmonary edema. Values are mean $\pm$ SD.

Light microscopy. Slowly reperfused ischemic lungs (Fig 3, A) demonstrated normal pulmonary architecture compared with normal/nonreperfused controls viewed at $\times 40$, $\times 100$, and $\times 400$ magnification. Rapidly reperfused ischemic lungs showed massive pulmonary edema with alveoli filled with proteinaceous edema fluid (Fig 3, B). In addition, there was extensive alveolar hemorrhage. Viewed at $\times 100$ or $\times 400$ magnification, circular blebs could be seen extending into the alveolar spaces, and they appeared to be filled with protein and edema fluid of a slightly different density to that in the

Fig 3. Light microscopy appearance of ischemic rat lung parenchyma after 10 minutes of reperfusion: slow and rapid reperfusion. **A**, There is overall preservation of the normal alveolar structure. The alveolar spaces (**A**) are free of edema fluid. Few residual erythrocytes (**RBC**) are present in the capillaries (**C**) of the interstitium (**I**). **B**, Note the difference in appearance of this sample in comparison with Fig 3, **A**. Note the edema fluid (*) filling up the alveolar spaces (**A**). Numerous circular blebs (**B**) are seen protruding from the alveolar wall into the alveolar spaces. Capillaries (**C**) in the interstitium (**I**) appear to be disrupted, and there is focal alveolar hemorrhage and spill-over (arrowheads) of erythrocytes into the alveolar spaces. (Hematoxylin and eosin stain; original magnification, $\times 325$.)

air spaces. These blebs were observed in almost every alveolus as shown in Fig 3, **B**.

Scanning electron microscopy. Slowly reperfused ischemic lungs had normal alveolar and capillary surfaces as seen on scanning electron microscopy (SEM; Fig 4, **A**) compared with normal lungs. Preparation artifact appears as a cleft on the alveolar surface in Fig 4, **A**. These clefts are also seen in normal/nonperfused lung. In contrast, the epithelial and endothelial surfaces in rapidly reperfused ischemic lungs were covered with proteinaceous edema fluid that made detailed assessment of the underlying air spaces and capillaries impossible (Fig 4, **B**).

Transmission electron microscopy. Slowly reperfused ischemic lungs (Fig 5, **B**) appeared normal compared with normal/nonreperfused lungs (Fig 5, **A**). All layers of the alveolar barrier seemed to be healthy, and endothelial

cell junctions were all intact. Rapidly reperfused ischemic lungs demonstrated numerous changes. Most striking were epithelial blebs of various sizes that could be seen protruding into the alveolar spaces (Fig 6). Higher magnification revealed these blebs to be a separation of the type I cell epithelial layer from an intact basement membrane (Fig 7). The blebs were filled with protein and edema fluid of a slightly different density to that in the air spaces. Occasionally, several blebs occurred in the same alveolus. Ruptures of the epithelial blebs could be identified in many locations resulting in the leakage of protein and edema fluid into the air spaces. The blebs showed considerable variability in size. Some of the blebs occupied almost the entire alveolar space, demonstrating the remarkable plasticity of the type I cell.

Massive alveolar edema filled air spaces completely in the rapidly reperfused ischemic lungs. Transmission

Fig 4. SEM of ischemic rat lung after slow and rapid reperfusion for 10 minutes. **A**, Alveolar spaces (A) and capillaries (C) within the interstitium (I) are within normal limits. The cleft in the alveolar surface is likely preparation artifact and is also seen in normal lung. **B**, Note the difference in appearance in comparison with Fig 4, A. This specimen shows the massive alveolar edema (*) represented as a floccular material on the surfaces of alveolar spaces. (Original magnification, $\times 2400$.)

electron microscopy (TEM) also demonstrated extensive interstitial edema and hemorrhage. Dissection of erythrocytes into the interstitial and alveolar spaces was clearly evident (Fig 8, A). Disruptions in the endothelial layer or basement membrane were not found as frequently, but complete disruption of all layers of the blood-gas barrier with erythrocytes traversing into air spaces was occasionally present (Fig 8, B). The endothelial layer appeared to be relatively free of injury, and no blebs of the endothelial layer were identified. These TEM findings in rapidly reperfused ischemic lungs are consistent with mechanical stress failure of the alveolar/capillary barrier as described by West and coworkers.¹⁰

Discussion

West and coworkers¹⁰ have described the 3 principal forces acting on the pulmonary capillary wall when it

experiences mechanical stress failure: (1) circumferential wall tension caused by the transmural pressure, (2) surface tension of the alveolar lining layer, and (3) tension of the tissue elements in the alveolar wall. During lung preservation a number of factors, such as ischemia or temperature, may reduce the threshold in the lung at which these forces adversely effect the alveolar/capillary barrier and result in mechanical failure with pulmonary edema at the time of reperfusion. We investigated the effect of slow versus rapid reperfusion on subsequent lung function and morphologic evidence to see whether slow reperfusion would ameliorate mechanical failure.

In the context of lung transplant-related ischemia/reperfusion injury, most studies suggest that the additional injury occurring during the reperfusion period occurs via oxidant mechanisms or via release of inflammatory mediators and results in endothelial cell dys-

Fig 5. A, TEM of normal lung. A type II pneumocyte (P) and erythrocytes (RBC) in an intact capillary (C) in the interstitium (I); intact alveolar spaces (A) are shown. (Original magnification, $\times 5960$.) B, Transmission electron micrograph of a portion of rat lung after slow reperfusion for 10 minutes. Note the overall normal appearance of this sample. A capillary (C) in the interstitium (I) is shown. Note that the capillary wall is intact and that tight junctions (arrowhead) are intact. The alveolar space (A) is normal and shows no edema. Original magnification, $\times 14,400$.)

function.¹² Our results suggest that the rapid acceleration of blood flow to the ischemic lung also plays an important role in reperfusion lung injury, resulting in mechanical stress failure of pulmonary capillaries and pulmonary edema, with most injury being seen in type I epithelial cells. Slow reperfusion of the ischemic lung protects against alveolar barrier disruption and epithelial bleb formation and results in good oxygenation. The fresh lung is also tolerant to rapid reperfusion and does not develop pulmonary edema as seen in ischemic lungs.

The problems of keeping the lung "dry" are appreciable. Even in the normal situation with a relatively low transcapillary pressure, the balance of forces is precarious. This is because the pulmonary microvasculature is loosely suspended in air over a large area with only a delicate tissue barrier for mechanical support.¹³ It is the capillary endothelium, the alveolar epithelium, pulmonary lymphatic drainage, and Starling forces across the endothelium that largely determine the fluid movements between lung compartments.^{14,15} The transplanted lung, unfortunately, has impaired lymphatic drainage, which is extremely important in regu-

lating fluid movement in the normal lung,¹⁵ and comes to depend on the integrity of the alveolar/capillary barrier and favorable Starling forces to prevent alveolar edema. It is the integrity of this barrier on which we focused our attention in this study.

Endothelial cells are sealed together by tight junctions; however, these "tight junctions" are somewhat leaky, allowing the transfer of smaller macromolecules, such as albumin.¹⁶⁻¹⁸ The endothelium therefore does not form a tight osmotically active barrier. However, type I cell intercellular junctions, which appear to be very tight, do prevent a free exchange of solutes between the interstitial and alveolar space.^{16,17,19} The epithelium is essentially impermeable to macromolecules such as albumin, and its permeability for electrolytes and water is much lower than that of the endothelium.¹⁷ Transudation across the alveolar epithelium is virtually zero unless the integrity of the barrier is compromised or the interstitial pressure increases above a critical level.¹⁶

In the present study the most conspicuous finding by TEM in rapidly reperfused ischemic lungs was bleb-

Fig 6. TEM of a portion of rat lung after rapid reperfusion for 10 minutes. Note the dramatic change with the presence of edema fluid (*) completely filling the alveolar spaces (A). Numerous cytoplasmic blebs (B) of different sizes protrude into the alveolar space. Interstitium (I). (Original magnification, $\times 3525$.)

Fig 7. Higher magnification TEM of rat lung after rapid reperfusion for 10 minutes with a close-up of an alveolar bleb (B). The bleb contains edema fluid that appears more concentrated in the alveolar space (A). Note the altered basement membrane (BM) at the base of the bleb and the plasma membrane of the type I epithelial cell (arrowheads). (Original magnification, $\times 25,600$.)

bing and disruption of the epithelial cell layer (ie, that part of the alveolar barrier whose integrity is central to keeping the alveoli dry). Overt endothelial defects were less frequently seen. Even in the immediate vicinity of

huge epithelial blebs, separated by the basement membrane only, endothelial linings were usually continuous with morphologically intact cell junctions.

The discrepancy in the extent of damage between

Fig 8. TEM of a portion of rat lung after rapid reperfusion for 10 minutes. **A**, Note changes in the interstitium (*I*) that is now filled with edema fluid. An erythrocyte (*RBC*) is present and free in the interstitium. The alveolar space (*A*) contains edema fluid. (Original magnification, $\times 3350$.) **B**, Several erythrocytes (*RBC*) are shown traversing from the capillary (*C*) into the alveolar space (*A*) through the disruption (arrowheads) in the alveolar/capillary barrier. (Original magnification, $\times 11,200$.)

epithelium and endothelium may be explained by structural differences in the cytoplasmic extensions and the cell junctions on the 1 hand¹⁹ and by the different repair potentials of the 2 cell layers on the other. Epithelial cell junctions are extremely tight, and stresses from rapid reperfusion may result in sudden over-distention and disruption of the delicate cell extensions rather than in the opening of intercellular junctions. By contrast, the tight junctions between endothelial cells are to some extent leaky,¹⁹ leaving a pathway for the passage of protein and blood cells without the destruction of endothelial cells. Edema fluid and blood cells thus dissect into the interstitial space resulting in interstitial edema, increasing interstitial pressure, and hemorrhage. As the edema increases, the epithelium is lifted from its basement membrane, forming the increasingly larger blebs. Blebs subsequently rupture, resulting in flooding of alveolar spaces and impaired gas exchange.

The high repair capacity of endothelial cells has been demonstrated in numerous animal experiments.²⁰⁻²² There is evidence to suggest that, in the setting of high pressure, pulmonary edema endothelial cells can move along their underlying matrix by rapid disengagement and reattachment of cell adhesion molecules, causing breaks to open or close within minutes when the pressure is decreased.²³ On the other hand, the repair of the epithelial layer, hence the re-establishment of a tight permeability barrier, requires more time.²⁴ Less extensive injuries to the epithelium may, however, be repaired in a similar fashion to the endothelium described earlier.²³

The increased variability in shunt fraction and pulmonary artery pressure seen in group 3 (ischemic lung, slowly reperfused) versus groups 1 and 2 (fresh lungs slowly and rapidly reperfused) is likely a result of ischemic preservation. Because ischemia is inevitable in clinical transplantation, we only studied ischemic

lungs for the ultrastructural assessment, knowing already from the physiologic assessment that fresh lungs functioned well after either slow or rapid reperfusion.

In lungs subjected to ischemia, our data suggest that the gradual reintroduction of blood flow over 10 minutes can make the difference between a lung that is completely functional and a lung that develops gross pulmonary edema within minutes of reperfusion. The mechanism by which slow reperfusion protects ischemic lungs is not clear. It may be that ischemia induces phenotypic changes in endothelial and type I epithelial cells that, during the early phase of reperfusion, result in increased permeability and pulmonary edema. These phenotypic changes may be alterations in the function of cell-to-matrix adhesion molecules and/or the integrity of the cell membranes themselves. Slow reperfusion over 10 minutes, with a gradual stepwise increase in pulmonary artery pressure, may have provided a brief but sufficient period of time for the resumption of a normal phenotype by endothelial and epithelial cells. During this time intercellular junctions likely regain their normal properties, and cell adhesion to basement membrane returns to a level sufficient to prevent epithelial bleb formation and subsequent rupture. The importance of the cell-cell and cell-matrix interactions in the acute respiratory distress syndrome has been described²⁵; however, the precise components in the basement membranes that contribute to cell activation or stabilization have not been clearly identified but include structural materials such as type IV collagen and laminin.²⁶

It has long been recognized that ischemia induces synthesis of numerous mediators of pulmonary vasoconstriction.^{27,28} Ischemia may also increase the critical opening pressure of pulmonary capillaries.^{10,29} It is likely that during the first few minutes of reperfusion, these vasoactive agents are present in high concentrations in the microvasculature of ischemic lungs. The slow reperfusion may have permitted the gradual washout of these mediators before the reperfusion flow reached its peak and the pulmonary artery pressure reached dangerous levels.^{10,29,30} This might have allowed for the progressive dilatation and recruitment of pulmonary vessels, increase in vascular surface area, and better distribution of blood flow.

In summary, we have shown that the rate of reperfusion after pulmonary ischemia is an important factor contributing to the severity of ischemia/reperfusion lung injury. Using an isolated rat lung reperfusion model, we were able to demonstrate that, in fresh lungs, hemodynamics and gas exchange were normal throughout a 2-hour assessment period after either slow or rapid reperfusion. However, in lungs subjected to 4 hours of 22°C

ischemia a simple modification where flow was restored gradually over 10 minutes yielded excellent gas exchange. Instantaneous or rapid reperfusion, as is current clinical practice, led to early pulmonary dysfunction in ischemic lungs. Ultrastructural assessment indicates that this injury is likely the result of rapid acceleration of pulmonary blood flow leading to mechanical stress failure in the alveolar/capillary barrier during the first few minutes of reperfusion.

A modification of clinical practice to the use of slow reperfusion may improve the function of transplanted lungs. The Toronto Lung Transplant Program has begun performing slow reperfusion as a result of this work, though no objective clinical data have yet been analyzed. Further large animal studies with more prolonged periods of observation may further elucidate the benefits of slow reperfusion.

We thank Ioan Mates, Julia Hwang, and Cameron Ackerly for technical assistance.

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