Identification of Optimal Conditions for Lung Graft Storage With Euro-Collins Solution by Use of a Rat Orthotopic Lung Transplant Model

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Background—Lung preservation disrupts normal vascular homeostasis, resulting in increased permeability, vasoconstriction, and endothelial cell adhesion for neutrophils. We hypothesized that a storage strategy that best preserves post–lung transplantation (LTX) vascular homeostasis might be organ and species specific. Because of the potential utility of a rat LTX model for developing improved lung preservation strategies, we have attempted to identify the optimal physical conditions for rat lung graft storage.

Methods and Results—Conditions that were tested included harvest inflation pressure (0, 10, or 20 mm Hg), inflation gas composition (100% N₂, room air, or 100% O₂), and storage temperature (4°, 10°, or 15°C). Modified Euro-Collins solution served as the base preservation solution for all experiments, with a preservation duration of 4 to 6 hours. Arterial oxygenation (PaO₂, mm Hg), pulmonary vascular resistance (mm Hg/mL per minute), recipient survival (%), and graft neutrophil infiltration (ΔAbs₄₆₀ nm/min) were measured 30 minutes after transplantation of the left lung and exclusion of the right lung from the circulation. All tested conditions significantly affect post-LTX vascular homeostasis. Inflation at 10 mm Hg pressure preserved lungs significantly better than did other pressures. There was a tendency for room air to improve all measured variables compared with 100% N₂ or 100% O₂ and a significant improvement in recipient survival with room air storage. Of the 3 storage temperatures investigated, 10°C storage provided the best preservation in terms of PaO₂, graft neutrophil infiltration, and survival.

Conclusions—We conclude that storage at 10°C, 10 mm Hg inflation pressure, with room air establishes optimal lung storage conditions with Euro-Collins solution in this rat LTX model. These data suggest that these conditions should be used to evaluate new and potentially improved preservation strategies. (Circulation. 1999;100[suppl II]:II-257–II-261.)

Key Words: grafting ■ lung ■ transplantation

Compared with outcomes after transplantation of other solid organs, the clinical experience with lung transplantation (LTX) has been dismal, with only ≈70% of patients receiving LTX surviving for 1 year.¹ One of the prime impediments to LTX is the extreme vulnerability of the lungs to ischemic injury during the ex vivo storage period. The limited ability that currently exists to preserve the lungs is manifest by an incidence of primary graft failure as high as ≈15%,¹ To improve current lung preservation strategies, it is necessary to have a reproducible animal model in which it is possible to rigorously test the effects of various perturbations of preservation conditions. The “ideal” physical conditions under which to store a lung graft ex vivo has been the subject of study in a number of laboratories. However, ideal conditions may vary between species and models. Important potential physical variables that may affect the success of LTX after lung graft storage include intratracheal inflation pressure, inflation gas composition, storage temperature, and others. Although each of these has been investigated in different models, there are no unified opinions regarding ideal conditions, particularly in a rat LTX model that has been used in numerous recent investigations.²⁻⁴ Even in the clinic, there is considerable variability in the storage conditions that are used for lung grafts. For example, one third of transplantation centers use 0° to 5°C for storage temperature, approximately one third use 5°C to 10°C, and 1 center reportedly uses 10° to 15°C. Generally, the donor lungs are ventilated with 100% oxygen before removal and are inflated to approximately two thirds of the total lung capacity before transport,³ although there is by no means unanimity in clinical practice. Significant variations are also reported in preservation solution composition, volume of flush solution infused, use of prostaglandins or steroids, and composition of the base preservation solution.⁶ Given the wide range of variability in clinical lung preservation strategies, one can imagine that the variations between preservation conditions in animal models are even more diverse, which make head-to-head comparisons between different preservation strategies nearly impossible.
The ability of a preservation solution to successfully improve lung preservation is based on the ability of the solution to maintain cellular integrity and viability of the parenchymal tissue as well as its ability to maintain normal vascular homeostatic mechanisms, such as inhibiting leukocyte adhesion, maintaining a relatively selective barrier to the transit of solutes, and displaying an anticoagulant phenotype to the vascular lumen. When these homeostatic mechanisms are disrupted, lung preservation is dismal. The end result of failed preservation is a vasoconstricted and edematous lung graft filled with clot and choked with neutrophils, which becomes a veritable factory for the synthesis of proinflammatory cytokines.

Given this vulnerability, how may physical conditions of lung graft storage be altered to optimize the result after transplantation? There are unique anatomic considerations in the lung that may permit modulating preservation strategy in ways that are not possible with other solid organs. For instance, the dual supply of oxygen (through the bronchial circulation and through inspired air through the trachea) and extremely high diffusing capacity of the lungs make it possible to insufflate the organ with high oxygen concentrations as a potential strategy to improve preservation. Second, because of the unique interface between the alveoli and the circulation, it is possible that high pressure insufflation may inhibit the egress of fluids and solutes into the hollows of the alveolar structures. Finally, although not unique to the lungs, the ex vivo period of storage facilitates manipulation of lung graft storage temperature, which may be important in terms of limiting the formation of microcrystalline ice within cells or which may inhibit the transcription and translation of deleterious proteins (such as adhesion receptors) during or shortly after the period of hypothermic storage. Because of the availability of inbred strains of rats, the high throughput, consistent reproducibility, and relatively limited expense of a rat LTX model, the current studies were undertaken to define optimal physical conditions for lung graft storage in this model. Defining these parameters should facilitate comparison between alternative preservation strategies that are currently in development or that become available in the future.

Methods

Graft Harvest and Storage

Inbred male Lewis rats (250 to 300 g) were used for all experiments according to a protocol approved by the Institutional Animal Care and Use Committee at Columbia University, in accordance with guidelines of the American Association for the Accreditation of Laboratory Animal Care. Donor rats were given 500 U of heparin intravenously, and the pulmonary artery (PA) was flushed with a 30 mL volume of preservation solution consisting of modified Euro-Collins preservation solution (Baxter Healthcare; modified by adding 10 mL of 10% magnesium sulfate and 50 mL 50% glucose solution per 930 mL). The preservation solution was infused with a stopcock and manometer device to ensure a constant infusion pressure of 20 mm Hg (1 mm Hg=133 Pa). Variables for these experiments included inflation gas composition (100% O<sub>2</sub>, room air, or 100% N<sub>2</sub>), intratracheal inflation pressure (0 mm Hg, 10 mm Hg, or 20 mm Hg), and graft storage temperature (4°, 10°, or 15°C). After lung harvest, a cuff was placed on each vascular and bronchial stump and the lung was submerged in preservation solution. For each tested variable, other variables were held constant; for the temperature groups, lungs were inflated with room air at 10 mm Hg pressure for 6 hours. Temperature was maintained as indicated in a circulating refrigerated/heated water bath with a thermostatic control. For the inflation gas composition experiments, lung storage temperature was 4°C; the inflation pressure was 10 mm Hg. For the inflation pressure experiments, preservation temperature was maintained at 4°C and the inflation gas was room air. For each of these latter 2 experiments, preservation duration was 4 hours. These storage durations were kept constant for each tested condition and were chosen for each on the basis of preliminary data that indicated the maximum ability to discriminate differences in preservation efficacy with the least number of experiments (for instance, extremely short preservation durations yielded consistently high survivals and would not permit us to discriminate differences without using an inordinate number of animals; similarly, preservation durations that were more prolonged resulted in high graft failure rates that also reduced the ability to discern differences between groups).

Graft Implantation

Sex/strain/size-matched rats were anesthetized, intubated, and ventilated with 100% O<sub>2</sub> with the use of a rodent ventilator (Harvard Apparatus). Orthotopic left LTX was performed through a left thoracotomy with the use of a rapid cuff technique for all 3 anastomoses, with warm ischemic times maintained <5 minutes. The hilar cross-clamp was released, reestablishing blood flow and ventilation to the transplanted lung. The right lung was then functionally removed from the recipient 15 minutes after reperfusion of the left lung by ligating the native right pulmonary artery (PA). Millar catheters (2F; Millar Instruments) were introduced into the main PA and left atrium, and a Doppler flow probe (Transonics) was placed around the main PA. This model was adopted based on published procedures.

Measurement of Lung Graft Function

Online hemodynamic monitoring was accomplished with the use of LabView and a Macintosh Ici computer. Measured hemodynamic parameters included PA pressure (mm Hg) and PA flow (mL/min). Arterial oxygen tension (PaO<sub>2</sub>, mm Hg) was measured during inspiration of 100% O<sub>2</sub> with a model ABL-2 gas analyzer (Radiometer A/S). Pulmonary vascular resistance (PVR) was calculated as (mean PA pressure−left atrium pressure)/mean PA flow (expressed as mm Hg/mL per minute). After baseline measurements, the native right PA was ligated and serial hemodynamic measurements were taken every 5 minutes until 30 minutes or recipient death. In addition to hemodynamic measurements, arterial blood for gas analysis was sampled at baseline and at the final time point.

Myeloperoxidase Assay

Thirty minutes after ligation of the native right PA, or at the time of recipient death, transplanted lungs were removed, rinsed briskly in physiological saline, and snap-frozen in liquid nitrogen until the time of myeloperoxidase assay, performed as described previously. Change in absorbance at 460 nm was measured over 1 minute (myeloperoxidase activity was expressed as ΔAbs<sub>460 nm</sub>/min).

Data Analysis

ANOVA was used to compare different conditions, with significant differences between groups detected with the use of Bonferroni post hoc comparison. Animal survival data were analyzed by contingency analysis with the use of the χ<sup>2</sup> statistic. Values are expressed as mean±SEM, with differences considered statistically significant at a level of P<0.05.

Results

Inflation Pressure

The first set of experiments was designed to determine the optimal inflation pressure in the rat LTX model. Three groups were studied: a hypoinflation group (0 mm Hg), a hyperinflation group (20 mm Hg), and an intermediate inflation.
group (10 mm Hg). Of these 3 groups, the group of lungs preserved at an intermediate inflation pressure fared the best in terms of arterial oxygenation (Table; n for each condition is shown in the denominator of Figures 1 to 3). Because posttransplantation PVR is an important marker of the success of lung preservation (grafts with lower PVRs fare better), the PVR was measured in the 3 groups after implantation of the lung isograft. Grafts preserved with hypoinflation or hyperinflation demonstrated significantly higher PVR than did those preserved at an intermediate level of inflation (Table). Another hallmark of early lung graft death is the accumulation of leukocytes in the newly reperfused graft, which causes inflammatory upregulation, microvascular obstruction, and tissue injury. In concordance with the other measures, the group of lungs preserved at an intermediate inflation pressure exhibited the lowest neutrophil accumulation of all groups as measured with a chromogenic assay to quantify myeloperoxidase activity (Table). Because all of these functional parameters are “summed” in this stringent in vivo model so that the recipient either survives or fails to survive the early posttransplantation period, survival was examined for the prespecified 30-minute duration of posttransplantation observation. In concordance with the functional results, recipients of those lungs preserved at an intermediate inflation pressure exhibited the highest survival of all groups (Figure 1).

Inflation Gas Composition
In the next set of experiments, the effects of inflation gas composition on lung preservation efficacy were tested. For these experiments, lungs were ventilated with either 100% N$_2$, room air, or 100% O$_2$ before clamping the mainstem bronchus. Subsequent to transplantation, arterial oxygenation, PVR, myeloperoxidase activity, and graft survival were measured in the same manner as was performed in the inflation pressure groups. Arterial oxygenation, PVR, and leukocyte accumulation in the room air group tended to be superior to those seen in the 100% nitrogen and 100% oxygen groups (Table). When survival of recipients was examined, the inflation gas composition was shown to have a significant effect. Those recipients of lungs inflated with room air exhibited higher survival than those of the nitrogen-inflated group. Again, a trend was seen toward superiority of room air over 100% oxygen in terms of effects on recipient survival after LTX (Figure 2).

Graft Storage Temperature
As a final parameter for investigation, the effects of storage temperature on lung graft function and recipient survival were examined. Three temperatures were studied: 4°C, 10°C, and 15°C. Arterial oxygenation was by far superior in the 10°C group (Table). Posttransplantation PVRs tended to be lowest in recipients of grafts stored at 10°C, and leukocyte accumulation was lowest in the 10°C-stored grafts compared with the other groups (Table). Survival rate in the 10°C group was significantly higher than both other temperature groups studied (Figure 3).

Discussion
To transplant a lung from a donor to a recipient, it is necessary to detach the organ from its native blood supply and to maintain the organ for a period of time under ex vivo conditions. Because of their exquisite sensitivity to ischemia and reperfusion injury and the relatively high incidence of primary graft failure, the success or failure of preservation is usually apparent to the operating surgeon within a short time after implantation. In the last several decades, a number of experimental strategies have been used to improve the quality of lung preservation, with strides remarkable enough in recent
years to make lung transplantation a clinical reality. However, because diverse animal models of lung preservation have been the driving force behind recent technical advances in LTX, diverse strategies for human lung preservation have evolved. Our group and a number of others have found a rat model of LTX to be especially useful for defining the role of specific endogenous molecules or pathways or for testing the effects of specific drugs on lung preservation. However, although separate studies have addressed the importance of a given physical condition for lung graft storage on posttransplantation lung function, the models have been diverse. The goal of the current studies was to identify preservation conditions that are optimal for lung graft storage in the rat model so that future studies and comparisons between experimental teams working on fundamentally dissimilar lung preservation issues may be compared.

The current studies identify 3 features of a rat lung storage strategy (using modified Euro-Collins solution) as optimal; inflation pressure of 10 mm Hg, inflation gas composition consisting of room air, and storage temperature of 10°C. These results are largely in agreement with similar studies in which separate conditions were examined in other models. For instance, recent reports indicate that increased ventilation volume during donor lung flush and hyperinflation during storage provide improved preservation. In these studies, the authors suggest that graft hyperinflation during storage may be beneficial because it prevents accumulation of serum proteins in the alveolar space and maintains surfactant activity, thereby improving early postoperative lung function. It appears that at a transpulmonary pulmonary pressure gradient of 15 cm H2O, sufficient oxygen is available from the airways and alveoli to permit ongoing ATP synthesis in excised canine lung lobes. On the other hand, excessive hyperinflation during donor lung flush and hyperinflation during storage may cause persistent atelectasis and barotrauma, which are associated with poor posttransplantation lung function. These data suggest the reason why in our model, an intermediate level of inflation pressure was optimal.

The second focus for our studies was the effects of inflation pressure on lung graft function after transplantation. Theoretically, it would be advantageous to prevent anoxia or the hypoxic component of ischemia because this is associated with significant inflammatory activation of the vascular endothelium (reviewed in Reference 16). In lung tissue, energy consumption remains unchanged until the alveolar Po2 declines to <1 mm Hg. Although the oxygen demand of lung cells for energy metabolism is low, oxygen will continue to be consumed when lungs are kept inflated with gas of low oxygen content during storage. Although it is easy to see how sufficient oxygen would improve pulmonary storage, there are theoretical concerns that especially in ischemic microenvironments, an overabundance of oxygen can drive reactions involving the production of reactive oxygen intermediates and cause superoxide-mediated lung injury. One report demonstrated that a free radical scavenger reduced pulmonary capillary permeability when lungs were exposed to 100% oxygen during preservation. Given this balance, in the rat LTX model, the optimal inflation gas composition was previously unknown. The current experiments indicate that room air gas provides the optimal balance of oxygen for lung graft storage. In a rabbit model of lung preservation, however, preservation with pure oxygen was superior to room air. Although the reason for the discrepancy between this study and ours is unclear, this comparison illustrates the need to ascertain optimal preservation conditions for any given model.

The final condition that we investigated in these studies was the effect of graft storage temperature on posttransplantation lung function. In general, temperatures are lowered during organ storage or during surgical procedures that may produce ischemia (such as cardiopulmonary bypass or cerebral aneurysm clipping) because this is a highly effective and easily reversible way to lower basal metabolism. However, there is a temperature beneath which cells may be injured, perhaps because of the formation of microcrystalline ice or other mechanisms by which cellular membrane structure and function are disrupted. In the cardiac and hepatic transplantation literature, reports indicate that 4°C is a more suitable preservation temperature than higher temperatures (such as 10°C to 12°C). In the pancreas, on the other hand, optimal preservation temperature appears to be from 7°C to 10°C; in a rat model involving preservation of heart/lung blocks that are reperfused ex vivo, 12°C preservation is optimal; in an ex vivo rabbit lung model, 10°C preservation was superior to both 4°C and 15°C. Taken together, these data indicate that the optimal storage temperature is organ- and model-specific. One of the reasons for differing temperature optima for different organs may be that the hypothermic heart, for instance, favors aerobic free fatty acid metabolism and thereby requires lower temperatures to prevent oxygen utilization by basal metabolism under preservation conditions in which oxygen is scarce. On the other hand, limited oxygen availability may be less of a problem for the preserved lung because the tracheobronchial tree and
alveoli present a substantial air/fluid interface so that oxygen is less scarce and aerobic metabolism may continue to a limited extent.

Although we have identified the conditions of inflation pressure, inflation gas composition, and storage temperature that provide optimal lung graft storage in the model under study, there are caveats that also should be stated. Other studies in large animals use more extended preservation durations, and there are a number of different lung preservation solutions that provide different levels of ischemic protection to the harvested and transplanted lungs (see Reference 26 for a comparison of several of these in the same rat lung transplantation model). Therefore, although the optimal conditions for lung graft storage we have identified apply to the orthotopic rat left LTX model with ischemic durations in the range used for this study (4 to 6 hours), it must be recognized that "optimal conditions" for lung graft storage are likely to be dependent on the species and model under study as well as the ischemic durations used. When taken in context, the results of the current study should be helpful in defining testing conditions to facilitate the development of improved lung preservation strategies.

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