RAFFINOSE IMPROVES THE FUNCTION OF RAT PULMONARY GRAFTS STORED FOR TWENTY-FOUR HOURS IN LOW-POTASSIUM DEXTRAN SOLUTION

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Objectives: The perfect strategy for pulmonary graft preservation remains elusive. Experimental work supports the use of perfusates, such as Euro-Collins, University of Wisconsin, and low-potassium dextran solutions. We use low-potassium dextran solution in our clinical program, but we aim for continued improvement. The trisaccharide raffinose has been shown to be responsible for the efficacy of University of Wisconsin perfusate in lung preservation. Raffinose is superior to a variety of other saccharides for this purpose. We tested the hypothesis that the addition of raffinose to low-potassium dextran solution might further improve graft function. **Methods:** In a randomized blinded study with a rat left lung transplant model, donor lungs were flushed with either standard low-potassium dextran solution or low-potassium dextran solution modified by the addition of 30 mmol/L raffinose ($n = 5$ for each group). Alprostadil (prostaglandin E_1 , 500 $\mu\text{g/L}$) was added to the perfusates in accordance with our clinical practice. Grafts were stored inflated at 4°C for 24 hours. After transplantation, recipients were ventilated with a fraction of inspired oxygen of 1 and a positive end-expiratory pressure of 2 cm H_2O . Graft function was evaluated by measuring oxygenation at 2 hours after graft reperfusion, peak airway pressure throughout the reperfusion period, and the wet/dry lung weight ratio. **Results:** The group receiving low-potassium dextran solution with raffinose demonstrated significantly higher oxygenation (oxygen tension, 370 ± 45 mm Hg vs 150 ± 64 mm Hg; $P = .0025$), lower peak airway pressures at 2 hours after lung reperfusion (11 ± 2.7 mm Hg vs 16 ± 2.4 mm Hg; $P < .001$), and a lower wet/dry weight ratio (4.7 ± 1.26 vs 11 ± 5.0 ; $P = .017$). **Conclusion:** Modification of low-potassium dextran solution with the trisaccharide raffinose resulted in a significant improvement in graft function in this model and merits further evaluation with respect to the mechanisms involved. (J Thorac Cardiovasc Surg 2000; 119:488-92)

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Many strategies of lung procurement are currently used worldwide, with no consensus regarding which is the most effective.¹ Safe graft ischemic times are generally considered to be around 6 to 8 hours irrespective of which technique is used, suggesting that none is superior to the others for short-term preservation of good-quality donor organs. Much experimental work has addressed this issue in the hope that a procurement technique might evolve that provides such satisfactory graft preservation as that experienced in liver and kidney transplantation. A modest reduction in lung graft failure and early mortality rates caused by ischemia-reperfusion injury would exert a significant effect on overall long-term survivals, particularly in the absence of an imminent treatment modality for bronchiolitis obliterans.

Low-potassium dextran (LPD) has been evaluated in experimental studies thoroughly over the past decade,²⁻⁸ but in a recent worldwide survey of lung procurement practice,¹ no center reported its use as a perfusate at that time. However, its use in clinical transplantation has now been reported.⁹ Since obtaining a license for its clinical use in Canada in early 1998, we have used LPD in our clinical program in over 50 consecutive cases with encouraging results. We strive continually for further improvement. The ability to preserve with confidence the more marginal lungs could potentially increase the pool of donors.

The trisaccharide raffinose is included in University of Wisconsin (UW) solution as an impermeant and has been shown to be largely responsible for the efficacy of UW solution as a lung storage medium.¹⁰ Furthermore, raffinose has been shown to be superior to a variety of other saccharides for this purpose.¹¹ Another study has documented the efficacy of a simple raffinose solution by using a porcine paracorporeal model with 24 hours of ischemia.¹² We tested the hypothesis that the addition of raffinose to LPD enhances its preservation properties, leading to improved graft function after transplantation of rat lungs after long-term preservation.

Material and methods

Preparation of modified LPD. Raffinose (17.86 g = 30 mmol/L) pentahydrate (Sigma Chemical Co, St Louis, Mo) was dissolved in 500 mL of LPD solution (Perfadex; Biophausia, Uppsala, Sweden) at room temperature and then reintroduced into the bag through a 0.22- μ m filter (Millex-GS; Millipore Corporation, Bedford, Mass). Tromethamine (INN: trometamol) was added to adjust the pH to 7.5. The resultant solution was colorless and indistinguishable from standard LPD solution. This enabled the experiments to be conducted in a blind fashion. The composition of the 2 solutions evaluated is shown in Table I.

Donor procedure. The Institutional Animal Care and Use Committee of the Toronto General Hospital reviewed and approved the protocol for this study. All animals received humane care in accordance with the "Guide for the care and use of laboratory animals" (NIH Publication No. 85-23, revised 1985).

Ten isogenic Lewis rats (Charles River Inc, Montreal, Canada) with an average body weight of 337 ± 12.6 g were anesthetized by means of an intraperitoneal injection of 1 mL of sodium pentobarbital (Somnotol; MTC Pharmaceuticals, Cambridge, Canada) and intubated through a tracheostomy with a 14-gauge intravenous catheter. Animals were connected to a volume-controlled ventilator (Harvard Rodent Ventilator, model 683, Harvard Apparatus Co, Inc, S Natick, mass) and ventilated with a fraction of inspired oxygen of 1, a tidal volume of 10 mL/kg at 75 breaths/min, and a positive end-expiratory pressure of 2 cm of H₂O. After this, a median

Table I. Composition of flush solutions

Component	LPD	LPD-R
Dextran 40	50 g/L	50 g/L
NaCl	8 g/L	8 g/L
KCl	400 mg/L	400 mg/L
MgSO ₄	98 mg/L	98 mg/L
Na ₂ HPO ₄	46 mg/L	46 mg/L
KHPO ₄	63 mg/L	63 mg/L
Glucose	910 mg/L	910 mg/L
Raffinose pentahydrate	—	17.86 g/L
Tromethamine	0.144 g/L	0.144 g/L
Osmolarity (measured)	292 mmol/L	306 mmol/L
pH	7.5	7.5

laparosternotomy was performed, and 300 USP of heparin (Hepalean; Organon Teknika, Toronto, Canada) was injected into the inferior vena cava. For the retrieval of the heart-lung block, the inferior vena cava was incised, the left atrial appendage was truncated, and a 14-gauge cannula was placed through a right ventricular outflow tractotomy into the main pulmonary artery. The lungs were flushed through this cannula with 20 mL of either LPD or LPD modified with 30 mmol/L raffinose (LPD-R) at 4°C. The flush solution also contained 500 μ g/L of alprostadil (prostaglandin E₁, Prostin VR; Upjohn, Don Mills, Canada). Immediately after the lungs had been flushed, the intratracheal tube was clamped to keep the lungs inflated for the time period of storage, and the heart-lung block was excised. Care was taken to maintain hypothermic conditions, during which semirigid cuffs prepared from a 14-gauge cannula were placed into the pulmonary artery, pulmonary vein, and main bronchus. In each case the vessel or bronchus was drawn through the center of the cuff, everted circumferentially around it, and secured with a 7-0 polypropylene ligature.

Recipient procedure. Recipient animals were anesthetized and intubated as described for donor animals. Animals' lungs were ventilated in a similar fashion. For measuring the airway pressure during transplantation and after graft reperfusion, a 3-way tap was inserted between the intratracheal tube and the ventilator circuit and connected to a pressure transducer. The condition of the recipient was monitored by blood pressure measurement through a 22-gauge cannula placed in the right carotid artery. A left-sided thoracotomy was performed through the 5th intercostal space. The left lung was mobilized by dividing the pulmonary ligament. A paper clip was placed on the left lung to facilitate retraction. The hilum of the left lung was dissected, and the pulmonary artery, pulmonary vein, and the left main bronchus were identified and isolated. All 3 structures were clamped by using microsurgical aneurysm clamps. All 3 structures were incised on their anterior aspect, and the 3 cuffs of the donor lung were placed into the equivalent recipient structures and fixed with a 7-0 polypropylene suture. After a standardized total warm ischemic time of 30 minutes, the transplanted lung was inflated, and blood was reintroduced by releasing the pulmonary vein followed by the arterial clamps. A 19-gauge drainage

Fig 1. Peak airway pressures (PAwP) in millimeters of mercury throughout the 2-hour period of reperfusion. Data points are expressed as the mean values $\pm$ SD. Overall, the 2 groups differed significantly ($P = .012$; 2-way ANOVA). However, the Student-Neuman-Keuls multiple comparison procedure revealed significant differences in peak airway pressures only at 120 minutes after graft reperfusion ($P < .001$) with 11 ± 2.7 mm Hg (LPD-R) versus 16 ± 2.4 mm Hg (LPD).

catheter (Butterfly-19; Abbott Laboratories Ltd, Saint Laurent, Canada) was placed into the left pleural space to avoid accumulation of fluid in the chest. The thoracotomy was closed loosely, and the recipient animal was ventilated with 100% oxygen at a rate of 75 breaths/min, a tidal volume of 10 mL/kg, and a positive end-expiratory pressure of 2 cm H_2O for 2 hours.

Graft assessment. Recipient arterial blood pressure was measured continuously and recorded at 15-minute intervals. Peak airway pressure was recorded before reperfusion; at 1, 5, and 15 minutes after reperfusion; and then every 15 minutes thereafter. Oxygenation of graft venous blood was assessed at the end of the 2-hour reperfusion period. Blood was sampled under direct vision by using a heparinized needle inserted distal to the anastomotic cuff and directed toward the donor lung. After 2 hours, the donor lung was excised, weighed, and desiccated to constant weight at 100°C. The wet/dry weight ratio was determined.

Statistical analysis. All data are expressed as mean values $\pm$ SD. An unpaired, 2-tailed, Student *t* test analysis was used to determine statistical significance between the 2 study groups regarding the blood gas data and the wet/dry graft weight ratio. To evaluate statistical difference between groups regarding the peak airway pressures over the 2-hour reperfusion period, a 2-way analysis of variance (ANOVA) for repeated measure was used. The SigmaStat version 1.0 (Jandel Scientific, San Rafael, Calif) software package was used for all statistical analyses.

Results

There were no statistically significant differences in donor or recipient body weights ($P = .12$ for the LPD-

R group and $P = .09$ for the LPD group). There were also no differences in the systolic arterial blood pressures of the recipients throughout the assessment period, as determined by using a rank-sum test ($P = .103$). Oxygenation 2 hours after graft reperfusion was significantly higher in the LPD-R group (370 ± 45 mm Hg vs 150 ± 67 mm Hg; $P = .0025$, Student *t* test). Pulmonary edema causes increased airway resistance, which is reflected in changes in peak airway pressures over the entire graft reperfusion period. Peak airway pressures differed significantly overall between groups ($P = .012$), as determined by using a 2-way ANOVA. Using the Newman-Student-Keuls test, we found that only at 2 hours after graft reperfusion were the differences between groups statistically significant ($P < .001$; 11 ± 2.7 mm Hg for the LPD-R group vs 16 ± 2.4 mm Hg for the LPD group, as shown in Fig 1). The increase in edematous fluid contained in the graft after reperfusion reflects the severity of lung injury. The wet/dry graft weight ratio was significantly lower in the LPD-R group at the end of the reperfusion period (4.7 ± 1.26 vs 11 ± 5.0 ; $P = .017$, Student *t* test). All data met normality and equal variance conditions, except the systolic arterial blood pressure data. Therefore those were analyzed by using a rank-sum test.

Discussion

In the pursuit of continued improvements in lung preservation, many models have been developed.^{13,14} Small animal models offer the advantage of lower cost and therefore the ability to screen different regimens of preservation before further evaluation in larger species. One disadvantage of using a small animal is the possible confounding factor of anastomotic variability in influencing the results. In this model the technique of semirigid connecting cuffs for all the anastomoses enables the possibility of variability in anastomoses to be largely discounted. We wished to inflict a major ischemic injury on the grafts, and therefore a period of 24 hours was chosen. Other studies with the rat had found 12 and even 6 hours of graft preservation to cause measurable reductions in graft function after transplantation, depending on the storage technique.^{15,16} Interspecies variation in susceptibility to ischemia is recognized, possibly because of widely differing circulating levels of xanthine oxidase,¹⁷ and thus caution is required in interpreting results of differing ischemic times in different species.

Single lung transplantation is the most commonly performed procedure for replacement for end-stage lung disease.¹⁸ To evaluate the ability of the transplanted lung to oxygenate blood, we sampled blood directly

from the pulmonary vein of the transplanted lung by means of needle aspiration distal to the anastomosis cuff. A concern may be the inadvertent sampling of left atrial blood. The randomized blind nature of this study mitigates this as a confounding factor. Repeated sampling throughout the study period would disrupt the pulmonary vein, and this study is limited by the single assessment after 2 hours. We chose not to occlude the hilum of the contralateral native lung for two reasons. First, in preliminary studies immediate contralateral hilar ligation often resulted in prompt death of the animal before any meaningful assessment of the graft could be carried out. Second, if the animal survives a few minutes of reperfusion, although reasonable oxygenation data might be obtained, the ability of the graft to adapt and undergo repair is still impossible to assess. Therefore by avoiding contralateral lung ligation, this more readily reflects the situation in clinical practice, and importantly, we were able to reliably assess the graft after a 2-hour period of reperfusion.

The oxygenation data is impressive; the group flushed with the LPD-R solution demonstrated clearly superior function. The actual value of airway pressure recorded is not an absolute reflection of changes in compliance of the donor lung. Given that a fixed tidal volume was used to ventilate both lungs through a single-lumen tube, the inevitable decrease in compliance of the donor lung will result in preferential ventilation of the native lung. This simply serves to underestimate the real changes in compliance of the graft. Therefore the significant difference in airway pressures that occurred reinforces our hypothesis that the LPD-R lungs were more compliant. A lower wet/dry weight ratio of the raffinose-modified group implies less pulmonary edema and hemorrhage.

Raffinose is a natural trisaccharide that is not found in human subjects. It is present in leguminous seeds and is formed from modified sucrose to which a galactose moiety is linked (α -Gal[1-6] α -Glc[1-2] β -Fru).¹⁹ It was included in UW solution by Belzer and Southard²⁰ for its properties as an osmotic impermeant that would neither diffuse nor be metabolized and thus aid in maintenance of the endothelial cell integrity of pancreas and liver grafts. It has also been used in studies of the renal proximal convoluted tubule to prevent cellular swelling.²¹ UW solution is complex and has been evaluated extensively as an alternative to Euro-Collins solution for lung preservation.^{15,16} Concern remains with regard to the high potassium composition of such a solution and its possible deleterious effect on the pulmonary endothelium.^{22,23} One study was able to demonstrate that the sequential removal of all the components of UW solution other than the phosphate

buffers and the raffinose resulted in no reduction in its efficacy in lung preservation.¹⁰ A subsequent study evaluated raffinose in comparison with iso-osmolar ionic-equivalent solutions of other saccharides, such as melezitose, trehalose, sucrose, fructose, and glucose, and raffinose was determined to be superior to these. Trisaccharides afforded superior graft preservation compared with disaccharides and monosaccharides.¹¹ On the basis of this, a simple solution of phosphate-buffered raffinose has been shown to be as effective as standard UW solution in a porcine model.¹²

The mechanisms by which raffinose exerted its impressive effect in this study are unclear. A concentration of 30 mmol/L was chosen because it had been used successfully in earlier studies with UW solution.^{10,11} We wished to exclude a major change in osmolarity as a potential cause of changes in graft function. Addition of raffinose resulted in only a slight increase in measured osmolarity (Table I), which was still within the normal physiologic range. We believe therefore that raffinose has inherent beneficial properties. The disaccharide trehalose has also been evaluated by other centers with promising results and has a sound theoretic basis for its potential role in organ preservation.²⁴⁻²⁷ It seems reasonable to suggest that trehalose may also improve the role of LPD solution.

We have extensively evaluated LPD solutions for lung preservation. We believe a low potassium concentration to be desirable, as a result of our work and that of other groups.^{3,6,7,23} We now use it clinically. Because many highly active programs are trying to extend donor organ use to include older and marginal organs, we believe the issue of lung preservation assumes increasing importance. Normal younger donor organs with relatively short ischemic times of around 6 to 8 hours appear to function satisfactorily, irrespective of the choice of perfusate. A recent report, however, documented increased mortality rates resulting from the combination of older donors and graft ischemic times greater than 7 hours.²⁸ However, there is a lack of consensus worldwide. A survey carried out in 1996 revealed that highly active and successful programs performing in excess of 40 transplants per year were using Euro-Collins, UW, and Wallwork solutions and donor core cooling. This implies that neither is obviously superior to the others for short-term preservation. However, we strive to improve our current standard in view of the long ischemic times required to procure organs across Canada. We envisage the development of a strategy that will enable rescue of marginal donors.

This study is our first attempt to improve our choice of perfusate. We are encouraged by our results. The fact that rat lungs stored for 24 hours are able to function to

any reasonable degree is an indicator of the efficacy of LPD alone. In one study rat lungs stored in UW solution for 24 hours functioned well only if the technique of controlled low-pressure reperfusion was applied.²⁹ In our model the clamp was simply removed from the hilum, making this a stringent test of the quality of preservation. That such an improvement was seen after the relatively simple modification with raffinose is remarkable. Ongoing work in our laboratory is now directed toward elucidating the mechanisms involved.

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