Effects of newly developed solutions containing trehalose on twenty-hour canine lung preservation

Trehalose is a nonreducing disaccharide that stabilizes the cell membrane under various stressful conditions. A previous study demonstrated that trehalose was effective in 12-hour canine lung preservation. We have developed new preservation solutions containing trehalose: an extracellular type ET-Kyoto solution (Na 100 mmol/L, K 44 mmol/L) and an intracellular type IT-Kyoto solution (Na 20 mmol/L, K 130 mmol/L). The composition of these solutions is identical except for the electrolyte content. We examined their efficacy in 20-hour lung preservation. Canine lungs were flushed with ET-Kyoto (group A, $n = 6$), with IT-Kyoto and prostaglandin E_1 (25 $\mu\text{g/kg}$) (group B, $n = 6$), or with Euro-Collins solution and prostaglandin E_1 (25 $\mu\text{g/kg}$) (group C, $n = 7$), and stored for 20 hours at 4°C. Left lung transplantation was performed and evaluated for up to 130 minutes. The flush time was similar in the three groups. Arterial oxygen tensions (inspired oxygen fraction = 0.5) in group A were uniformly excellent (303.3 ± 7.0 mm Hg 70 minutes after reperfusion and 303.0 ± 19.6 mm Hg 130 minutes after reperfusion) and significantly higher than in group B (202.6 ± 32.0 mm Hg, $p < 0.05$, and 197.8 ± 44.0 mm Hg, $p = 0.054$, respectively) or group C (185.9 ± 23.0 mm Hg, $p < 0.01$, and 155.7 ± 36.3 mm Hg, $p < 0.05$, respectively). Peak inspiratory pressure in group A was significantly lower than in groups B and C ($p < 0.05$). Wet/dry weight ratio in group A was significantly lower than in groups B ($p < 0.05$) and C ($p < 0.01$). Histologic and scanning electron microscopic examinations showed better preservation in group A than in groups B and C. We conclude that ET-Kyoto is superior to IT-Kyoto and to Euro-Collins solution for 20-hour lung preservation. (*J THORAC CARDIOVASC SURG* 1994;108:92-8)

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Although lung transplantation has been established as a therapeutic option for patients with end-stage lung disease, a shortage of suitable donor lungs limits its widespread application. With the growing experience in bilateral sequential lung transplantation,¹ ischemic times of more than 6 hours are now commonplace in clinical practice. Improved methods of lung preservation would increase the supply of transplantable lungs and reduce acute and chronic complications. In liver transplantation,

University of Wisconsin solution (UW), which can provide reliable preservation for more than 24 hours,² has been applied clinically and is widely established. Most clinical lung transplantation programs have preserved donor lungs with prostaglandin (PGE_1 or PGI_2) pretreatment followed by pulmonary arterial flushing with Euro-Collins solution (EC).³ However, a reliable clinical technique of lung preservation beyond 9 hours has not been achieved yet. In animal experiments, a number of investigators have studied extensively the effectiveness of various solutions, such as EC, UW, and low-potassium dextran, in lung preservation for more than 18 hours,⁴⁻⁶ but the results are not satisfactory enough to be applied directly to clinical situations.

Trehalose ($\text{C}_{12}\text{H}_{22}\text{O}_{11}$) is a nonreducing disaccharide (1- α -D-glucopyranosyl-1- α -D-glucopyranoside) with a molecular weight of 342 and consists of two D-glucose moieties connected by a 1,1-linkage. It exists in many

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Table I. Composition of preservation solutions

	ET-K solution	IT-K solution	EC solution
Na (mmol/L)	100	20	10
K (mmol/L)	44	130	115
Cl (mmol/L)	—	—	15
Gluconate (mmol/L)	100	106	—
Phosphate (mmol/L)	25	25	58
Bicarbonate (mmol/L)	—	—	10
Glucose (gm/L)	—	—	35
Trehalose (gm/L)	41	41	—
Hydroxyethyl starch (gm/L)	30	30	—
Osmolarity (mOsm/L)	366	370	355

prokaryotes, fungi, yeasts, some desert plants, and insect body fluid.⁷ In the human body, trehalose yields glucose on hydrolysis by trehalase. Trehalose has been shown to stabilize or protect cell membrane structures under various environmental stresses, such as desiccation, freezing, and high temperatures.⁷⁻¹¹ The effectiveness of trehalose in canine lung preservation has been examined in our laboratory with the use of a modified EC solution in which 3.5% or 7% trehalose replaces glucose. The composition of the solutions is identical except for the saccharide (glucose or trehalose). We¹² have shown that EC containing trehalose provides significantly better preservation than standard EC (containing glucose) for 12-hour cold storage. Although EC containing trehalose shows good results in 12-hour lung preservation, reliable 20-hour preservation has not been achieved (unpublished data). Several components other than trehalose are needed to improve lung preservation. We have developed two new types of preservation solution that contain 4.1% trehalose, hydroxyethyl starch, and gluconate (Table I). ET-Kyoto solution (ET-K) is an extracellular type of solution with high sodium and low potassium content (Na 100, K 44 mmol/L), and IT-Kyoto solution (IT-K) is an intracellular-type solution (Na 20, K 130 mmol/L). In the present study, the efficacy of ET-K and IT-K for 20-hour lung preservation was examined in canine lung allotransplantation.

Materials and methods

Animals. Pairs of size-matched adult mongrel dogs weighing 8.3 to 14.4 kg were assigned randomly to three groups. In group A ($n = 6$) lungs were flushed with ET-K, in group B ($n = 6$) with IT-K, and in group C ($n = 7$) with EC, and they were stored at 4° C in their respective solutions. In groups B and C, PGE₁ pretreatment was used before the flushing. The composition of the solutions is shown in Table I. ET-K and IT-K solutions were generously provided by Roussel Morishita Co. Ltd., Osaka, Japan, and EC solution was supplied by the Green Cross Corporation, Osaka, Japan.

Anesthesia. Induction and maintenance of anesthesia were

Table II. Donor data

	Group A	Group B	Group C
CIT (min)	1193.0 ± 4.5	1199.0 ± 4.0	1197.7 ± 12.0
WIT (min)	65.3 ± 5.5	65.0 ± 2.0	59.7 ± 6.5
Flush time (sec)	72.5 ± 6.9	83.7 ± 4.0	65.7 ± 3.8
PaO ₂ (mm Hg)*	311.8 ± 21.8	286.9 ± 7.5	313.3 ± 7.7
Paco ₂ (mm Hg)*	23.9 ± 3.0	23.2 ± 1.1	26.1 ± 1.0
PIP (cm H ₂ O)†	17.2 ± 0.5	17.7 ± 0.5	16.4 ± 0.8
PVR (dynes · sec · cm ⁻⁵)	402.0 ± 112.4	435.5 ± 71.2	273.1 ± 39.0

All values are mean ± standard error of the mean. CIT, Cold ischemic time; WIT, warm ischemic time; PaO₂, arterial oxygen tension; Paco₂, arterial carbon dioxide tension; PIP, peak inspiratory pressure; PVR, pulmonary vascular resistance.

*Arterial oxygen and carbon dioxide tensions during bilateral ventilation with an inspired oxygen fraction of 0.5.

†Peak inspiratory pressure of the recipient before the operation.

performed as described previously.¹²⁻¹⁴ The dogs' lungs were ventilated with an inspired oxygen fraction of 0.5 at a tidal volume of 20 ml/kg, a respiration rate of 15 breaths/min, and a positive end-expiratory pressure of 5 cm H₂O. Anesthetic gas of nitrous oxide was used with 0.5% to 2.0% halothane during the operation.

Donor procedure. A right femoral venous catheter (Swan-Ganz catheter; Baxter Healthcare Corp., Edwards Div., Irvine, Calif.) and an arterial catheter were introduced. Arterial blood gas analysis, peak inspiratory pressure, and systemic and pulmonary hemodynamics were recorded. Pulmonary vascular resistance (PVR) was calculated as follows: $PVR = ([PAP - PCWP]/CO) \times 80 \text{ dyne} \cdot \text{sec} \cdot \text{cm}^{-5}$ (PAP, mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; CO, cardiac output). After median sternotomy, the azygos vein was divided and the superior and inferior venae cavae, aorta, and pulmonary artery were encircled. After a heparin injection (200 units/kg), the main pulmonary artery was cannulated with a 5 mm aortic arch cannula through a 3-0 Prolene pursestring suture (Ethicon, Inc., Somerville, N.J.). In groups B and C, a large bolus of PGE₁ (25 µg/kg) was injected into the right ventricular outflow. When the systemic pressure declined by at least 40%, the superior and inferior venae cavae and aorta were divided and the proximal pulmonary artery was ligated. The left atrial appendage was amputated, and the lungs were inflated to a maximum inspiratory pressure until all atelectasis was eliminated. The pulmonary artery was flushed by gravity from a height of 50 cm with cold (4° C) perfusate 70 ml/kg (ET-K, IT-K, or EC). Ventilation of the lungs was continued during the pulmonary artery flush and the duration of flushing was recorded. The defect in the cannula insertion site of the pulmonary artery was closed after removal of the cannula, and the left atrial appendage was ligated. Under the endotracheal pressure of 20 cm H₂O, the trachea was clamped and the heart-lung block was excised with minimal handling of both lungs. The block was then placed in a sterile plastic bag containing 1000 ml of the cold corresponding solution and stored at 4° C for 20 hours. After the 20-hour storage, the right lateral basal segment (S⁹) of the donor lung was excised for scanning electron microscopic examination.

Recipient procedure. Recipients were anesthetized, and Swan-Ganz and arterial catheters were introduced as in the donor procedure. Blood gases, peak inspiratory pressures, and

Fig. 1. Arterial oxygen tension (PaO_2) of donor and transplanted lungs (mean $\pm$ standard error). * $p < 0.01$, ET-K versus EC; $p < 0.05$, ET-K versus IT-K. ** $p < 0.05$, ET-K versus EC. FiO_2 , Inspired oxygen fraction.

Fig. 2. Peak inspiratory pressure (PIP) of transplanted lungs (mean $\pm$ standard error). * $p < 0.05$, ET-K versus IT-K; ** $p < 0.05$, ET-K versus IT-K and EC. *Preop*, Before operation on recipients.

systemic and pulmonary hemodynamics were recorded. After pneumonectomy, single left lung transplantation was performed as described previously.¹²⁻¹⁴ Anastomosis was performed in the order of the left atrium, the left main bronchus, and the pulmonary artery. The left pulmonary artery was anastomosed during ventilation of both lungs. At 40, 70, and 130 minutes after reperfusion, the right pulmonary artery was clamped for 5 min-

utes and blood gas analysis, peak inspiratory pressure, pulmonary artery pressure, systemic blood pressure, and heart rate were recorded. Pulmonary capillary wedge pressure and cardiac output were measured 130 minutes after reperfusion. After the final assessment the dogs were killed. The apical posterior ($S^1 + 2$) and lateral basal (S^9) segments of the transplanted lung were excised and examined histologically. The apical posterior

(S¹⁺²) and anterior medial basal (S⁸) segments of the transplanted lung were excised and dried at 70° C for 72 hours, and the wet/dry weight ratio was calculated.

Statistical analysis of the data was performed by analysis of variance, Scheffe's multiple comparison test, and paired, two-tailed *t* test. A *p* value less than 0.05 was considered significant. All data are expressed as mean $\pm$ standard error of the mean.

All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Results

Donor data. Cold and warm ischemic times were similar in the three groups. The flush time was also similar in all groups and the quality of flush was equivalent in all groups according to subjective criteria such as rapid blanching and absence of mottling. No significant differences were detected among the three groups with regard to arterial oxygen and carbon dioxide tensions, peak inspiratory pressure, and pulmonary vascular resistance of the donor lungs before harvest (Table II).

Recipient data. All animals but one could survive the final assessment. One animal in group C died after the assessment at 70 minutes after reperfusion.

Arterial blood gas analysis. In group A, the arterial oxygen tension of the transplanted lung with 5 minutes' occlusion of the right pulmonary artery 40, 70, and 130 minutes after reperfusion were uniformly excellent (289.4 ± 5.7 , 303.3 ± 7.0 , and 303.0 ± 19.6 mm Hg, respectively), and they were significantly higher than in groups B and C after 70 minutes' reperfusion ($p < 0.05$ versus group B, 202.6 ± 32.0 mm Hg; $p < 0.01$ versus group C, 185.9 ± 23.0 mm Hg) and higher than in group C after 130 minutes' reperfusion ($p = 0.054$ versus group B, 197.8 ± 44.0 mm Hg; $p < 0.05$ versus group C, 155.7 ± 36.3 mm Hg) (Fig. 1). In group A, the arterial carbon dioxide tension of the transplanted lung 40, 70, and 130 minutes after reperfusion were 26.6 ± 2.5 , 25.5 ± 2.3 , and 25.1 ± 1.9 mm Hg, respectively; in group B they were 28.9 ± 4.6 , 30.5 ± 5.8 , and 30.3 ± 5.7 mm Hg; and in group C, 27.5 ± 3.3 , 25.6 ± 2.0 , and 28.6 ± 2.5 mm Hg, respectively. No significant difference in arterial carbon dioxide tension after reperfusion was detected among the three groups.

Peak inspiratory pressure. In group C, the peak inspiratory pressure increased significantly with the duration of reperfusion ($p < 0.05$, 40 minutes versus 130 minutes), whereas in groups A and B, the pressures were almost constant after reperfusion. The peak inspiratory pressure in group A was significantly lower than in group

Fig. 3. Wet/dry weight ratio of transplanted lungs (mean $\pm$ standard error). * $p < 0.01$, ET-K versus EC; $p < 0.05$, ET-K versus IT-K.

B after 40 minutes' reperfusion (group A, 13.0 ± 1.3 cm H₂O; group B, 19.2 ± 2.0 cm H₂O; $p < 0.05$) and lower than in groups B and C after 70 minutes' reperfusion (group A, 13.0 ± 1.4 cm H₂O; group B, 18.3 ± 0.9 cm H₂O; group C, 17.2 ± 0.9 cm H₂O; $p < 0.05$) and after 130 minutes' reperfusion (group A, 13.1 ± 1.2 cm H₂O; group B, 19.1 ± 1.4 cm H₂O; group C, 18.5 ± 1.4 cm H₂O; $p < 0.05$) (Fig. 2).

Pulmonary vascular resistance. In group A, the pulmonary vascular resistance of the transplanted lung 130 minutes after reperfusion was 1022.6 ± 209.3 dyne $\cdot$ sec $\cdot$ cm⁻⁵. The corresponding values were 1476.1 ± 174.0 in group B and 1296.5 ± 213.4 in group C. No significant difference was detected among the three groups.

Wet/dry weight ratio. The wet/dry weight ratio of the transplanted lung was 5.72 ± 0.21 in group A, significantly lower than in group B (6.52 ± 0.17 , $p < 0.05$) and in group C (7.00 ± 0.26 , $p < 0.01$) (Fig. 3).

Histologic examination. Histologic studies demonstrated almost normal structures and no sign of pulmonary edema in group A. Two of six animals in group B and one of the seven in group C showed no histologic evidence of edema; the remaining four dogs in group B and six in group C showed thickening of the alveolar septum (mild pulmonary edema).

Scanning electron microscopic examination. Scan-

Fig. 4. Representative scanning electron microscopic findings of preserved lungs. Normal canine pulmonary arterial endothelium with intact architecture (**D**). Scanning electron micrograph of endothelial surface in group A (**A**) showed almost normal structures preserved. Significant endothelial swelling was evident in group B (**B**) and disruption of endothelial cell structures in group C (**C**). (Lines = 10 μ m.)

ning electron microscopy revealed almost normal endothelial structures in group A after 20 hours of storage (Fig. 4, *A* and *D*), whereas significant endothelial swelling was detected in group B (Fig. 4, *B*) and disruption in group C (Fig. 4, *C*).

Discussion

Among the strategies to increase the supply of transplantable lungs is the development of methods to improve lung preservation that minimize ischemic lung injury. Extending preservation time for lung grafts to more than 20 hours would permit semiselective surgery in clinical lung transplantation. Clinically, the most widely used method of organ preservation is hypothermic pulmonary arterial flush with EC after pretreatment with prostaglandin. The limit of the safe ischemic time of this method is considered to be approximately 9 hours.³ Recently, Hardesty and associates¹⁵ reported that UW provided a comparable preservation with EC despite its longer ischemic time. Puskus and colleagues⁴ reported that in 18-hour canine lung preservation with either low-potassium dextran or EC with PGE₁, no significant difference in

transplanted lung function was found between the groups. Aeba and colleagues¹⁶ investigated the effectiveness of UW in 12-hour rat lung preservation without prostaglandin. No significant difference was detected between UW and EC. Oka and coworkers¹⁷ reported that low-potassium dextran was superior to UW and EC for 30-hour rabbit lung preservation with PGE₁ and that no significant difference in efficacy existed between UW and EC. Recently, Kawahara and colleagues⁵ reported that UW with PGE₁ provided better survival than EC with PGE₁ in 24-hour canine lung preservation. The results of experimental lung preservation with UW are controversial, and it is still unclear whether UW is definitely superior to EC.

We investigated the effectiveness of two new types of solution, ET-K and IT-K, which contain 4.1% trehalose, hydroxyethyl starch, and gluconate. Trehalose stabilizes or protects the cell membrane structure under various stressful conditions such as desiccation, freezing, and high temperatures.⁷⁻¹¹ Crowe and associates⁸ reported that trehalose binds to the polar head of membrane phospholipid and prevents fusion and gelatinization of the cell membrane during dehydration. We¹² described previous-

ly the effect of trehalose on 12-hour lung preservation. During cold storage, lung injury seems to be caused by ischemia and hypothermia, which can destroy the cell membrane structure and its function. Scanning electron microscopy showed that damage to endothelium in groups A and B was less than to that in group C. Trehalose may produce a stable environment around the endothelial cell membrane and act as a thermoprotectant against lung injury.

It is still unclear whether a high-potassium concentration solution for the lung is beneficial. In experimental lung preservation, Yamazaki and colleagues¹⁸ reported that an extracellular solution (low-potassium dextran) was superior to an intracellular solution (EC) for 18-hour preservation. However, most clinical organ transplant programs have used an intracellular solution containing a high-potassium concentration (EC or UW) for organ preservation. Intracellular solutions have been considered to induce pulmonary vasoconstriction during flushing, causing interruption of homogeneous flushout.³ Therefore, a vasodilator (PGE₁ or PGI₂) is currently used during organ procurement to prevent vasoconstriction caused by a high-potassium solution. In the present study, an extracellular solution (ET-K), even without PGE₁, provided significantly better preservation than intracellular solutions (IT-K and EC) with PGE₁. The oxygen tension of transplanted lungs in group A was uniformly excellent and significantly higher than that in groups B and C, although the difference between groups A and B did not reach statistical significance at 130 minutes after reperfusion ($p = 0.054$). The impairment of lung function after preservation with intracellular solutions may be due not only to damage during flushing but also to injury during hypothermic storage.

ET-K and IT-K also contain gluconate and hydroxyethyl starch. Gluconate was used as an anion in the place of chloride. Chloride ion passes freely through the cell membrane to draw water into the cell, but the cell membrane is much less permeable to gluconate, which has a greater molecular weight (MW 196) than chloride (MW 35.5).¹⁹ This limited passage may help to reduce cell swelling under hypothermic conditions. Hydroxyethyl starch is believed to create osmotic pressure, to prevent interstitial edema, and to facilitate vascular flushout of organs for preservation.²⁰

We have demonstrated that ET-K provides reliable 20-hour lung preservation in dogs, even without PGE₁. Our comparison of ET-K and IT-K suggests that a low-potassium solution has a more beneficial effect on lung preservation. Further studies are needed

to examine the efficacy of ET-K for longer periods of preservation, to evaluate the importance of the various components, and to compare ET-K with UW. It is expected that ET-K may be of clinical use and may help in achieving the goal of elective lung transplantation.

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