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Evaluation of a new solution containing trehalose for twenty-hour canine lung preservation

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Abstract We examined the efficacy of two new preservation solutions containing trehalose – an extracellular type (ET-K) of solution and an intracellular type (IT-K) of solution – in relation to that of Euro-Collins (EC) solution in 20-h canine lung preservation. Canine lungs were flushed with one of the three solutions ($n = 5$ for each solution) after pretreatment with PGE_1 (20 μg/kg) and were stored for 20 h at 4 °C. The left lungs were transplanted and evaluated to 6 h post transplant. In the ET-K group, the arterial oxygen tension after reperfusion was significantly higher than in the IT-K and EC groups. The pulmonary vascular resistance, wet/dry weight ratio, and histological evaluation of each transplanted lung in the ET-K group were also better than in the IT-K and EC groups. This indicates that ET-K solution is useful for 20-h preservation of canine lung grafts.

Key words Lung preservation, dog, trehalose · Preservation, lung, trehalose · Trehalose, lung preservation

Introduction

Lung transplantation is usually performed for patients with end-stage lung disease. However, the frequency with which it is carried out is limited by the lack of suitable donor lungs. Clinically, the best method for lung preservation has not been established. In clinical practice, it is difficult to preserve a lung longer than 9 h. This is considered the maximum safe ischemic time [6]. Many studies employing various lung preservation solutions, such as the Euro-Collins (EC) solution, University of Wisconsin (UW) solution, and low-potassium-dextran (LPD) solution, have been reported [10, 18]. Nevertheless, their significance is unclear.

Recently, new preservation solutions containing 4.1 % trehalose, hydroxyethyl starch (HES), and gluconate have been developed. One is an extracellular type (ET-K) solution, containing 100 mmol/l sodium and 44 mmol/l potassium. Another is an intracellular type (IT-K) solution, containing 20 mmol/l sodium and 130 mmol/l potassium. The Kyoto University group previously investigated the effectiveness of the new solutions, especially ET-K solution, in 12-h and 20-h canine lung preservation studies [1]. However, they chose a 130-min postoperative follow-up period to estimate the preserved lung function. In this study, we selected a 6-h follow-up period after reperfusion to investigate the effectiveness of the new preservation solutions and we examined whether either solution was more beneficial than EC solution for 20-h lung preservation in canine lung allotransplantation.

Materials and methods

Animals and preservation solutions

Fifteen pairs of size-matched adult mongrel dogs weighing 10–20 kg were divided randomly into three groups of five pairs each. One dog in each pair was randomly selected as the donor and the other as the recipient. The solutions for lung flushing (at 4 °C) and

Table 1 Composition of the preservation solutions (*EC* Euro-Collins, *IT-K* intracellular solution, *ET-K* extracellular solution)

	EC	IT-K	ET-K
Na (mmol/l)	10	20	100
K (mmol/l)	115	130	44
Cl (mmol/l)	–	–	–
Gluconate (mmol/l)	–	106	100
Phosphate (mmol/l)	58	25	25
Bicarbonate (mmol/l)	10	–	–
Glucose (g/l)	35	–	–
Trehalose (g/l)	–	41	41
Hydroxyethyl starch (g/l)	–	30	30
Osmolarity (mosmol/l)	355	370	366

preservation were: ET-K solution ($n = 5$), IT-K solution ($n = 5$), and EC solution ($n = 5$). The compositions of the solutions are shown in Table 1.

In all groups, the donor organs were pretreated with prostaglandin E_1 (PGE_1) before flushing. The ET-K and IT-K solutions were generously provided by Roussel Morishita (Osaka, Japan) and the EC solution by the Green Cross (Tokyo, Japan). All animals received humane care in compliance with the "Principles of Laboratory Animal Care" published by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" proposed by the National Institutes of Health (National Institutes of Health Publication No. 86–23, revised 1988).

Anesthesia

Basal anesthesia consisted of 10 mg/kg ketamine hydrochloride injected intramuscularly, then 15 mg/kg sodium thiopental injected intravenously. The trachea was then intubated with a tube with an internal diameter of 7.5 mm. Ventilation was controlled with a respirator (model SN-480-3, CLEA, Tokyo, Japan) with the following setting: tidal volume 20 ml/kg, respiratory rate 15/min, and positive end-expiratory pressure 5 cm H_2O. Both donor and recipient lungs were ventilated with great care; we ensured conditions as identical as possible. The anesthetic gas mixture consisted of 1 % halothane and equal amounts of oxygen and nitrous oxide.

Donor procedure

The donor was placed in a supine position, and a 7 Fr Swan-Ganz catheter (Baxter Healthcare, Edwards Division, Irvine, Calif., USA) was inserted through the right femoral vein to continuously measure the heart rate (HR) as well as the systemic and pulmonary hemodynamics. An arterial catheter was introduced for arterial blood gas analysis.

A midline sternal and pericardial incision was made. The azygos vein was divided, and the superior and inferior venae cavae, aorta, and pulmonary artery were encircled and taped. After the right main pulmonary artery was clamped for 10 min, a blood gas analysis was made and mean pulmonary arterial pressure (PAP), pulmonary capillary wedge pressure (PCWP), and cardiac output (CO) were recorded. The pulmonary vascular resistance (PVR) was calculated as follows: $PVR = ([PAP\text{-}PCWP]/CO) \times 80$ dyne · s · cm^{-5}. The right main pulmonary artery was then released, and heparin (200 units/kg) was injected intravenously. The pulmonary artery trunk was cannulated with a 5-mm aortic arch cannula that was fixed with a 3-0 prolene pursestring suture (polypropylene monofilament, Ethicon), and a bolus of PGE_1 (20 μg/kg) was injected into the right ventricular outflow. When the systemic pressure had sunk to at least 40 %, the superior and inferior venae cavae and the aorta were dissected, and the proximal portion of the pulmonary arterial trunk was ligated. The left atrial appendage was cut to exclude the reperfusion solution. The pulmonary artery was flushed by gravity from a height of 50 cm with 70 ml/kg (ET-K, IT-K, or EC) cold (4 °C) perfusate, and the bilateral lungs were inflated to a maximum inspiratory pressure until no remaining atelectasis could be seen. During pulmonary artery flushing, ventilation of the lungs was continued. After removal of the cannula, the site where the cannula had been inserted into the pulmonary artery was closed, and the left atrial appendage was ligated. The trachea was clamped under an endotracheal pressure of 20 cm H_2O, and the heart and lungs were excised en bloc. They were placed in a sterile plastic bag containing 1000 ml of the corresponding cold solution and stored at 4 °C for 20 h.

Recipient procedure

Recipients were anesthetized and ventilated; Swan-Ganz and arterial catheters were then introduced in the same way as for the donors. The recipients were placed in the right lateral position and a posterolateral thoracotomy was performed in the left fifth intercostal space. After left pneumonectomy, the left lung was transplanted according to the method of Haverich and colleagues [11] and Veith and colleagues [22], as follows. The left atrium was anastomosed with continuous everting mattress sutures with 4–0 prolene. Pulmonary arterial and bronchial anastomoses were accomplished with continuous over-and-over sutures with 5–0 prolene and 4–0 Maxon absorbable sutures (monofilament polyglyconate, Davis and Geck), respectively. The left pulmonary artery was anastomosed during ventilation of both lungs. At 1, 2, 4, and 6 h after reperfusion, the right pulmonary artery was clamped for 10 min. Blood gas analysis was made and PAP, systemic blood pressure, and HR were recorded. The PCWP and CO were measured 6 h after reperfusion. After the final assessment, the dogs were sacrificed. The apical posterior (S 1 + 2) and dorsal basal (S 10) segments of the transplanted lung were excised and stained with hematoxylin and eosin for histological evaluation. The apical posterior (S 1 + 2) and anterior medial basal (S 8) segments of the transplanted lung were excised and dried at 70 °C for 72 h and the wet/dry weight ratio was calculated.

Statistical analysis

We used an analysis of variance (ANOVA), Scheffe's multiple comparison test, and Student's paired, two-tailed *t*-test for the statistical analysis of the data. A P value below 0.05 was considered significant. All data were expressed as means ± standard error of the mean.

Results

Donor data

The cold ischemia, warm ischemia, and flushing times were similar in all groups (Table 2). Among the three groups, there were no significant differences for arterial oxygen and carbon dioxide tensions, PAP, and PVR of the donor lungs after 10-min clamping of the right main pulmonary artery before harvesting (Table 2).

Table 2 Donor data. All values are means ± standard error of the mean. (*EC* Euro-Collins, *IT-K* intracellular solution, *ET-K* extracellular solution, *CIT* cold ischemia time, *WIT* warm ischemia time, PaO_2 arterial oxygen tension, $PaCO_2$ arterial carbon dioxide tension, *PAP* pulmonary arterial pressure, *PVR* pulmonary vascular resistance)

	EC group	IT-K group	ET-K group
CIT (min)1197.8 ± 4.5	1198.4 ± 6.2	1195.2 ± 3.7	
WIT (min)	64.7 ± 4.2	63.5 ± 3.3	67.8 ± 4.6
Flush time (s)	76.2 ± 3.9	75.9 ± 5.3	70.4 ± 4.4
PaO_2 (mm Hg)[a]	212.4 ± 21.5	230.3 ± 10.9	216.2 ± 5.3
$PaCO_2$ (mm Hg)[a]	45.3 ± 4.0	37.9 ± 3.1	42.3 ± 4.3
PAP (mm Hg)[b]	20.0 ± 4.2	17.0 ± 2.6	17.7 ± 2.5
PVR (dyne · sec · cm^{-5})[b]	327.3 ± 39.1	356.3 ± 76.8	315.6 ± 47.6

[a] Obtained after 10-min clamping of the right main pulmonary artery with an inspired oxygen fraction of 0.5
[b] Obtained after 10-min clamping of the right main pulmonary artery

Table 3 Assessment of transplanted lung function. All values are means ± standard error of the mean (*ET-K* extracellular solution, *IT-K* intracellular solution, *EC* Eurocollins, PaO_2 arterial oxygen tension, $PaCO_2$ arterial carbon dioxide tension, *PAP* pulmonary arterial pressure, *PVR* pulmonary vascular resistance)

PaO_2 (mm Hg)	1 h	2 h	4 h	6 h
ET-K group	233.7 ± 18.9	247.6 ± 18.3	251.3 ± 13.7	245.9 ± 21.0
IT-K group	184.7 ± 33.0	152.2 ± 16.5	113.3 ± 10.9	121.2 ± 48.6
EC group	190.6 ± 27.6	186.4 ± 29.0	115.6 ± 21.2	107.8 ± 13
$PaCO_2$ (mm Hg)	1 h	2 h	4 h	6 h
ET-K group	36.9 ± 16.6	35.7 ± 11.4	32.6 ± 7.0	32.4 ± 7.8
IT-K group	35.4 ± 8.8	36.5 ± 10.0	39.0 ± 10.1	47.8 ± 5.2
EC group	26.6 ± 14.2	44.2 ± 16.0	45.6 ± 15.2	42.0 ± 12.3
PAP (mm Hg)	1 h	2 h	4 h	6 h
ET-K group	21.0 ± 4.9	20.6 ± 2.0	18.8 ± 2.3	18.7 ± 3.7
IT-K group	18.5 ± 6.1	17.9 ± 12.5	24.5 ± 8.1	25.8 ± 11.6
EC group	19.0 ± 4.8	19.8 ± 7.7	20.9 ± 5.5	26.5 ± 7.0
PVR (dyne · sec · cm^{-5})	Donor	6 h after reperfusion		
ET-K group	315.6 ± 47.6	417.4 ± 34.1		
IT-K group	356.3 ± 76.8	773.0 ± 189.4		
EC group	327.3 ± 39.1	786.3 ± 108.3		
Wet/dry ratio	6 h after reperfusion			
ET-K group	5.40 ± 0.14			
IT-K group	6.44 ± 0.21			
EC group	6.98 ± 0.07			

Recipient data

All but two animals survived the final assessment. One animal in the EC group and another in the IT-K group died of severe pulmonary edema after the assessment at 6 h after reperfusion. The assessment of transplanted lung function is shown in Table 3.

Arterial blood gas analysis

The arterial oxygen tension of the transplanted lung with 10-min clamping of the right pulmonary artery in the ET-K group was uniformly excellent at 1, 2, 4, and 6 h after reperfusion. These values were significantly higher than in the IT-K group 2 h after reperfusion ($P < 0.01$), higher than in the IT-K and EC groups 4 h after reperfusion ($P < 0.01$), and higher than in the EC group 6 h after reperfusion ($P < 0.05$; Fig. 1). In the EC group, the donor data recorded after 10-min clamping of the right pulmonary artery showed a significant decrease in arterial oxygen tension compared to the tension 6 h after reperfusion ($P < 0.01$). The results of arterial carbon dioxide tension of the transplanted lung 1, 2, 4, and 6 h after reperfusion in the ET-K, IT-K, and EC groups are shown in Table 3. No significant difference was detected among the three groups (Fig. 2).

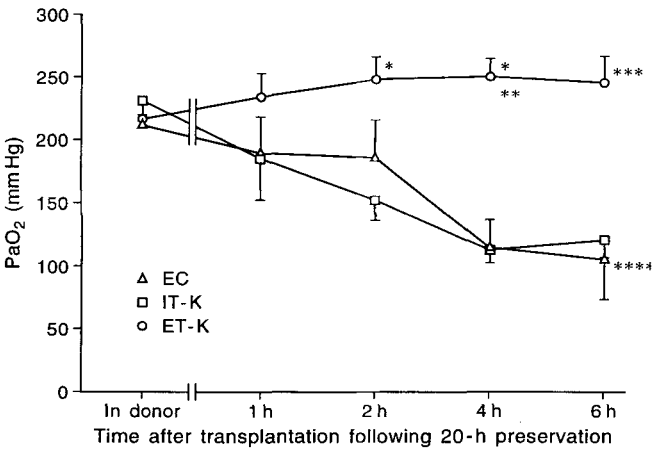

Fig. 1 Arterial oxygen tension (PaO_2) of the donor and of the transplanted lungs (means ± standard error of the mean). Inspired oxygen fraction (FiO_2) = 0.5 *1 $P < 0.01$ for IT-K vs ET-K; *2 $P < 0.01$ for EC vs ET-K; *3 $P < 0.05$ for EC vs ET-K (ANOVA); *4 $P < 0.01$ for EC: donor vs 6 h after reperfusion (Student's two-tailed t-test)

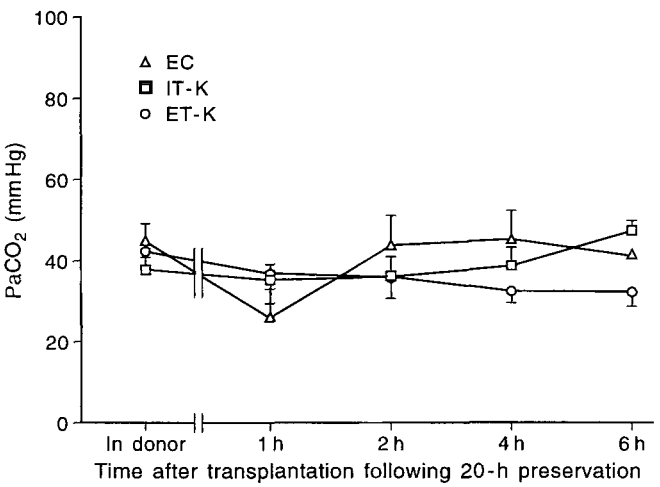

Fig. 2 Arterial carbon dioxide tension ($PaCO_2$) of donor and of transplanted lungs (means ± standard error of the mean). No significant difference was detected among the three groups

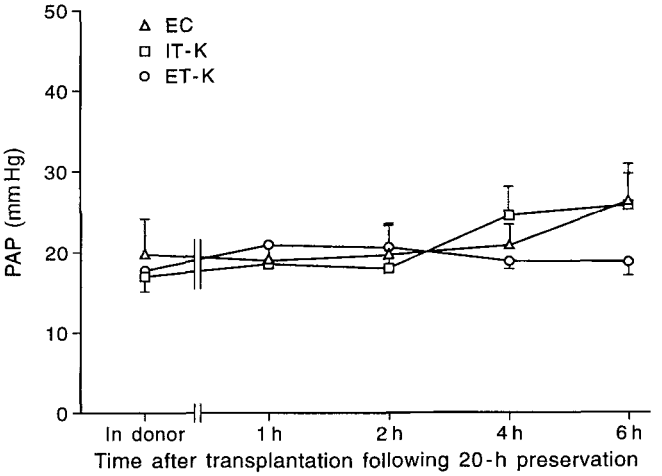

Fig. 3 Pulmonary arterial pressure (PAP) of the donor and transplanted lungs (means ± standard error of the mean). No significant difference was detected among the three groups

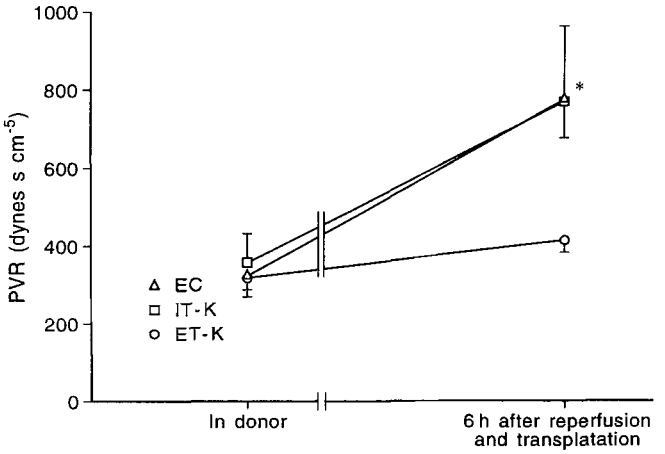

Fig. 4 Pulmonary vascular resistance (PVR) of donor and of transplanted lungs 6 h after reperfusion (means ± standard error of the mean) * $P < 0.05$ for EC: donor vs 6 h after reperfusion (Student's two-tailed t-test). No significant difference was detected among the three groups (ANOVA)

Pulmonary artery pressure

In the ET-K group, the PAP of the transplanted lung with 10-min clamping of the right pulmonary artery 1, 2, 4, and 6 h after reperfusion was almost constant (Table 3). No significant difference in PAP was detected among the three groups after reperfusion (Fig. 3).

Pulmonary vascular resistance

The PVRs of the transplanted lungs 6 h after reperfusion are shown in Table 3. No significant difference was detected among the three groups (Fig. 4). In the EC group, the donor data recorded after 10-min clamping of the right pulmonary artery showed a significant increase in PVR compared to the PVR 6 h after reperfusion ($P < 0.05$; Fig. 4).

Wet/dry weight ratio

The wet/dry weight ratios of the transplanted lungs are shown in Table 3. The ratio for the ET-K group was significantly lower than that for the IT-K and EC groups ($P < 0.01$; Fig. 5).

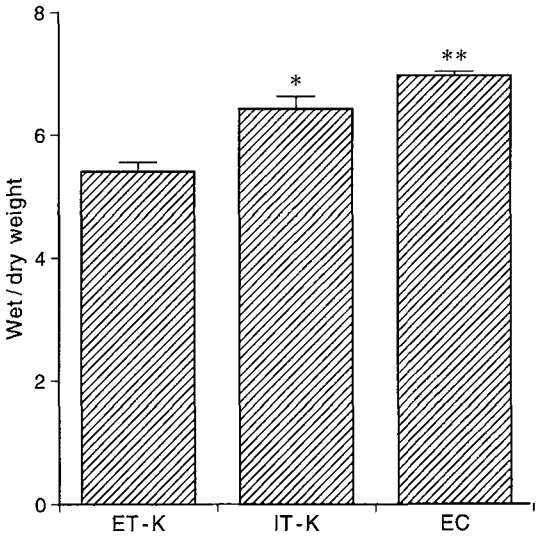

Fig. 5 Wet/dry weight ratio of donor and transplanted lungs (means ± standard error of the mean). $^{*}P < 0.01$ for EC vs ET-K; $^{**}P < 0.01$ for IT-K vs ET-K

Histological evaluation

Four of the five animals in the EC group showed severe interstitial edema, thickening of the alveolar septum, and many intra-alveolar red blood cells and lymphocytes. Interstitial and alveolar hemorrhage, in addition to edematous change, were the most severe changes in all three groups (severe pulmonary edema). All of the lungs in the EC group had pulmonary edema, resulting in seepage of the fluid within the bronchi. This caused greater impedance to the pressurized inflation. Four of the five animals in the IT-K group showed diffuse alveolar damage and interstitial edema (mild pulmonary edema). One dog in the EC group and another in the IT-K group died; they had severe pulmonary edema. In the ET-K group, a histological examination of all transplanted lungs showed normal structure and no signs of pulmonary edema (Fig. 6).

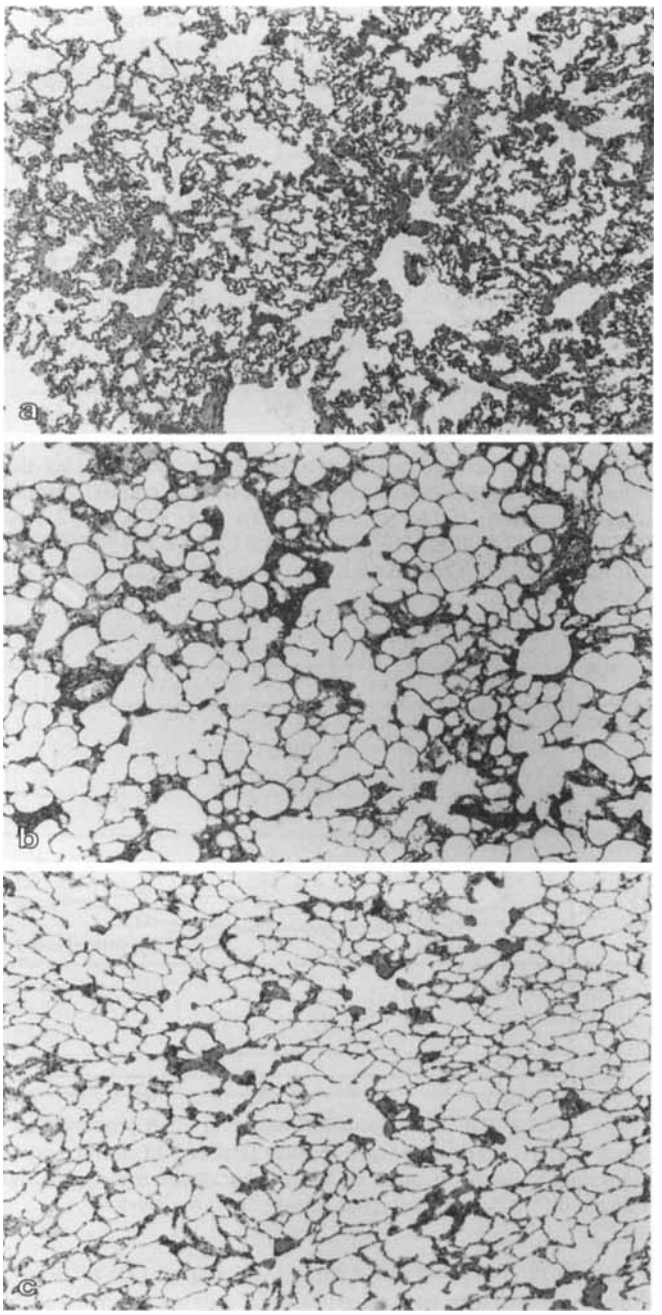

Fig. 6 a–c Photomicrographs of transplanted lungs: **a** severe pulmonary edema is observed in four of the five dogs in the EC group; **b** mild pulmonary edema is observed in four of the five dogs in the IT-K group; **c** all transplanted lungs show normal structure and no sign of pulmonary edema in the ET-K group (H&E, × 40)

Discussion

Most clinical lung transplantation programs have preserved donor lungs with EC (hypothermic pulmonary arterial flushing) after pretreatment with prostaglandins (PGE_1 or PGI_2) [6]. Many studies with various lung preservation solutions such as EC, UW, and LPD have been reported [10, 18]. Nevertheless, no completely satisfactory solution for use in the practical clinical situation has been developed. Recently, new preservation solutions containing 4.1 % trehalose, HES, and gluconate have been developed. These include both an extracellular (ET-K) and an intracellular (IT-K) solution. In this study, the oxygen tension of the transplanted lungs in the ET-K group was uniformly excellent and significantly higher than in the EC group at 4 h ($P < 0.01$) and at 6 h ($P < 0.05$) after reperfusion.

Cold storage means that the lung has been exposed to hypoxic and hypothermic conditions. These lead to cell damage and ultimately to cell death because the structure of the cell membrane and its function are destroyed [2].

Bando and colleagues have studied the role of trehalose in lung preservation [1]. They suggest that trehalose produces a stable environment around the endothelial cell membrane and that it acts as a thermoprotectant against lung injury. Their ET-K group had significantly higher oxygen tension in the transplanted lungs at 130 min after reperfusion than their IT-K and EC groups [1]. However, there were no significant differences among these three groups in the early follow-up period after reperfusion. While the difference between the ET-K group and the EC group was not statistically significant at 1 and 2 h after reperfusion, the former group showed uniformly excellent results. This suggested that ET-K solution has a more beneficial effect than EC during the 6-h postoperative follow-up period.

Trehalose stabilizes or protects the cell membrane structure under stressful conditions such as desiccation, freezing, and high temperatures by binding to the polar head of phospholipids in the lipid bilayer of the cell membrane [7, 8, 23, 24]. In addition, trehalose is incorporated between the polar head groups of the phospholipids, thereby maintaining a specific distance between the molecules that inhibits gelatinization and subsequent dysfunction of the bilayer under stressful conditions [9, 15, 19]. Moreover, trehalose functions as an osmoregulatory agent in the cytoplasm [14–16, 20] and acts to preserve enzyme activity [5].

In the ET-K and IT-K solutions, gluconate was used (100 mmol/l and 106 mmol/l, respectively) instead of chloride. The molecular weight of gluconate (196) is much greater than that of chloride (35.5). Therefore, the chloride ion passes freely through the cell membrane to draw water into the cell, but the cell membrane is much less permeable to gluconate. Under hypothermic conditions, this phenomenon may help to reduce cell swelling [2].

ET-K and IT-K solutions also contain HES (30 gm/l) to prevent the expansion of extracellular space and to facilitate vascular flushout of organs for preservation [3]. Hirata and colleagues reported that trehalose was effective in 12-h canine lung preservation [12]. More recently, Bando and colleagues reported the effectiveness of ET-K for 20-h canine lung preservation [1]. However, they chose a 130-min post-transplantation follow-up period to estimate the preservation quality, and short observation times might have underestimated the damage resulting from reperfusion injury. Hooper and coworkers reported that a 4-h follow-up period for lung preservation seems appropriate for assessing the severity of reperfusion injury [13]. Mills et al. reported that 4 h after reperfusion the arteries appear to be less constricted and have a more intact basement membrane [17].

In the present study, we selected a 6-h follow-up period for lung preservation. We also investigated the effectiveness of ET-K (an extracellular solution) and of IT-K (an intracellular solution) after pretreatment with a bolus intra-arterial injection of PGE_1. The effectiveness of a high-potassium concentration in the solution for lung preservation is still unclear. Yamazaki and colleagues reported that an extracellular solution (LPD) was superior to an intracellular solution (EC) for 18-h preservation [25]. However, clinically, intracellular solutions containing high-potassium concentrations (EC or UW) have generally been used for organ preservation. Intracellular solutions are considered to cause pulmonary vasoconstriction during pulmonary flushing. Pulmonary vasoconstriction may interrupt homogeneous flushout and impair the uniform distribution of the flushing solution, resulting in adequate preservation [14, 21, 25]. Therefore, prostaglandins (PGE_1 or PGI_2), which induce vasodilation, are widely used clinically during pulmonary flushing to prevent vasoconstriction [1, 4]. We anticipated that the addition of PGE_1 would make IT-K as effective as ET-K. However, the arterial oxygen tension of transplanted lungs in the ET-K group was significantly higher than that in the IT-K group at 4 h after reperfusion ($P < 0.01$). Although no significant differences were detected between the ET-K group and the It-K group in arterial carbon dioxide tension, PAP, or PVR, the ET-K group uniformly yielded the best results at all times examined. Our comparison of ET-K and IT-K solutions suggested that a low concentration of potassium is beneficial for lung preservation. One reason why IT-K with PGE_1 was not more effective than ET-K for lung preservation might be that the effectiveness of PGE_1, its dose range, and delivery route has not yet been established [4]. Further investigations with suitable pretreatment of PGE_1 will ensure the efficacy of an intracellular type of preservation solution. A second reason is that not only reperfusion injury, but also damage during hypothermic storage caused by the destruction of various ion-pump activities may affect lung function after transplantation [1, 17, 25].

We conclude from this study that ET-K solution is significantly better at preserving lungs than IT-K and EC solutions at 6 h after reperfusion. Moreover ET-K solution with PGE_1 is significantly better than IT-K solution with PGE_1. In the present study, no statistically significant differences were seen between IT-K and EC solutions. However, our results indicate that ET-K solution is useful for 20-h cold preservation of lung grafts. Further studies evaluating these solutions are necessary, and the importance of various components of the solutions should also be clarified. ET-K solution shows great promise for clinical use in lung transplantation.

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