Impaired endothelium-derived hyperpolarizing factor-mediated relaxation in porcine pulmonary microarteries after cold storage with Euro-Collins and University of Wisconsin solutions

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Background: Endothelium plays an important role in mediating the function of transplanted organs. The widely used University of Wisconsin solution impairs the endothelium-derived hyperpolarizing factor-mediated relaxation in coronary arteries, but little is known about effects of lung preservation on endothelium-derived hyperpolarizing factor-mediated endothelial function. This study examined the effect of organ preservation solutions on the endothelium-derived hyperpolarizing factor-mediated relaxation in the pulmonary microarteries (diameter 200 to 450 μ m).

Methods: Two segments (1 as control) from the same microartery were allocated in 2 chambers of a myograph. After incubation with hyperkalemia (potassium 115 mmol/L), University of Wisconsin, or Euro-Collins solution (at 4°C for 4 hours), the endothelium-derived hyperpolarizing factor-mediated relaxation was induced by bradykinin (-10 to -6.5 log M, $n = 8$) or calcium ionophore (A_{23187} , -9 to -5.5 log M, $n = 7$) in U_{46619} (-7.5 log M) precontracted rings in the presence of indomethacin (7 μ mol/L), N^G -nitro-L-arginine (300 μ mol/L), and oxyhemoglobin (20 μ mol/L).

Results: Exposure to hyperkalemia and storage with Euro-Collins or University of Wisconsin solution significantly decreased the relaxation to bradykinin ($51.9 \pm 8.4\%$ vs $60.3 \pm 6.1\%$, $P = .02$ or $49.3 \pm 7.3\%$ vs $65.2 \pm 3.5\%$, $P = .04$) or A_{23187} ($12.5 \pm 0.02\%$ vs $33.8 \pm 0.07\%$, $P = .02$ or $13.2 \pm 0.03\%$ vs $31.0 \pm 0.05\%$, $P = .03\%$).

Conclusions: Endothelium-derived hyperpolarizing factor plays an important role in porcine pulmonary microarteries, and the endothelium-derived hyperpolarizing factor-mediated relaxation is impaired when the lung is preserved with University of Wisconsin or Euro-Collins solution. This impairment may affect the lung function during the reperfusion period after lung transplantation.

During the preservation period of the donor organ, the vascular endothelium is in direct contact with preservation solutions. The effect of organ preservation solutions on the endothelium is therefore an important issue in organ transplantation surgery. In fact, the effect of hyperkalemia, one of the major components in organ preservation solutions, and the effect of the solutions have been investigated in several recent studies.¹⁻¹⁰ It has been suggested that University of Wisconsin (UW) solution reduces the endothelium-dependent relaxation or causes endothelial dysfunction.^{4,7,8,10}

Endothelium is an important modulator of vascular tone. Endothelium-dependent relaxation is known to be the effect of a variety of endothelium-derived relaxing factors (EDRFs). These are endothelium-derived nitric oxide (NO),¹¹ prostacyclin (PGI₂), and endothelium-derived hyperpolarizing factor (EDHF).^{6-9,12-17} Because of the complexity of the endothelial function, it is necessary to study the effect of preservation solutions on individual EDRFs to explore the best method for preservation of the endothelium. Euro-Collins (E-C) or UW solution has been used for clinical lung transplantation. However, the effect of E-C and UW on the EDHF-mediated endothelial function in the pulmonary artery has not been reported.

Because potassium (K⁺) at high concentrations (hyperkalemia) is a key component in organ preservation solutions for transplantation or in cardioplegic solutions, we have performed a series of experiments to examine the effect of hyperkalemia on endothelial function. Our previous studies have demonstrated that when the production of NO and PGI₂ is inhibited, the residual relaxation (mediated by EDHF) is significantly reduced by exposure to hyperkalemia (K⁺ concentration of 20 to 50 mmol/L).^{3,6,9} The mechanism of the impaired EDHF-mediated relaxation after exposure to hyperkalemia is related to the inhibition of K⁺ channels and prolonged depolarization of the smooth muscle membrane potential.^{6,9}

In E-C or UW solution, concentrations of K⁺ is as high as 115 or 125 mmol/L. The extremely high K⁺ concentration in E-C and UW solutions is a major concern with regard to endothelial preservation.¹⁰ However, the composition of E-C or UW is complex and the effect of these organ preservation solutions may therefore be complicated by their multiple components. In fact, the effect of storage of the lung with E-C and UW solutions on the EDHF-mediated relaxation in the pulmonary artery is unknown.

We therefore designed the present study to examine, in the porcine pulmonary microartery, (1) whether the EDHF-mediated relaxation exists and (2) the effect of hyperkalemia and cold storage with E-C or UW solution, similar to the clinical setting, on the EDHF-mediated endothelial function and the contractility of the artery.

Methods

Microarterial Preparation

Fresh porcine lungs collected from a local slaughterhouse were placed in a container filled with cold Krebs' solution and immediately transferred to the laboratory. Upon receipt of the lung, intralobular pulmonary microarteries (usually small branches of the upper lobe artery, diameter 200 to 450 μ m) were carefully dissected and removed under a microscope. The vessels were cleaned of fat and connective tissue and cut into cylindrical rings of 2-mm length under a microscope. The Krebs' solution was aerated with a gas mixture of 95% O₂–5% CO₂ at 37°C and had the following composition (in millimoles per liter): NaCl: 118.4;

KCl: 4.7; MgSO₄·7H₂O: 1.2; KH₂PO₄: 1.2; NaHCO₃: 25; (+)-glucose: 11.1; and CaCl₂·2H₂O: 2.5. This gives the following final molar concentration (in millimoles per liter): 143.4 Na⁺, 5.9 K⁺, 2.5 Ca²⁺, 1.2 Mg²⁺, 128.7 Cl[–], 25 HCO₃[–], 1.2 SO₄^{2–}, 1.2 H₂PO₄[–], and 11.1 glucose. During the above procedure, the endothelium was intentionally preserved by cautiously dissecting and mounting the rings.

Normalization

After the rings were mounted in a 2-channel myograph (model 500A; J.P. Trading, Aarhus, Denmark), a previously described method^{4,6} was used to normalize vascular rings under a condition simulating the transmural pressure encountered in vivo in the pulmonary microartery. Briefly, the artery rings were progressively stretched until the passive transmural pressure reached 40 mm Hg. The internal circumference was then set to a normalized value, estimated to be equivalent to 90% of the circumference at a passive transmural pressure of 40 mm Hg. The transmural pressure at this point is about 30 mm Hg. This pressure was maintained throughout the experiments.

Protocol

After the normalization procedure, the microarterial rings were equilibrated for at least 20 to 45 minutes.

Precontraction

The vessel was contracted with thromboxane A₂ mimetic (U₄₆₆₁₉, –7.5 log M). This dose was chosen from our pilot experiment in which the effective concentration causing 50% of the maximal response (EC₅₀, see "Data Analysis" below) for U₄₆₆₁₉-induced contraction was 7.4 $\pm$ 0.4 log M, calculated from the concentration-response curves in porcine pulmonary microarteries (n = 8; taken from 8 porcine lungs). U₄₆₆₁₉ at –7.5 log M caused a stable submaximal contraction of all rings.

The following inhibitors were used: cyclooxygenase inhibitor indomethacin (INDO), 7 μ mol/L; the nitric oxide (NO) synthesis inhibitor, N^G-nitro-L-arginine (L-NNA), 300 μ mol/L; and a potent NO scavenger oxyhemoglobin (HbO), 20 μ mol/L.

Role of EDHF-Mediated (INDO-, L-NNA-, and HbO-Resistant) Relaxation in Porcine Pulmonary Microarteries by Bradykinin or Calcium Ionophore

A₂₃₁₈₇

With the presence of INDO, L-NNA, and HbO, concentration-relaxation curves to bradykinin (BK; –10 to –6.5 log M) or A₂₃₁₈₇ (–9 to –5.5 log M) were established when the U₄₆₆₁₉-induced contraction reached a plateau. This relaxation is considered to be related to EDHF because both NO and PGI₂ are abolished.¹²⁻¹⁴

Two rings taken from the same microartery were mounted in the 2 chambers and equilibrated for 45 minutes. The normalization procedure was then performed. INDO (7 μ mol/L), L-NNA (300 μ mol/L), and HbO (20 μ mol/L) were then added into 1 chamber and the other was used as control. After the precontraction with U₄₆₆₁₉ (–7.5 log M), cumulative concentration-relaxation curves to BK (n = 8) or A₂₃₁₈₇ (n = 7) were established.

TABLE 1. Components of University of Wisconsin (UW) and Euro-Collins (E-C) solutions

Components	UW	E-C
Na ⁺	29 mmol/L	10 mmol/L
K ⁺	125 mmol/L	115 mmol/L
Mg ²⁺	5 mmol/L	
Cl ⁻		15 mmol/L
HCO ₃ ⁻		10 mmol/L
H ₂ PO ₄ ⁻	25 mmol/L	15 mmol/L
HPO ₄ ²⁻		42.5 mmol/L
Pentafraction	50 g/L	
Lactobionic acid	35.83 g/L	
Raffinose	17.83 g/L	
Adenosine	1.34 g/L	
Allopurinol	0.136 g/L	
Total glutathione	0.922 g/L	
Glucose		195 mmol/L
Osmolarity	320 mOsm/kg	375 mOsm/kg

Effect of Hyperkalemia or Preservation Solutions (E-C or UW) on the EDHF-Mediated Relaxation by BK or A₂₃₁₈₇

The effect of hyperkalemia. For group I, 2 rings taken from the same microartery were mounted in the 2 chambers. After normalization procedure, 1 ring was incubated with hyperkalemia (K⁺ 115 mmol/L) for 1 hour at 37°C and the other was used as control (Krebs' solution). The rings were then repeatedly washed with Krebs' solution and incubated with INDO, L-NNA, and HbO. In the U₄₆₆₁₉-induced precontraction, cumulative concentration-relaxation curves to BK (group Ia, n = 8) or A₂₃₁₈₇ (group Ib, n = 7) were established.

In the hyperkalemic solution, K⁺ 115 mmol/L was used to replace the equivalent Na⁺ in the Krebs' solution.

Effect of E-C solution on the EDHF-mediated relaxation. For group II, 2 rings taken from the same microartery were mounted in the 2 chambers. One ring was incubated with E-C solution and the other with Krebs' as control for 4 hours at 4°C (in a refrigerator). After storage for 4 hours, both rings were repeatedly washed with Krebs' solution and the normalization procedure was performed. The rings were incubated with INDO, L-NNA, and HbO. In the U₄₆₆₁₉-induced precontraction, cumulative concentration-relaxation curves to BK (group IIa, n = 8) or A₂₃₁₈₇ (group IIb, n = 7) were established.

Effect of UW solution on the EDHF-mediated relaxation. For group III, the protocol was the similar to that in group II, except that the ring was incubated in UW solution instead of E-C solution.

Effect of UW and E-C solutions on the contractility of the pulmonary microartery. For group IV, 2 rings taken from the same microartery were mounted in the 2 chambers. One ring was incubated with E-C (group IVa) or UW solution (group IVb) and the other with Krebs' as control at 4°C (in a refrigerator). After storage for 4 hours, both rings were repeatedly washed with Krebs' solution and the normalization procedure was performed. Cumulative concentration-contraction curves to U₄₆₆₁₉ (-10 to -6.5 log M) were then established (n = 8).

Figure 1. Mean concentration-relaxation (% of contraction by U₄₆₆₁₉) curves for bradykinin (BK, n = 8) or A₂₃₁₈₇ (n = 7) with or without indomethacin (INDO, 7 μmol/L), N^G-nitro-L-arginine (L-NNA, 300 μmol/L), and oxyhemoglobin (HbO, 20 μmol/L). Two artery rings were taken from the same artery and allocated into the paired groups. Vertical error bars are 1 SEM. *P < .05, paired t test.

The compositions of E-C and UW solution are shown in Table 1.

In all experiments, only 1 concentration-relaxation (contraction) curve was obtained from each pulmonary ring. A mean concentration-relaxation curve was calculated from a group of experiments. During the experiment, the myograph chambers were continuously bubbled with a gas mixture of 95% O₂ and 5% CO₂.

Data Analysis

Relaxation was expressed as the percentage decrease in isometric force induced by U₄₆₆₁₉. The effective concentration of BK that caused 50% of maximal relaxation was defined as EC₅₀. The EC₅₀ was determined from each concentration-relaxation curve by a logistic, curve-fitting equation: $E = MA^P / (A^P + K^P)$, where E is response, M is maximal relaxation, A is concentration, K is EC₅₀ concentration, and P is the slope parameter. From this fitted equation, the mean EC₅₀ ± SEM was calculated for each group.

TABLE 2. Resting force (mN) in porcine pulmonary microarteries

Group	Bradykinin	Control	P	95% CI	Group	A ₂₃₁₈₇	Control	P	95% CI
Ia	1.1	1.1	.9	−0.3-1.9	Ib	1.1	1.2	.2	−0.4-0.1
IIa	1.0	1.0	.9	−0.4-0.4	IIb	1.3	1.3	.8	−0.2-0.2
IIIa	1.0	1.2	.2	−0.1-0.3	IIIb	1.3	1.1	.1	−0.5-0.1

Values are means $\pm$ SE. See "Methods" for the details of the groups. Two microarterial rings were taken from the same artery and allocated into the paired groups. *P* value was from the paired *t* test.

Statistical Analysis

Data were expressed as mean $\pm$ SEM and were analyzed with ANOVA (followed by Scheffé *F* test) or *t* test when appropriate. Values of *P* less than .05 were considered significant.

Drugs

Chemicals used and their sources were as follows: BK, A₂₃₁₈₇, L-NNA, Indo, HbO (Sigma Chemical Co, St Louis, Mo), U₄₆₆₁₉ (Cayman Chemical, Ann Arbor, Mich). L-NNA (dissolved in distilled water) and INDO (dissolved in ethanol) were stored at 4°C. The solutions of U₄₆₆₁₉, HbO, and BK were held frozen until required. The source of E-C solution (marketed as Euro-Collins Solution) is Fresenius AG (Homburg, Germany) and of UW solution (marketed as Via Span), The DuPont Merck Pharmaceutical Co (Wilmington, Del).

Results

Resting Force

After normalization, the arterial rings were set at 90% of the diameter equivalent to 40 mm Hg. This passive transmural pressure was 27.9 ± 0.4 mm Hg (*n* = 131), which is in physiological range. With regard to the resting force, there were no significant differences between the arteries exposed to hyperkalemia, E-C, UW, and control (Krebs') solution (*P* > .5). Table 2 gives the details of the resting force in these pulmonary microarteries.

U₄₆₆₁₉-Induced Contraction Force

In all porcine pulmonary microarterial rings, U₄₆₆₁₉ (−7.5 log M) induced a rapidly developed and stable tension (ranging from 2 to 3.5 mN), which was not significantly different compared with the control in all groups.

Role of EDHF-Mediated Relaxation Induced by BK or A₂₃₁₈₇

In control rings, BK or A₂₃₁₈₇ induced $97.1 \pm 2\%$ (EC50 -7.18 ± 0.40 log M, *n* = 8) or $73.7 \pm 7.6\%$ (EC50 -6.45 ± 0.43 log M, *n* = 7) relaxation. With the presence of INDO, L-NNA, and HbO, this relaxation (mediated by EDHF) reduced to $69.6 \pm 6.3\%$ (*P* < .003) with EC50 of -7.54 ± 0.18 log M (*P* = .39) or $38.7 \pm 4.3\%$ (*P* < .02) with EC50 of -6.34 ± 0.17 log M (*P* = .9) (Figure 1). These experiments demonstrate that EDHF plays a role in the pulmonary microarteries.

The effect of hyperkalemia. In group I, after exposure to hyperkalemia, the EDHF-mediated relaxation was significantly

reduced in either BK (group Ia: $38.6 \pm 3.0\%$ vs $59.1 \pm 7.4\%$ in control; 95% CI: 3.8% to 37.2%; *P* < .02) or A₂₃₁₈₇ (group Ib: $22.7 \pm 0.03\%$ vs $36.6 \pm 0.02\%$ in control; *P* < .01) subgroup (Figure 2).

With regard to the EC50, the exposure to hyperkalemia did not change the EC50 in either BK (group Ia: -7.41 ± 0.15 vs -7.52 ± 0.12 log M, *P* = .45) or A₂₃₁₈₇ (-6.84 ± 0.37 vs -6.16 ± 0.44 log M, *P* = .58) subgroup.

Effect of E-C solution on the EDHF-mediated relaxation. Similarly, in group II, after exposure to E-C solution, the EDHF-mediated relaxation was significantly reduced in either BK (group IIa: $51.9 \pm 8.4\%$ vs $60.3 \pm 6.1\%$ in control; 95% CI, 1.1% to 15.7%, *P* < .02) or A₂₃₁₈₇ (group IIb: $12.5 \pm 0.02\%$ vs $33.8 \pm 0.07\%$ in control; 95% CI: 0.05% to 0.37%, *P* < .02) subgroup (Figure 3).

With regard to the EC50, the exposure to E-C solution did not change the EC50 in either BK (group IIa: -7.41 ± 0.23 vs -7.51 ± 0.28 log M, *P* = .62) or A₂₃₁₈₇ (group IIb: -6.22 ± 0.46 vs -6.4 ± 0.28 log M, *P* = .68) subgroup.

Effect of UW solution on the EDHF-mediated relaxation. Again, in group III, after exposure to UW solution, the EDHF-mediated relaxation was significantly reduced in either BK (group IIIa: 49.3 ± 7.3 vs $65.2 \pm 3.5\%$ in control; 95% CI, 0.4% to 31.4%; *P* < 0.04) or A₂₃₁₈₇ (group IIIb: $13.2 \pm 0.03\%$ vs $31.0 \pm 0.05\%$ in control; *P* < 0.03, Figure 4) subgroup.

With regard to the EC50, the exposure to E-C solution did not change the EC50 in either BK (group IIIa: -7.69 ± 0.20 vs -7.34 ± 0.20 log M, *P* = .1) or A₂₃₁₈₇ (group IIIb: -7.32 ± 0.88 vs -6.66 ± 0.43 log M, *P* = .44) subgroup.

Effect of UW and E-C solution on the contractility of the pulmonary microartery. Compared with Krebs' solution, after incubation with E-C or UW solution for 4 hours, the U₄₆₆₁₉ (−10 to −6.5 log M)-induced maximal contraction was significantly decreased (group IVa: 1.5 ± 0.3 vs 2.4 ± 0.4 , 95% CI: 0.2% to 1.5, *P* < .01; group IVb: 1.2 ± 0.2 vs 2.2 ± 0.3 mN, 95% CI: −1.94 to −0.16, *P* < .02; Figure 5). However, there were no changes with regard to the EC50. After incubation with E-C solution, the EC50 was -7.53 ± 0.36 log M, compared with control of -7.69 ± 0.16 log M (*P* = .2), whereas after incubation with UW solution, the EC50 was -6.44 ± 0.46 log M vs -6.95 ± 0.43 log M in control (*P* = .43).

Figure 2. Mean concentration-relaxation (% of contraction by U_{46619}) curves for BK ($n = 8$) or A_{23187} ($n = 7$) in the presence of $7 \mu\text{mol/L}$ INDO, $300 \mu\text{mol/L}$ L-NNA, and $20 \mu\text{mol/L}$ HbO after exposure to hyperkalemia ($K^+115 \text{ mmol/L}$) or Krebs' solution (control) at 37°C for 1 hour. Two artery rings were taken from the same artery and allocated into the paired groups. Vertical error bars are 1 SEM. * $P < .05$, paired t test.

Figure 3. Mean concentration-relaxation (% of contraction by U_{46619}) curves for BK ($n = 8$) or A_{23187} ($n = 7$) in the presence of $7 \mu\text{mol/L}$ INDO, $300 \mu\text{mol/L}$ L-NNA, and $20 \mu\text{mol/L}$ HbO after exposure to Euro-Collins (E-C) or Krebs' solution (control) at 4°C for 4 hours. Two artery rings were taken from the same artery and allocated into the paired groups. Vertical error bars are 1 SEM. * $P < .05$, paired t test.

Discussion

The present study has demonstrated that (1) EDHF-mediated (INDO-, L-NNA-, and HbO- resistant) endothelium-dependent relaxation exists in the porcine pulmonary microartery; (2) after exposure to hyperkalemia ($K^+115 \text{ mmol/L}$), the major component in organ preservation solutions, the relaxation is reduced; (3) the preservation of lungs in hypothermic organ preservation solutions (E-C or UW solution) for the clinically relevant period reduces the EDHF-mediated relaxation; and (4) the storage may also affect the contractility of the microartery.

EDHF-Mediated Endothelial Function Exists in the Pulmonary Microcirculation

Unlike NO and PGI_2 , which have been well studied, the nature of EDHF has not been finally identified, although most re-

cently the cytochrome P450-monooxygenase metabolites of arachidonic acid have been suggested to be EDHF.¹⁵ EDHF induces vascular smooth muscle relaxation via hyperpolarization of the smooth muscle cells, which involves potassium (K^+) channels.^{6,9,12,15-17} However, all of these EDRFs are released in response to the increase of intracellular (cytosolic-free) calcium concentration in the endothelial cell.⁶

There is evidence that NO and EDHF are two primary mechanisms of endothelium-dependent relaxation.¹⁸ However, it is unclear whether EDHF plays a role in the pulmonary microcirculation. We have previously demonstrated that to study EDHF, it is essential to have all NO production eliminated by not only NO synthesis inhibitors such as L-NNA, but also NO scavenger HbO.¹²⁻¹⁴ Therefore, in the present study, L-NNA and HbO were present in all experiments to study EDHF. Under such circumstance, BK or

Figure 4. Mean concentration-relaxation (% of contraction by U_{46619}) curves for BK ($n = 8$) or A_{23187} ($n = 7$) in the presence of $7 \mu\text{mol/L}$ INDO, $300 \mu\text{mol/L}$ L-NNA, and $20 \mu\text{mol/L}$ HbO after exposure to University of Wisconsin (UW) or Krebs' solution (control) at 4°C for 4 hours. Two artery rings were taken from the same artery and allocated into the paired groups. Vertical error bars are 1 SEM. * $P < .05$, paired t test.

A_{23187} induced 69.6% or 38.7% relaxation. These two agonists induce endothelium-dependent relaxation through different mechanisms. BK-induced release of endothelium-derived relaxing factors is receptor-dependent as BK stimulates B_2 receptors on the endothelial cell to release a number of relaxing factors.¹⁹⁻²¹ Additionally, BK induces vasodilatation through a potassium channel-dependent mechanism via endothelium.²² In contrast, A_{23187} is a calcium ionophore that directly increases free Ca^{2+} in the endothelial cells and triggers the cascade of events in endothelial cell activation, leading to the release of endothelium-derived vasodilators.²³ The fact that in the present study both BK and A_{23187} induced a significant relaxation when the production of NO and PGI_2 were abolished suggests the existence and the role of EDHF in the pulmonary microvasculature.

Figure 5. Mean contraction force (mN) to U_{46619} (-10 to $-6.5 \log \text{M}$) after incubation with Euro-Collins (E-C) (A) or University of Wisconsin (UW) solution (B) and Krebs' solution (Control) at 4°C for 4 hours. Two artery rings were taken from the same artery and allocated into the paired groups. * $P < .05$, $n = 8$, respectively, paired t test.

Hyperkalemia Exposure Reduces EDHF-Related Relaxation and Possible Mechanism

High potassium concentration is one of the major components in either cardioplegia or organ preservation solution. It has been repeatedly demonstrated that hyperkalemia may affect the endothelial function. We have previously demonstrated that hyperkalemia affects the coronary endothelial function in either the large conductance^{3,6,9} or the micro-(resistance) artery⁴ through the EDHF mechanism. This is because hyperkalemia depolarizes the membrane potential of the smooth muscle of the coronary artery that interacts the hyperpolarization by EDHF. In the present study, we have for the first time demonstrated that hyperkalemia also affects the EDHF pathway in the pulmonary microarteries. This shows that the effect of high potassium on the EDHF mechanism is independent to the type of vessel. This phe-

nomenon has clinical importance as high potassium is contained in many clinically used solutions, as mentioned previously.

The Effect of E-C and UW Solutions on the EDHF-Mediated Relaxation in the Pulmonary Microarteries

E-C and UW solutions are of the intracellular type solutions and are clinically used most frequently in lung transplantation, with E-C and UW being used in 77% and 25% of centers.²⁴ E-C and UW solutions are designed for cold storage of the organs. Hypothermic flush perfusion is the method most commonly used for pulmonary preservation in clinical practice to limit ischemic injury to the lung, although some studies have shown that lung preservation is superior when a more moderate hypothermia with a temperature of 10°C is used.²⁵ However, because of the concerns regarding the deleterious effects of flush and storage temperatures greater than 10°C and the difficulties in maintaining this temperature during the procurement procedure, clinical flush perfusion continues to be performed at the lower temperatures. In lung transplantation surgery, most flush solutions are administered at 4°C so that metabolism is slowed by nearly 80% to 90%. We therefore studied the effect of the preservation with E-C and UW solutions at 4°C.

Further, preservation of the lung should involve both parenchyma and vasculature of the lung. Preservation of pulmonary endothelium has been looked at as a factor for better lung preservation.²⁶⁻²⁸ However, although endothelium-dependent relaxation in pulmonary arteries has been studied,²⁶⁻²⁸ due to the complexity of the endothelial function, previous studies only looked at the function of endothelium as a whole and there have been no reports on the effect of preservation methods by using either E-C or UW solution on the particular EDHF-mediated endothelial function.

As hyperkalemic solutions, the concentration of potassium in the E-C is as high as 115 mmol/L, and in UW solution, 125 mmol/L. As mentioned previously, such high potassium concentrations may affect EDHF-mediated relaxation and this has been demonstrated in the coronary arteries.^{3,4,6-8} However, the components in both E-C and UW solutions are complex (see Table 1) and these components in the solutions may interact each other. It is therefore difficult to predict the final action of the solution on the endothelial function. Moreover, the effect of either E-C or UW solution in the pulmonary microarteries has not been reported. The present study clearly demonstrates that the EDHF-mediated relaxation induced by either BK or A23187 is reduced after the cold storage with either E-C or UW solution for a clinically relevant period (4 hours). This fact should be taken into account in the clinical lung preservation for transplantation to overcome this shortage of the preservation method.

Study Limitations

The present study is an in vitro experimental investigation. In this model, the individual EDRFs may be accurately separated and studied in microarteries. However, in vivo animal or human studies may provide further insights on the impairment of the EDHF-mediated function in the pulmonary circulation.

Conclusions

In summary, the present study demonstrated that hypothermic storage with hyperkalemic organ preservation solutions such as E-C or UW solutions impair a special pathway of the endothelial function (ie, the EDHF-pathway in the pulmonary microarteries). Because the microarteries are the major resistance arteries in the pulmonary circulation, the reduced EDHF-mediated relaxation in the microcirculation after the storage may impair the flow capacity of the pulmonary artery by increasing the pulmonary arterial resistance during the reperfusion period. This may have adverse effects in the clinical lung transplantation. The present study therefore opens a new area of the lung preservation in transplantation surgery. Better preservation methods should be explored to further improve the results of lung transplantation surgery.

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