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<td>Kobayashi, Masahiro</td>
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NAOSITE: Nagasaki University’s Academic Output SITE
A 24 Hour Lung Storage Using Low Potassium UW Solution

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The availability of low potassium UW (University of Wisconsin) solution was experimentally evaluated by using model of isolated donor lung in dogs. Isolated lung was prepared by flushing with low potassium UW solution, stored at 4 °C for 24 hours.

The experiment was divided into the following five groups. G I (Group I): control, G II: stored with Euro-Collins solution, G III: stored with original UW solution, G IV: stored with low potassium UW solution (K⁺ = 30 mEq/l) and G V: stored with lower potassium UW solution (K⁺ = 4 mEq/l). Pulmonary vascular resistance (PVR) showed 117 ± 17 in G I , 271 ± 1.11 in G II , 140 ± 36 in G III , 8.7 ± 31 in G IV , 83 ± 24 in G V at 120 min after reperfusion. PVR in G IV and V were significantly lower than that in G III immediately after reperfusion.

The pulmonary extravascular water content (PEWC) showed 82.2 ± 2.2 in G I , 89.6 ± 0.8 in G II , 85.6 ± 1.6 in G III , 84.2 ± 1.3 in G IV , 86.3 ± 0.7 in G V . PEWC in G II , III , IV and V were apparently higher than that in G I and PEWC in G IV was the lowest. The histologic findings of stored lungs indicated the patterns of lung edema. The lipoperoxide in the blood of stored lungs was significantly higher than that of G I at 120 min. There was no significant difference among G III , IV and V . The tissue malondialdehide (MDA) levels in G IV and V were lower than that in G III with not statistically significant difference.

In conclusion, it was apparent that 30 mEq/L of low potassium concentration was the best to preserve a donor lung function for a 24 hour storage on the basis of a result of reperfusion model experiment.

Introduction

The donor lung storage was limited to four to six hours by means of immerse cooling and a possible long-term storage has been required for the development of further beneficial methods.

It is reported that UW solution is of great value in preservation of a donor lung in our institute. There are many reports that the K⁺ concentration is effective in approaching the composition of the extracellular fluid. The purpose of this study is to clarify as to whether low potassium UW solution is available for a 24 hour storage.

Material and Methods

1) Laboratory study

Thirty-four adult mongrel dogs weighting 8-11 kg were prepared and provided from the Laboratory Animal Center for Biochemical Research of Nagasaki University School of Medicine.

2) Operative method: The basic preparation has been previously described in detail.

The animals were anesthetized with intravenous administration of sodium pentobarbital, 30 mg/kg. They were intubated orotracheally and ventilated with 100% O₂ using a volume-cycled ventilator (Harvard-ventilator) at a tidal volume of 300 ml and rate of 14 cycles/min. A median sternotomy incision was made, and the great vessels were dissected. After the right main bronchus was clamped, the airway and flow rate were measured. Fifty-hundred units of heparin sodium was administered intravenously, and both lungs were removed with the heart.

3) Wash out method

After both lungs and the heart were excised, the left lung was isolated and a polyethylene cannula was secured in the left pulmonary artery. An endotracheal tube (8.5 mm in diameter) was placed in the left main bronchus. The lung was flushed with approximately 250-300 ml of 4-6 °C EC or UW or low potassium UW solution through the left pulmonary artery at a hydrostatic pressure of 25 cmH₂O. During flushing, the left lung was ventilated with room air using a Harvard ventilator at a tidal volume of 200 ml and rate of 10 cycles/min.
4) preservation

After flushing, the left lung was inflated to 40% of the endotidal volume, and the left main bronchus was clamped. The graft was placed in a plastic box filled with the same flush solution, and stored in a refrigerator at 4-6 °C for 24 hours.

5) reperfusion

After a hypothermic preservation period, the isolated left lung was reperfused for 120 min.

An arterial cannula was connected to a reservoir, and the pulmonary vein was left open allowing lung perfusate to drain freely into the reservoir. The left lung was ventilated with room air at a tidal volume of 200 ml and a rate of 10 cycles/min.

As a perfusate, fresh allogeneous blood, which had been drawn from the femoral vessel was used. The perfusate was pumped continuously by a roller pump at a flow rate of 100 ml/min, and maintained at 37 °C by a small temperature-controlled warm bath (Fig. 1).

6) experimental groups

The animals were divided into the following five groups according to the preservation solutions.

- Group 1. nonpreserved lungs (control) (n = 7)
- Group 2. lungs were flushed with EC solution and cold stored (4-6°C) in the same flush solution for 24 hr (N = 7)
- Group 3. lungs flushed and cold-stored with UW solution for 24 hours (n = 7)
- Group 4. lungs flushed and cold-stored with modified UW solution (K⁺:30 mEq/L) for 24hr (n = 7)
- Group 5. lungs flushed and cold-stored with modified UW solution (K⁺:4 mEq/L) for 24hr (n = 6)

The composition of the preservation solution is shown in Table 1.

### Table 1. Composition of preservation solution

<table>
<thead>
<tr>
<th>Component</th>
<th>UW (mM)</th>
<th>UW K⁺ 30 mEq/L (mM)</th>
<th>UW K⁺ 4 mEq/L (mM)</th>
<th>Euro-Collins (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K lactobionate</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Na lactobionate</td>
<td>-</td>
<td>100</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>KCl</td>
<td>-</td>
<td>-</td>
<td>15</td>
<td>42.5</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>25</td>
<td>25</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>NaH₂PO₄, 2H₂O</td>
<td>-</td>
<td>-</td>
<td>21</td>
<td>-</td>
</tr>
<tr>
<td>NaCO₃</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10</td>
</tr>
<tr>
<td>MgSO₄, 7H₂O</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.5%</td>
</tr>
<tr>
<td>Raffinose</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>Glutathione</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Adenosine</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Penicillin</td>
<td>200,000</td>
<td>200,000</td>
<td>200,000</td>
<td>-</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>16 mg</td>
<td>16 mg</td>
<td>16 mg</td>
<td>-</td>
</tr>
<tr>
<td>Insulin</td>
<td>40 U</td>
<td>40 U</td>
<td>40 U</td>
<td>-</td>
</tr>
<tr>
<td>Hydroxyethyl starch</td>
<td>50 g</td>
<td>50 g</td>
<td>50 g</td>
<td>400 U</td>
</tr>
<tr>
<td>Na⁺</td>
<td>30 mEq/L</td>
<td>130 ± 7 mEq/L</td>
<td>143 ± 4 mEq/L</td>
<td>10 mEq/L</td>
</tr>
<tr>
<td>K⁺</td>
<td>120 mEq/L</td>
<td>29 ± 1 mEq/L</td>
<td>4 ± 0.3 mEq/L</td>
<td>115 mEq/L</td>
</tr>
<tr>
<td>Osmotic P.</td>
<td>320 mOsm/L</td>
<td>342 ± 11 mOsm/L</td>
<td>339 ± 14 mOsm/L</td>
<td>355 mOsm/L</td>
</tr>
<tr>
<td>pH (room temp.)</td>
<td>7.4</td>
<td>7.4</td>
<td>7.4</td>
<td>7.2 - 7.5</td>
</tr>
</tbody>
</table>
8) Data

Blood gases, the count of white blood cells, and the amount of serum malondialdehyde were measured during the reperfusion period every thirty minutes. Pulmonary arterial pressure and airway pressure were monitored, and static lung compliance and pulmonary vascular resistance were calculated as follow:

1. Static lung compliance:
The static lung compliance was calculated as tidal volume/pressure (ml/cmH2O) at 1.2 sec of the end-inspiratory plateau.

2. Pulmonary vascular resistance:
The pulmonary vascular resistance was calculated as pulmonary artery pressure/flow rate (mmHg/L/min)

3. Serum malondialdehyde (MDA):
The serum malondialdehyde was measured by thiobarbituric acid (TBA) assay by means of Lipoperoxide-Test (Wako).

4. Lung tissue lipid peroxidation:
Lung tissue lipid peroxidation was measured as TBA reactive material. The assay was performed during the reperfusion period every sixty minutes. A specimen was homogenized to yield a 10% homogenate. Then 0.2 ml of homogenate was added to 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (pH3.5), and 1.5 ml of 0.8% TBA. The mixture was diluted up to 4 ml with distilled water, heates at 95 °C for 60 minutes, and then cooled. 1 ml of water and 5 ml of butanol/pyridine (15:1, vol. vol) were added. The solution was centrifuged at 3,000 rpm for 10 min.

The fluorescent intensity of the top layer was read in fluorospectrophotometry, excitation 515 nm and emission 553 nm. The lipoperoxide concentration (nmol/l) was determined by reference to a standard MDA.

Homogenate protein content was measured by Lowry's method, then TBA reactive material was expressed as nanomoles per mg protein.

5. The wet/dry weight ratio:
After the 120 min reperfusion, lung water in the left lower lobe was determined as follows:

Reperfused lungs were weighed and placed in a desicator at 110 °C for 48hr, after that time, and the dry lungs weighed. Then lung water was calculated by the following formulas:

\[(\text{Wr-Wd})/\text{Wr, where} \]

\[\text{Wr = weight of reperfused lobe} \]

\[\text{Wd = blood free dry weight of the lobe} \]

6. Histology:
All reperfused pulmonary lobes were fixed in formalin and stained with hematoxylin and eosin for histologic examination.

7. Statistics:
The results are given as means ± SEM. Statistical evaluation was made using the Mann-whitney's U-test. A value < 0.05 was considered statistically significant.

Results

1. Blood gas analysis: There was not statistically significant difference in PaO2 between each groups and there was no time-course change in PaO2 between each groups and there was no time-course change in PaO2 with a 30 minute elapse of time.

2. Blood examination: The leukocyte counts of each groups were significantly lower than those of the control at 30 min.

3. Intratracheal pressure, air flow volume: The intratracheal pressure in G II was higher than that in the other four groups immediately after reperfusion. In contrast, at 120 min it reached at a significantly high level. However, there was no statistically significant difference between G III and IV, V.

Mongrel dogs (average body weigh 10 kg)

Pentobarbital 30 mg/kg iv

Harvard ventilator (35 ml/kg 14f FiO2 = 1.0)

Midsternotomy heparin 3 mg/kg iv
evacuate blood

en bloc removal of the heart-lung

Isolation of left lung, PA cannulation

reperfusion

Condition of ventilator: VT = 20 ml/kg/breath, RR = 10/min, Room air

Conditions of reperfusion: Roller pump 100 ml/min, 37 °C, 2 hours

Measurements: WBC, PaO2, PA pressure, PVR, Airway pressure

Static lung compliance, Extravascular lung water

Serum lipid peroxide tissue lipid peroxide

Fig. 2. Protocol of the experiment

4. Statistic lung compliance: The value of statistic lung compliance in G II was higher than that in the other four groups immediately after reperfusion, at 120 minutes, it showed a significantly higher value. However, there was no statistically significant difference in the values of statistic lung compliance between G III and IV, V.

5. Pulmonary pressure (PAP), pulmonary vascular resistance (PVR): The PVR in G II showed a higher level as compared with those in the other four groups and those in IV, V were lower than that in G III. At 120 min, these were obviously lowered.

6. Serum lipoperoxide: The levels of serum lipoperoxidate in G II -V were higher than that in G I at 120 min. However, there was not statistically significant difference among G II to V (Fig. 3)

7. Tissue lipoperoxide: The MDA levels in G III, IV, V were significantly lower than that in G II. There was not significant difference between G III and G IV, V (Fig. 4).
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Table 2. Result after a 120 min perfusion

<table>
<thead>
<tr>
<th></th>
<th>Group 1 (n = 7)</th>
<th>Group 2 (n = 7)</th>
<th>Group 3 (n = 7)</th>
<th>Group 4 (n = 7)</th>
<th>Group 5 (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (10^9/L)</td>
<td>3400 ± 1100</td>
<td>2800 ± 950</td>
<td>3400 ± 2000</td>
<td>1800 ± 640</td>
<td>3100 ± 2200</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>143 ± 12.6</td>
<td>166.4 ± 21.1</td>
<td>162.4 ± 9.5</td>
<td>150.7 ± 19.9</td>
<td>150.0 ± 15.1</td>
</tr>
<tr>
<td>PVR (mmHg/L/min)</td>
<td>117 ± 17.1</td>
<td>271 ± 111.1</td>
<td>140 ± 38.0</td>
<td>87 ± 31.0</td>
<td>83 ± 24.0</td>
</tr>
<tr>
<td>AWP (cmH₂O)</td>
<td>16.0 ± 3.6</td>
<td>30.4 ± 8.6</td>
<td>19.9 ± 3.4</td>
<td>19.7 ± 3.8</td>
<td>21.2 ± 3.8</td>
</tr>
<tr>
<td>Cst (ml/cmH₂O)</td>
<td>15.8 ± 1.5</td>
<td>9.7 ± 2.5</td>
<td>12.8 ± 2.0m</td>
<td>13.1 ± 2.3</td>
<td>15.1 ± 3.3</td>
</tr>
<tr>
<td>Wr (g)</td>
<td>34.0 ± 9.0</td>
<td>77.9 ± 27.1</td>
<td>45.6 ± 23.4</td>
<td>50.1 ± 11.1</td>
<td>50.7 ± 16.1</td>
</tr>
<tr>
<td>Wd (g)</td>
<td>6.1 ± 1.7</td>
<td>7.9 ± 2.4</td>
<td>6.5 ± 3.3</td>
<td>8.0 ± 2.2</td>
<td>7.0 ± 2.5</td>
</tr>
<tr>
<td>(Wr-Wd) × 100/Wr (%)</td>
<td>82.2 ± 1.2</td>
<td>89.6 ± 0.8</td>
<td>85.8 ± 1.6</td>
<td>84.2 ± 1.3</td>
<td>86.3 ± 0.7</td>
</tr>
</tbody>
</table>

PVR: pulmonary vascular resistance  AWB: air way pressure  Cst: static lung compliance  Wr: wet lung weight  Wd: dry lung weight

p < 0.01; *: p < 0.05; " vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. ', ' vs. '

8. The lung wet/dry weight ratio: The lung wet/dry weight ratio in G II was apparently higher than those in the other groups. As compared with G III, IV and V, that in G IV was obviously lower that those in the other two groups (Table 2).

9. histologic examination: Fig. 5 showed a histologic pattern in G I, revealing almost the same as the normal. Fig. 6 showed a histologic findings in G II after perfusion, showing the findings of thickened alveolar septum, accumulation of exudate in the alveolar space, atelectasis, congestion of small vessels and perivascular bleeding.

82.2 ± 1.2
85.8 ± 1.6

Fig. 3. Serum lipoperoxide
Fig. 4. Tissue lipoperoxide

Fig. 5. Histology in Group I, demonstrating almost the same finding as the normal

Fig. 6. Histology: lung in Group II after perfusion (HE staining × 20)
congestion in the small vessels, perivascular bleeding with which the finding of lung edema was compatible. On the other hand, Fig 7A, B and C were the histologic findings of G III, IV and V, showing almost the normal lung structures.

Fig. 7. Histologic finding in Group III (A), IV (B) and V (C) after perfusion, showing almost the normal finding (HE staining ×20)

Discussion

It is generally said that a safe-time limitation of a donor lung storage is limited to four to six hours. Prolonged storage method is now necessary to extend a clinical use. A donor lung storage by cooling has experimentally been widespread as reported by Blumenstock. Tsuji, who had emphasized that 1) it is needed for washing out using infusate with optimal osmotic pressure after isolation of a donor lung and 2) for inflating a donor lung during the period of storage. Collins reported that preservation solution with high concentration of potassium inhibits a loss of potassium ion and Collins solution which resemble a constitution of intracellular fluid has been established as one of the preservation solutions. He succeeded in a 30 hour storage of the kidney. Thereafter, Dreikorn developed EC solution and it had made it possible to storage the kidney up to 48 hours and the liver up to eight hours respectively.

On the other hand, the intracellular fluid has been used in the field of lung storage research. Veith modified original UW solution to 120 mEq/l of Na+ and 30 mEq/l of K+ and utilized it to heart transplantation with a favorable result. On the other hand, Starkey et al indicated that flushing from the pulmonary artery with a large amount of solution resembling intracellular fluid induces vasoconstriction at the initial phase. Richard reported that flushing from the pulmonary artery with cold EC solution causes increased PVR and decreased pulmonary compliance. Unruh clarified that PVR increased in the response to infusion of cold EC solution, which results in vasoconstriction and/or occlusion by direct action of EC solution using reperfusion model of left lower lobe in dogs.

Recently, vasodilatation agents such as PGE or prostacyclin have become widely used to prevent vasoconstriction by intracellular fluid infusion. Handa et al have been developed Ep solution (Na+:115 mEq/l, K+:26.5 mEq/l) with colloid osmolar pressure and buffer action, which are essential in lung preservation and a 96 hour lung preservation was progressed by using Ep solution. Kawahara also clarified that original UW solution is of great value in a 24 hour donor lung preservation.

In this study, low PVR of the stored lung at 120 min after reperfusion was obtained by using low potassium UW solution in spite of not significant difference in the intracheal pressure and static lung compliance as compared with the use of original UW solution. It means that vasoconstriction by low K+ containing solution is slight as compared with that by high K+ one although there is not significant difference in histologic findings between both
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solutions. Further accumulated studies are needed for assessing how much vasoconstriction caused by high potassium containing solution is influential on graft failure. EVLW was much more reduced by 30 mEq/L of low K+ containing solution than by 4 mEq/L at 120 min after reperfusion despite an increase in EVLW even by low potassium UW solution.

A few reports explain the optimal K+ concentration. As indicated by this result, 30 mEq/L potassium ion concentration is of great value in prolongation of lung preservation time. The PaO2 is one of the monitors to indicate the function of a donor lung. However, the drawback of isolated lung perfusion model fails to measure PaO2, because there is nothing to deoxygenate in the circuit.

It is accepted that reperfusion injury is caused by oxygen free radicals, prostaglandin metabolites and calcium influx. In addition, it has been elucidated that the addition of oxygen free radical scavenger, calcium antagonist, and lipoxygenase inhibitors is influential on graft failure. UW solution contains glutathione, which is oxygen free radical scavenger and allopurinol which inhibits xanthine oxidase.

In this study, the serum lipid peroxide was measured to assess as to which effect represents in the amount of the endothelium of the pulmonary artery. As a result, there were not statistically significant differences in lipoperoxide among EC, UW and low potassium UW groups. In contrast, at 120 min after reperfusion it in UW and low potassium UW groups was significantly lower than that in EC group.

Miya reported that the serum MDA is one of the monitors, which evaluates the degree of tissue damage by oxygen free radical. Ide also clarified that the measurement of lipoperoxidate of the lung tissues is more valuable in assessing damage to the lung rather than that of pulmonary vein. It is generally known that free oxygen radical is generated by activated xanthine oxidase, at reperfusion generation of free oxygen radical plays an important role in reperfusion injury. It is assumed that UW and low K+ UW solutions serve as inhibition of not only generation of lipoperoxide in the lung but generation of free oxygen radical of granulocytes at reperfusion.

In the circulation model used in this study, the leukocyte counts at the initial stage of reperfusion was significantly diminished. It is obvious that trapping of leukocytes takes place not only in the circuit but in the lung.

As reported by Belzer, the addition of oxygen free radical scavenger such as SOD is essential so that a prolonged donor lung storage time may be obtained to facilitate a clinical use little by little.

Acknowledgement

I would like to express deep appreciation to Prof. Masao Tomita, The First Department of Surgery, Nagasaki University School of Medicine and Dr. Katsunobu Kawahara and other staff of Department of their cooperation and also appreciate for kindness of animal supply from the Laboratory Animal Center for Biochemical Research of Nagasaki University School of Medicine.

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References


