Reperfusion injury and the effect of leukocyte depletion on a storage lung
—Comparison of UW solution and Euro-Collins solution—

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Summary: Using isolated perfusion model of a donor lung preserved for 24 hours, the efficacy of UW solution in lung preservation was evaluated in comparison with Euro-Collins (E-C) solution as well as the inhibitory effect of leukocyte depletion on post-ischemic reperfusion injury. The following results were obtained.

1) After 24 hours of preservation, the lung functions were maintained more favorably in UW solution when compared with the results obtained with E-C solution, indicating that UW solution the best for a long-term preservation of a donor lung.

2) By depleting leukocytes at the time of reperfusion, it was possible to minimize the reperfusion injury.

3) The model used in the present study was available for simple and useful means of evaluating the validity of lung preservation solution and the intensity of reperfusion injury.

Introduction

Lung transplantation was first applied for clinical use in 1963 by Hardy,[1] and since Cooper et al[2] in 1983 reported a long time survival case. Lung transplantation has been drastically facilitated. This, however, caused a shortage of donor lung procurement as in the case of other organs. In this regard, it is believed that the development of a preservation solution which can maintain viability of a donor lung makes it possible to presure greatly for a long time. When compared with conventional preservation solutions, UW (University of Wisconsin) solution, a new preservation solution recently developed by Belzer et al[3] enables organs such as the liver, kidney and pancreas[3-5] to preserve for longer time.

As a post-transplant problem, organ injury by the active oxygen generating at the time of post-ischemic reperfusion has recently been attracting attention, and leukocyte (polynuclear leukocyte) has been pointed out as one of the active oxygen sources. Therefore, an isolated perfusion model was used for the evaluation of viability of dog's lung preserved for 24 hours, so that the efficacy of lung preservation was compared between UW and Euro-Collins (E-C) solutions. And also the purpose of this study is to investigate the inhibitory effect of leukocyte depletion from the postischemic reperfusion injury.

Materials and Methods

Thirty-five mongrel adult dogs weighing 8-15 kg were used. These dogs were placed under intravenous anesthesia by pentobarbital (25 mg/kg) for endotrachial intubation. Using Harvard Ventilator, the respiration was adjusted under the following ventilation conditions; $F_iO_2 = 1.0$, tidal volume 15 ml/kg, 14 times/min. Midline sternotomy was conducted, and after intravenous injection of heparin (3 mg/kg), the heart and lungs were extracted en bloc. Then the left lung alone was isolated. A tube with the internal diameter of 8.5 mm was inserted into the trachea while a 16 Fr cannula was ensured the pulmonary artery. The lung was flushed with 250-300 ml of 4 °C E-C solution or UW solution through the pulmonary artery cannula at hydrostatic pressure of 25 cm H_2O, while the left lung was ventilated by room air (200 ml/ventilation, 10 times/min). After completion of flush out, the left lung was inflated with room air to 40% of the total lung capacity, at which state the left main bronchus was clamped and placed in a plastic container filled with same flush solution for 24 hours of immersion at 4-6 °C . After 24 hours, the left lung was transferred to another plastic container and placed under ventilation by room air (200 ml per ventilation, 10 times/min). The roller pump was then washed with the fresh whole blood taken from the femoral artery of a donor dog and kept warm at 37 °C in a warm bath or the blood from which leukocyte was depleted by passing an Imuguard IG-400 (Thermo Co.) filter. Using this roller pump, reperfusion was then conducted at a flow rate of 100 ml/min for 120 minutes. In carrying out reperfusion, the flow rate was gradually increased to the prescribed rate in approximately 5 minutes from the start (Fig. 1).

A. Test groups

The dogs were divided into the following 5 groups.

Group I : No-preservation group (n=7)
 After extracting the left lung, ventilation and reperfusion were immediately conducted.

Group II : After preservation in E-C solution for 24 hours, the left lung was reperfused with whole blood (n = 7).
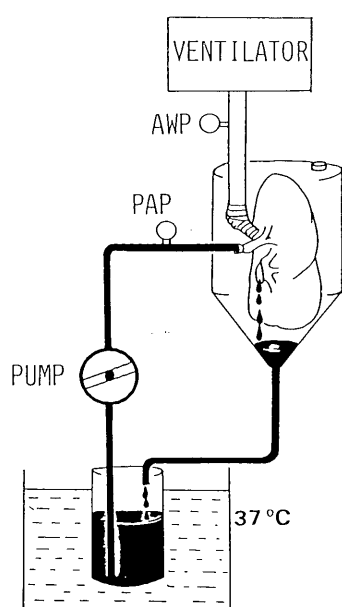
Fig. 1. Schema of isolated perfusion lung model
AWP: Airway Pressure, PAP: Pulmonary arterial pressure

Group Ⅲ : After preservation in E-C solution for 24 hours, reperfusion was conducted with leukocyte-depleted blood. (n = 7)

Group Ⅳ : After preservation in UW solution for 24 hours, the left lung was reperfused with whole blood (n = 7).

Group V : After preservation in UW solution for 24 hours, reperfusion was conducted with leukocyte-depleted blood (n = 7).

B. Measurement items

1. Peripheral leukocyte count

The blood from femoral artery before reperfusion (before and after filtration in the case of Group Ⅲ and V) and that from the pulmonary vein every 30 minutes during the reperfusion process were taken for determination of leukocyte count by automatic hemocalculator.

2. Arterial blood gas analysis

The blood was taken from each group before reperfusion (blood from femoral artery, $F_iO_2=1.0$) and every 30 minutes during reperfusion (blood from pulmonary vein, room air) for determination.

3. Pulmonary vascular resistance

The pulmonary arterial pressure was continuously recorded on a polyrecorder. With the pulmonary vein open and the pressure at 0 mmHg, the pulmonary vascular resistance was calculated from the average pulmonary arterial pressure/flow rate (mmHg/L/min).

4. Airway pressure, Total static compliance of the lung (C_{ST}) Inspiratory flow speed, airway pressure and ventilation volume were simultaneously recorded before and immediately after reperfusion as well as every 30 minutes during

the reperfusion. At the end of inspiration, the air flow was adjusted to zero for 1.2 second, and using this final pressure (P), C_{ST} was calculated as the ventilation volume/P (ml/cmH_2O).[6] In determining the value before reperfusion, the right main bronchus was clamped before extraction of the heart and lungs, and the measurement was taken while ventilating the left lung under the same conditions as applied at the time of reperfusion.

5. Extravascular lung water

The left lower lobe was weighed (wet weight) immediately after the completion of reperfusion. This was then dried for 48 hours at 100 ℃ using an electric automatic oven, after which it was measured again (dry weight). The evaluation was made by calculating the wet/dry weight ratio.

6. Lipid peroxide (serum, tissue)

Malondialdehyde (MDA) was measured by TBA method (by Yagi[7] method in the case of serum and by the method of Okawa et al.[8] in the case of tissue). Blood from the pulmonary vein was taken every 30 minutes while tissue was taken before preservation, after 24 hours of preservation, and after 120 minutes of reperfusion. The MDA value of the tissue was corrected by the protein content determined by Lowry method for evaluation.

7. Pathohistological examination

The left upper lobe after 120 minutes of reperfusion was fixed in 10% formalin for hematoxylin eosin staining.

8. Statistical analysis

The values obtained were expressed in Mean ± SD, and a significant difference was assessed by Wilcoxon's test. A difference of p < 0.05 was regarded as statistically significant.

Results

1. Peripheral leukocyte count (Fig. 2)

In Group Ⅲ and V (leukocyte-depleted group), the values obtained after filtration were almost similar (respectively
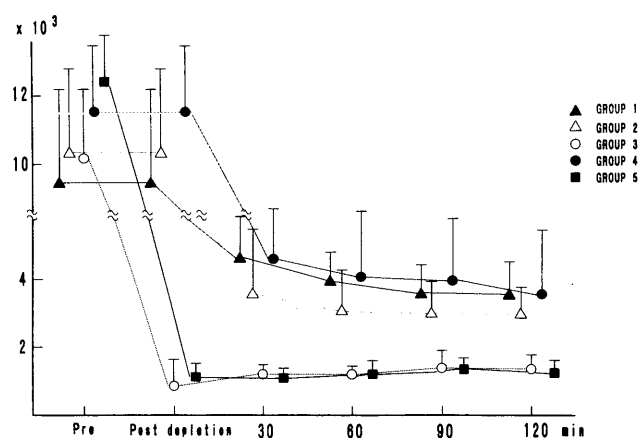

Fig. 2. Peripheral leukocyte count

9.8 and 7.9% of the prefiltration values), and any fluctuation was hardly observed 30 minutes after the start of reperfusion and onward. The values in Group I , II and IV (whole blood group) 30 minutes after the start of reperfusion were reduced to respectively 48.0, 32.6 and 39.1% of the prereperfusion values, and a significant decrease was observed in Group II compared with Group I (p < 0.05).

Compared with the whole blood groups, significantly lower values were observed in the leukocyte-depleted groups up to 120 minutes after the start of reperfusion (p <

0.01).

2. Arterial blood gas analysis (Table 1)

Compared with the values before reperfusion, PaO_2: in each group 30 minutes after the start of reperfusion significantly decreased, but hardly any fluctuation was observed thereafter. No significant difference was noted between the groups, either.

Table 1. Blood gas analysis -PaO_2- (mmHg)

	Pre	30	60	90	120
Group 1	264.5 ± 63.4	134.1 ± 14.1	132.3 ± 21.7	138.8 ± 16.7	143.3 ± 12.6
Group 2	366.0 ± 91.3	151.2 ± 6.9	157.3 ± 23.9	161.1 ± 22.6	166.4 ± 21.2
Group 3	329.7 ± 83.3	158.2 ± 7.5	155.3 ± 11.1	158.1 ± 9.9	155.9 ± 10.0
Group 4	324.2 ± 103.0	155.7 ± 18.9	154.8 ± 17.2	149.9 ± 6.8	162.4 ± 9.5
Group 5	288.5 ± 54.0	158.7 ± 14.7	157.0 ± 18.8	156.9 ± 18.9	152.6 ± 17.4

3. Pulmonary vascular resistance (Fig. 3)

Compared with other 4 groups, Group II demonstrated significantly higher values immediately after reperfusion (p < 0.05). The 120 minute value was 117.5 ± 17.2 (mmHg/L/min, the same below) in Group I , 270.8 ± 111.3 in Group II , 152.5 ± 39.8 in Group III , 140.1 ± 37.5 in Group IV and 150.1 ± 33.6 in Group V , with Group II showing approximately twice higher value than the other 4 groups. However, there was no significant difference between Group III , IV , V and Group I .
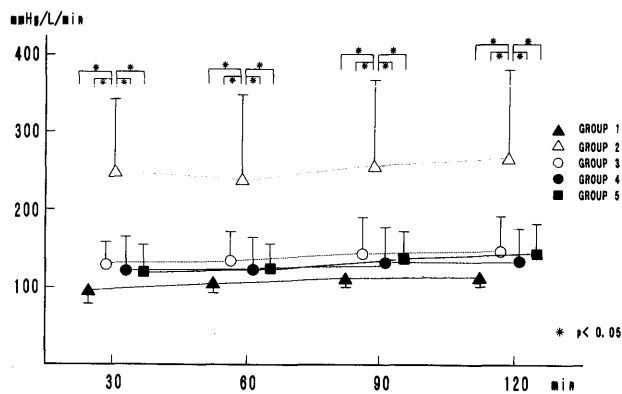

Fig. 3. Pulmonary vascular resistance

4. Airway pressure (Fig. 4)

Compared with other groups, the value was already significantly higher in Group II immediately after reperfusion. While a time course increase thereafter was observed in Group II , only a slight increase occurred in the other 4 groups.
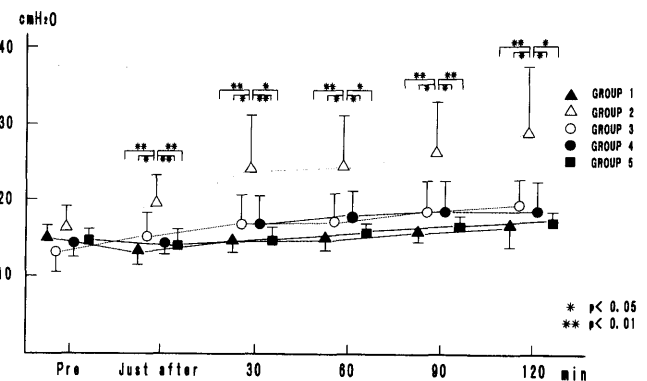

Fig. 4. Airway pressure

5. Total static compliance of the lung (Fig. 5)

The total static compliance of the lung significantly decreased in Group II compared with Group I , IV , V immediately after reperfusion. The 120 minute value was 15.8 ± 1.7 (ml/cmH2O, the same below) in Group I , 9.7
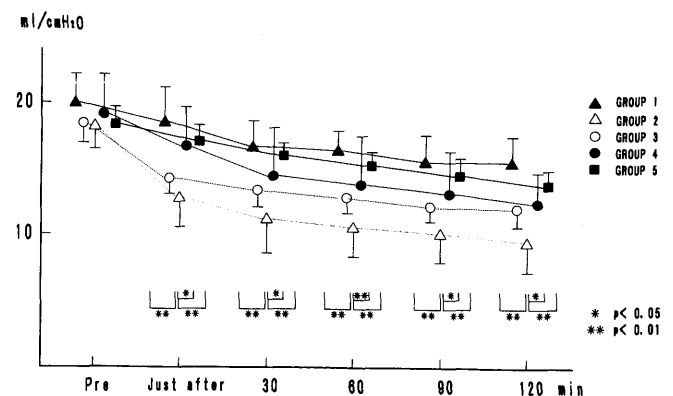

Fig. 5. Total static lung compliance (C_{ST})

± 2.5 in Group II , 12.4 ± 1.8 in Group III , 12.8 ± 2.0 in Group IV and 14.2 ± 0.8 in Group V , indicating that the compliance was maintained in these groups in the order of Group I , V , IV , III and II . There was no significant difference between Group III , IV , V and Group I .

6. Extravascular lung water (Fig. 6)

The wet/dry weight ratio was 5.6 ± 0.4 in Group I , 9.7 ± 0.7 in Group II , 8.0 ± 0.8 in Group III , 7.1 ± 0.7 in Group IV and 6.7 ± 0.6 in Group V . The values demonstrated by Group II , III , IV and V were higher than the Group I value. Furthermore, Group II demonstrated a significantly higher value compared with that shown by Group III , IV and V (p < 0.01). The Group V value was significantly lower than the Group III value (p < 0.05).
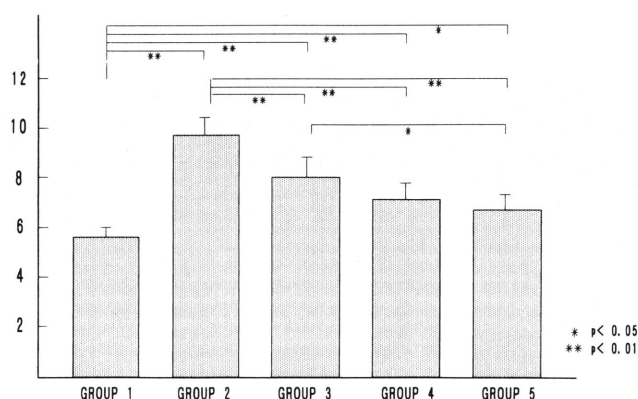
Fig. 6. Extravascular lung water (Wet/Dry weight ratio)

7. Lipid peroxide (Fig. 7, 8)

The serum MDA of Group II and IV perfused with whole blood tended to show higher values compared with Group I , III and V 30 minutes after the start of reperfusion and onward, and the value became significantly higher compared to that shown by Group I and V 120 minutes afterwards (p < 0.05). The tissue MDA preserved for 24 hours significantly increased in each group compared to the value before reperfusion (p < 0.01). It was 2150 ± 285 (nmol/g protein, same below) in E-C groups (Group II and III) and 1739 ± 378 in UW groups (Group IV and V), indicating a significantly lower value for the latter (p < 0.01). The 120 minute value was 682 ± 48 in Group I , 882 ± 60 in Group II , 781 ± 90 in Group III , 850 ± 150 in Group IV and 705 ± 151 in Group V .

A significantly high value was observed in Group II and IV compared with Group I (p < 0.01). Group II demonstrated a significantly higher value than those shown by Group III and V (p < 0.05).

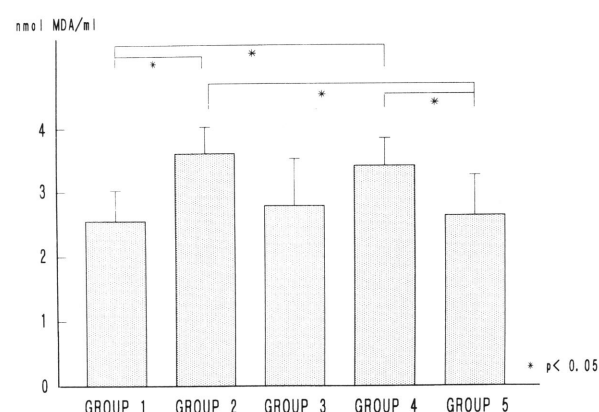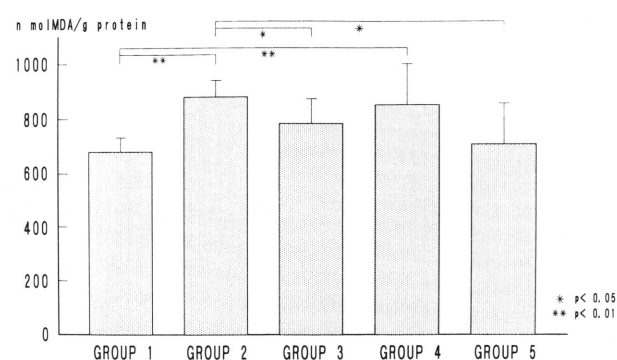**Fig. 7.** Lipid peroxide in blood plasma

Fig. 8. Lipid peroxide in tissue

8. Pathohistological finding (Fig. 9)

In Group II , hypertrophy of alveolar septum, atelectasis, fluid leak into and its retention in alveoli, congestion of small vessels, hemorrhage in the periphery of vessels were observed, indicating a remarkable pulmonary edema. Though much milder, the above findings indicating pulmonary edema were also observed in Group III . On the other
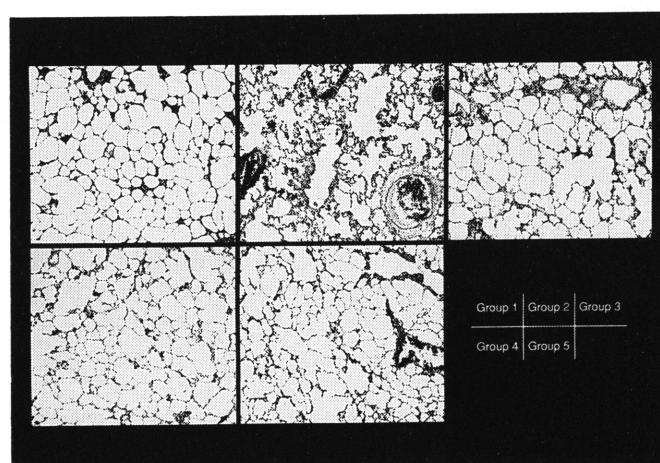
Fig. 5. Photomicrographs of reperfused lungs (Hematoxylin and Eosin x20)

hand, almost normal pulmonary tissue image as demonstrated by Group I was also observed in Group IV and V .

Discussion

The lung anatomically and physiologically, consists of pulmonary capillaries, interstitial tissue and alveoli. As such structure is vulnerable to edema, it is more difficult to preserve the lung compared to other organs. Various preservation methods including freezing, persistent perfusion, high pressure oxygen, simple immersion methods or combination of these methods have been applied in the past. Considering the ease in transfer and simplicity in handling, however, the simple immersion & cooling preservation has become the mainstream at present. The maximum limit of lung preservation under ordinary temperature is reportedly 3-4 hours in the inflated state. On the other hand, ever since Blumenstock[9] applied first cold-preservation for the purpose of prolonged lung preservation time by suppressing the oxygen consumption of the tissue under low temperature, various preservation solutions have been in use. Crane et al.[10] reported their success in 24 hour preservation in Collins-Sacks solution, and Toledo-Pereyra et al.[11] in 24-72 hour preservation in modified silicagel fraction, both using animals. However, in clinical cases, a 6-8 hours duration of preservation is still a safe limit by using the present preservation solution. Therefore, the development of a better preservation solution is strongly desired. E-C solution used in the present study is a solution developed for preservation of the kidney and has the composition similar to that of intracellular fluid. This solution has been widely used for the preservation of various organs including the lung. UW solution was a recently developed solution also having the composition similar to that of intracellular fluid (Table 2). It is more likely to be used for preservation of the liver, kidney and pancreas. Reports have been made on the efficacy of UW solution not only in animal experiments but also in clinical cases.[12, 13] However, any study has been hardly made on the efficacy of UW solution in lung preservation. Though UW solution is composed of various constituents, major constituents and their roles may be summarized as follows:[14, 15]

(1) Lactobionate: A comparatively large anion with the molecular weight of 358. It is difficult for this substance to penetrate the cellular membrane. With raffinose (molecular weight 595), it inhibits enlargement of cells under low temperature.

(2) Phosphate: As a buffer to hydrogen ion, it prevents intracellular acidosis.

(3) Hydroxyethyl starch: Steady and harmless colloid with the mean molecular weight of 250,000. It stays on the vascular floor to prevent edema in the interstitial tissue.

(4) Glutathione, allopurinol: They prevent injury caused by oxygen free radicals generated under ischemic condition.

Whether these constituents are all indispensable or not is not yet to be clarified. However, the importance of lactobionate has been widely recognized. In the test conducted by the author, the lung preserved in UW solution had lower pulmonary vascular resistance, less extravascular lung water and higher C_{ST} as compared to the one preserved in E-C solution. The advantage over the UW-preserved lung was demonstrated by less pulmonary edema, which was not contradictory to the pathohistological findings. When Compared with the non-preserved lung, however, extravascular lung water was increased, indicating that this method was not complete to prevent the cell injury caused by reperfusion after ischemia. It has been reported that MDA, a metabolite of lipid peroxide, increased in the tissue of the preserved-lung. In our experiment as well, a significant in crease in the pulmonary tissue MDA was observed after 24 hours of preservation when compared to the value before preservation. This result revealed that active oxygen is generated from the oxygen in alveoli during the preservation of the lung which is well pneumatic by nature. The active oxygen generated in the preserved-lung enhances the adhesion of leukocytes to vascular endothelial cells at the time of reperfusion and causes leukocyte plugging, which

Table 2. Composition of UW and E-C solutions

	UW	EC
Impermeants	Raffinose (30 mmol/L) Lactobionate (100 mmol/L)	Glucose (194 mmol/L)
H+Buffers	KH₂PO₄ (25 mmol/L)	KH₂PO₄ (15 mmol/L) K₂HPO₄ (42 mmol/L)
Colloids	Hydroxyethyl starch (5g/dL)	NaHCO₃ (10 mmol/L)
Metabolites and others	MgSO₄(5 mmol/L) Adenosine (5 mmol/L) Glutathione (3 mmol/L) Allopurinol (1 mmol/L) Insulin (40 U//L) Dexamethasone 8 mg/L)	KCL (15 mmol/L)
mosm/L	320	355
Na (mmol/L)	30	10
K (mmol/L)	120	117
pH	7.4	7.2

results in increased peripheral vascular resistance and concurrently injury to vascular endothelial cells, consequently causing pulmonary edema. In fact, abundant leukocyte infiltration was noted in the interstitial tissue of the lung after reperfusion with whole blood in the E-C preservation groups, showing a histologic finding of histologically proving the leukocyte trapping. When compared with the lung preserved with E-C solution, the tissue MDA was significantly lowed in the lung preserved in UW solution. This was attributable to the anti-oxidizing effect of glutathione and allopurinol contained in UW solution, suggesting that these antioxidants were of great use in the lung preservation. Recent studies reported that UW solution with extracellular fluid composition was more effective,[16] which warrants further study in the future.

The phenomenon that an organ in ischemic state is further injured by resumption of blood flow, in other words, post-ischemic reperfusion injury, is an important item to consider in organ preservation. There still remains much to be explained with regard to the mechanism. Since Granger et al[17] suggested that active oxygen play an important role in reperfusion injury of the small intestine, his theory has been widely proven in various organs including the heart,[18] liver[19] and kidney.[20] In 1966, Zimmerman et al. perfused the heart of rat which had been perfused at 37 °C with Ca^{2+} free solution for more than 4 minutes and then reperfused it with a solution containing Ca^{2+}. They reported that after the above treatment, a phenomenon called calcium paradox[21] in which myocardial necrosis was observed without resumption of the pulsation occurred. The cause for such phenomenon was the violent Ca^{2+} overload at the time of reperfusion, which was assumed to be a factor causing reperfusion injury after ischemia. The inhibitory effect by Ca^{2+} antagonist on this phenomenon has also been reported.[22] Hearse et al.[23] described oxygen paradox and calcium paradox as "facets of the same problem". Also reported is the involvement of active oxygen in Ca^{2+} overload.[24] It is obvious that active oxygen is a key mediator in post-ischemic reperfusion injury. Hypoxanthine-xanthineoxidase system has been known as a source generating active oxygen.[25] Under persisting ischemia, ATP is mainly produced through the action of adenylate kinase. This enzyme produces ATP and AMP from 2 molecule substance ADP. AMP therefore tends to accumulate in an ischemic tissue and, as a result, hypoxanthine via adenosine has become increased. At the same time, xanthine dehydrogenase is converted to xanthine oxidase under ischemic state. With hypoxanthine and oxygen as the substrate, O_2-is generated. Allopurinol is a specific inhibitor of xanthine oxidase and its effect against post-ischemic reperfusion injury has been reported.[26] However, xanthine oxidase activity differs depending on animal species and organ.[27] Post-ischemic reperfusion injury occurs in the liver and lung with high activity as well as in the heart and brain with lower activity. It is widely known that xanthine oxidase

alone cannot explain the mechanism. As an active oxygen generating source, attention at present is focused on NADPH oxidase of granulocyte.[28]

$$NADPH + H^+ + 2O_2 - NADP^+ + 2O_2^- + 2H^+$$
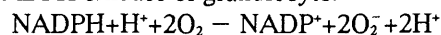
As shown in the above formula, it is assumed that NADPH derived from glucose decomposition via pentose-phosphate pathway reduces oxygen by 1 electron by catalys is of NADPH oxidase in the cellular membrane of granulocyte to produce O_2^-, and then H_2O_2 is produced by non-enzymatic heterogeneous reaction. It was reported that the removal of leukocytes at the time of reperfusion actually inhibited the production of active oxygen, thereby reducing reperfusion injury.[29,30] In the present study as well, pulmonary function was more favorably maintained immediately after reperfusion in the lung perfused with leukocyte-depleted blood with significantly lower extravascular lung water when compared to the lung perfused with whole blood. Serum and tissue lipid peroxide value was also lower in the former, indicating the serious involvement of active oxygen derived from leukocyte in the cell injury at the time of reperfusion. Regarding the lungs preserved in UW solution, there was no difference in extravascular lung water volume between the one perfused with leukocyte-depleted blood and the other perfused with whole blood. This was attributable to the antioxidizing effect of glutathione and allopurinol contained in UW solution. Glutathione mainly deals with H_2O_2 using glutathion peroxidase as a catalyst, but its involvement in NADPH oxidase has also been recognized.

The isolated lung perfusion model used in the present study had a demerit that deoxygenation was not divised in the circuit. However, it is sure that this model provides a great benefit and convenience in evaluating the efficacy of the preservation solution.

Acknowledgement

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