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Allo-transplantation of the Lung Preserved 24 Hour with UW Solution and the Preventive Effect of Flush with Leukocyte-depleted Blood before Reperfusion.

Noriaki Itoyanagi

The First Department of Surgery, Nagasaki University School of Medicine
Address to correspondence to Itoyanagi. The 1 st Department of Surgery.
Nagasaki University school of Medicine. Sakamoto 1-7-1, 852, Nagasaki city, Japan.

Abstract: The purpose of this study was to evaluate the possibility of clinical use of a 24 hour preserved donor lung with UW solution and the effects of the flush with Leukocyte depleted before reperfusion.

The left canine lung was used for allotransplantation and dogs were divided into 4 groups. The donor lungs preserved for 24 hours with UW solution (Group 1 and 2) or EC solution (Group 3 and 4) were transplanted in mongrel dogs. Moreover, the flush with Leukocyte-depleted blood for 30-60 minutes was performed in Group 2 and 4. The severity of reperfusion injury at 60 minutes of reperfusion was assessed and the graft funchion was observed for 14 days.

The PaO_2 value at 60 minutes of reperfusion was 345 ± 132, 261 ± 161, 312 ± 120, 152 ± 146 Torr, static compliance was 31.2 ± 4.5, 27.8 ± 5.0, 22.2 ± 9.0, 17.6 ± 6.1, and dynamic compliance was 13.9 ± 1.9, 14.2 ± 1.9, 10.9 ± 1.8, 11.7 ± 1.5 ml/cmH_2O, respectively. There were no significant differences among the groups. And no significant deterioration was seen in these parameters as compared with the figures before harvesting. Pulmonary vascular resistance (PVR) was 1824 ± 650, 2100 ± 564, 3830 ± 1549, 4553 ± 1819 dyne*sec*cm^{-5} respectively. Group 1 and 2 showed significantly lower PVR than Group 3 and 4.

Tissue Lipid Peroxide was 0.75 ± 0.15, 0.85 ± 0.21, 1.52 ± 0.85, 0.70 ± 0.27 nmolMDA/mg pt, and the ability of superoxide generation of neutrophils (SOX) was 4697 ± 1886, 4466 ± 1760, 6934 ± 156, 3125 ± 725 respectively at 60 minutes of reperfuison. Group 1, 2 and 4 showed better results than Group 3. There was a significant decrease in SOX of Group 4 as compared with Group 3.

Recipients were administered Cyclospoline A (20 mg/kg/day) and Azachiopurine (2 mg/kg/day). The survival rates were 100% (6/6), 57% (4/7), 0% (0/4), 0% (0/4) respectively. And 4 of 6 in Group 1 and 3 of 7 in Group 2 functioned well with PaO_2 of 354 ± 66, 317 ± 178 Torr at sacrifice (10 to 21 POD). And a slight rise in PVR was recognized in both groups as compared with the figures before harvesting. (2524 ± 894, 2947 ± 381 respectively)

Histological examination after 60 minutes of reperfusion revealed mild interstitial edema in Group 1 and 2. Severe alveolar edema, marked vascular congestion, and perivascular extravasation were seen in Group 3 and 4. But the grade of vascular congestion was slightly lower in Group 4 as compared with that in Group 3. At sacrifice survivors showed rejection in three dogs and none in four dogs, in which interstitial thickening, diffues perivascular cuffing was seen. There was no significant difference between Group 1 and 2.

The data suggest that UW solution may prepare the 24-hour-preserved donor lung for succesful lung transplantation. And the flush with leukocyte depleted blood showed attenuation of the tissue lipid peroxidation and superoxide generation of neutrophils in the lung preserved with EC but not with UW. The flush with leukocyte-depleted blood may play a role in attenuation of neutrophils related reperfusion injury.

Introduction

Lung transplantation in human being was first performed in 1963. After the development of Cyclosporine, lung transplantation has become recognized as the only possible way of therapy for many patients suffering from the end-stage of lung diseases.

However, the shortage of suitable donor organs is one of the restricting factors in clinical application of lung transplantation. Recent studies focused on the technique of longer preservation and better quality of the donor lung.

In recent years, there has been growing evidence that University of Wisconsin solution is superior to Euro-Collins solution for preservation of the liver, the kidney and the pancreas. But there were a few reports about the donor lung.

In our previous study, We have demonstrated the superiority of UW solution to EC solution for 24-hour lung preservation in vitro canine model.[1] In this model lower PVR and better compliance were observed, whereas PaO_2 values were not significantly better. The estimation of oxygenation is controversial about this model because of no oxygen consumption. And the duration of reperfusion was limited. The mutual factors between recipients and grafts such as the circulating neutrophils' effects to the endothelium were unclear. And the survival times depending on the following reperfusion injury, implantation response and immunological rejection were not estimated.

Therefore, the lst purpose of this study was to clarify the possibility of 24 hour preservation with UW solution in vivo model, though it is costly and time consuming.

It is well known that the important role of leukocytes and its production of toxic metabolites in reperfusion injury.[2-5] It contributes to damage to the pulmonary vascular endothelium, which results in leukocyte sequestration, vasospasm, hemorrhage and lung edema.[4,6] The other factors such as Leukotriene, and Thromboxane affect the advances in lung injury.

Therefore, peripheral blood leukocyte depletion after ischemia is useful to attenuate the reperfusion injury.[5,7]

In the lung preserved for 24 hours, Breda[7] demonstrared that reperfusion with leukocyte-depleted blood resulted in excellent functional and histological recovery. Moreover, Timothy[9] demonstrated that reperfusion injury was minimized when leukocytes were returned to perfusate after 60 minutes but not after 5 to 30 minutes. It meant the importance of the initial 60 minutes of reperfusion.

This study was attempted to elucidate the fact that flushing with Leukocyte-depleted blood for initial 60 minutes of reperfusion may ameliorte the reperfusion injury. The 2nd purpose of this study is to evaluate if the flush with Leukocyte-depleted blood for initial 60 minutes ameliorates reperfusion injury.

Materials and Methods

(1) Study groups

Dogs were divided into the four groups. Adult mongrel dogs were undergone allotransplantation. The canine lungs were preserved for 24 hours with either cold UW solution (Group 1 and 2) or EC solution (Group 3 and 4). In Group 2 and 4, after transplantation the lungs were flushed with leukocyte-depleted blood during the initial 30 to 60 minutes before reperfusion.

(2) University of Wisconsin solution;

UW solution (ViaSpan (TM)) was kindly provided by Du Pont Pharmaceuticals. Table 1 depicts the individual component of UW solution and their concentrations.

Table 1. UW cold storage solution

Substance	Concentration/l
K^+-lactobionate	100mmol
KH_2PO_4	25mmol
$MgSO_4$	5mmol
Raffinose	30mmol
Adenosine	5mmol
Glutathione	3mmol
Insulin	100U
Peniciline	200,000U
Dexamethasone	8mg
Allopurinol	1mM
Hydroxyethyl Strach	50g
pH (at room temperature)	7.4
Na^+	30mmol/l
K^+	120mmol/l
Osmolarity	320-330mOsm/l

(3) Leukocyte-depleted blood;

Leukocyte-depleted blood was prepared by filtration through an Imugard TF-IG400Y (Terumo Corp., Tokyo, Japan). A total of 400 ml of Blood was collected from the donor dog and stored at 4 ℃ after filtration. Blood was collected through a large bore cannula inserted into the right atrium just before flush of cold storage solution. And leukocyte-depleted blood was flushed after reaching the room temperature. The flush of the transplanted lung with leukocyte-depleted blood was performed through the feeding tube inserted into the PA. A maximum hydrostatic pressure was less than 25 cmH_2O. As a result, it took about 30 to 60 minutes for complete flushing. The perfusate from atrial cuff was abandoned.

(4) Anesthesia;

All animals received human care in compliance with the "Guide for the Care and Use of Laboratory Animals of Nagasaki University". All animals were anesthetized with intravenous administration of pentobarbital of 25 mg/kg (Nembutal injection (R)) and intubated, ventilated at a fixed FIO_2 of 1.0, a tidal volume of 30 ml/kg and the respiratory rate of 14 breaths/min using Harvard ventilator. A 5 Fr-thermodilution catheter (TD catheter, TERUMO) was introduced from the left external juglar vein to the main PA before thoracotomy was made.

(5) Unilateral pulmonary artery occlusion test (UPAO test);

UPAO test was performed before harvesting, at 60 minutes of reperfusion and after mean 14 days if survived.

After occlusion of the right PA and the main bronchus by clamps, the cardiac output was calculated by the Thermodilution meter MTC-6100 (Nihon Koden Co.). The pressure was measured by using polygraph RMP 6004S (Nihon Koden Co.). Left PV pressure was measured by insertion of 19-gauge needle direcly. Pulmonary vascular resistance (PVR) was calculated as (PAp-PVp) *80/CO (dyne*sec*cm^{-5}). Concerning the intratracheal pressure, the peak pressure and the pressure at 1.4 sec of the end-inspiratory plateau (EIP) were measured with the small catheter located within the airway. The static lung compliance was calculated as the pulmonary flow volume/EIP (ml/cmH_2O). The dynamic lung compliance was also calculated as the pulmonary flow volume/peak pressure (ml/cmH_2O).

Blood sample for gas analysis was taken from the left atrium after 10 minutes' occlusion. As described in detail below, tissue lipid peroxide (LPO), wet/dry ratio of the tissue, the ability of superoxide generation (SOX) of the circulating neutrophils was also measured at the study state of UPAO test.

(6) Donors procedure;

The donor dogs weighing 10.3 ± 1.3 kg (range 8 to 11.5 kg) were anesthetized as described before.

After median sternotomy, sling loops were passed around the left subclavian artery, the ascending aorta, the innominate artery, the superior and inferior venae cavae. And the both sides of PA and right main bronchus were isolated from slings.

UPAO test was performed as described above. After administration of 5000 U Heparin, a 5-0 Proline purse-string suture was located in the main PA to introduce 5 Fr feeding tube. After the inferior and superior cavae were ligated, the right PA was ligated, and the main PA was clamped. Then prostaglandin E1 of 100 microgram was administered through the feeding tube to prevent vascular spasm and make distribution of solution valid.

The left lung was flushed with 300 ml to 350 ml of a cold storage solution, UW or EC, at a hydrostatic pressure of less than 25 cmH_2O. The left atrium was opened to permit drainage of the perfusate. During this flush, ventilation of the left lung was continued.

Then, the heart and the lungs were excised. The trachea was dissected free in the superior mediastinum and then clamped and divided with an inflation of 60 percent end-tidal volume. These were put in a sterile vinyl bag filled with the same cold storage solution and stored at 4-6 ℃ for 24 hours in a refrigerator. Throughout the preparation, utmost care was taken to prevent lung injury from any compression or manipulation of lung tissues.

Specimen as control weighing about 3 g obtained from the right lower lobe was divided into 3 pieces. The first piece was immediately frozen at liquid nitrogen for tissue lipid peroxide measurement. The 2nd piece was fixed in 20 % formalin solution and stained with hematoxylin and eosin for histological examination. The 3rd piece was weighed accurately to know wet weight (W). And after desiccation at 100 ℃ for 24 hours it was weighed again as dry weight (D). Lung D/W ratio was determined.

(7) Recipients Procedure;

On the next day, a weight- and stature-matched dog was anesthetized and a 5 Fr-thermodilution catheter was introduced as described above. Under right lateral position, a left thoracotomy was performed, then the pericardium at pulmonary hilus was incised. Then the right PA and main bronchus were isolated as previously descrived for the UPAO test. For left pneumonectomy, the left PA and PV were divided distal to the hilum after clamping, and the left bronchus was divided at the level of the upper lobe bronchus.

Then the preserved lungs and heart were taken out from the vinyl bag, trimmed to suit for anastomosis, the left PA was divided long, and the left bronchus was divided as keeping 2 rings, and the left atrium was divided to form the atrial cuff.

The atrial cuff was sutured with a 5-0 Proline (Ethicon Inc.) in a fashion of horizontal everting mattress suture. The PA was sutured with 6-0 Proline in a fashion of over and over continuous running suture. The bronchus was sutured with 4-0 Proline in the same fashion. During these procedures the lung was covered with cold wet gauze.

After all anastomoses were completed in Group 1 and 3, all clamps were released and reperfusion was started as 0 minute. In Group 2 and 4, flush with Leukocyte-depleted blood was performed just after anastomosis through a feeding tube inserted into the PA. Anastomosis of the Left atrium was opened for drainage of perfusate. After the flush was completed, within 30 to 60 minutes, PA clamp was released as 0 minute of reperfusion. PGE1 of 100 microgram was administered after reperfusion started. After 60 minutes of reperfusion UPAO test was performed as previously described. Then the specimen of the left lower lobe, about 3 g, were obtained and divided into 3 pieces for histological examination and measurement of LPO and D/W ratio. The incision was closed with 5-0 Nylon. When hemostasis was insured, the thorax was closed leaving a 20 Fr thoracic drainage tube for evacuation.

(8) Post-operative Care;

All dogs were extubated 4-6 hours after operation, and thoracic drainage tube was removed if air leakage was not recognized. From the day of surgery, prophylactic antibiotics, 1 g of cephems, were administered intramuscularly. And immunosuppressant Azathioprine at a dose of 25 mg/body, and Cyclosporine at a dose of 20 mg/kg were administered orally every day until they were sacrificed.

Chest X-rays were taken on the postoperative 1, 3, 5, 7, 10, 14th day if survived. The chest X-ray films were graded as follows. Score 0; normal, Score 1; almost normal, Score 2; mild infiltrating shadow, Score 3; moderate infiltrating shadow, Score 4; severe infiltrating shadow.

The survivors were anesthetized on the mean 14th day. Lungs were exposed and inspected through a median sternotomy. After macroscopic examination, UPAO test was carried out if possible. And animals were sacrificed to take samples for histological examination.

(9) Lung tissue lipid peroxides:

This assay was performed about Donor's right lower lobe as control, immediately after teh UPAO test of 60 minutes of reperfusion. Lipid peroxide was measured by estimation of Malondialdehyde (MDA). The MDA was measured as thiobarbituric acid (TBA) activity using the methods of Okawa,[8] as follows. A lung specimen of 1.0 g previously frozen at liquid nitrogen was homogenized in the 9 fold weight of cold saline to yield the 10% homogenate. And 0.2 ml of the homogenate was added with 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of 20% acetic acid (PH 3.5), 0.6 ml of distilled water and 1.5 ml of 0.8% TBA.

The mixture was heated at 95 ℃ for 60 minutes. After being cooled, 1.0 ml of distilled water and 5 ml of n-butanol/piridyne (15:1, vol/vol) were added. The solution was centrifuged at 3000 rpm for 10 minutes. The fluorescent intensity of the supernatant was measured with excitation of 515 nm and emission of 553 nm by using Spectrophotofluorometer RF5000. (Shimazu Cop, Tokyo, Japan). The lipid peroxide concentration was determined by reference to a standard fluid of 0.5 nmol 1, 1, 3, 3-tetra methoxypropane that yields 0.5 nmol of MDA. The MDA levels were calculated as nmol/g tissue. The protein content of the homogenate was determined by the Lowry's method.[9] Lipid peroxide was expressed as nmol MDA/mg tissue protein.

(10) Assay of oxidative product formation by peripheral blood neutrophils by Flow Cytometry[10]:

Ability of intracellular generation of superoxide by peripheral neutrophils at 0 minute and 60 minutes of reperfusion was measured using flow cytometry.

DCFH-DA (2', 7'-Dichlorofluorescein-Diacetate) is a nonfluorescent compound, diffused into the cells, hydrolyzed by intracellular esterases to DCFH (2', 7'-Dichlorofluorescein) that is nonfluorescent fluorescein analog, and thereby trapped within the cells, oxidized to the highly fluorescent DCF (Dichlorofluorescein) in the presence of hydrogen peroxide which is generated by neutrophils stimulated by phorbol myristate acetate (PMA) undergoing a respiratory burst. This reaction was employed to measure hydrogen peroxide released by neutrophils.

0.1 ml heparinized whole blood obtained at 0 min and 60 min of reperfusion was added to 1.9 ml of DCFH-DA (5 micro mol/l), and incubated for 15 minutes at 37 ℃ , and 0.5 ml of 0.7% EDTA (=20 mM/l), 0.15 microgram of PMA, were and incubated for 25 minutes at 37 ℃ as stimuted samples. Controls did not include PMA as unstimualated samples but other procedure was the same. After adding 2 ml of PBS, samples were centrifuged at 1300 rpm for 10 minutes, and 2 ml of ammonium chloride (0.82%) was added and left for 10 minutes to hemolyze. After centrifuging at 1300 rpm for 10 minutes, supernatant was removed leaving the pellet. Then 2 ml of PBS was added to suspend neutrophils. Neutrophils' fluorescence was determined by using a SPECTRUM Ⅲ flow cytometer (Ortho Diagnostic System).

Individual neutrophils were discerned by the combination of forward scattered and right angle scattered laser light at the quadrant. The fluorescent intensity was graded 250 channel depending on its intensity. And the gain setting was set up for 95% of unstimulated neutrophils to be included with a range of No. 0 to No. 10 channels. On these conditions stimulated neutrophils were analyzed. The percentage populations that registered at over No. 10 channel and the mean channel number was determined. And percentage population multiplying the mean channel numbers was expressed as the generation ability of hydrogen peroxide of neutrophils, and it correlates with the ability of superoxide generation of neutrophils. Numerable neutrophils in each measurement was more than 2000.

(11) Pathological estimation after 60 minutes and at sacrifice;

Specimens obtained after 60 minutes of reperfusion and at sacrifice were fixed with a 20 % formaline and stained with hematoxyline and eosin. Pathology of lung tissues at the 60 minutes of reperfusion was estimated in terms of reperfusion injury. And specimens at the sacrifice were examined with respect to the degree of lung edema, pneumonia, rejection according to Veith classification. (Grade 1; earliest evidence, Grade 2; extension, Grade 3; well established, Grade 3+; preinfarction, Grade 4; end stage)

(12) Statistical analysis;

All values were expressed as mean ± standard error. Statistical analysis unpaired t was applied for comparison between Groug 1 and 2, 3 and 4 about data at 60 minutes of reperfusion. In each group, comparison of data before harvesting (pre) and data at 60 minutes of reperfusion or on sacrifice were analyzed by paired t test. P values of less than 0.05 were considered to be statistical significant.

Results

Twenty-one adult mongrel dogs underwent left allo transplantation. Mean body weight of donor and recipient dogs were 10.4 ± 2.2 kg and 11.1 ± 1.7 kg. Average cold ischemic time was 22.4 ± 1.1 hour. Average total ischemic time was 24.4 ± 1.2 hour. There was no significant difference among groups. The flush with cold storage solution during harvesting was performed within 30-40 minutes. The average anastomotic time at transplantation was 46.6 ± 10.3 minutes. In Group 2 and 4, The flushing procedure with leukocyte-depleted blood took about 30 to 60 minutes.

As shown in Table 2, all animals (n=6) in Group 1 survived. Four of 6 survived 10, 14, 14 and 15 days showing good graft condition. But remaining 2 survived for 8 and 15 days and showed diffuse graft hepatization. In Group 2(n = 7), two of 7 died of respiratory failure due to severe lung edema. Another dog died of cardiac failure after the operation. Three of other 4 survived 12, 17, 21 days showing good graft condition. Another dog survived for 21 days until sacrifice, showing a good chest X-ray figure but increasing infiltrating shadow from the 14th day, revealed obstructive pneumonia due to bronchial anastomotic stenosis at sacrifce. Therefore, three of 7 could

Table 2. Postoperative course

Group		cause of death	date of sacrifice	macroscopic graft condition
Group. 1:	No. 1	sacrifice	14d	good
	No. 2	sacrifice	15d	oeganized
	No. 3	sacrifice	8d	oeganized
	No. 4	sacrifice	15d	good
	No. 5	sacrifice	14d	good
	No. 6	sacrifice	10d	good
Group. 2:	No. 1	sacrifice	17d	good
	No. 2	sacrifice	21d	good
	No. 3	sacrifice	18d	obstructive peumonia
	No. 4	card. fail.	3h	mild lung edema
	No. 5	sacrifice	12d	LUL good, LLL organized
	No. 6	res. fail.	12h	severe lung edema
	No. 7	res. fail.	12h	severe lung edema
Group. 3:	No. 1	resp. fail.	12h	severe lung edema
	No. 2	resp. fail.	11h	moderate lung edema
	No. 3	sacrifice	14d	LLL partialy good
	No. 4	bleeding	6d	good
Group. 4:	No. 1	resp. fail.	44h	lung edema
	No. 2	resp. fail.	4h	lung edema
	No. 3	emerciation	6d	LLL partialy good
	No. 4	card. arrest	1h	severe lung edema

abbreviation
resp. fail.; respirotory failure.
card. fail.; cardiac failure.
bleeding; bleeding from the digestive tract.
LLL; left lower lobe

complete the UPAO test. In Group 3 (n = 4), two of 4 died of respiratory failure due to lung edema. Another one died of bleeding from the digestive tract on the 6th day, although the graft was in good condition at autopsy. One survived for 14 days, showing diffuse hepatization of the graft except in the lower lobe. In Group 4, three of 4 dogs died of severe lung edema after the operation. Another dog died of emaciation on the 6th day. The lung was almost organized but the part of the lower lobe was keeping aeration. There was no dog which could achieve the UPAO test prior to sacrifice in Group 3 and 4. The survival rates in each group were 100 (6/6), 57 (4/7), 25 (1/4), 0 (0/4)%. Graft survival rates were 66 (4/6), 57 (4/7), 0 (0/4), 0 (0/4)%, respectively. The survival rates of the UW groups (1 and 2) were apparently better than those of EC groups (3 and 4), but the effect of the flush with Leukocyte-depleted blood was not apparent.

A 60 minutes' reperfusion has resulted in a moderate lung edema in Group 1 and 2. In contrast, uneven distribution of bloos flow was seen at the early phase of reperfusion in Group 3 and 4. And a large amount of foamy bloody fluid was obtained in the tracheal tube because of severe lung edema. All data at 60 minutes of reperfusion were summarized in Table 3.

Under the condition of FiO_2=1.0, RR=14, TV=30 ml/kg PaO_2 tension of PV blood was 345 ± 132, 261 ± 161, 312 ± 120, 152 ± 146 Torr respectively, and these values didn't differ statistically among all groups. In each group, there was no statistical difference when compared with a donor lung function before harvesting. But slight decrease was found in Group 2 and 4.

Dynamic compliance was 13.9 ± 1.9, 14.2 ± 1.9, 10.9 ± 1.8, 11.7 ± 1.5 ml/cmH_2O. Static compliance was 31.2 ± 4.5, 27.8 ± 5.0, 22.2 ± 9.0, 17.6 ± 6.1 ml/cmH_2O at 60 minutes of reperfusion respectively. Significant difference was not seen among all groups. But when compared with that of harvesting, a significant decrease was observed in

Table 3. Results after 60 min of reperfusion on UPAO test (meam ± SD)

Variables		Group 1	Group 2	Group 3	Group 4
PaO_2 (Torr)	pre	417.7 ± 12.0^a	389.6 ± 66.1^a	433.5 ± 47.0^a	362.3 ± 83.6^a
	60 min	344.6 ± 132.4^b	261.0 ± 161.2^b	312.0 ± 119.9^b	152.3 ± 145.9^b
$PaCO_2$ (Torr)	pre	29.3 ± 4.5^a	31.4 ± 9.6^a	22.2 ± 1.5^a	27.4 ± 8.7^a
	60 min	31.6 ± 5.2^{bc}	37.5 ± 4.0^{bd}	25.4 ± 6.1^b	28.7 ± 12.1^b
PVR (dyn*sec*cm^{-5})	pre	1350 ± 279^a	1374 ± 1175^a	1539 ± 518^a	1152 ± 627^a
	60 min	1824 ± 650^{be}	2100 ± 564^b	3830 ± 1549^{bf}	4553 ± 1818^b
Dynamic CL (ml/cmH_2O)	pre	14.1 ± 1.2^a	19.0 ± 2.3^a	13.5 ± 2.9^a	15.9 ± 2.7^a
	60 min	13.9 ± 1.9^b	14.2 ± 1.9^b	10.9 ± 1.8^b	11.7 ± 1.5^b
Static CL (ml/cmH_2O)	pre	31.4 ± 7.8^a	35.5 ± 4.5^i	25.7 ± 4.5^a	27.7 ± 3.3^a
	60 min	31.2 ± 4.5^b	27.8 ± 5.0^j	22.2 ± 9.0^b	17.6 ± 6.1^b

CL; Compliance.
^{a}; no significant difference; ^{a} vs. ^{b}. (in each variable)
^{b}; no significant difference; ^{a} vs. ^{b}. (in each variable)
^{c}; $p < 0.05$; ^{c} vs. ^{d}
^{d}; $p < 0.05$; ^{d} vs. ^{c}
^{e}; $p < 0.05$; ^{e} vs. ^{f}
^{f}; $p < 0.05$; ^{f} vs. ^{e}
^{g}; $p < 0.01$; ^{g} vs. ^{h}
^{h}; $p < 0.01$; ^{h} vs. ^{g}
^{i}; $p < 0.01$; ^{i} vs. ^{j}
^{j}; $p < 0.01$; ^{j} vs. ^{i}

Table 4. Results after 60 min of reperfusion (meam ± SD)

Variables		Group 1	Group 2	Group 3	Group 4
Tissue LPO	pre	1.20 ± 0.76[a]	1.64 ± 0.61[c]	2.28 ± 0.69	1.29 ± 0.25
(nmolMDA/mg pt)	60 min	0.73 ± 0.15[b]	0.85 ± 0.21[d]	1.52 ± 0.85	0.70 ± 0.27
Serum-LPO	0 min	42.7 ± 17.4	100.0 ± 52.3	50.8 ± 28.2	50.9 ± 14.0
(pmolMDA/mg pt)	60 min	49.1 ± 19.2[e]	77.9 ± 25.3[f]	47.2 ± 17.0	36.7 ± 13.3
WBC	0 min	6316 ± 3451	8343 ± 2693[g]	8750 ± 550	15100 ± 10970
(/μl)	60 min	6233 ± 2339	6429 ± 3245[h]	7375 ± 1883	7600 ± 4984
PLT	0 min	20.5 ± 4.7	20.1 ± 6.4	21.2 ± 9.8	19.3 ± 9.3
(10^4/μl)	60 min	20.3 ± 2.1	19.5 ± 6.3	20.2 ± 11.3	21.7 ± 11.9
	rate	1.04 ± 0.22	1.00 ± 0.38	0.94 ± 0.17	1.15 ± 0.27
Superoxide	0 min	4605 ± 1273	4704 ± 1575	6375 ± 3341	4138 ± 1403
production (sox)	60 min	4697 ± 1886	4466 ± 1760	6934 ± 1560[i]	3125 ± 725[j]
	rate	1.00 ± 0.23	0.97 ± 0.29	1.33 ± 0.57	0.81 ± 0.20
D/W ratio (%)	pre	18.6 ± 0.5	18.8 ± 1.6[k]	18.2 ± 0.7	19.1 ± 1.5
	60 min	12.9 ± 2.3	13.1 ± 1.4[l]	10.4 ± 6.2	12.7 ± 2.2

[a]; $p < 0.05$; [a] vs. [b]
[b]; $p < 0.05$; [b] vs. [a]
[c]; $p < 0.05$; [c] vs. [d]
[d]; $p < 0.05$; [d] vs. [c]
[e]; $p < 0.05$; [e] vs. [f]
[f]; $p < 0.05$; [f] vs. [e]
[g]; $p < 0.05$; [g] vs. [h]
[h]; $p < 0.05$; [h] vs. [g]
[i]; $p < 0.01$; [i] vs. [j]
[j]; $p < 0.01$; [j] vs. [i]
[k]; $p < 0.05$; [k] vs. [l]
[l]; $p < 0.05$; [l] vs. [k]

Group 2 ($p < 0.0078$).

PVR at 60 minutes of reperfusion were 1824 ± 650, 2100 ± 564 with UW solution showing significantly lower PVR than lungs preserved with EC solution ($p = 0.019$). There was no significant difference between Group 1 and 2, 3 and 4.

The values of Tissue LPO, Leukocyte and Platelet counts, ability of Superoxide generation of circulating neutrophils (SOX) and D/W ratio were summarized in Table 4.

Tissue LPO was 0.75 ± 0.15, 0.85 ± 0.21, 1.52 ± 0.85, 0.70 ± 0.27 nmol MDA/mg pt. SOX was 4697 ± 1886, 4466 ± 1760, 6934 ± 156, 3125 ± 725. D/W ratio (%) was 12.9 ± 2.3, 13.1 ± 1.4, 10.4 ± 6.2, 12.7 ± 2.2 respectively. Group 1, 2 and 4 showed better results than Group 3. There was statistical difference in SOX between 3 and 4 ($P < 0.01$). Concerning the flush with Leukocyte-depleted blood it seemed to inhibit the generation of tissue lipid peroxide and superoxide despite deterioration of PaO_2, PVR and lung compliance in EC groups (Group 4) but not in UW groups (Group 2).

PaO_2 value at sacrifice was 354.2 ± 65.5, 317.0 ± 177.5 Torr in Group 1 ($n = 4$) and Group 2 ($n = 3$). $PaCO_2$ was 33.8 ± 3.9, 40.4 ± 13.0 Torr resprectively. There was no significant difference between two groups keeping a good donor lung function as compared with that before harvesting. In contrast, PVR was 2524 ± 894 and 2947 ± 381 dyne*sec*cm^{-5}, which rose significantly in both groups ($p = 0.031$, $p = 0.0256$ respectively). Dynamic and static comliance fell significantly in Group 1 ($p = 0.0175$). D/W ratio was elevated in both groups insignificantly, meaning recovery from lung edema.

Table 5. Results of UPAO test at sacrifice (meam + SD)

Variables	Group 1	Group 2
number	4/6	3/7
PaO_2 (Torr)	354.2 ± 65.5	317.0 ± 177.5
$PaCO_2$ (Torr)	33.8 ± 3.9	40.0 ± 13.0
PVR (dyn*sec*cm^{-5})	2524 ± 894[ab]	2947 ± 381[ac]
Dynamic compliance (ml/cmH_2O)	8.3 ± 4.7[a]	8.3 ± 2.9
Static compliance (ml/cmH_2O)	34.7 ± 7.9[d]	22.8 ± 4.6[e]
D/W ratio (%)	16.9 ± 2.0	14.9 ± 1.5

[a] ; $p < 0.05$; [a] vs. pre in the same Group.
[b] ; $p < 0.05$; [b] vs. [c]
[c] ; $p < 0.05$; [c] vs. [b]
[d] ; $p < 0.05$; [d] vs. [e]
[e] ; $p < 0.05$; [e] vs. [d]

Histological examination about 60 minutes of reperfusion showed mild interstitial edema in Group 1 and 2. But severe alveolar edema, marked vascular congestion, perivascular edema and alveolar and perivascular extravasation were seen in Group 3 and 4. And vascular congestion was slightly better in Group 3 as ompared with those in Groug 4. (Fig. 1; 60 min in Group 1, Fig. 2; 60 min in Group 2, Fig. 3; 60 min in Group 3, Fig. 4; 60 min in Group 4)

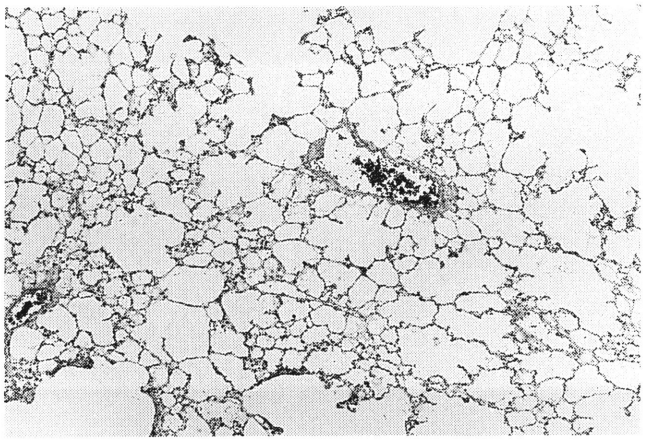

Fig. 1. 60 minutes in Group 1. (Preserved with UW solution for 24 hours and reperfused for 60 minutes.)
Mild interstitial edema was seen. Alveolar structure was preserved well.

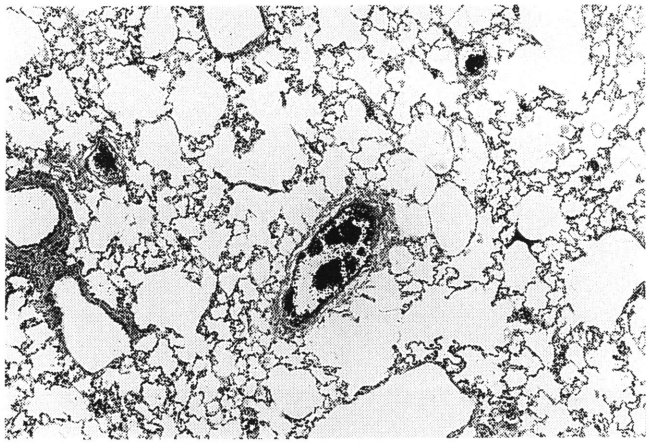

Fig. 2. 60 minutes in Group 2. (Preserved with UW solution for 24 hours and flushed with WBC depleted blood and reperfused for 60 minutes.)
Mild interstitial edema was seen.

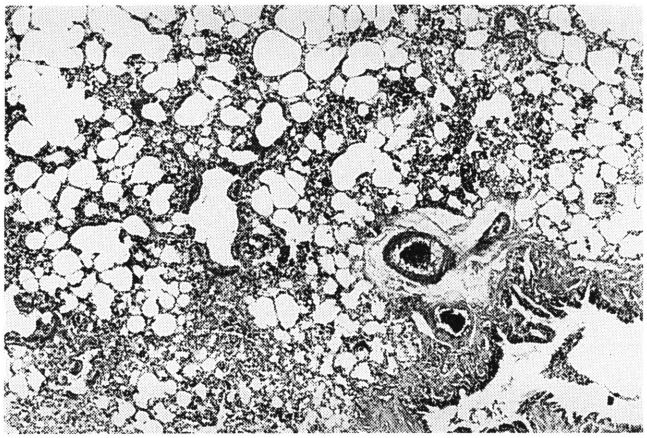

Fig. 3. 60 minutes in Group 3. (Preserved with EC solution for 24 hours and reperfused for 60 minutes.)
Severe alveolar edema, congestion, perivascular edema and extravasation were seen.

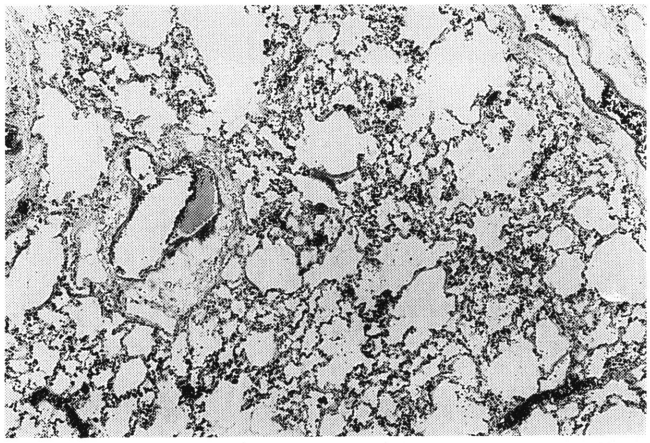

Fig. 4. 60 minutes in Group 4. (Preserved with EC solution for 24 hours and flushed with WBC depleted blood and reperfused for 60 minutes.)
Severe alveolar edema, congestion, perivascular edema extravasation were seen.

Among survivors, specimens of 14 POD showed rejection in 1 in Group 1 and 2 in Group 2 (all grade 2 in Veith classification). But others didn't have any evidence of rejection and showed interstitial thickening and diffuse cuffing around the vessels (Fig. 5; 14 POD, Group 1). These seemed to cause rising of PVR. There was no discriminate difference between Group 1 and 2.

On chest X-ray films (Fig. 6) there was no sign of increasing pulmonary infiltration in 5 out of the 6 animals in Group 1 and all in Group 2. The lung transplants were considered susceptible to the most severe lung edema. No recovery from the infiltrating shadow on the first day of transplantation was observed in this series (2 in Group 1, 2 in Group 2), which implied the irreverssible change of the donor lung.

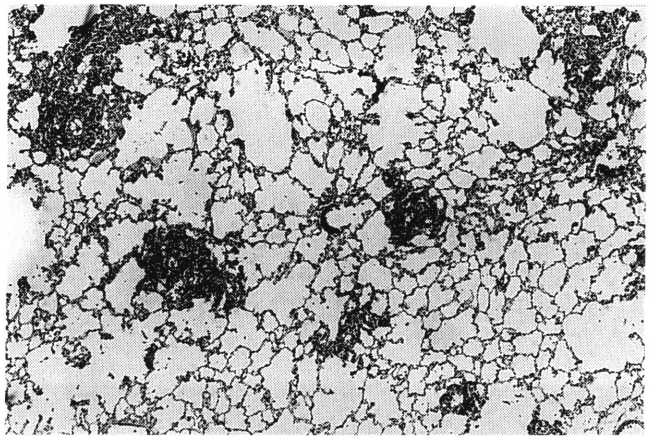

Fig. 5. 14 POD in Group 1.
Diffuse cuffing and slight thickening was seen.

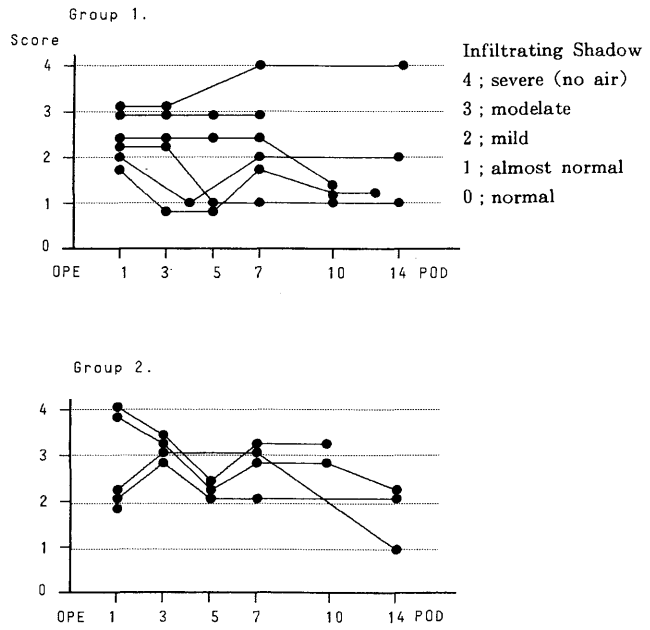

Fig. 6. Post operative Chest X-rays

Discussion

The introduction of UW solution developed by Belzer in 1986 has revolutionized organ procurement and preservation.

Clinically Anthony M. D' Alessnadro reported[11] that the mean preservation time of the liver, the pancreas and the kidney has been extended up to 12.6 ± 4.5, 16.7 ± 4.4, 18.3 ± 4.3 with UW solution respectively. Moreover according to the experimental results, the time limits of successful preservation with UW solution were said 48 hour,[12] 72 hour,[13] 72 hour[14] respectively.

UW solution contains several unique, beneficial components.[15] An impermeable anion or Lactobionate and a trisaccharide or Raffinose with a large molecular weight are active osmotic agent which minimize hypothermic-induced cell swelling. Hydroxyethyl starch creates colloidal osmotic pressure and prevents expansion of the interstitial space. Glutathione and allopurinol prevent injury from oxygen-free radicals and suppress the generation of hydrogen peroxide and the lipid peroxidation. Adenosine is a substrate for ATP synthesis. The independent efficacy of each of these substances remains unclear.

In this study, the functions of lung grafts preserved with UW solution were satisfactory at 60 minutes of reperfusion compared with that before harvesting. And all recipients survived and the donor lungs were maintained well in 4 of 6 for 8 to 15 days.

In contrast, the donor lungs preserved with EC solution failed to retain their function until the sacrifice. Especially two of 4 died of respiratory failure due to progressive pulmonary edema. Though PaO_2 at 60 minutes was satisfactory, PVR was apparently higher than that in UW solution. It indicates that UW solution was able to preserve better endothelial condition or microcirculation but EC was not. It was assumed that microcirculation of the donor lung indicated the good quality of the donor lung and the long survival after transplantation.

EC and UW solutions are intracellular type solutions with high-potassium and low-sodium content. A high-potassium solution is based on the Collins' belief[16] that it prevents cell swelling and potassium ion leakage from preserved cells. However Per Ola Kimblad[17] showed that high potassium caused strong vasoconstriction in the lung preserved with EC solution.

Oka[18] demonstrated that UW and EC solution showed high PA pressure at reperfusion when compared with "low potassium" UW solution. In contrast a gradual decrease in PA pressure was seen after 5 minutes of reperfusion after using UW solution.

It was thought that when UW solution is used a rise in PVR was possibly due to vasoconstriction or high viscosity and not due to severe endothelial damage or dysfunction. However when EC solution is used a rise in PVR might occur not only due to vasoconstriction but also irreversible endothelial cell damage or dysfunction, as confirmed histologically in this study.

Viscosity and oncotic pressure of EC and UW solution were measured at room temperature. Viscosity ratio to normal saline was 1.51, 2.41 and Oncotic pressure was 3.7, 24.4 mmHg respectively. These values of UW solution were high as compared with those of EC solution. Therefore the viscosity and oncotic pressure were not the main factors which affect a rise of PVR.

This study shed a light on the fact that UW solution have a possibility of preservation of the lung for 24 hours.

In this study high values of tissue lipid peroxide were measured in lungs preserved with EC, while lower values were obtained in the groups with UW, EC solution and flushing with leukocyte-depleted blood. And a significant decrease in the superoxide generation of neutrophils was seen with leukocyte-depleted blood. It is accepted that peroxidation of membrane lipids should be done by free radicals, thereby, causing membrane disruption.

It is well known that Oxygen-free radicals are the key mediator of ischemia-reperfusion injury. There are many reports that SOD,[2,3] Dimethlthiourea[19] etc were effective to diminish the reperfusion injury.

McCord[20] indicated that injury induced by oxygen-free radicals may be extremely important in the lung. Because endogenous xanthine oxidase activity is relatively high in the lung compared with that of the kidney and the liver, injury to the lung by oxygen free radicals may be extremely crucial.

It is believed that Oxygen-free radical generation at reperfusion takes place at first in endothelial cells,[21] then is released[22] out of cells even if neutrophils would absent.

As another source of oxygen free radicals, NADPH oxidase system of leukocytes is important. Superoxide is produced in the following formula. ($NADPH+2O_2 \rightarrow NADP^+ +2O_2^- +H^+$). And hydrogen peroxide is produced by dispropornative reaction.

Leukocytes stimulated by a variety of agents exert a cytotoxic effects on endothelial cells. The literature on the subject is growing rapidly and it has been obvious that endothelial cell killing offers a better correlation with generation of oxygen-free radicals, especially the generation of Hydrogen peroxide.[23] Moreover, Hydrogen peroxide is said to stimulate the synthesis of PAF of endothelial cells.[24]

Adhesion of leukocytes to venules is an early and crucial step in the development of an inflammatory response[25] including the reperfusion injury. Endothelial cells contribute to this adhesion with several cell surface molecules that bind various leukocyte population. Different endothelial cell adhesion molecules are present during the time of an inflammatory response leading to sequential recruitment of leukocyte population into an inflammatory site. A key role of these adhesion molecules had been reported[26] in acute rejection of cardiac allotransplantation, but few reports have been made concerning reperfusion injury and rejection in lung transplantation.

F M. Williams[4] reported that microvascular plasma protain leakage was provoked after the adhesion and accumulation of neutrophils.

As described above, peripheral leukocytes play an important role in the progression of reperfusion injury. The circulating neutrophils release free radicals and other vasoactive mediators, and cause endothelial damage, leukocyte sequestration, vasospasm, hemorrhage and increase of permeability, resulting in lung edema.

Therefore, peripheral blood leukocyte depletion after ischemia helps to minimize the reperfusion injury.[5,7,27] In heart transplantation, the initial reperfusion with leukocyte-depleted blood reduced injury, demonstrating the importance of the initial period of reperfusion to attenuate the injury.[27]

Concerning the lung, Breda[7] demonstrated that a donor lung preserved for 24 hours followed by reperfusion with leukocyte-depleted blood verified excellent functional and histological recovery. Moreover, Timothy[5] demonstrated that reperfusion injury with leukocyte-depleted blood was aggravated when leukocytes were returned to the perfusate at 5 or 30 minutes, in contrast, minimal changes were seen when leukocytes returned after 60 minutes of reperfusion. Readdition of leukocytes after 60 minutes of reperfusion did not cause significant damage to the lung.

It meant the importance of leukocyte depletion at the first 60 minutes of reperfusion. These findings strongly suggested that the majority of lung destruction might occur in the first one hour of reperfusion by leukocytes, or there might exist an initiating mechanism of advancing sequential lung injury. For instance, PAF, complements, Leukotriene B_4, and activation of adhesion molecules have been associated with reperfusion injury. These factors argument the importance of the initial 60 min of reperfusion.

Our hypothesis has been that the flush with leukocyte-depleted blood might ameliorate the reperfusion injury and permit the recovery of endothelial cells instead of the attack by leukocytes.

The effect of the flush with leukocyte-depleted blood for initial 60 minutes of reperfusion must be emphasized.

This study clarified that a significant decrease in the superoxide generation of neutrophils and slight decrease of tissue lipid peroxide were observed with leukocyte-depleted blood in the donor lungs preserved with EC.

It is suggested that the flush with leukocyte-depleted blood may have a role of attenuation of leukocyte-related reperfusion injury.

In UW groups, no beneficial effect was observed. It may be based on the antioxidants like allopurinol and glutathione which is included in UW solution perviously and work to prevent reperfusion injury.

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