

Efficacy of a Rinse Solution for Prevention of Reperfusion Injury in Canine 24-hour Cold Preserved Lungs

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The efficacy of a rinse solution for prevention of ischemia-reperfusion injury in the lung preserved for 24 hours was evaluated by using the canine isolated lung perfusion model. The heart and the lungs were harvested and preserved for 24 hours at 4-6 °C in modified Euro-Collins (EC) solution. Prostaglandin E1 (100 µg) was administered into the pulmonary trunk prior to flushing canine lungs with preservation solution. The next day, left lungs were isolated with a perfluorochemical (FC-43) or Carolina rinse II solution. Immediately after rinsing, the lung was reperfused with homologous venous blood (400 ml) for 120 min. in the experimental pump circulation system.

The lungs were classified into the following six groups: Group 1 (n = 5), without rinsing (control group), Group 2 (n = 6), rinsing with oxygenated FC-43 solution (250 ml), Group 3 (n = 4), rinsing with non-oxygenated FC-43 solution (250 ml), Group 4 (n = 5), rinsing with pluronic F 68 solution (250 ml), Group 5 (n = 4), rinsing with the Carolina Rinse Solution II (250 ml) and Group 6 (n = 4), rinsing with the Carolina Rinse Solution II (250 ml) in addition with hydroxyethyl starch (50 g/l). Pulmonary vascular resistance and lung water volume (wet-dry/wet ratio) were lower and dynamic lung compliance was higher in Group 2 than in other groups. The tissue myeloperoxidase level in Group 2 was significantly lower than those in Groups 4, 5 and 6 ($p < 0.05$).

Histologically, severe marked lung edema was observed in Groups 1, 5 and 6. The lung showed almost normal architecture in Group 2. In conclusion, terminal rinsing with oxygenated FC-43 solution immediately before blood reperfusion is useful for prevention of ischemia-reperfusion injury after 24-hour cold ischemic storage in modified Euro-Collins solution.

Introduction

In lung preservation, ischemia-reperfusion injury is dependent on preservation time and preservation solution. Locke et al.¹⁾ reported the successful 6-hour canine lung preservation using Euro-Collins solution confirmed the superiority to topical cooling alone, and then Euro-Collins has become the standard pulmonary flush solution in practice of clinical lung and heart-lung transplantation²⁾. Recently many investigators has studied how the storage interval of the lung preservation could be extended. The

duration of lung allograft preservation has been lengthened but the storage intervals of at least 12 hours are now regarded as a safe limit of lung preservation³⁾⁻⁵⁾.

FC-43 solution, one of perfluorochemicals, is an artificial blood substitute which has a high-oxygen-carrying capacity. Further studies have revealed that the solution had an anti-neutrophilic actions and kept microvascular permeability and resulted in limiting myocardial reperfusion injury in the experimental models⁶⁾⁷⁾.

Carolina rinse solution was developed in 1990 to minimize reperfusion injury in the liver transplantation⁸⁾ and improved graft function after transplantation. Carolina rinse solution II was a modified solution reducing some components of carolina rinse solution to establish more adequate effects.

In this study, we evaluated the efficacy of a rinse solution for ischemia-reperfusion injury of canine 24-hour cold ischemic stored lungs using the canine isolated lung perfusion model.

Materials and methods

(1) Experimental animals

Twenty-eight adult mongrel dogs weighting 7 to 12 kg, were prepared and provided from the Laboratory Animal Center for Biochemical Research of Nagasaki University School of Medicine.

All animals received human care in compliance with the "Guide for the Care and Use of Laboratory Animals of Nagasaki University". After overnight fast, the animals were premedicated with intramuscular ketamine hydrochloride at 10 mg/kg and atropine sulfate at 0.03 mg/kg, and anesthetized with intravenous administration of sodium pentobarbital (25mg/kg). The animals were intubated with an endotracheal tube and ventilated at a fixed FIO₂ of 1.0, a tidal volume (TV) of 35 ml/kg and the respiratory rate (RR) of 14 breaths/min using volume-cycled ventilator (Harvard-animal-ventilator). Median sternotomy was performed, and the both subclavian arteries, the innominate artery and the superior vena cava

were exposed and looped. The right main bronchus was clamped, a tidal volume and ventilatory rate were decreased to 20 ml/kg and 10 breath/min, and then the airway pressure was measured. 500 units/kg of heparin sodium was injected intravenously and 100 microgram of the prostaglandin E 1 was administered through the main pulmonary artery. A silastic 24 Fr. catheter was inserted through the pulmonary trunk. After ligation of the left subclavian artery, the innominate artery and the superior vena cava, both lungs were flushed with 500 ml cold EC solution at the pressure of 10 mmHg through the catheter placed in the pulmonary trunk. During the flushing, ventilation was continued. The left atrium was opened to permit drainage of the perfusate. Immediately after the flushing, the heart and the lungs were harvested and immersed in a sterile vinyl bag filled with the same cold storage solution keeping these distensibility at their inflation of 80 % endotidal volume and stored at 4-6°C for 24 hours in a refrigerator.

The animals were classified into the following six groups according to a rinse solution.

Group 1 (n = 5): not rinsed (control group).

Group 2 (n = 6): rinsed with oxygenated perfluorocarbon emulsion (FC-43).

Group 3 (n = 4): rinsed with non-oxygenated FC-43.

Group 4 (n = 5): rinsed with Pluronic F68 solution.

Group 5 (n = 4): rinsed with the Carolina Rinse Solution II.

Group 6 (n = 4): rinsed with the Carolina Rinse Solution II containing with HES (50 g/L).

Oxygen pressure of oxygenated and non-oxygenated FC-43 was 217 ± 28 mmHg and 95 ± 5 mmHg, respectively. Rinse solutions were warmed at 37°C.

(2) Oxygenation of FC-43

The FC-43 emulsion was mixed in the Erlenmeyer flask and oxygen (5L/min) was blown on the surface of FC-43 emulsion in the Erlenmeyer flask for over 10 minutes. The circulation tube was put into the flask and filled with the emulsion, and then rinsing was performed. The non-oxygenated FC-43 was maintained in a sealed vinyl chloride pack without air.

(3) Procedure of rinse and reperfusion

After hypothermic preservation for 24 hours, the left lung was divided. A pulmonary arterial cannula was connected to the reservoir filled with rinse solution which was warmed at 37°C, and pulmonary vein was left open allowing lung perfusate to drain freely into a waste bottle. The isolated left lung was rinsed with 250 mL solution by a roller pump at a flow rate of 10 mL/kg/min for 10 minutes. The flow rate was gradually increased for the

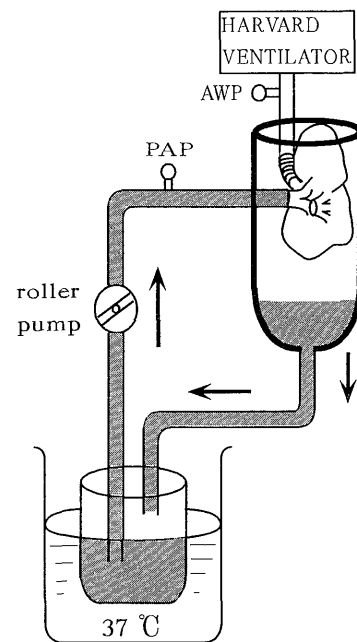


Fig. 1. Isolated lung perfusion model (PAP: pulmonary artery pressure, AWP: air way pressure)

initial 2 minutes. During rinsing, the lung was ventilated with room air at a tidal volume of 20 mL/kg and at a rate of 10 breath/min, and the same ventilating condition was continued through the subsequential blood reperfusion. After rinsing, the isolated left lung was reperfused with allogeneous blood for 120 minutes (Fig.1). The pulmonary artery cannula was connected to the reservoir containing allogeneous blood, and the lung perfusate from the pulmonary vein was drained into the reservoir.

(4) Monitoring of isolated lung function

The lung perfusate for the initial 5 minutes was collected and blood gas analysis was performed. When reperfusion was finished, the lungs were reperfused with 100 mL of the second allogeneous venous blood and blood gas analysis was performed. During the reperfusion, blood samples were taken from the pulmonary vein for the blood gas analysis and count of blood red cells, leukocytes and platelets at 5, 10, 30, 60 and 120 min. The pulmonary arterial pressure and the airway pressure were monitored, the dynamic and static lung compliance and the pulmonary vascular resistance were calculated; the dynamic lung compliance: tidal volume/pressure (mL/cmH₂O) at end-inspiratory plateau, the static lung compliance: tidal volume/pressure (mL/cmH₂O) at 1.4 sec of the end-inspiratory plateau, pulmonary vascular resistance: pulmonary artery pressure/flow rate (mmHg/L/min). Tissue TBA (lipid peroxidation) and myeloperoxidase were measured before and after reperfusion. After reperfusion, lung water volume was measured.

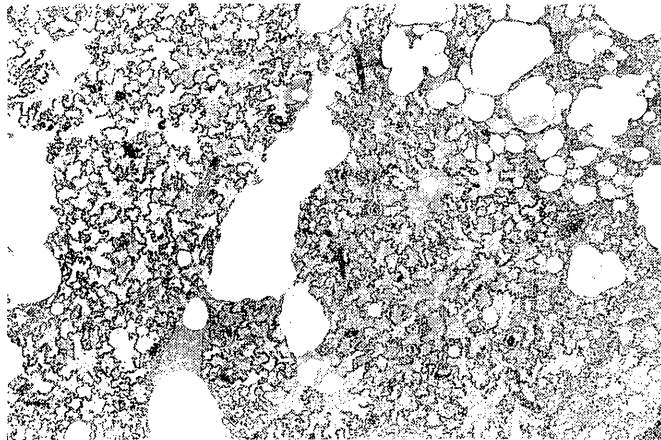


Fig. 2. Photomicrogram of the lung in Group 1 (HE staining $\times 16$). Severe alveolar edema with thickness of the alveolar septa can be seen.

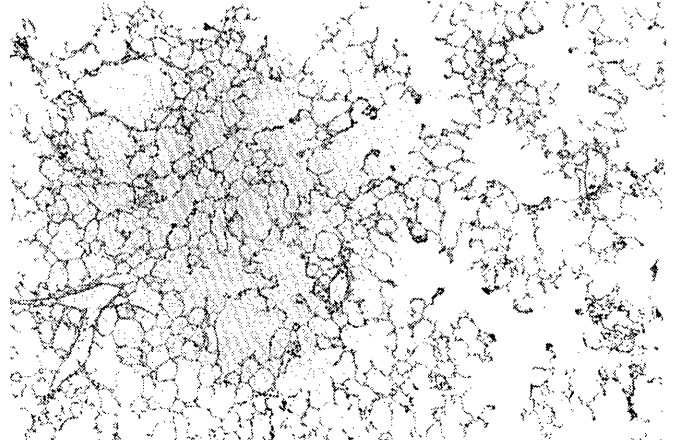


Fig. 3. Photomicrogram of the lung in Group 2 (HE staining $\times 16$). Histologically, the lung appears normal architecture.

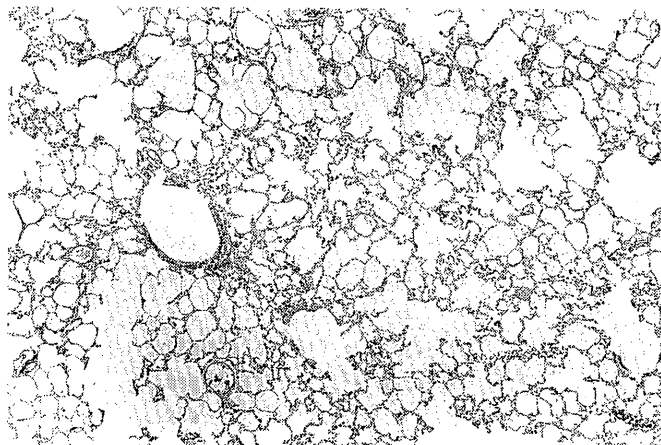


Fig. 4. Photomicrogram of the lung in Group 3 (HE staining $\times 16$). Histologically, the lung appears normal architecture.

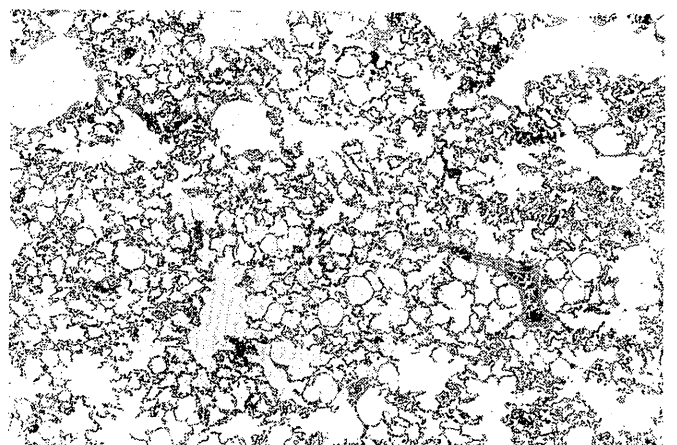


Fig. 5. Photomicrogram of the lung in Group 4 (HE staining $\times 16$). Mild intraalveolar and intraseptal bleeding can be seen.

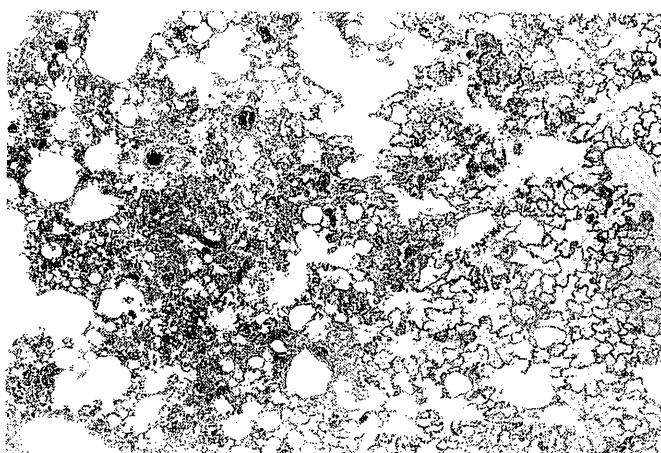


Fig. 6. Photomicrogram of the lung in Group 5 (HE staining $\times 16$). Marked alveolar and perivascular extravasation and ragged alveolar septa can be seen.

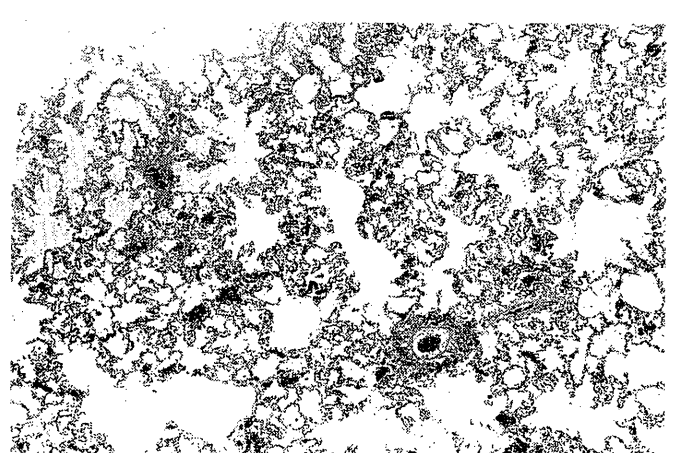


Fig. 7. Photomicrogram of the lung in Group 6 (HE staining $\times 16$). Marked alveolar and perivascular extravasation and ragged alveolar septa can be seen.

Lung tissue lipid peroxidation

Lung tissue lipid peroxidation was evaluated as TBA reactive material⁹⁾. The assay was performed immediately before and after reperfusion. A 0.3 g lung specimen was frozen in liquid nitrogen. The frozen specimen was homogenized and added with cold saline to make a 10% homogenate. A 0.2 mL of the homogenate was added and mixed 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 mL TBA. The mixture was heated at 95°C for 60 minutes. A 1.0 mL of distilled water and 5 mL of n-butanol/pyridine (15:1, vol/vol) were added to the mixture after cooling. The mixture 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 RF 5000 (Shimadzu Cop, Tokyo, Japan). The lipid peroxide concentration was determined by reference to a standard fluid of 0.5 nmol 1, 1, 3, 3-tetramethoxypropane that yields 0.5 nmol of malondialdehyde (MDA). The protein content of the homogenate was determined by the Lowry's method¹⁰⁾. Tissue lipid peroxidation was evaluated as MDA nmol/mg tissue protein.

Lung tissue myeloperoxidase

Myeloperoxidase (MPO) can be used as a marker for tissue neutrophils which was trapping in the tissue during reperfusion¹¹⁾. Lung tissues were homogenized and suspended by a 0.5% hexadecyltrimethylammonium bromide (HTAB) (Sigma chemical Co., St. Louis, MO) in 50 mM potassium phosphate buffer, pH 6.0, to yield the 5% homogenate. The suspension (1 ml) was centrifuged at 40,000 × g for 15 minutes, and then the supernatant (0.1 ml) was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, containing 0.167 mg/mL o-dianisidine dihydrochloride (Sigma Chemical Co.) and 0.0005% hydrogen peroxide (WAKO pure chemical industries, LTD. Osaka, Japan). The mixture was assayed by spectrophotometry. The change of absorbance at 460 nm was measured with (Double-beam spectrophotometer, UV-150-02. Shimadzu Seisakusho, Kyoto, Japan). MPO concentration was determined by reference to a standard human MPO. One unit of MPO activity was defined as that degrading one micromole of peroxide per minute at 25°C.

Lung water volume

The left lower lobe was divided and weighed and placed in a desiccator at 160°C for 48 hrs after reperfusion, and then the dry lung was weighed. Lung water volume was determined by the (wet-dry)/wet weight ratio in the left lower lobe.

Histological examination

The reperfused lungs were fixed in 20% formalin, paraffin embedded, and standard hematoxylin and eosin-stained slides were prepared.

(5) Statistical analysis

Data were compared using the unpaired Wilcoxon test. A P-value of less than 0.05 was considered statistically significant.

Results

There was no significant difference in the rinse pressure among Groups 2, 3, 4, 5 and 6 (Table 1).

Lung reperfusion time was 64.2 ± 34.6 , 120 ± 0 , 97.5 ± 39.0 , 52.8 ± 35.2 , 85.5 ± 36.2 and 62.4 ± 16.3 minutes in Group 1, 2, 3, 4, 5 and 6 each. Successful reperfusion for the entire 120 minutes was accomplished in all of the lungs in Group 2 (Table 2).

There was no significant difference in PaO₂ and dynamic lung compliance among the groups.

The PVR and the lung (wet-dry)/wet weight ratio were lower in Group 2 than in the other five groups ($p < 0.01$).

The t-LPO was higher in Group 3 than in Groups 1, 4, 5 and 6 ($p < 0.05$).

The tissue MPO level was lower in Group 2 than in Groups 4, 5 and 6 ($p < 0.05$).

Histologically, severe lung edema was observed in the lungs in Groups 1, 5 and 6 (Fig.1, 5 and 6), and bleeding was observed in Group 4 (Fig.4). Microscopic findings of the lung in Groups 2 and 3, showed almost normal lung architecture (Fig.2, 3).

Discussion

Our data demonstrates that the oxygenated FC-43 may be useful for prevention of ischemic reperfusion injury of canine 24-hour cold preserved lung.

FC-43 (The Green Cross Corporation, Osaka, Japan) is an emulsion of perfluorocarbon which has the high oxygen solubility (Table 3). Perfluorocarbons have been initially developed as artificial blood substitutes because the emulsion has a high affinity for oxygen and releases oxygen in a linear fashion unaffected by temperature¹²⁾. Pluronic F-68 is the agent that is used to stabilize the emulsion in which the perfluorocarbon is suspended.

The experimental studies with perfluorocarbon emulsion are mainly for the organs' preservation for transplantation. Perfluorochemicals have significantly prolonged the preservation and prevent the reperfusion injury of the heart⁶⁾¹³⁾, the liver¹⁴⁾, the lung¹⁵⁾¹⁶⁾ and the pancreatic¹⁷⁾

Table 1. Results after 120 minutes perfusion(mean ±SD)

	Group 1 (n = 5)	Group 2 (n = 6)	Group 3 (n = 4)	Group 4 (n = 5)	Group 5 (n = 4)	Group 6 (n = 4)
Rinse P.(mmHg)		6.8±2.4	7.8±2.9	11.8±3.5	7.9±4.1	13.6±5.1
ΔPO ₂ (mmHg) at initial 5 minute	66.3±43.5	83.0±38.7	39.3±48.1	65.1±12.5	43.6±32.8	34.2±12.4
ΔPO ₂ (mmHg) at additional venous blood	37.7±31.4	68.6±32.6	66.6±26.1	61.6±21.5	48.1±34.4	46.9±22.4
Dynamic LC (mL/cmH ₂ O)	7.30±1.39	10.33±2.31 ^a	8.92±2.56	5.97±1.89 ^b	6.28±1.48 ^c	7.32±1.37 ^d
PVR (mmHg/L/min)	322.30±190.52 ^e	84.26±33.26 ^f	171.19±104.77 ^g	357.85±166.52 ^h	474.10±219.10 ⁱ	430.22±88.74 ^j
WD ratio	0.9055±0.0067 ^k	0.8683±0.0210 ^l	0.9256±0.0230 ^m	0.9133±0.0092 ⁿ	0.9217±0.0217 ^o	0.9213±0.0108 ^p
t-LPO (nmol/mg protein)	0.507±0.225 ^q	1.776±0.689	1.241±0.142 ^r	0.541±0.326 ^s	0.441±0.119 ^t	0.547±0.261 ^u
t-MPO (unit/g tissue)	358.5±125.9	240.0±94.0 ^v	275.1±42.8	450.0±345.2 ^w	399.4±106.4 ^x	454.7±215.7 ^y

abbreviation

- Rinse P.; Rinse Pressure
- ΔPO₂; gradient of blood oxygen pressure between pulmonary vein and pulmonary artery
- LC; Lung Compliance
- PVR; Pulmonary Vascular Resistance
- WD ratio;(wet lung weight-dry lung weight)/(wet lung weight)
- t-LPO; tissue lipid peroxidation
- t-MPO; tissue myeloperoxidase

p < 0.01; a vs. b and d; f vs. e, h, i and j; e vs. i and j; g vs. i and j; h vs. i and j; l vs. k, m and p; r vs. u and t.
p < 0.05; a vs. c; l vs. o; r vs. q and s; v vs. w, x and y.

Table 2. Lung reperfusion time (minute)

	Group 1 (n = 5)	Group 2 (n = 6)	Group 3 (n = 4)	Group 4 (n = 5)	Group 5 (n = 4)	Group 6 (n = 4)
No.1	50	120	120	120	120	35
No.2	120	120	30	51	93	71
No.3	45	120	120	20	104	77
No.4	21	120	120	43	25	67
No.5	85	120		30		
No.6		120				
mean±SD	64.2±34.6 ^a	120±0 ^b	97.5±39.0 ^c	52.8±35.2 ^d	85.5±36.2 ^e	62.5±16.3 ^f

p < 0.05; b vs. a, b vs. d, b vs. e, b vs. f
no significant difference; b vs. c

grafts. Tabayashi et al.¹³⁾ reported that the oxygenated fluorocarbon solution gave superior myocardial protection during 2 hours of ischemic arrest and postischemic left ventricular hemodynamics, and water content was significantly lower in the oxygenated fluorocarbon group than in the nonoxygenated crystalloid cardioplegia. Kawamura et al.¹⁷⁾ reported that a 2-layer (Euro-Collins'/Perfluorochemical) cold storage method for the preservation of canine pancreas extended the storage interval for 72-hour and that the functional success rate after transplantation was 100 % and biopsies of the graft showed almost normal

architecture in exocrine and endocrine tissues. These papers showed that one of the perfluorochemical action, an oxygen-supplying agent, was utilized for the ischemic organs and the tissues were kept in almost normal functions and structures. Tuula S. Kurki et al.¹⁶⁾ reported that oxygenated FC-43 solution was useful for lung preservation in pigs. After donor lung was preserved with oxygenated FC-43 for 6 hours, single lung allotransplantation was performed and they concluded that oxygenated FC-43 donor lung preservation was superior in functional recovery in pulmonary gas exchange during

Table 3. Composition of preservation solution and rinse solution

	Euro-Collins (mM)	FC-43 (mM)	F-68 (mM)	Carolina Rinse II (mM)
FC-43		20 %		
Pluronic F68		2.56%	2.56%	
NaCl		102	102	102
KCl	15	4.6	4.6	4
K ₂ HPO ₄	42.5			
KH ₂ PO ₄	15			
NaCO ₃	10			
NaHCO ₃		2.5	2.5	
CaCl ₂		2.5	2.5	3
MgCl ₂		2.1	2.1	
Sodium lactate				28
Glucose	3.5%	1.8 %	1.8 %	
Adenosine				0.1
Alloprinol				1
Desferrioxamine				1
Gluthatione				3
Hydroxyethyl starch		30g/L	30g/L	(additional HES) 50g/L
Na +	10 mEg/L	104.5 mEg/L	104.5 mEg/L	102 mEg/L
K +	115 mEg/L	4.6 mEg/L	4.6 mEg/L	4 mEg/L
Osmotic pressure	335 mOsm/L	309 mOsm	309 mOsm	278 mOsm/L
pH (room temp.)	7.2 - 7.5	7.3 - 7.5	7.3 - 7.4	7.4

HES: Hydroxyethyl starch
temp.: temperature

reperfusion compared with the lung function preserved with Euro-Collins solution. Lehtola and colleagues¹⁹⁾ demonstrated that electron microscopic findings of the lungs preserved with oxygenated FC-43 for 6 hours showed better-preserved alveolar epithelium lining comparing with the findings of the lungs preserved with modified Euro-Collins solution.

The perfluorochemicals have not only high oxygen solubility but beneficial effects on prevention of ischemia-reperfusion injury. Many reports indicated that the perfluorochemicals reduced neutrophil chemotaxis, adherence, degranulation and superoxide production⁷⁾¹⁸⁾¹⁹⁾ and kept the microvascular permeability²⁰⁾⁻²²⁾. Virmani et al.¹⁸⁾ reported oxypherol (perfluorotributylamine) inhibited human neutrophil function in vitro. Neutrophils treated with oxypherol-treated caused nearly 90% suppression of chemotactic response to zymosan-activated serum calculated by comparing the control response. Neutrophils exposed to oxypherol were stimulated by exposure to phorbol myristate acetate and superoxide release of the neutrophils was reduced to nearly 80% by oxypherol exposure compared with buffer response. Joseph E. Hall et al.²⁰⁾ reported that perfluorocarbon-related changes in canine lobar permeability were determined by measuring the pulmonary filtration coefficient (Kf). In the isolated canine right lower lobe model, Kf value was not affected by FC-43 infusion, the mean Kf values in FC-43 infused group and bovine serum albumin solution group were 0.075

and 0.070 mL/min/torr/100 g each.

In the experimental liver transplantation, Carolina rinse solution was developed for the use as a rinse after storage to prevent lethal injury to sinusoidal endothelial cells⁹⁾. Wensi Gao et al.²³⁾ reported that in the Lewis rat livers which were implanted after 12-hour cold storage in University of Wisconsin solution and rinsed with Carolina rinse solution prior to reperfusion the survival time was improved as compared with the livers rinsed with Ringer's solution. They also reported that adenosine was an essential component of Carolina rinse and they simplified Carolina rinse and formulated Carolina rinse II with a minimum of ingredients²⁴⁾. It contains electrolytes similar to plasma, antioxidants against oxygen radical and vasodilator to improve microcirculation. In this study, Carolina rinse II solution was not effective to prevent lung edema in canine 24-hour cold preserved lungs.

The tissue lipoperoxidation (MDA) and MPO were significantly lower in the lungs rinsed with oxygenated FC-43 than those in the lungs rinsed with non-oxygenated FC-43 or Carolina rinse II solution. Lipid peroxidation, the oxidative deterioration of polyunsaturated fatty acids, is a widely accepted mechanism for cellular injury, especially ischemia-reperfusion injury. Activated xanthine oxidase, which catalyzes the reaction of hypoxanthine with oxygen, produces xanthine and superoxide radicals. The hypoxanthine substrate accumulates in ischemic tissue. In this study, MDA reactants production was reduced in the

lungs rinsed with FC-43 after reperfusion and it suggested that FC-43 may prevent superoxide radical generation.

Bradley et al.¹¹⁾ reported that MPO was a marker for tissue neutrophil content and skin MPO content which might be useful as a measure of the neutrophil inflammatory response in a variety of clinical and experimental states. Goldblum et al.²⁵⁾ reported that lung MPO reflected granulocytes which were not freely circulating but were sequestered or marginated in the lung. In this study, MPO level was higher in the lungs rinsed with Carolina rinse II solution than the lungs rinsed with oxygenated FC-43. Therefore, Carolina rinse II solution may induce neutrophils chemotaxis and adherence response compared with FC-43.

Oxygenated FC-43 solution was superior to non-oxygenated FC-43 in lung water volume. It is suggested that FC-43 provides oxygen to the endothelium so that the anaerobic state changed into the aerobic state. Superoxide and other noxious metabolite generated at preservation were enzymatically removed from the endothelium. In consequence, the pulmonary permeability did not increase and ischemia-reperfusion injury to the lungs were protected.

In summary, emphasis has been placed on the terminal rinsing with oxygenated FC-43 prior to reperfusion to serve as maintenance of the pulmonary function after reperfusion and also to allow for prolongation of lung preservation time. The efficacy of this solution is based on the action of perfluorocarbon and oxygen delivery.

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