Acta Med. Nagasaki 34 : 177−187

Experimental study on active oxygen species to warm ischemic lung. − Availability of GSH, SOD, and alloprinol −

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Received for publication, December 26, 1987

SUMMARY : Ischemic-reperfusion injury caused by oxygen-drived free radicals is one of the great problems to be solved for successful lung transplantation and preservation. This study was performed to clarify the effect of the free radical scavenger, reduced glutathione (GSH), superoxide dismutase (SOD), and alloprinol for warm ischemic lung, and to elucidate changes of active oxygen species production in neutrophils.

<**Method** > Sixty-three mongrel dogs underwent hilar stripping following left thoracotomy. The left pulmonary artery, pulmonary vein, and left main bronchus were clamped for 2-3 hours. To reduce the free-oxygen radicals, alloprinol (30mg/kg /day, for 3 days peros), GSH (50mg/kg, I. V.) administration and perfusion through the pulmonary artery with 4℃ Euro-Collins' solution including GSH (1mg/*l*), SOD (15mg/ml), and alloprinol (20mg/*l*) were performed. At the time of right pulmonary artery occlusion test, the blood gas analysis of arterial blood and measurement of left pulmonary arterial pressure were done on the pre-ischemic period, immediately after and one hour after declamping.

At the same time, active oxygen species production in neutrophils was evaluated using flow cytometric procedure.

<**Results**> 1) The good pulmonary gas-changing function remained after ischemic period by administration and perfusion with free radical scavenger GSH, SOD, and alloprinol.

2) Changes of pulmonary arterial pressure at right pulmonary artery occulision test was not statistically significant.

3) Production of active oxynene species in neutrophils was increased after ischemic period.

In conclusion, all of GSH, SOD, and alloprinol are effective in elminating ischemia-reperfusion injury to warm ischemic lung.

INTRODUCTION

Since 1965, clinical application of lung transplantation has been made in 3 patients in Japan.[1) 2)] However, its outcome failed to obtain long survival due to immunologic rejection. On the contrary, in Europe and the united states of America there are quite a few prolonged survivors following transplantation including renal transplantation with an did of development of cyclosporine A.[3)]

Therefore, reperfusion injury is a great concern to success of clinical lung transplantation except for immunologic response. There are many reports in the pathogenesis of reperfusion syndrome. Great concern is focused on an activation of active oxygen species[4) 5) 6)] to

prevent damage to warm ischemic donor lung. The purpose of this study is to clarify the role of free radical scavenger such as GSH, SOD and alloprinol to elimenate reperfusion injury at transplantation by means of an evaluation of inhibited generation of free radical in peripheral neutrophils.

MATERIAL AND METHOD

1) Animal

Sixty-three Mongrel dogs weighing from 8 to 14 kg in body weight were used. All dogs were supplied from Laboratory Animal Center for Biomedical Reserch, Nagasaki University School of Medicine.

2) Experimental Method

Dogs were anesthetized with 25mg/kg of pentobarbital sodium, intubated intratracheally and ventilated with Harvard Ventilator, 250-350ml in tidal volume, 12-14/min in respiratory rate. Cannulation into the femoral artery was performed to measure the systemic blood pressure continuously. Left thoracotomy was made at the 5th ICS. The left main pulmonary artery and left main bronchus were isolated from the surrounding tissue. A procedure of hilar stripping , which is composed of dividing branches of the vagus, bronchial artery and lymphatic channels.

After heparization of 100mg/kg, the left main pulmonary artery, vein and left main bronchus were separately clamped to make a model of warm ischemic lung for 2-3 hours. After ralease of clamps with elapsed time of 2-3 hours blood flow was recirculated. In infusion groups prior to restart of blood flow, a small catheter (1.9mm I.D.) was introduced into the pulmonary artery to monitor the infusion pressure of 40cmH_2O and also a small hole was made in the left atrial wall so as to allow perfusate to flow out.

At the time of completion of perfusion prior to restoration of blood circulation, the monitoring catheter was withdrawn and the left atrial wall was repaired.

At the first hour following blood recirculation, PA pressure was measured and blood samples from PA, PV and femoral A were prepared for blood gas analysis and assessment of free radical in neutrophils.

3) Measurement of generation of free radical in neutrophils.

The measurement was in accordance with modified BASS' method[7] (Fig. 1) by using Spectrum III (ORTHO Co.). As a stimulator to neutrophils, phorbol myristate acetate (PMA) was used.

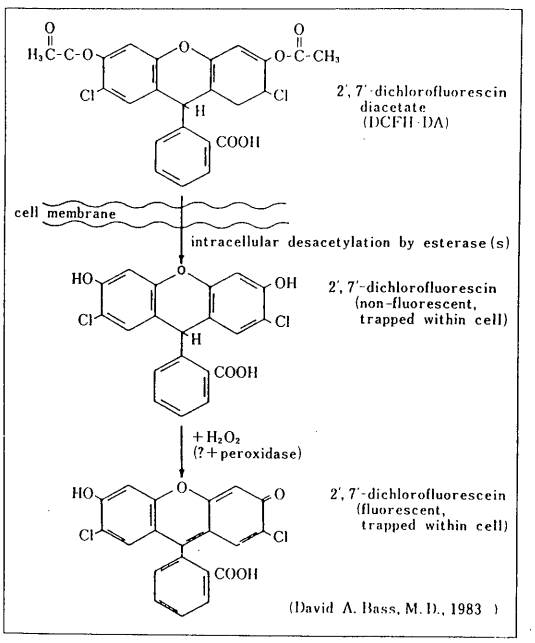

Fig. 1. Principle of evaluation of active oxygen species production

<Measurement steps>

1) 0.1ml Blood samples were taken, 1.9ml of 0.5M 2-7-dichlorofluorescin diacetate (DCFH-DA) was added, incubated at 37℃ for 15 min.

2) 0.5ml of EDTA adjusted to 24mM with PBS were added to prevent aggregation of neutrophils.

3) and incubated at 37℃ for 25 min in addition to 10μl of PMA (15μg/ml)

4) washed with PBS and centrifused at 1300 rpm for 10 min.

5) hemolysed with lysing solution of 0.87% NH_4Cl

6) washed with PBS and centrifused at 1300 rpm for 10 min

7) the pellet is suspended with 10% gel-Hanks'

solution

8) analysed with the use of spectrum Ⅲ. As a negative control, the peripheral blood from a dog was used and the positive zone (B%) was adjusted to be 2-5% of B-start at the resting phase and generation of active oxygen species was calculated as being B% after stimulation with PMA.

<Experimental groups>

The groups in the experiment were divided into 7.

group Ⅰ : simple warm ischemia (n=13)

group Ⅱ : intravenously given GSH (50mg/kg) at induction of anesthesia and prior to recirculation (n=8)

group Ⅲ : orally given alloprinol (30mg/kg) 3 days before the experiment (n=10)

group Ⅳ : perfusion group with Euro-Collins' solution (E-C solution) (n=8)

group Ⅴ : perfusion group with E-C solution +GSH (1mg/ml) (n=8)

group Ⅵ : perfusion group perfused with E-C solution+SOD (15mg/*l*) (n=8)

group Ⅶ : group perfused with E-C solution + Alloprinol (20mg/*l*) (n=8). (Alloprinol supplied from Jap Welcome Co.)

<Statistical analysis>

The values were expressed as the means ± standard deviation (SD). The difference among means was determined to be significant with Wilcoxon-t test.

RESULTS

1) PA pressure (Table 1, Fig. 2)

The PA pressures in all groups were elevated after temporary contralateral PA blockage. There was no statistically significant difference among the groups.

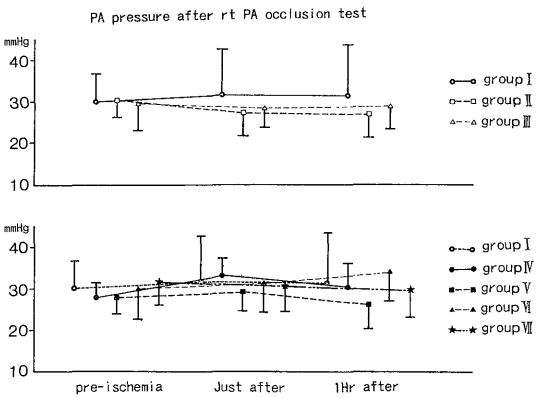

Fig. 2. Pulmonary artlrial pressure after rt PA occulusion test

2) arterial PaO_2 (Table 2, Fig. 3)

The levels of paO_2 in group Ⅱ, Ⅲ were superior to that in group Ⅰ and also those in group Ⅴ, Ⅵ, Ⅶ were better than in group Ⅳ immediately and one hour after restoration of blood circulation. Statistically significant differences were calculated in group Ⅱ immediately after and one hour after and in group Ⅲ immediately after recirculation ($P<0.05$) and in group Ⅲ one hour after recirculation ($p<0.01$) and as compared with the control in group Ⅴ, Ⅶ one hour after ($p<0.05$) respectively.

3) arterial $PaCO_2$ (Table 3, Fig. 4)

The levels of $PaCO_2$ were increased by temporary contralateral PA block test. Its grade of group Ⅱ Ⅲ and Group Ⅴ Ⅵ Ⅶ was depressed as compared with that of group Ⅰ and Ⅳ, in particular there is significant difference between group Ⅱ, Ⅴ and the control groups.

Table 1. Pulmonary arterial pressure (mmHg)

	group Ⅰ	group Ⅱ	group Ⅲ	group Ⅳ	group Ⅴ	group Ⅵ	group Ⅶ
pre-ischemia	19.7± 9.0	21.7± 4.1	18.4± 4.8	19.5± 2.8	19.0± 4.0	20.8± 5.7	21.6± 4.7
r. PA occ. pre.	30.1± 6.5	30.4± 3.8	29.6± 6.2	27.9± 3.3	27.9± 4.1	29.9± 7.3	31.3± 5.2
immediately after	20.6± 7.8	18.9± 4.2	19.8± 5.6	19.8± 2.9	18.9± 3.8	22.1± 7.9	21.1± 3.9
r. PA occ. im. after	31.1±11.7	27.4± 5.6	28.3± 4.7	33.1± 4.2	29.3± 4.6	31.4± 7.3	30.6± 6.1
1 Hr after	22.6± 8.0	20.6± 4.6	20.8± 5.6	20.0± 3.8	19.0± 3.1	23.9± 5.4	20.6± 3.0
r. PA occ. -1Hr after	31.4±12.2	27.0± 5.1	28.9± 5.5	30.4± 5.6	26.3± 6.0	34.0± 6.8	29.5± 6.7

Table 2. Blood gas alalysis-PaO_2- (mmHg)

	group I	group II	group III	group IV	group V	group VI	group VII
pre-ischemia	424.9± 75.5	455.8± 48.4	473.5± 58.3	469.5± 46.3	441.0±113.2	462.8± 32.1	459.7± 67.2
r. PA occ. pre.	223.4±109.3	230.4±106.0	312.6±164.0	275.3±103.5	249.0± 90.6	222.8±126.3	177.3±112.3
immediately after	357.7± 97.4	495.5±102.8	438.6±136.6	452.7±136.3	440.8±132.6	399.7±100.7	459.9±141.9
r. PA occ. im. after	86.4± 44.2	225.2±118.3	236.4±166.0	193.5±113.9	252.3±138.9	230.4±109.7	294.7±151.9
1 Hr after	155.3± 88.8	356.2±173.2	390.0±119.1	340.3± 85.3	373.0±104.2	354.4±116.6	367.5±184.7
r. PA occ. -1Hr after	92.0± 30.8	182.6± 97.6	215.0±100.0	113.6± 47.3	237.6±155.2	198.9±148.0	232.5±184.5

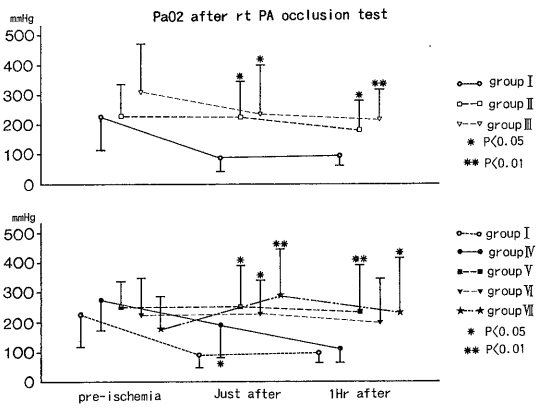

Fig. 3. PaO_2 after rt PA occulusion test

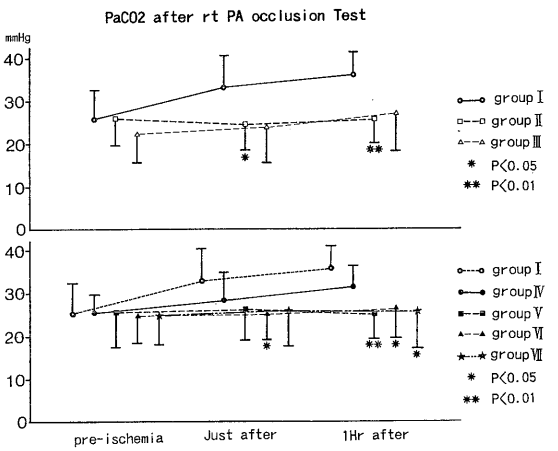

Fig. 4. $PaCO_2$ after rt PA occultsion test

Table 3. Blood gas analysis-pressure (mmHg)

	group I	group II	group III	group IV	group V	group VI	group VII
pre-ischemia	26.9± 9.3	17.9± 3.3	18.9± 4.3	19.2± 2.1	18.4± 5.0	18.2± 5.5	18.2± 3.1
r. PA occ. pre.	25.7± 6.9	26.1± 6.7	22.2± 6.5	25.4± 4.3	25.5± 8.4	24.7± 6.4	24.8± 6.8
immediately after	23.8± 6.4	19.9± 4.7	19.5± 5.0	23.3± 7.6	19.4± 4.4	20.3± 5.1	19.2± 5.1
r. PA occ. im. after	33.2± 7.3	24.5± 6.1	23.9± 8.4	28.4± 6.5	26.2± 7.1	25.3± 5.9	26.1± 8.5
1 Hr after	29.2± 8.7	22.1± 5.7	22.4± 6.3	27.3± 6.4	22.2± 4.0	23.2± 4.4	23.9± 7.5
r. PA occ. -1Hr after	36.0± 5.1	25.8± 5.6	27.0± 8.8	31.5± 4.9	25.1± 6.0	26.8± 7.3	25.5± 8.5

4) Intrapulmonary shunt (Table 4, Fig 5)

The intrapulmonary shunting rate was calculated as the following equation[8], $Qs/Qt = 0.03\ (PAO_2 - PaO_2)\ /4.5 + 0.003\ (PAO_2 - PaO_2)$, wherein $FiO_2 = 1$, $SaO_2 = 100\%$, $CaO_2 - CvO_2 = 4.5$ vol%.

The intrapulmonary shunting rates were figured out during performing temporary contralateral PA block test Intrapulmonary shunt lessened in groups in which protective drugs for storaged lungs were used, in particular, were of great benefit in group II immediately and one hour after recirculation ($P < 0.01$).

5) Generation of free radical in neutrophils (Table 5, Fig. 6)

Fig. 7 revealed a cytogram of Spectrum III, in

Table 4. Blood gas analysis-shut ratio- (%)

	group I	group II	group III	group IV	group V	group VI	group VII
pre-ischemia	23.32±2.97	23.04±4.10	22.53±5.22	20.32±3.67	22.27±3.73	23.20±5.33	25.03±4.17
immediately after	28.10±1.36	23.17±4.63	25.23±4.75	24.71±4.63	21.92±5.64	22.96±4.34	20.12±6.20
1 Hr after	27,80±0.99	24.95±3.67	24.94±1.46	27.47±1.52	22.46±6.55	23.96±5.80	22.65±8.15

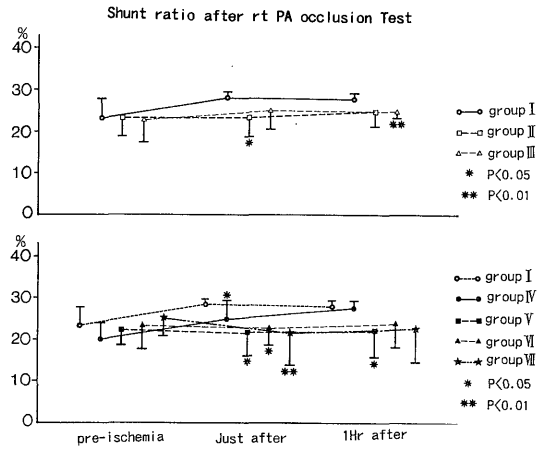

Fig. 5. Intrapulmonary shunt

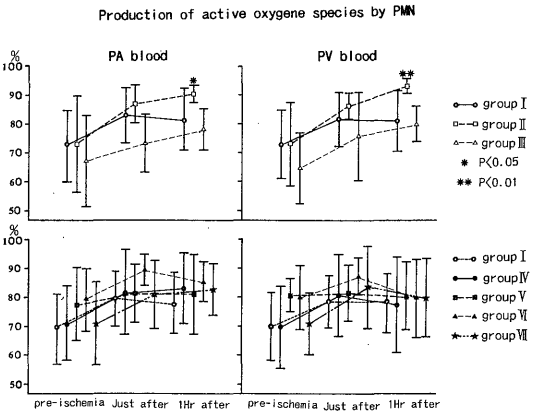

Fig. 6. Generation of free radical in neutrophils

Table 5. Production of active oxygene species by PMN (%)

	group I	group II	group III	group IV	group V	group VI	group VII
pre-ischemia (PA)	72.7±12.0	73.1±16.8	67.3±15.7	71.0±12.9	77.8±12.4	79.2±10.7	72.0±13.3
pre-ischemia (PV)	72.9±12.1	73.1±14.8	64.7±12.3	69.8±14.1	80.9± 5.5	79.9±11.1	70.7±10.7
immed. after (PA)	83.0± 9.3	86.9± 6.6	73.6± 9.8	82.0±14.8	81.3±10.3	89.3± 4.8	80.9±11.9
immed. after (PV)	81.9± 9.6	86.4± 4.3	76.0±12.4	80.7±14.1	81.7± 9.8	87.5± 6.4	83.7±11.8
1 Hr after (PA)	81.5±10.7	90.3± 3.1	78.1± 7.2	83.1±12.4	80.9±13.9	85.6± 6.9	82.8± 8.9
1 Hr. after (PV)	81.1±10.2	92.9± 1.8	80.1± 5.9	77.5±16.6	86.7±11.5	87.0± 6.7	79.9±13.5

which the ordinate represented the size of cells obtained by the foward light scatter method, on the other hand, the abscissa presented inner structure of cells obtained by the 90 degree light scatter method.

Fig. 8-A demonstrated a histogram at the resting states and Fig. 8-B showed a histogram after stimulation by PMA. It showed that neutophils distributed regularily. In every group, generation of free radical was accerelated after ischemia. However, there was no significant difference in generation of free radical between the blood sumpling from pulmonary arteries and veins.

As compared with the control, generation of free radical one hour after recirculation was pronounced in group II, although there was no definite difference in the other groups.

DISCUSSION

Since lung transplantation has first been made in clinical application by HARDY[9], scores of patients were reported. However, cyclosporin offered insight into the significant effect on suppression of immune response. In

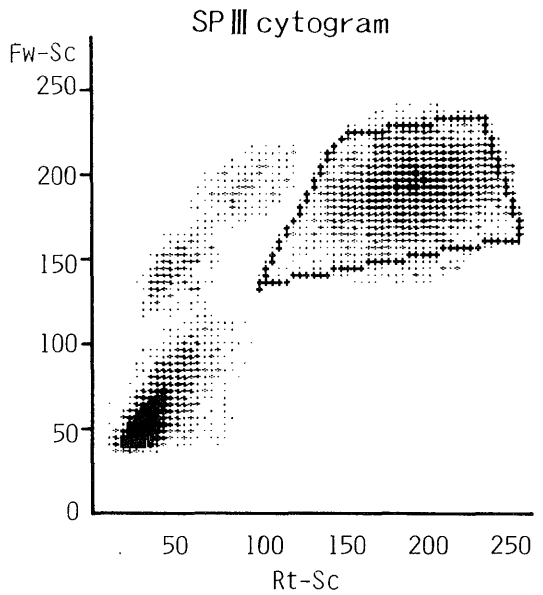

Fig. 7. Cotogram of spectum III

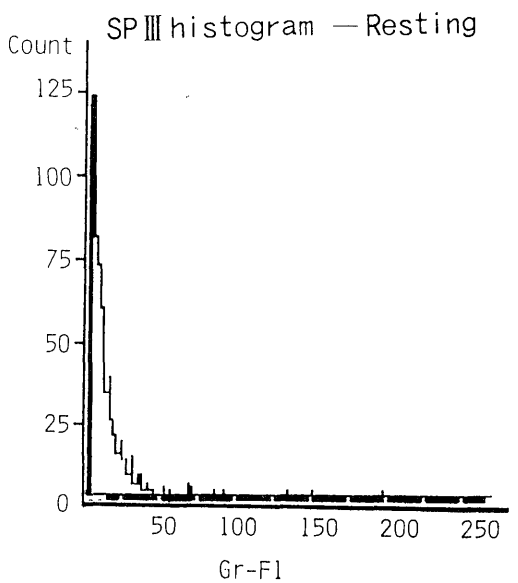

Fig. 8-A Histogram of spectum III (Resting)

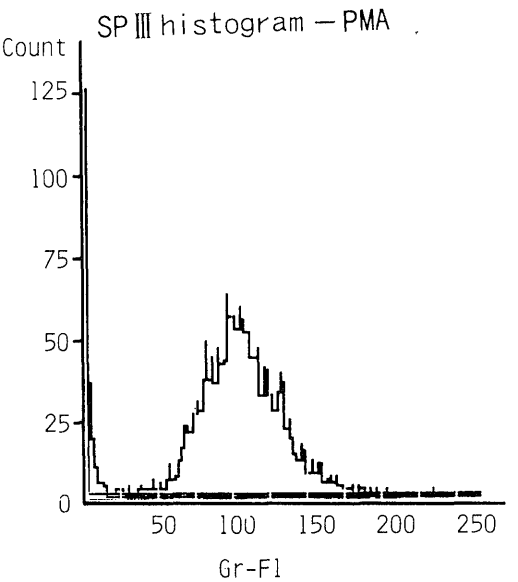

Fig. 8-B. Histogram of spectrum III (PMA stimulation)

consequence Copper reported prolonged survivors with lung allograft in clinical use.

To expect an adequate function of lung allografts immediately after lung transplantation, reperfusion injury should be minimized during storage of a donor lung. MCCORD[4] explains that ischemic damage to a donor lung is based on increased permeability of the vessel wall and subsequent edema. Furthermore cytotoxic ferment breaks away from the inside of cells to the blood.

On the other hand, it is accepted that reperfusion injury is mainly caused by free radicals. [4)-6) 10) 11)]

WEISS [10] suggested that main damage to cellular structure and function is associated with action of free radicals. HAGLUND *et al* [11] suggested that organ damage of storage lungs is due to not only ischemia but reoxygenation. JACKSON *et al* [12] reported that free radicals allow destruction of protein, membrane and nucleic acid and causes stagnation, incomplete reexpansion of donor lung, thickness of alveolar septum and fibrosis in the pulmonary parenchym.

A damage to a donor lung caused by free redicals offers a great problem on ischemia during storage of a donor lung and restoration of blood circulation after completion of transplantation procedure.

Lung edema which takes place in early stage after lung transplantation is referred as reimplantation response[13) 14)] and main cause is ischemia and denervation. However, it still

remains unsettled.

<Alloprinol>

In 1968 MCCORD [15] pointed out that most important source of free radicals is xanthine oxidase system as shown in Fig. 9[16], when the reactions of ATP, ADP, AMP to hypoxanthine progress, O_2 supply by blood recirculation makes O_2^- by a reaction of hypoxanthine to Xanthine. It needs for Xanthine oxidase (XOD) which contains abundantly in organs.

Alloprirol is an inhibitor of XOD, which is used for gout.

There are some reports on the effects of alloprinol with respect to the heart[4) 17) 18)] small interstine[4) 19)] and liver[4)], although it is scanty concerning the lung.

HASHIMOTO [20] reported that XOD activities in various organs are as follows, 19.5munit/g in the brain, 240.0 in the liver, 185.1 in the lung, 26.3 in the heart, 225.4 in the spleen, 122.3 in the kidney 630.4 in the pancreas 708.0 in the small intestine 95.2 in the colon 147.2 in the adrenal grand respectively. KINASEWITZ [21] also cited that alloprinol effectively inhibited increased permeability of vessel walls in the pulmonary capillary. From the facts, alloprinol is one of the effective inhibitors against ischemia and reperfusion injury.

It is well known that the protective role of alloprinol in ischemia and reperfusion injury is required for alteration to oxyprinol in vivo and it consumes some time interval. Therefore, it is recommended that alloprinol should be administered in advance.

In this study, it is substantiated that the use of alloprionl prior to 3 days is of great benefit to eliminate damage of ischemia and reperfusion injury to a donor lung as compared with the control and a result of group Ⅷ which was intraoperatively perfused with protective drugs.

<GSH>

GSH acts as one of radical scavengers and cooperates with glutathione perioxidase (GSH-Px) to eliminate an action of active oxygen species[22) 26)], which deals with H_2O_2 in the cells and/or mitochondria[23)] and plays a key role in acting as ground substance[24)] with an aid of NADPH and NADH actions (Fig. 10).

In the hypoxic circumstances, a shortage of ATP supply results in a decrease in GSH synthesis and a delay in dealing with active oxygen species.[25)] It is a confirmed fact that hypoxia in the brain reduces the amount of GSH[27)], and it is suggested that GSH plays an important role in removing active oxygen species.

On the other hand, it is well known that a

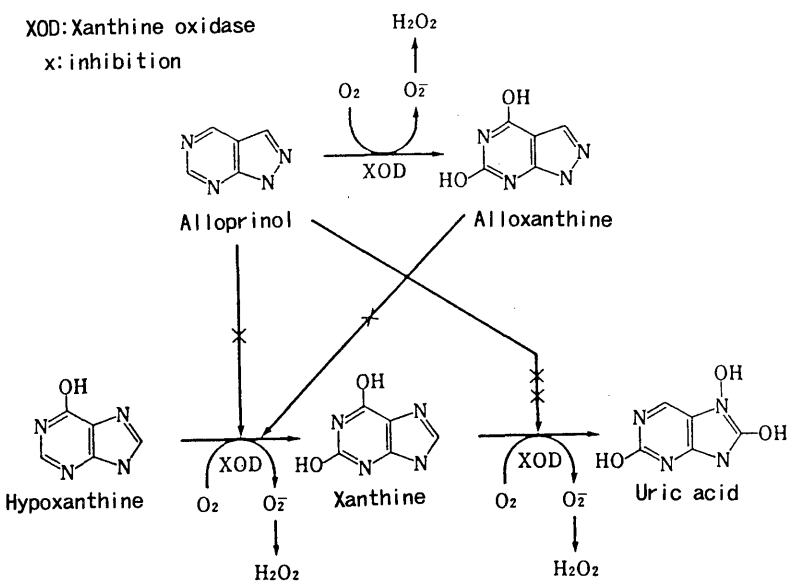

Fig. 9. Reaction of Xanthine

Cat:catarase

GR :glutathione reductase

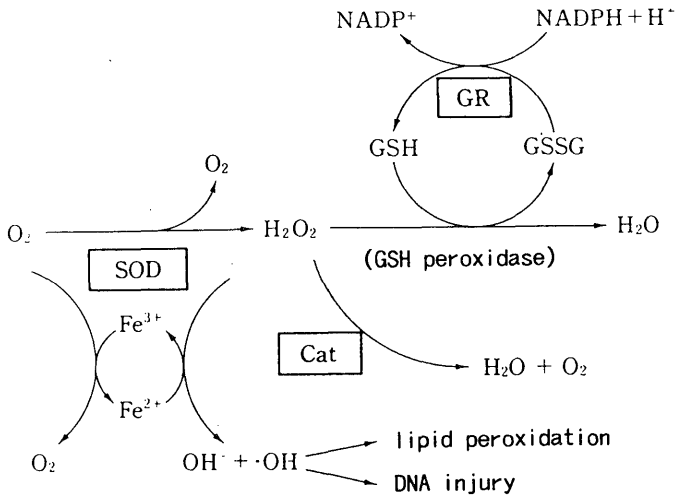

Fig. 10. Reaction of glutathione

shortage of GSH in the lung facilitates oxygen intoxication.[28]

DAWSON[29] explained a merit of the use of exogenous GSH in the experiments of isolated lung and/or isolated lung cells in the hypoxic-c ircumstances to prevent is chemic damage to pulmonary parenchym. It is also clarified that damage to the epithelial cells of the gut is protected by exgenous GSH, and administration of GSH contributes to elimination of active oxygen species.[30]

It is dubious as to whether intravenous administration of GSH is absorbed in the reticuloendothelial system or not. It is accepted that the half life of GSH is just 3 min and its action reduces to 90% at 10 min and during that time the content of GSH in the liver and kidney does not vary in rats. In human being the half life is 2 min when given intravenously and it is quickly metabolized.

When GSH is dissolved, it converts into three amino-acids of cystine, glycine and glutamic acid and is resynthesized to GSH in the liver and carried via blood circulation.

In this study, it is ascertained that GSH is of great help to reduce ill effects of ischemic damage and reperfusion injury on a storage lung. The reasons are due to direct protective action of GSH and/or resynthesized GSH in the liver from amino-acids of dissolved GSH.

It is said that antioxidation by the help of GSH is established with GSH, intracellularily synthesized, which plays a key role in contributing to dealing with glutathione peroxidase (GSH-Px) in the plasma.[31) 32]

<SOD>

Since MCCORD *et al*[33] discovered SOD in 1969, advances in basic study on SOD have been established. SOD includes the types of Mn, Fe and Cu, Zn. In mammalias, SOD exists in the Cu, Zn type in the cells and the Mn type in the mitochondria.

In the human body, SOD is distributed in varying patterns[34] (Table. 6). The main action is promotion of a reaction from O_2^- to H_2O_2. Therefore, final product of O_2^- should be removed in humans.

SOD increases with advancing age. However, this tendency toward an increase in SOD is much more potent in neonates than in older patients[35]. In the experiment with the rat lung which has high resistance to oxygen activity, the following fact is clarified that type II alveolar cells increase in number and SOD is activated at the same time[36].

However, it is doubtful if intravenous

Table. 6. Cu, Zu-SOD and Mn-SOD in human organs (mg/kg)

	Cu, Zn-SOD	Mn-SOD
liver	490	390
lung	254	25
kidney (cortex)	164	142
RBC	157	0
uterus	148	26
brain	136	55
heart	119	97
muscle	107	52
thyroid	103	65
testis	76	22
spleen	71	22
lymph node	58	71
fatty tissue	8	3

administration of SOD is helpful to reduce O_2 intoxication because of a short halftime. It is reported that 55% of SOD is excreted in the urine 2 hours after I.V. administration and 29 % accumulates in the renal cortex[37]. In this study, direct administration by perfusion was performed to maintain its action as long as possible in order to expect a role of SOD.

Needless to say, there are many devices to keep long-standing action of SOD. For example, it is a trial product of included SOD to liposome which shows a high affinity to the cell membrane containing a rich lipoprotein so that it is easy for SOD to be taken into the cells, and attention is paid to prolong halflife time. FREEMAN[38] reported that by using vascular endothelium which is highly sensitive to oxygen intoxication, SOD included in liposome provided a 95% resistance to oxygen, and it is confirmed that in in-vivo experiment it made a half time of circulating SOD prolonged and achieved inhibition of a 100% O2 intoxication with an aid of high activity of SOD.

<Neutrophils>

The main source of active oxygen species is neutrophils. Ischemia causes active arachidon acid reaction and consequently free radicals are generated. In addition destructive product of fibrin, production of leukotrien B_4, platelet activating factor (PAF) and so on activate neutrophile in the reaction circle of generation of free redicals.[39]

In this study, the levels of free radicals were kept high on account of stimulation of ischemia at the time of start of recirculation and by activated neutrophils at one hour after recirculation. The method of measuring free redicals according to BASS[7] by using flow cytometry is of great benefit in terms of simplicity and accuracy.

In the experiment regarding ischemic hearts[40] it is reported that, when recirculation was performed with free-neutrophil blood, the ischemic area in the heart was reduced and minimized and lessened the degree of infiltration of neutrophils around the tissues by adding SOD, suggesting some parts of SOD to neutrophils.

In this study, the author emphasized that intravenous administration of GSH offered insight into the effect that given GSH accelerates GSSG reaction circle by facilitating NADPH reaction, and consequently leads to an increase in O_2^- generation.

Needless to say, O_2^- generated in the body is eliminated by the help of GSH action, therefore damage of O_2^- is minimized. On the other hand, in the perfusion groups, activation of O_2^- was lower than those in the other groups. It is more likely for GSH to be not in contact with the membranes of neutrophils in the perfusion groups. It is different from in I. V given groups.

As for the pressure of the pulmonary artery, it was a tendency to be lower in the groups in which protective drugs were used. However, it was of no statistical significance. It seems to be associated with the slight degree of reversible alteration of perivascular edema and thrombosis, reflecting no ominous effects on pulmonary vascular resistance.

SUMMARY

The study aimed at an inhibition of free radicals to prevent warm ischemic damage to a donor lung.

1) SOD, GSH and alloprinol play a protective role in preventing warm ischemic damage and in eliminating alveolar-capillary block

2) These drugs were significantly of no use to reduce the pressure of the pulmonary artery following lung transplantation

3) Generation of active oxygen species in neutrophils increased by an addition to ischemia and start of recirculation, in particular, that in the GSH-given group was much more prominent than those in the other groups.

ACKNOWLEDGEMENT

The author would like to thank Prof Dr Masao TOMITA, First Department of Surgery, Nagasaki University School of Medicine for his advice and revision and thank Dr Katsunobu KAWAHARA and the doctors for the willing co-operation and technical assistance in this study.

I also appreciate for kindness of animal supply from the Labolatory Animal Center for Biomedical Reserch of Nagasaki University School of Medicine and thank to the members of Blood Transfusion Service of Nagasaki University Hospital. This work was partially supported by a Grant-in-Aid for general scientific reserch from the Ministry of Education of Japan.

REFFERENCE

1) Y.TSUJI : Experimental study on lung transplantation : *Jap. J. transplant.* : **1** : 236-240, 1966.
2) Y.HAYATA *et al* : Clinical manufestation of lung transplantation : *Jap. J. Chest Disease* : **27** : 99-102, 1968.
3) R.Y. CALNE, D.J.G. WHITE, S.THIRU, *et al* : Cyclosporine A in patients receiving renal allografts from cadaver doners : *Lancet* : **2** : 1323-1327, 1987.
4) J.M. MCCORD : Oxygen-derived free radicals in post-ischemic tissue injury : *The new Engl. J. of Med.* : **312**, 159-163, 1985.
5) S.L.MARKLAND : Oxygen toxicity and protective systems : *Clin. Toxicology* : **23**, 4-6 : 289-298, 1985.
6) I.A.CLARK : Tissue damage caused by free oxygen radicals : *Pathology* : **18** : 181-186, 1986.
7) D.A.BASS, J.W.PARCE, L.R.DECHATELET, *et al* : Flow cytometric studies of oxdative product formation by neutrophils -A graded response of membrane stimulation : *The J. of Immunology* : **130, 4** : 1910-1917, 1983.
8) Y.OHSAKI, T. IRIE : Shunt : *Training of examination for respiratory function* : **86-91**, 1983, Chuugai shuppan Co.LTD, Tokyo.
9) J.D.HARDY *et al* : Lung homotransplantation in man-Report of the initial case : *JAMA* : **186** : 1065-1074, 1963.
10) A.J.WEISS : Oxygen, ischmia and inflammation : *Acta Phisio. Scand.* : **548** : 9-37, 1986.
11) U.HAGLUND, O.LUNDGREN : Intestinal ischemia and shock factors : *Fed. Proc.* : **37** : 2729-2733, 1978.
12) R.M.JACKSON : pulmonary oxygen toxicity : *Chest* : **88,6** : 900-905, 1988.
13) S.S.SIEGELMAN, S.B.P.SINHA, F.J.VEITH : Pulmonary reimplantation response : *Ann. Surg.* : **177, 1** : 30-36, 1973.
14) M.G.EHRIE, J.D.CRAPO, N.C. DURHAM, *et al* : Reimplantation response in isografted rat lungs. -Analysis of causal factors- : *J. Thorac. Cardiovasc. Surg.* : **87** : 702-711, 1984.
15) J.M. MCCORD, I.FRIDOVICH : The reduction of cytochrome C by milk Xanthine oxidanse : *J. Biol. Chem.* : **243** : 5753-5760, 1968.
16) Y. OOYANAGI : Introduction : *Superoxide and Medicine* : **1-22**, 1981, Kyouritsu Shuppan Co.LTD, Tokyo.
17) D.J.CHAMBERS, M.V.BRAIMBRIDGE, D.J. HEARSE : Free radicals and Cardioplegia alloprinol and oxypurinol reduce myocardial injury following ischemic arrest : *Ann. Thorac. Surg.* : **44** : 291-297, 1987.
18) K. BANDO, M.TAGO, S. TERAMOTO : Prevention of free radical induced myocardial injury by alloprinol -Experimental study in cardiac preservation and transplantation : *J. Thorac. Cardiovasc. Surg.* : **95** : 465-476, 1988.
19) D.A.PARKS, G.B.BULKLEY, D.N.GRANGER *et al* : Ischemic injury in the cat intestine : *Gastroenterelogy* : **82** : 9-15, 1982.
20) S. HASHIMOTO : A new spectrophotametric assay method of Xanthine oxidase in crude tissue homogenate : *Anal. Biochem.* : **62** : 426-435, 1974.
21) G.T.KINASEWITZ, L.J.GROOME, R.P. MARSHALL *et al* : Effect of hypoxia on permeability of pulmonary endotherium of canine visceral pleura : *Appl. Physiol.* : **61** : 554-560, 1986.
22) K.NISHIKIORI, H.MAENO : Glutathione for medical treatment : *Protein, Nucleic Acid, Enzyme* : **33, 9** : 1625-1631, 1988.
23) K.TAKAHASHI : Seren and glutathione peroxidase : *Protein, Nucleic Acid, Enzyme* : **33, 9** : 1945-1504, 1988.
24) N.S. KOSOWER, E.M. KOSOWER : *Free radicals in Biology* : (ed W.A.Pryor) : **12** : 58-84, 1976, Achademic Press.

25) N.TATEISHI, T. HIGASHI, N. TANIGUCHI : Metabolic-map of glutathione : *Protein, Nucleic Acid, Enzyme* : **33, 9** : 1358-1364, 1988.
26) T,YUSA : Free radical scavenger and pulmanary disease : *Annual Review of Resp.* : **79-87**, 1988.
27) A.J.L.COOPER, W.A.PULSINELLI, T.E.DUFFY : Glutathione and ascorbate during ischemia and postischemic reperfusion in rat brain : *J.of Neurochemistry* : **35, 5** : 1242-1245, 1980.
28) S.M.DNEKE, B.A.LYNCH, B.L.FANBURG : Transient depletion of lung glutathione by diethylmaleate enhances oxygen toxicity : *J.Appl. Physiol.* : **58** : 571-574, 1985.
29) J.R. DAWSON, K. VAHAKANGAS, B. JERNSTRON *et al* : Glutathione conjugation by isolated lung cell and isolated, perfused lung-Effect of extracellular glutathione : *Eur.J.Biochem.* : **138** : 439-443, 1984.
30) L.H.LASH, T.M.HAGEN, D.P.JONES : Exogenous glutathione products intestinal epitherial cells from oxidative injury : *Proc. Natl. Acad.Sci. USA* : **83** : 4641-4645, 1986.
31) M. INOUE : Membranous transportation of glutathione and its metabolism : *Glutathione* : **43-66**, 1985 : Koudansha Co. LTD, Tokyo.
32) K.R.MADDIPATI, L.J.MARNETT : Characterization of the major hydroperoxide reducing activity of human plasma. Purification and properties of a selenium dependent glutathione peroxide : *J. Biol. Chem.* : **262** : 17398-17403, 1987.
33) J.M. MCCORD, I. FRIDOVICH : SOD an enzimic function for erythrocuprein : *J. Biol. Chem.* : **25** : 6049-6055, 1969.
34) S. MARKLUND : Distribution of Cu, Zn-SOD and Mn-SOD in human tissue and extracellular fluid : *Acta Phisiol. Scand.* : **492** : 19-23, 1980.
35) Y. KOBAYASHI, T. USUI : Clinical meaning of superoxide dismutase : *Jap. J. Metabolism* : **15** : 23-32, 1978.
36) B.A.FREEMAN, B.J.MASON, M.C.WILLIAMS *et al* : Antioxididant enzyme activity in alveolar type II cells after exposure of rats to hyperoxia : *Exp. Lung Res.* : **10** : 203-222, 1986.
37) Y.SHIOKAWA : Free radical and anti-inflammatory drugs. : *Active Oxygen Species* : **318-333**, 1987 : Ishiyaku shuppan Co.LTD, Tokyo.
38) B.A.FREEMAN, J.F.TURRENS, Z.MIRZA, *et al* : Modulation of oxidant lung injury by using liposome intrapped superoxide dismutase and catalase : *Fed. Proc.* : **44** : 2591-2595,1985.
39) E. OKABE, H. ITOH, Cardiac ischemia -Free radical as mediator of ischemia-reperfusion injury.- : *Jap. J. Clin. Med.* : **46, 10** : 2190-2195, 1988.
40) M.L.HESS, *et al* : Identification of Hydrogen peroxide and hydroxyl radicals as mediators of leukocyte-induced myocardial dysfunction : *Adv. Myocardiol* : **5** : 159-175, 1985.