

Cytoprotective roles of GSH, SOD and solcoseryl against ischemic damage and reperfusion injury to warm ischemic lung. — Study of Canine warm ischemic lung. —

*Hideki TANIGUCHI, Katsunobu KAWAHARA, Tomonori NAKASONE
Hideki IKARI, Seiji HONJOH, Hiroshi HISANO, Takao TAKAHASHI
Hiroyuki KUSANO, Hiroyoshi AYABE, and Masao TOMITA

*The First Department of Surgery
Nagasaki University School of Medicine*

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SUMMARY: This study was performed to clarify the roles of reduced glutathion (GSH), superoxide dismutase (SOD), and solcoseryl in ischemic damage and reperfusion injury to the warm ischemic lungs of experimental animals.

Fifty-one warm ischemic canine lungs were made by hilar stripping and clamping of the left PA, PV and bronchus for 2-3 hours. In the Non-perfusion group, GSH (50mg/kg, I.V.: Group II) and solcoseryl (50mg/kg, I.V.: Group III) were administered. In the perfusion group, Euro-Collins (E-C) solution (20ml/kg) was perfused (Group IV) and GSH (1mg/ml in E-C solution: Group V) and SOD (15mg/l in E-C solution: Group VI) were used for anti-oxidative drugs. The pulmonary arterial pressure and aortic pressure were measured and also blood gas analysis was made during the preischemic period, immediately after, one hour and 3 days after reperfusion. Small parts of pulmonary tissues were taken for pathological examination one hour and 3 days after reperfusion. Chest X-ray films were taken at 3 days after the operation.

GSH, SOD, and solcoseryl effectively act as scavengers of active oxygen species (AOS), especially in terms of oxygenation. In the group with anti-oxidative drugs, cytoprotective effects of the pathological and chest X-ray findings on ischemic damage and reperfusion injury were much more manifest rather than those in control groups.

INTRODUCTION

For better organ preservation, ischemic damage and reperfusion injury are serious problems. The major factor contributable to the injury is activation of free radicals. McCORD¹⁾ reported that postischemic tissue injury was caused by secondary edema, which was associated with an increase in membranous permeability.

This study was conducted to clarify the beneficial effects of the use of GSH, SOD, and

solcoseryl on ischemic and reperfusion injury to warm ischemic lung. It was clarified that these drugs play a key role in improving the pulmonary function of the warm ischemic lung after reperfusion, in particular, with regard to oxygenation.

MATERIALS AND METHODS

Fifty-one mongrel dogs were used for this study. They were obtained from the Laboratory Animal Center for Biomedical Research of

Nagasaki University School of Medicine.

Under general anesthesia using intravenously pentobarbital sodium (25mg/kg), a thora-

cotomy was made via the 5th intercostal space and a warm ischemic lung model was made by hilar stripping and clamping of the left pulmo-



Fig. 1-A Score 3

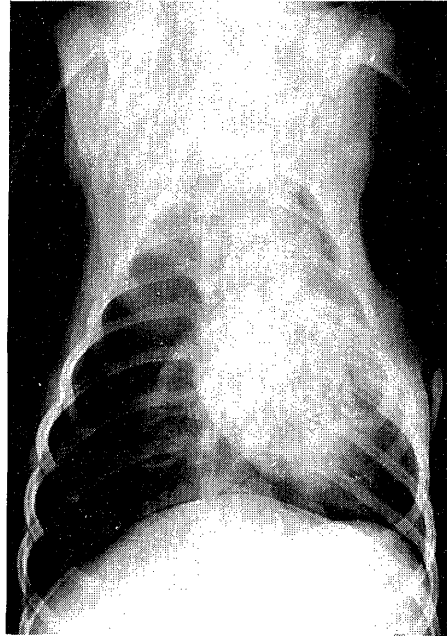


Fig. 1-B Score 2

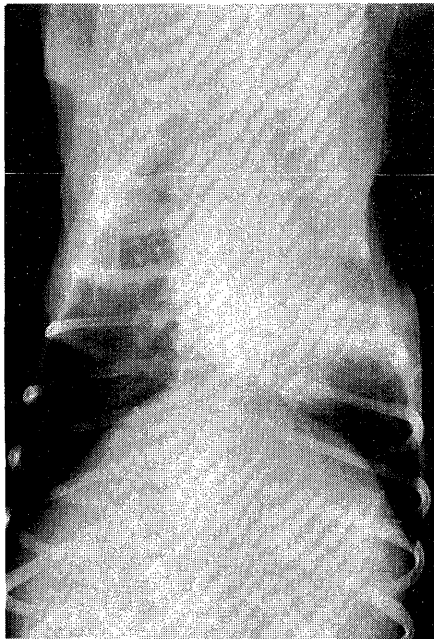


Fig. 1-C Score 1

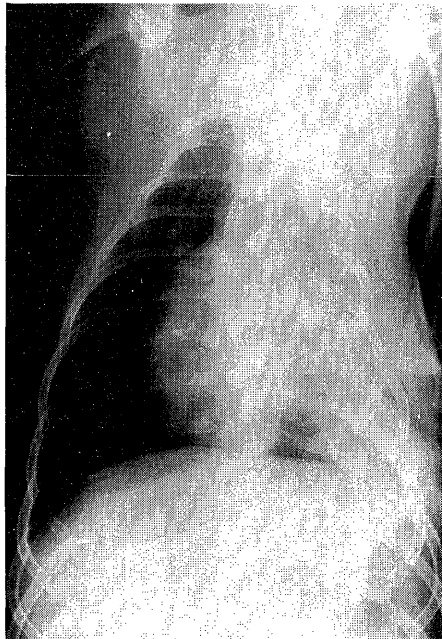


Fig. 1-D Score 0

Fig.1. Score from 0 to 3 by Chest X-ray findings.

nary artery, pulmonary vein, and bronchus. Heparine (100mg/kg) was intravenously administered before clamping and 2-3 hours after clamping, reperfusion was initiated after declamping. In the perfusion group, perfusion was achieved through a cut-down tube (1.9mm I.D.) in the left pulmonary artery at a pressure of 40cmH₂O. The perfusate was overflowed from the small incised wall of the left atrium. Before reperfusion, the opening sites of the left pulmonary artery and left atrium were sutured with 6-0 proline. The aortic pressure and pulmonary arterial pressures were measured and blood gas analysis was made during the pre-ischemic time, immediately after, one hour and 3 days after reperfusion. The findings of chest X-ray films and pathology of lung tissues

were graded as follows.

(X-ray : **Fig. 1**)

Score 0: severe infiltrating shadow.

Score 1: moderate infiltrating shadow.

Score 2: mild infiltrating shadow.

Score 3: normal.

(Pathology : **Fig. 2**)

Score 0: severe destruction of the alveolar structure and edema.

Score 1: moderate destruction of the alveolar structure and edema.

Score 2: mild destruction of the alveolar structure and edema.

Score 3: normal.

The dogs were divided into 6 groups.

— Non perfusion groups —

Group I: simple warm ischemic group. (n=13)

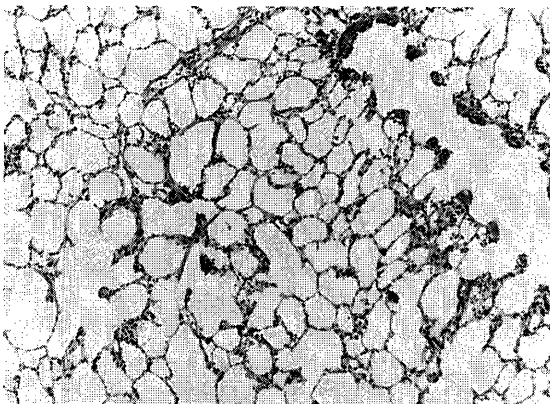


Fig.2-A Score 3

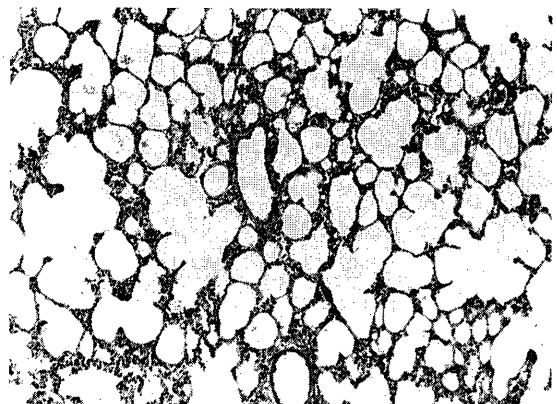


Fig.2-B Score 2

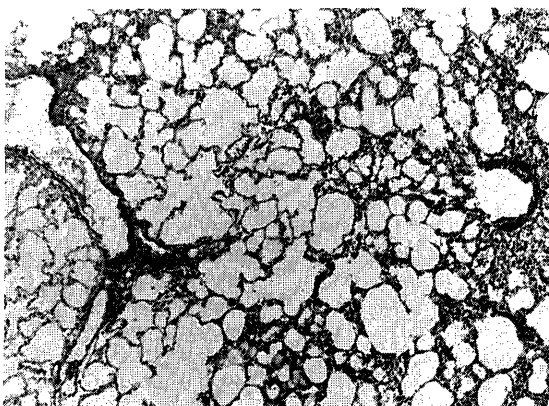


Fig.2-C Score 1

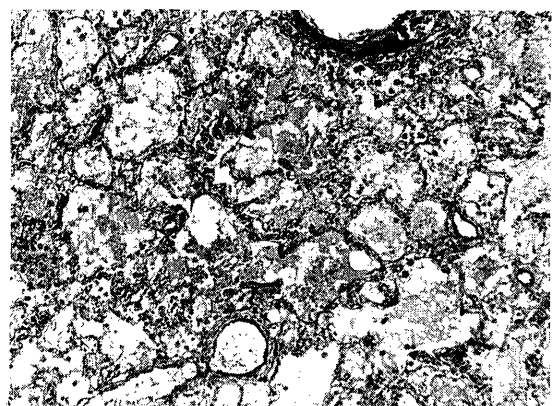


Fig.2-D Score 0

Fig.2. Grading from score 3 to 0 by microscopic findings.

Group II : GSH (50mg/kg) (I. V.) group. (n=8)
 Group III : solcoseryl (50mg/kg)(I. V.) group. (n=6)

— Perfusion groups —

Group IV : Perfusion with E-C solution (20ml/kg) (n=8)

Group V : Group IV+GSH (1mg/ml) (n=8)

Group VI : Group IV+SOD (15mg/l) (n=8)

RESULTS

1) Pulmonary Arterial pressure after the right PA occlusion test (Fig. 3)

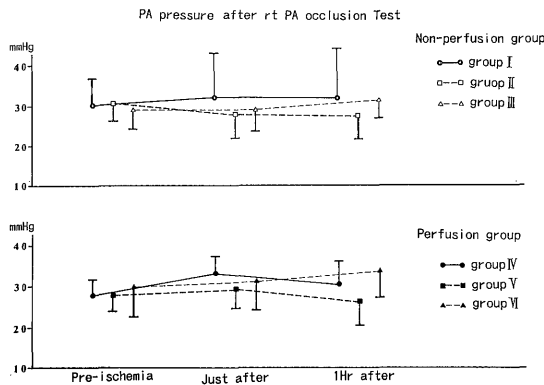


Fig. 3. Changes in PA pressure after unilateral PA occlusion test on the right side. Between non and perfusion groups.

The PA pressure after the right pulmonary artery occlusion test was elevated in all groups. The level of PA pressure in the group with anti-oxidative drugs was lower than that of the control groups, but there was no significant difference between two groups.

2) PaO₂ after the right PA occlusion test (Fig. 4)

The values of PaO₂ in arterial blood in Groups II and III were much more satisfactory than those in Group I, and better in Groups V and VI than Group IV. In Group II, PaO₂ values immediately after one hour and 3 days after reperfusion were significantly higher (p<0.05) than those in Group I. In the perfusion groups, PaO₂ in Group V was significantly better than that in Groups I and IV (controls).

3) PaCO₂ after the right PA occlusion test (Fig. 5)

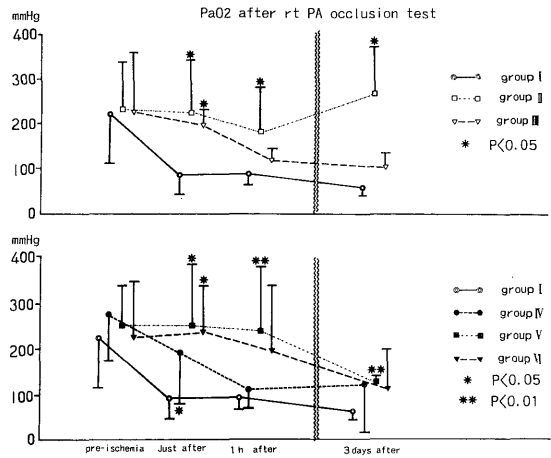


Fig. 4. Changes in PaO₂ after unilateral PA occlusion test on the right side among each a group.

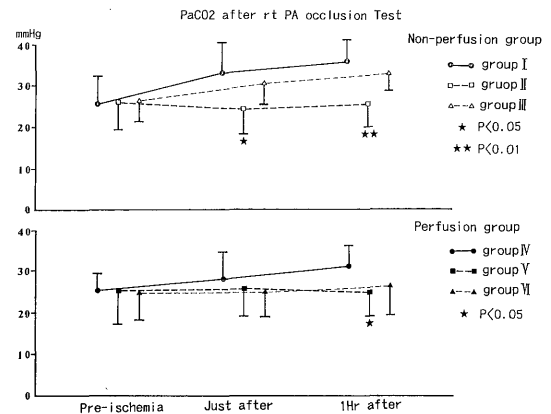


Fig. 5. Changes in PaCO₂ after unilateral PA occlusion test on the right side among each a group.

PaCO₂ after the right PA occlusion test increased in all groups, to a lesser extent in Groups II, III, and V, VI than in Groups I and IV. In particular, Groups II and V, that significantly varied with the control group.

4) Shunt Ratio after the right PA occlusion test (Fig. 6)

The shunt ratio was calculated as follows : $QS/QT = 0.003 (PAO_2 / -PaO_2) / 4.5 + 0.003 (PAO_2 - PaO_2)$ SaO₂ was taken as 100% because FiO₂ was 1.0, and CaO₂ - CvO₂ was assumed to be 4.5 vol%.

The results are shown in Fig. 6. These parameters in the group with drugs remained favorable as compared with control groups.

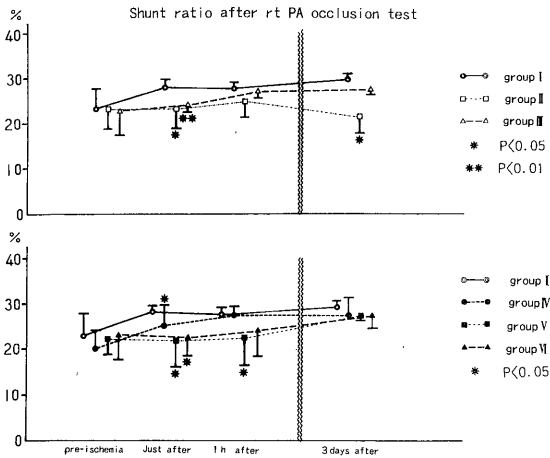


Fig. 6. Changes in Shunt ratio after unilateral PA occlusion test on the right among each a group.

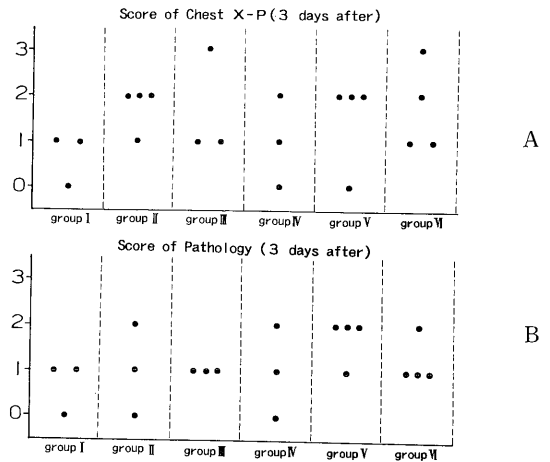


Fig. 7. Changes in the grading represented by score of chest X-ray and pathologic findings among each a group.

Table 1. Scores of mean values and SD based on chest X-ray and Pathological findings

Groups	group I	group II	group III	group IV	group V	group VI
X-P score	0.67 ± 0.58	1.75 ± 0.50	1.67 ± 1.15	1.00 ± 1.00	1.50 ± 1.00	1.75 ± 0.96
Path. score	0.67 ± 0.58	1.50 ± 0.58	1.00 ± 0.00	1.00 ± 1.00	1.75 ± 0.55	1.25 ± 0.55
Total	0.67 ± 0.52	1.67 ± 0.52	1.33 ± 0.82	1.00 ± 0.89	1.50 ± 0.93	1.50 ± 0.76

Especially in Groups II (just after and 3 days after) and V (just after and one hour after) there were statistically significant differences ($P < 0.05$). In group III, the shunt ratio was significantly kept much more satisfactory than that in Group I ($P < 0.01$).

5) Chest X-ray findings (**Fig. 7A**)

Chest X-ray findings were evaluated 3 days after the operation. As shown in **Fig. 7A**, the scores of Group I (control) were between 0 and 1 while those of anti-oxidative drug-administered groups were satisfactory.

6) Pathological findings (**Fig. 7B**)

Three days after reperfusion, pulmonary tissues were taken by thoracotomy for pathological examination. As shown in **Fig. 7b**, the scores of the drug-administered groups were by far more excellent than those of control groups. The mean values and standard deviations of scores for chest X-ray finding and pathological damage are shown in **Table 1**.

DISCUSSION

It is accepted that ischemia and reperfusion injury are mainly caused by oxygen-derived free radicals.²⁾³⁾ In the field of transplantation, this injury is a serious problem for organ preservation and reoxygenation. Especially in lung transplantation, pulmonary edema which often occurs on days 3 to 5 after transplantation, so-called reimplantation response (PIR), may possibly arise from ischemia and reperfusion injury and denervation.⁴⁾⁵⁾ GSH itself is a radical scavenger and has a function to erase AOS with a help of the action of glutathion peroxidase (GSH-Px).⁶⁾ Dawson *et al*⁷⁾ reported that extracellular glutathion conjugation was of great benefit to protect tissues from damage by using isolated perfused rat lung and isolated lung cells. In 1969 McCord *et al*⁸⁾ first discussed the function of SOD. SOD is an enzyme which facilitates the conversion from O_2^- to H_2O_2 and/or scavenges O_2^- . But its half-life is very short,

and therefore, the intravenous administration of SOD is of doubtful value. In this study, the use of SOD was directly applied to the lung. The result was satisfactory. Recent studies focus on liposome-trapping SOD which shows a long-acting effect. The author substantiated cytoprotective effects of GSH, SOD and allopurinol on warm ischemic lung⁹). It is well known that solcoseryl acts as a scavenger of AOS. However, the mechanism to protect the lung from the injury is still unknown. Solcoseryl has a suppressive action on the production of AOS in PMN. In this study, inhibitory effects of solcoseryl on AOS production appeared to be the same as those of SOD or GSH. Hess *et al*¹⁰) in a study on ischemic hearts stressed that reperfusion with less leukocyte blood greatly helps to reduce the extent of cardiac infarction, and also SOD lessened the area of infarction and inhibited the infiltration of leukocytes into infarcted tissues.

CONCLUSION

All of GSH, SOD and solcoseryl play a role in reducing ischemia and reperfusion injury of warm ischemic lung, particularly in terms of oxygenation.

ACKNOWLEDGMENTS

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