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AMELIORATION OF ISCHEMIA-REPERFUSION INJURY BY HUMAN THIOREDOXIN IN RABBIT LUNG

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Human thioredoxin is a polypeptide with thiol groups, possessing reducing activity, which is proved to have the ability to reduce active oxygens. This study evaluated the effect of human thioredoxin on the ischemia-reperfusion lung injury and the roles of human thioredoxin on active oxygens by chemiluminescence examination. The left hilum of the lung of Japanese white rabbits was occluded for 110 minutes and then reperfused for 90 minutes. Ten, 30, 60, and 90 minutes after reperfusion the right hilum was occluded for 5 minutes and the pulmonary functions of the left lung were examined. The animals were divided into four groups, three ischemia groups and a sham group (without occlusion; $n = 6$). The ischemia groups received human thioredoxin, 60 mg/kg ($n = 10$), *N*-acetylcysteine, 150 mg/kg ($n = 7$), or saline solution (control, $n = 10$) during reperfusion. Three rabbits in the human thioredoxin group and the control group were used to measure active oxygens with a cypridina luciferin analog. An additional group of reperfused lungs ($n = 3$) that were given superoxide dismutase after 110 minutes of ischemia was established to identify chemiluminescence examination. Compared with the sham group, reperfusion after 110 minutes of ischemia produced a significant lung injury in the control group. Among the ischemia groups, the human thioredoxin group showed significantly higher arterial oxygen tension at 30, 60, and 90 minutes after reperfusion than the control group, although there was no significant

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difference between the *N*-acetylcysteine and control groups. Histologically, intraalveolar exudation, interstitial thickening, and cellular infiltration were seen in the control group, whereas in the thioredoxin group alveolar structure was well preserved. In the measurement of active oxygens the chemiluminescence in the human thioredoxin group was less than that in the control group and as little as that in the group administered superoxide dismutase. We concluded human thioredoxin attenuated ischemia-reperfusion injury by involving active oxygens in rabbit lungs. (J Thorac Cardiovasc Surg 1997;113:1-9)

The injury that occurs after blood flow is restored to an ischemic organ has been termed ischemia-reperfusion injury and represents a complex multifactorial phenomenon. Organ dysfunction caused by ischemia-reperfusion is a pathophysiologic event with broad clinical relevance. In the lung it is characterized by deterioration of gas exchange, decreased static compliance, and pulmonary edema owing to enhanced vascular permeability.¹⁻³ Early dysfunction of reperfused lung after ischemia remains a crucial problem in clinical situations, such as lung transplantation. The mechanism of this ischemia-reperfusion injury has been extensively investigated^{4,5} and many studies have suggested that active oxygens, including free radicals, play a pivotal role in its pathogenesis.⁶⁻⁹ Many attempts have been made to ameliorate ischemia-reperfusion lung injury by using radical scavengers, such as superoxide dismutase (SOD),¹⁰ catalase,¹¹ and iron-chelating agents.¹²

Thioredoxin is a polypeptide with reducing activity mediated by thiol groups. Human thioredoxin (hTRX) consists of 104 amino acids with a molecular weight of 12,000 D.¹³ hTRX has the ability to reduce active oxygens in vitro¹⁴ and to affect intracellular redox reactions. Recently, we¹⁵⁻¹⁷ reported the protective effect of hTRX against ischemia-reperfusion lung injury in rats and dogs. There is also a report that hTRX enhances the intracellular redox activity in lymphocytes by increasing uptake of *l*-cystine, an oxidized form of *l*-cysteine, which is a precursor of glutathione.¹⁸ However, how hTRX protects against ischemia-reperfusion lung injury and whether hTRX has an effect against active oxygens in reperfused lung were not clear.

Because active oxygens are highly reactive chemical species, it is not easy to detect them, especially in in vivo models. Recently, Nakano¹⁹ reported that one of the cypridina luciferin analogs, 2-methyl-6-[*p*-methoxyphenyl]-3,7-dihydro-

imidazo[1,2-*a*]pyrazin-3-one (MCLA) was a very sensitive and specific luminescence probe to detect active oxygens. MCLA reacts with O₂⁻ or ¹O₂ to emit light, which could be detected from the surface of the lung by a sensitive photon counter.²⁰ The objective of this study was to examine the protective effect of hTRX on ischemia-reperfusion lung injury in a rabbit model and to investigate the role of hTRX on active oxygens with a chemiluminescence method.

Materials and methods

Animals. Male Japanese white rabbits weighing 3.0 to 3.5 kg, which were certified to be free of specific pathogens, were used. They received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

hTRX. Cyclic deoxyribonucleic acid of hTRX was expressed in *Escherichia coli*, and hTRX protein accumulated in the bacterial cells was purified by ion-exchange chromatography. Then it was fully reduced by treatment with dithiothreitol. The resulting sample was more than 99% pure, as determined by densitometer analysis after sodium dodecyl sulfate-polyacrylamide gel electrophoresis and silver staining. The content of bacterial endotoxin was less than 4 pg/mg protein according to the quantitative chromogenic *Limulus* amoebocyte lysate method (Ajinomoto Co., Inc., Kawasaki, Japan).¹⁴

Surgical preparation. The rabbits were premedicated with intramuscular injections of atropine sulfate (0.15 mg) and ketamine chloride (150 mg) and anesthetized with intravenous injection of thiopental (25 mg/kg). A tracheostomy was performed, and a 4.5F endotracheal tube was placed that permitted ventilation with a ventilator (Shinano Industrial Company, Kyoto, Japan) at 30 breaths/min, a tidal volume of 10 ml/kg, and a positive end-expiratory pressure of 2 cm H₂O. The inspiratory fraction of oxygen was 1.0. Ringer's lactate solution was infused and anesthesia was maintained by continuous infusion of 25 mg thiopental per hour and intravenous injections of 0.8 mg pancuronium bromide every 30 to 60 minutes. A warming mat was placed under the animals to maintain body temperature. A 20-gauge Surflood tube (Terumo Co., Tokyo, Japan) was placed in the femoral artery to monitor

the arterial pressure. The sternum was incised horizontally, and the thorax was opened bilaterally at the fourth intercostal space. After removal of mediastinal tissues and pericardiotomy, the inferior pulmonary ligaments were divided and the left pulmonary hilum was stripped. After an intravenous injection of heparin sodium (1000 U/kg), the left hilum, which included the left main bronchus and pulmonary artery and veins, was occluded with a rubber clip (Double Soft Jaw Handleless Clamp, model CV 5114, Baxter Healthcare Corp., Irvine, Calif.) to produce ischemia. Ischemia was imposed on the lung in the deflated state. After 110 minutes of occlusion, the rubber clip was released and the left lung was reperfused and reventilated for 90 minutes.

Experimental protocol. Thirty-six animals were divided into four groups, three ischemia groups and a sham group. The ischemia groups were the hTRX group ($n = 10$), which received hTRX 60 mg/kg dissolved in saline solution, the NAC group ($n = 7$), which received *N*-acetylcysteine (NAC) 150 mg/kg dissolved in saline solution, and the control group ($n = 10$), which received saline solution. The sham group ($n = 6$) consisted of rabbits in which the left hilum was not occluded; after 110 minutes of bilateral ventilation the left lung was examined with saline solution to be compared with the control group. The pharmacologic agents were administered continuously through a central vein over 60 minutes, starting 5 minutes before the beginning of reperfusion.

Three rabbits in the hTRX group and the control group were used to evaluate active oxygens by measuring chemiluminescence of reperfused lungs. As a positive control, after 110 minutes of ischemia, an additional group of reperfused lungs was given SOD (Copper-zinc SOD from bovine erythrocytes, Sigma Chemical Co., St. Louis, Mo.), 5 mg/kg (5600 U/mg) ($n = 3$), in the same way as the other groups to identify chemiluminescence examination.

Physiologic examination. A 5F flow-directed balloon catheter was inserted through the femoral vein, and its tip was placed in the main pulmonary artery with a 20 ml/hr infusion. Baseline measurement of arterial pressure, pulmonary arterial pressure, arterial blood gas analysis, peak inspiratory airway pressure, and core temperature were made in both lungs before the hilar occlusion. Arterial pressure and pulmonary arterial pressure were measured with a pressure transducer (Nihon Koden, Tokyo, Japan). Arterial blood gas analysis was conducted with an ABL 300 analyzer (Radiometer A/S, Copenhagen, Denmark). Peak inspiratory pressure was measured with a calibration manometer (Siemens AG—Bereich Medizinische Technik, Erlangen, Germany). The core temperature was measured with a thermometer (COM-1, Baxter). The surgical field was covered with wet gauze and plastic wrap to maintain body temperature and humidity during ischemia. After 110 minutes of ischemia the accessory lobe was pulled out from the dorsal side of the inferior vena cava and placed in the right pleural cavity so that the right hilum could be clamped completely. Ten, 30, 60, and 90 minutes after reperfusion, arterial pressure, pulmonary arterial pressure, arterial blood gases, and peak inspiratory pressure were measured in the left lung alone after the right hilum had been clamped intermittently for 5 minutes with a forceps. The animals were put to death

after the measurements had been made for 90 minutes. Ninety minutes after the beginning of reperfusion or immediately after death from respiratory failure, the upper half of the left lower lobe was weighed as a wet lung and then stored at 70° C for 72 hours, at which time the wet/dry weight ratio was calculated.

Measurement of lipid peroxides. The upper lobe was homogenized and its content of malondialdehyde, which is formed by the degradation of lipid peroxides, was measured. Malondialdehyde reacts with thiobarbituric acid and produces a red substance, the absorbance A535 of which can be measured by means of a spectrophotometer.

Histologic examination. The lower half of the lower lobe of the left lung was stained with hematoxylin-eosin and examined histologically.

Chemiluminescence examination. The light emission from the surface of the lung was measured by a photomultiplier (R1332, Hamamatsu Photonics, Hamamatsu, Japan), responsive in the range of 350 to 650 nm. The sensitivity of the photomultiplier was detected by Hastings's method and found to be 1 cps = about 1500 photons per second at a 10 cm distance from the window of the photomultiplier.²⁰ The central venous lines were kept by a 20-gauge Surflew tube and a 4F tube in the femoral vein and the internal jugular vein, respectively, with a total infusion of 10 ml/hr. After the left hilar dissection, the rabbit was placed in the right semi-decubitus position into a special light-tight box so that the left lung was positioned 10 cm under the window of the photomultiplier. The body temperature was kept with the warming mat at 38° C, which was checked with a thermometer inserted into the chest wall on the left side. The tubes from the respirator, the arterial line, two central venous lines, the heating pad, and the thermometer were connected outside through the side holes of the instruments. The lung surface was exposed through a 3 cm hole in a black cloth, which covered the whole animal. The photon was detected continuously for 5 seconds.

MCLA (Tokyo Kasei, Tokyo, Japan) was dissolved in saline solution at a concentration of 300 μ mol/ml, which was based on $\epsilon_{430 \text{ nm}} = 9600 \text{ mol}^{-1} \cdot \text{cm}^{-1}$. After the bolus injection of 400 nmol/kg, the MCLA was infused through the femoral vein at a rate of 400 nmol/kg until the end of the experiment. When MCLA was injected into the rabbit, a strong burst of luminescence, greater than the natural luminescence, was detected from the lung surface. Then the luminescence decreased and remained constant during continuous infusion of MCLA. The left hilum was occluded with the clip more than 20 minutes after the bolus injection when the luminescence became constant. During ischemia the thorax was covered with transparent plastic wrap. After 110 minutes of ischemia, the clip was released and the left lung was reperfused and reventilated bilaterally. Luminescence was expressed as ratios of photon numbers to the baseline, which was the mean number of the photons measured during 2 minutes just before ischemia when the photons were constant.

Statistical analysis. All values are expressed as mean $\pm$ standard error of the mean. Comparisons were made by means of analysis of variance and Students' *t* test. Statistical significance was taken as $p < 0.05$. Chemilumines-

Fig. 1. Arterial oxygen tension (P_{aO_2}) at baseline and after reperfusion. P_{aO_2} in the control group was significantly lower after reperfusion than in the sham group. In the hTRX group P_{aO_2} was significantly higher at 30, 60, and 90 minutes after reperfusion than in the control group. Values are mean $\pm$ standard error of the mean. * $p < 0.05$ versus control; ** $p < 0.01$ versus control.

Table I. Survival to 90 minutes after reperfusion, mean arterial pressure, and body temperature on physiologic examination

	Survival	Arterial pressure (mm Hg)*		Temperature ($^{\circ}$ C)*	
		Baseline	90 min	Baseline	90 min
hTRX	7/7	76.5 $\pm$ 4.5	57.1 $\pm$ 3.6	38.3 $\pm$ 0.1	36.0 $\pm$ 0.2
NAC	7/7	77.2 $\pm$ 2.8	61.1 $\pm$ 7.4	38.6 $\pm$ 0.2	36.3 $\pm$ 0.3
Control	5/7	75.7 $\pm$ 5.9	67.0 $\pm$ 5.5	38.5 $\pm$ 0.1	36.3 $\pm$ 0.3
Sham	6/6	67.5 $\pm$ 5.6	66.4 $\pm$ 8.9	38.3 $\pm$ 0.3	36.2 $\pm$ 0.4

Values are mean $\pm$ standard error of the mean. hTRX, Human thioredoxin; NAC, N-acetylcysteine.

*There were no significant differences between the control and the sham groups or among the three ischemia groups.

cence data are shown graphically for each group, and areas above the baseline for each group are expressed as mean $\pm$ standard error of the mean.

Results

Thirty-nine experiments were performed. Hypotensive rabbits with systolic pressures less than 60 mm Hg during ischemia were excluded before any drugs were added ($n = 3$), because it was not our purpose to assess circulatory impairment resulting from ischemia alone. Among 36 experiments, two of the seven rabbits in the control group died less than 90 minutes after the beginning of reperfusion—one at 28 minutes and the other at 62 minutes. The last blood gas analysis showed low oxygen tension in these rabbits. The other rabbits in the hTRX, NAC, and sham groups survived for 90 minutes after reperfusion (Table I).

Arterial oxygen tension (Fig. 1). The arterial oxygen tensions at baseline and 90 minutes after

reperfusion in the control group were 505.3 ± 16.3 and 82.6 ± 11.2 mm Hg, respectively. In the sham group the values were 525.3 ± 16.6 and 501.1 ± 26.7 mm Hg, respectively. The differences were significant at 10 ($p < 0.05$), 30, 60, and 90 minutes ($p < 0.01$). In the hTRX group the oxygen tensions were 531.6 ± 5.8 and 296.4 ± 71.9 mm Hg, respectively, and in the NAC group they were 527.9 ± 10.4 and 183.8 ± 51.6 mm Hg, respectively. In the hTRX group arterial oxygen tension was significantly higher than in the control group at 30, 60, and 90 min ($p < 0.05$). No other significant differences were observed among the experimental groups.

Pulmonary functions of reperfused lung (Table II). Pulmonary arterial pressure in the control group was higher than that in the sham group at 30, 60, and 90 minutes ($p < 0.05$) after reperfusion, but there were no significant differences among the three ischemia groups. Peak inspiratory pressure in the control groups was not significantly different

Table II. Pulmonary functions of reperfused lungs

Group		Baseline	10 min	30 min	60 min	90 min	W/D ratio
hTRX	PAP (mm Hg)	18.4 ± 0.7	20.2 ± 2.3	20.5 ± 3.1	21.4 ± 2.0	23.5 ± 2.1	7.68 ± 0.41
NAC	PIP (cm H ₂ O)	14.4 ± 0.7	40.1 ± 2.3	40.2 ± 4.9	33.2 ± 1.2	33.1 ± 1.2	
	PAP	18.0 ± 1.1	20.8 ± 2.3	22.4 ± 3.0	25.2 ± 1.8	24.5 ± 1.5	8.24 ± 0.44
Control	PIP	14.7 ± 0.8	33.1 ± 2.9	34.2 ± 3.9	34.1 ± 2.9	37.1 ± 4.7	9.20 ± 0.57†
	PAP	17.5 ± 1.2	19.1 ± 1.6	21.6 ± 1.9*	25.4 ± 2.3*	25.0 ± 1.9*	
Sham	PIP	14.3 ± 0.5	33.5 ± 2.9	29.6 ± 1.4	31.1 ± 2.0	33.0 ± 1.9	5.85 ± 0.21
	PAP	14.7 ± 1.5	16.0 ± 1.4	16.3 ± 1.0	16.8 ± 1.3	16.3 ± 1.7	
	PIP	15.3 ± 0.8	33.6 ± 2.1	32.8 ± 1.7	30.0 ± 1.2	29.6 ± 1.1	

Values are mean ± standard error of the mean. hTRX, Human thioredoxin; NAC, N-acetylcysteine; PAP, mean pulmonary arterial pressure; PIP, peak inspiratory airway pressure; W/D, wet/dry weight ratio.

* $p < 0.05$ versus sham.

† $p < 0.01$ versus sham.

from that in the sham group and there were no significant differences among the ischemia groups. The wet/dry ratio in the control group was significantly higher than in the sham group ($p < 0.01$). The wet/dry ratio in the hTRX group tended to be lower than in the control group ($p = 0.056$), but the difference was not significant. The lipid peroxides in the tissues after reperfusion were 52.7 ± 6.1 , 61.4 ± 8.5 , 52.7 ± 6.1 , and 55.8 ± 16.1 nmol/gm in the hTRX, NAC, control, and sham groups, respectively. There were no significant differences between the control and sham groups or among the ischemia groups.

Histologic examination (Fig. 2). Intraalveolar exudation was noted, the interstitium was thickened, and neutrophil infiltrations were marked in the control group. Alveolar exudate was little in the NAC group and minimal in the hTRX group. Alveolar architecture was well preserved in both the NAC and the hTRX groups.

Chemiluminescence examination. The time courses in photon ratios in three cases in the control, hTRX, and SOD groups are shown in Fig. 3. After hilar occlusion, luminescence decreased because blood flow had been stopped; then, after reperfusion, it recovered to the value before ischemia. In the control group the photon ratio gradually increased to 2.5 or larger within 30 minutes and continued until 90 minutes after reperfusion in all three cases. The photon ratios did not reach 2 within 90 minutes after reperfusion in either the SOD group or the hTRX group. The areas above the baseline, the integral of the photon ratios, in the SOD and hTRX groups are significantly lower than in the control group ($p < 0.05$) (Fig. 4).

Discussion

hTRX was first discovered as an adult T-cell leukemia-derived factor produced by a cell line derived from adult T-cell leukemia infected by human T-cell leukemia virus type 1 (HTLV-1).^{21, 22} Subsequent purification and gene cloning proved that hTRX is a protein with a molecular weight of 12,000 D, composed of 104 amino acids,¹³ and that it is a human homolog of bacterial coenzyme thioredoxin.²³ It is widely distributed in the human body, and it is induced by a variety of stresses including x-ray, ultraviolet, hydrogen peroxide, mitogen, and viral infection.²⁴ hTRX has a -Cys-Gly-Pro-Cys-amino acid sequence, which is a conserved sequence forming active site of thioredoxin of all species including eukaryotic and prokaryotic organisms.²⁵ It exhibits various biologic activities, such as reducing activity through proton donation, active oxygen-reducing activity,¹⁴ cytokine-like activity,²⁶ and regulation of gene expression.²⁷ It is thought that hTRX can act as a redox regulating factor.

Our study was undertaken to determine the effect of hTRX on ischemia-reperfusion injury and on active oxygens in an in vivo rabbit lung model. An ischemic time of 110 minutes was chosen, because it produced reliable injury. In this model, arterial oxygen tension in the control group was significantly lower than that in the sham group. This phenomenon appeared to be caused by increased vascular permeability and pressure, because in the control group pulmonary arterial pressure and the wet/dry ratio were significantly higher than those in the sham group and pulmonary edema was histologically observed.

Fig. 2. Microscopic findings of lungs after 110 minutes of hilar occlusion and 90 minutes of reperfusion (original magnification $\times 100$, hematoxylin-eosin staining). **A**, Control group; **B**, NAC group; **C**, hTRX group. In the control group, intraalveolar exudation was noted, the interstitium was thickened, and neutrophil infiltrations were marked. In the hTRX group, the exudate was minimal and the alveolar architecture was well preserved.

Fig. 3. Time courses of chemiluminescence from the lung surface before and after the MCLA infusion with 110 minutes of ischemia and 90 minutes of reperfusion. The left hilum of the lung was occluded after the high chemiluminescence caused by a bolus injection (arrows) of MCLA became constant. The photon ratio in the control group was 2.5 or higher after reperfusion. The ratios in the SOD and hTRX groups were less than 2 after reperfusion.

NAC is a reducing agent as well as a glutathione precursor. It has a thiol group like hTRX and is already in clinical use for the treatment of acetaminophen intoxication²⁸ or hemorrhagic cystitis owing to alkylating agents,²⁹ the efficacy of which is thought to be a radical scavenging action.³⁰ Therefore the protective effect of hTRX was compared with that of NAC. NAC was administered at a dose of 150 mg/kg, which has been reported to provide salutary effects in experimental animals^{30,31} over a period of 60 minutes, starting 5 minutes before reperfusion. hTRX was administered at a dose of 60 mg/kg per hour in the same manner as NAC, which is equivalent on the basis of weight per hour to the one used in our previous experiment.¹⁶ We demonstrated that hTRX attenuated ischemia-reperfusion lung injury but NAC failed to provide protection in the rabbit model. Decreases of the arterial oxygen tension in the TRX group were significantly less than in the control group ($p < 0.05$), but the arterial oxygen tension levels in the NAC group were not significantly different from those in the control group. Our previous *in vivo* study in dogs revealed the protective effect of NAC on ischemia-reperfusion lung injury similar to that of hTRX. There are a number of possible explanations for the apparent discrepancy between these studies. (1) The rabbit species has a much lower level of xanthine oxidase

Fig. 4. The areas above the baseline after reperfusion in the control, SOD, and hTRX groups ($n = 3$, each group). Areas in the SOD and hTRX groups were significantly lower than in the control group. * $p < 0.05$ versus control.

than the dog.⁶ Therefore, in rabbits, active oxygens through xanthine-xanthine oxidase pathway, which NAC was supposed to act on, might be less involved in the ischemia-reperfusion injury than in dogs. (2) The dose and duration of NAC and hTRX might be

different. NAC was administered for 60 minutes instead of 30 minutes, which had been used in our previous study,¹⁶ although either has shown the effectiveness in the previous experiments.^{29,31} (3) The lung injury model was different. It is conceivable that lung injury in this study was more severe than that in our previous canine model and thus NAC may not have been able to attenuate the injury of a greater insult.

Previously we reported a new method for measuring active oxygens that makes use of chemiluminescence of cypridina luciferin analogs, 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-a]pyrazin-3-one (CLA) and MCLA.¹⁹ MCLA reacts with O_2^- or 1O_2 to yield a dioxetane analog that decarboxylates and protonates to an excited carbonyl compound that emits light strongly near 465 nm during its return to ground state.³² The luminescence level in the hTRX group was significantly lower than that in the control group and appeared to be similar to the level of the SOD group used as a positive control. These results suggested that hTRX may reduce the generation of active oxygens or eliminate them, which would result in the attenuation of ischemia-reperfusion injury in rabbit lungs. Because no significance was seen even between the control and the sham groups in our measurements of lipid peroxides, the chemiluminescence method appeared to be sufficiently sensitive to detect active oxygens.

Our study does not elucidate the mechanism by which hTRX reduced active oxygens. It is likely that hTRX reduced active oxygen by scavenging action. There are some reports that suggest possible mechanisms of hTRX. Mitsui, Harakawa, and Yodoi¹⁴ have proved that hTRX can reduce active oxygens induced by xanthine-xanthine oxidase in vitro. Iwata and associates¹⁸ demonstrated that the administration of hTRX increases the intracellular cystine uptake in cultured cells, suggesting that hTRX increase intracellular level of glutathione, which is one of the most important antioxidants. Our colleague demonstrated that treatment with hTRX and *L*-cysteine attenuates ischemia-reperfusion injury in isolated rat lungs.³³ These studies support the hypothesis that hTRX might reduce active oxygens by increasing glutathione synthesis in pulmonary cells. Furthermore, thioredoxin-dependent peroxide reductase that uses thioredoxin as the immediate hydrogen donor was identified from yeast recently.³⁴ Thioredoxin may be involved in thioredoxin-dependent radical-scavenging system with this protein.

Further studies are needed to determine the relationship among hTRX, active oxygens, and ischemia-reperfusion lung injury.

In conclusion, our study indicated that hTRX ameliorates ischemia-reperfusion injury in *in vivo* rabbit lungs, and this effect of hTRX may involve reducing active oxygens. It is hoped that these results will help clarify the mechanism of ischemia-reperfusion lung injury.

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