

Trauma Does Not Aggravate Deleterious Effects of Ischemia Reperfusion Injury on the Lung

Makbule Ergin,¹ Ali Yeginsu,¹ Huseyin Ozyurt,² Cigdem Elmas,³
Ali Akbas,² Guleser Caglar Goktas³

Abstract

Objectives: Our purpose was to investigate the effects of ischemia-reperfusion injury on traumatized lungs.

Materials and Methods: Twenty-four Wistar rats were used in the study. Rats were randomly divided into 4 groups. In the control group (group 1), only anesthesia and ventilation were used. In group 2, only lung ischemia-reperfusion injury was instituted. In group 3, only blunt chest trauma was instituted. And in group 4, lung ischemia-reperfusion injury, consisting of 24 hours after the constitution of blunt chest trauma, was used. Lung damage and systemic inflammation parameters were evaluated.

Results: All parameters (alveolar degeneration grades, alveolar macrophage and lymphocyte counts, antioxidant enzyme activities, cytokine levels, and bronchoalveolar lavage fluid albumin level) were higher in all groups than they were in the control group ($P < .05$). Only tissue malondialdehyde, tissue interleukin-8, and serum glutathione peroxidase levels were significantly higher in the lung ischemia-reperfusion group than they were in the trauma group ($P < .05$). The trauma + ischemia-reperfusion group showed no significant difference when compared with the only ischemia-reperfusion or only trauma groups in any parameters ($P > .05$).

Conclusions: The findings showed that lung trauma does not aggravate the deleterious effects of lung ischemia-reperfusion injury.

Key words: Lung transplant, Lung contusion, Donor lung

Lung ischemia-reperfusion injury is a complex phenomenon characterized by activation of inflammatory cells, increased proinflammatory cytokine expression, production of reactive oxygen species, and other molecule activations (eg, adhesion molecules, complements, and transcription factors), which may result in lung cellular injury, pneumocyte necrosis, and apoptosis.

Clinically, pulmonary thromboendarterectomy, cardiopulmonary bypass, and particularly, thoracic organ transplants are well-known procedures that make the lungs exposed to ischemia-reperfusion. Lung transplant still is the best option for treating end-stage lung diseases.

Donor lung quality is a major determinant for a successful lung transplant. Unfortunately, several lung donors die from various traumas, and various degrees of lung injuries result from blunt chest trauma in these patients.

It is not ideal, but traumatized lungs have sometimes been implanted to recipients because of the shortage of donor lung availability. Logically, in such cases, with compared with nontraumatized patients, poorer results would be expected. However, some data show that outcomes are not significantly different between traumatized and nontraumatized donors (1). So far, this issue has not been clearly investigated.

In this study, our aim was to investigate the influences of lung trauma on lung ischemia-reperfusion injury in a rat lung ischemia, reperfusion injury model.

Materials and Methods

Animals

Twenty-four male Wistar rats weighing 280-320 g were used. The study was approved by local ethics

From the ¹Gaziosmanpasa University School of Medicine Department of Thoracic Surgery, ²Department of Biochemistry, Tokat, and the ³Gazi University School of Medicine Department of Histology and Embryology, Ankara, Turkey.

Address reprint requests to: Ali Yeginsu, Gaziosmanpasa Universitesi Tip Fakultesi Gogus Cerrahisi AD. 60100, Tokat, Turkey

Phone: +90 356 2129500 Fax: +90 356 2133179 E-mail: yeginsu@hotmail.com

Experimental and Clinical Transplantation (2010) 1: 49-54

committee and financially supported by the scientific research board of the university. All animals received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources (2).

Animal groups

1. Control group (n=6): Only anesthesia and ventilation were performed 5-hour period.
2. Trauma group (n=6): Lung contusion was instituted under general anesthesia.
3. Lung ischemia-reperfusion injury group (n=6): Rats were anesthetized and ischemia-reperfusion injury was created in the left lung.
4. Trauma-plus-lung-ischemia-reperfusion injury group (n=6): Rats were anesthetized, and a lung contusion was created. After 24 hours of lung trauma, lung ischemia-reperfusion injury was done on the left lung.

Constitution of lung trauma

Rats were anesthetized with 30 mg/kg thiopentone ip (pental sodium, IE Ulagay, Istanbul, Turkey). The experimental lung contusion model described by Raghavendran and associates (3) was done to create a lung contusion with a special setup. Briefly, a 500-g, iron, cylindrical weight was dropped from 50 cm onto the chest. Approximately 2.45 joule of impact energy was achieved according to the formula, $E=mgh$, where "m" is the mass of cylinder (in kilograms), "g" is gravitational acceleration (9.8 m/s^2), "h" is the height of the weight above the platform (in meters).

Constitution of the lung ischemia-reperfusion injury

Rats were anesthetized with 50 mg/kg thiopentone ip, and left hemithorax was shaved. Animals were intubated by tracheostomy and connected to a rodent ventilator (Harvard instrument, ASVP, USA). Animals were ventilated with room air (10-cm H_2O peak inspiratory pressure, 4-cm H_2O positive end expiratory pressure, 60 breaths/min frequency). Atropine 0.4 mg IM was given. A left, dorsolateral, thoracotomy, in the fourth intercostal space was performed. The left lung was mobilized atraumatically, and the mediastinum was dissected around the left lung hilus. Fifty units heparin was given via the penile vein. Five minutes later, animals underwent 2 h of warm lung ischemia by clamping

the bronchus, pulmonary artery, and veins of the left lung in an inflated state, using a microvascular clamp. During the operation, the left hemithorax was covered by a moist tissue to minimize evaporative loss. After 2 hours of ischemia, the clamp was removed, and reperfusion was allowed. At the end of 3 hours of reperfusion, a laparosternotomy was performed. Two- to 4-mL blood samples were taken, and then the inferior vena cava and right and left atrial appendages were cut. The lung was flushed through pulmonary artery, with a 20-mL saline solution, at 20-cm H_2O pressure. The lung-heart block was removed, and bronchoalveolar lavage fluid was taken from the left lung. One half of left lung was kept in -80°C for biochemistry, and the remainder was kept in a 10%-formalin solution for histomorphometric studies.

Preparation of lung tissue homogenate

Lung tissues were stored at -80°C until analysis. After the lung tissues had been weighed, they were homogenized in 5 volumes of ice-cold tris-HCl buffer (50 mM, pH 7.4) containing 0.50 mL/L of Triton X-100. Homogenization was performed for 2 minutes at 13 000 rpm with an Ultra-Turrax T25 basic (IKA, Staufen, Germany). All procedures were performed at 4°C . Homogenate, supernatant, and extracted samples were prepared, and the following analyses were performed on the samples with commercial chemicals supplied by Sigma (St. Louis, MI, USA).

Histomorphometry

Lung tissue samples were kept in 10%-formalin solution and embedded in paraffin blocks. All specimens were sampled with 4- μm serial cuts (Leica SM 2000, Germany) and dyed with hematoxylin and eosin. The sections were evaluated under the light microscope (Leica DM 4000 B and Leica DFC 280 Plus camera; Weetlar, Germany) by a histologist who was blinded to the study. Alveolar degeneration (differences in infiltration, terminal bronchial wall structure, the terminal bronchial debris, respiratory bronchial wall structure, respiratory bronchial debris, alveolar structure, and edema in vascular wall) were evaluated using semiquantitative scores between 0 and 5 (0, no changes; 1, focal slight changes; 2, multifocal slight changes; 3, multifocal significant changes; 4, diffuse light changes; 5, diffuse significant changes). Cells were counted in 10 sections of each sample, and were randomly chosen from 10 different

areas, using $\times 400$ magnification. Photos taken with the Leica Q Win V3 Plus Image program were used for counting. Lymphocytes were detected with a dark, round nucleus, and a narrow cytoplasmic area around their nucleus. Free macrophages were counted in alveolar and bronchial spaces.

Antioxidant enzyme activities and lipid peroxidation

Superoxide dismutase, glutathione peroxidase activities, and malondialdehyde levels were studied in serum and lung tissue homogenate. Total (Cu-Zn) superoxide dismutase (EC 1.15.1.1) activity was determined according to the method of Sun and associates (5). The superoxide dismutase activity is expressed as unit per gram protein. Glutathione peroxidase activity was measured by the method of Paglia and associates (6). Activity is expressed as unit per gram protein. The tissue thiobarbituric acid-reactive substance level was determined by a method (7) based on reaction with thiobarbituric acid at 90-100°C. Results are expressed as nmol per gram wet tissue, according to the standard graphic, prepared from measurements with a standard solution (1,1,3,3-tetramethoxypropane).

Cytokine levels

Interleukin-8 (IL-8) and TNF- α levels were measured in serum and lung tissue homogenate. Interleukin-8 and TNF- α levels were measured with enzyme-linked immunosorbent assay (ELISA) method. Data are expressed as pictogram per milliliter.

Bronchoalveolar lavage albumin

Bronchoalveolar lavage albumin fluid level was measured by immunoturbidimetric method with Roche/ Hitachi (Roche Diagnostics GmbH D-68298 Mannheim, Germany) Modular P autoanalyzer. Data were recorded as gram per deciliter.

Statistics

Statistical analyses were performed with SPSS software for Windows (Statistical Product and Service Solutions, version 15.0, SSPS Inc, Chicago, IL, USA). Data were expressed as mean $\pm$ standard deviation (SD). Kruskal-Wallis and 1-way ANOVA were used for nonparametric and parametric comparisons among the groups. Post hoc multiple comparisons were calculated with Mann-Whitney *U*

and Tukey tests. A *P* value less than .05 was accepted as statistically significant.

Results

Histomorphometric studies

Degeneration grades, and macrophage and lymphocyte counts, were higher in all groups compared with the control group ($P < .05$). There was no significant difference among lung ischemia-reperfusion injury, trauma, and trauma + lung ischemia-reperfusion injury groups. Microscopic views are shown in Figures 1-4.

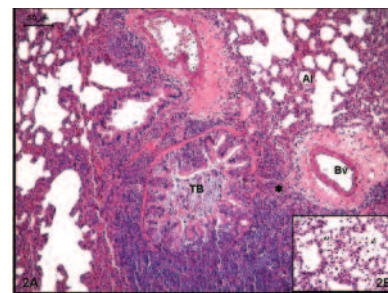


Figure 1. Control group. Normal tissue structure was seen. Terminal bronchioles (TB), respiratory bronchioles, alveolar (Al), and blood vessels (Bv) have normal structure (hematoxylin-eosin 1A $\times 100$, 1B $\times 400$).

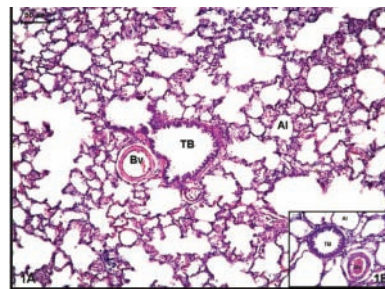


Figure 2. Ischemia reperfusion group. A diffuse lymphocytic infiltration (*) was present in lung tissue. Histologic structure was damaged. Infiltrative cells were increased, and muscle layer thickened in terminal bronchioles (TB). Bronchial lumen has cellular debris. Respiratory bronchioles were degenerative. Vascular smooth muscle cell increase and pericapillary edema were seen (hematoxylin-eosin 2A $\times 100$, 2B $\times 400$).

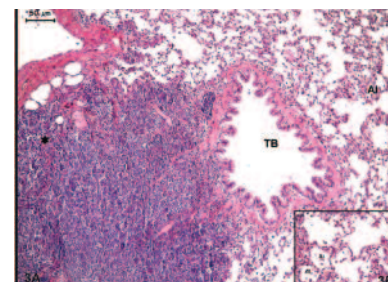


Figure 3. Trauma group. Histologic changes were similar to IR group. Lymphocyte infiltration (*) was obvious in entire lung. There was cell debris in terminal and respiratory bronchioles. Alveolar walls were degenerative. Increases in vascular smooth muscle cells and pericapillary edema were seen (hematoxylin-eosin 2A $\times 100$, 2B $\times 400$).

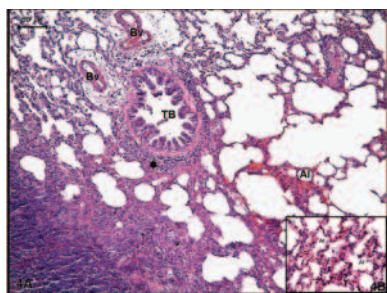


Figure 4. Trauma plus ischemia-reperfusion group. Lymphocytic infiltration was manifest in the entire lung. Terminal bronchiolar walls have patchy degeneration areas. Broad cell infiltration in alveolar area was noted. Patchy hemorrhages were seen interalveolar areas and in alveolar lumen. Perivascular lymphocytic infiltration and cellular debris in the terminal bronchiolar lumen were noted (hematoxylin-eosin 4A $\times 100$, 4B $\times 400$).

Oxidative stress and lipid peroxidation

Antioxidant enzyme activities and malondialdehyde levels in lung tissue and serum were significantly higher in all groups when compared with the control group ($P < .05$). In lung ischemia-reperfusion injury group, tissue malondialdehyde levels and serum glutathione peroxidase levels were significantly higher when compared with the trauma group ($P < .05$). In trauma + lung ischemia-reperfusion injury group, there was no significant difference as compared with lung ischemia-reperfusion injury group, and trauma group ($P > .05$).

Cytokines

IL-8 and TNF- α levels were higher in all groups than they were in the control group. In the lung ischemia-reperfusion injury group, tissue IL-8 level was significantly higher than the trauma group ($P < .05$). There was no significant difference between lung ischemia-reperfusion injury, and trauma + lung ischemia-reperfusion injury groups; and between trauma and trauma + lung ischemia-reperfusion injury groups.

Bronchoalveolar lavage albumin

Bronchoalveolar lavage albumin fluid albumin level was significantly higher in all groups compared to the control group ($P < .05$). There was no statistically significant difference among lung ischemia-reperfusion injury, trauma, and trauma + lung ischemia-reperfusion injury groups. Statistical analyses are shown in tables 1 and 2

Discussion

In this study, we saw that some parameters in the traumatized lung were lower than the nontraumatized

Table 1. Statistical comparison of all groups.

Groups	Control	LIRI	Trauma	Trauma+	P value
LIRI					
Alveolar degeneration	0.0	3.3 $\pm$ 1.0	3.3 $\pm$ 1.5	3.6 $\pm$ 0.8	.003*
Alveolar macrophage	28.1 $\pm$ 3.5	117.6 $\pm$ 14.8	110.6 $\pm$ 9.4	108.8 $\pm$ 23.5	.003*
Tissue lymphocyte	3.8 $\pm$ 2.1	21.6 $\pm$ 4.4	20.4 $\pm$ 4.0	21.0 $\pm$ 1.7	< .001*
Tissue MDA [†]	3.1 $\pm$ 1.3	8.1 $\pm$ 0.9	6.5 $\pm$ 3.9	6.9 $\pm$ 2.0	.002*
Tissue SOD [‡]	45.3 $\pm$ 4.0	76.5 $\pm$ 11.9	71.5 $\pm$ 8.8	77.8 $\pm$ 17.9	.005*
Tissue GPX [‡]	14.0 $\pm$ 3.1	41.5 $\pm$ 11.9	32.4 $\pm$ 7.0	37.5 $\pm$ 9.0	< .001*
Tissue IL-8 [§]	158 $\pm$ 29	613 $\pm$ 151	362 $\pm$ 117	476 $\pm$ 121	.001*
Tissue TNF- α [§]	127 $\pm$ 11	220 $\pm$ 19	214 $\pm$ 33	238 $\pm$ 22	< .001*
Serum MDA	1.1 $\pm$ 0.1	1.7 $\pm$ 0.2	1.5 $\pm$ 0.1	1.5 $\pm$ 0.1	.001*
Serum SOD	45.1 $\pm$ 10.6	79.2 $\pm$ 7.1	70.5 $\pm$ 16.9	73.2 $\pm$ 16.0	.011*
Serum GPX	57.5 $\pm$ 10.5	155.2 $\pm$ 27.3	106.4 $\pm$ 33.0	120.7 $\pm$ 42.2	.002*
Serum IL-8	154 $\pm$ 25	612 $\pm$ 106	675 $\pm$ 127	652 $\pm$ 174	.004*
Serum TNF- α	133 $\pm$ 35	260 $\pm$ 40	240 $\pm$ 59	297 $\pm$ 108	.003*
BAL albumin	6.6 $\pm$ 2.4	14.4 $\pm$ 3.4	13.8 $\pm$ 3.8	15.5 $\pm$ 3.3	.001*

*Statistically significant.

Abbreviations: BAL, bronchoalveolar lavage; MDA, malondialdehyde; SOD, superoxide dismutase; GPX, glutathione peroxidase; IL-8, interleukin-8; TNF- α , tumor necrosis factor alpha; LIRI, lung ischemia-reperfusion injury.

[†]Nanomoles per gram of wet tissue.

[‡]Unit per gram of protein.

[§]pg/mL.

Table 2. P values of posthoc comparisons.

Groups	1 vs 2	1 vs 3	1 vs 4	2 vs 3	2 vs 4	3 vs 4
Alveolar degeneration	.002*	.002*	.002*	.937	.589	.699
Alveolar macrophage	.002*	.002*	.002*	.394	.394	.699
Tissue lymphocyte	< .001*	< .001*	< .001*	.971	.985	1.000
Tissue MDA	.002*	.002*	.004*	.015*	.394	.937
Tissue SOD	.002*	.002	.004*	.394	.937	.485
Tissue GPX	< .001*	.006*	.001*	.268	.841	.721
Tissue IL-8	.002*	.002*	.002*	.026*	.180	.132
Tissue TNF- α	< .001*	< .001*	< .001*	.978	.526	.313
Serum MDA	.001*	.036*	.007*	.277	.683	.879
Serum SOD	.002*	.026*	.009*	.589	.310	.589
Serum GPX	.002*	.015*	.002*	.041*	.132	.818
Serum IL-8	.002*	.002*	.002*	.310	.818	1.000
Serum TNF- α	.019*	.057	.002*	.952	.790	.482
BAL albumin	.003*	.006*	.001*	.999	.946	.829

Abbreviations: BAL, bronchoalveolar lavage; GPX, glutathione peroxidase; IL-8, interleukin-8; MDA, malondialdehyde; SOD, superoxide dismutase.

*Statistically significant.

lung when they were exposed to ischemia-reperfusion injury; but the results were not significantly different. Therefore, it will be speculative to conclude that lung trauma protects the lung against lung ischemia-reperfusion injury. Our study clearly shows that blunt chest trauma does not aggravate the deleterious effects of lung ischemia-reperfusion injury. A possible explanation for these results may be a mechanism of preconditioning. A sublethal trauma may have caused an inflammatory response rendering the lungs resistant to subsequent ischemia-reperfusion injury.

It is well-known that trauma is a source of stress and causes an inflammatory response. Animal lung contusion studies showed that leukocyte activation and infiltration, inflammatory cytokine and chemokine levels, and oxidative stress parameters increased after lung traumas. Trauma activates some immune cells also (8-10).

Preconditioning is a defense mechanism rendering an organism more resistant to severe stress. First recognized by Murry (11) as ischemic preconditioning, many forms of preconditioning have been defined, such as thermal, pharmacologic, gas inhalation, and toxic (12-14). The protective mechanism of preconditioning has not exactly been lightened. Activation of some cell membrane (adenosine, α -adrenergic, bradykinin, and opioid receptors), administration of endogenous triggers (free radicals, nitric oxide, calcium), activation of intracellular signaling pathway (protein kinase C, tyrosine kinase, mitogen-activated protein kinase), and affecting, related, end effectors (metabolism, protein synthesis, K-ATP channels, Na-K pump, cytoskeleton) have been reported as investigated mechanisms (13). We suggest that a traumatic stress can activate all of these mechanisms, and in our study, lung trauma triggered one or more mechanisms mentioned above. In addition, tissue ischemia, developing after lung contusion, also may have contributed to consist of an ischemic preconditioning process in the lungs.

Actually, it has been anticipated that transplanting of a traumatized donor lung results in a greater number of early and late postoperative complications, but this topic has never been particularly investigated (15). In contrast, many authors reported that there was no significantly difference in early posttransplant outcomes, mortality, and survival between traumatized and nontraumatized donors (1, 15-17). These papers may support the data of our study; however, none of these papers was prospectively designed for isolated traumatized lungs, and multiple factors such as brain death, immunity, treatment options, and others may have affected the outcomes.

Our study was designed to show only the effects of ischemia-reperfusion on the traumatized lung, but not to show the effects of immunity and other factors. Some limitations are present in our study. First, the study was not experienced in a transplant model. So, the results may not reflect clinical outcomes. Second,

the effects of brain death were not included in this study. Third, preoperative, and postlung functions and oxygenation were not studied. We did not know how rats were physiologically influenced from the procedures. Subsequently, our study did not answer the question of whether we could safely use a traumatized donor lung in a transplant or not. Further studies are needed for certain recommendations.

In conclusion, lung trauma does not significantly aggravate the deleterious effects of ischemia-reperfusion on the lungs. Stress preconditioning mechanisms may contribute to this condition.

References

1. Waller DA, Thompson AM, Wrightson WN, et al. Does the mode of donor death influence the early outcome of lung transplantation? A review of lung transplantation from donors involved in major trauma. *J Heart Lung Transplant*. 1995;14(2):318-321.
2. Guide for the care and use of laboratory animals. Bethesda, MD: National Institutes of Health, 1985; Publication No. 85-23.
3. Raghavendran K, Davidson BA, Helinski JD, et al. A rat model for isolated bilateral lung contusion from blunt chest trauma. *Anesth Analg*. 2005;101(5):1482-1489.
4. Weibel ER, Losa G, Bolender RP. Stereological method for estimating relative membrane surface area in freeze-fracture preparations of subcellular fractions. *J Microsc*. 1976;107(3):255-266.
5. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clin Chem*. 1988;34(3):497-500.
6. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*. 1967;70(1):158-169.
7. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. In Packer L, Glazer AN, eds. *Methods in Enzymology*. Vol 186. Oxygen radicals in biological systems. New York, NY Academic Press; 1990:407-421.
8. Hoth JJ, Stitzel JD, Gayzik FS, et al. The pathogenesis of pulmonary contusion: an open chest model in the rat. *J Trauma*. 2006;61(1):32-44; discussion 44-45.
9. Keel M, Ecknauer E, Stocker R, et al. Different pattern of local and systemic release of proinflammatory and anti-inflammatory mediators in severely injured patients with chest trauma. *J Trauma*. 1996;40(6):907-912; discussion 912-914.
10. Motycka V, Grofová Z, Havlíček K, Siller J, Fortová M, Linda B. Blunt thoracic injury and the oxidation stress [Czech]. *Rozhl Chir*. 2006;85(8):404-407.
11. Murry CE, Jennings RB, Reimer KA. Preconditioning with ischemia: a delay of lethal cell injury in ischemic myocardium. *Circulation*. 1986;74(5):1124-1136.
12. Luh SP, Yang PC. Organ preconditioning: the past, current status, and related lung studies. *J Zhejiang Univ Sci B*. 2006;7(5):331-341.
13. McCormick PH, Chen G, Tlerness S, Kelly CJ, Bouchier-Hayes DJ. Clinically relevant thermal preconditioning attenuates ischemia-reperfusion injury. *J Surg Res*. 2003;109(1):24-30.
14. Mallard C, Hagberg H. Inflammation-induced preconditioning in the immature brain. *Semin Fetal Neonatal Med*. 2007;12(4):280-286.
15. Straznicka M, Follette DM, Eisner MD, Roberts PF, Menza RL, Babcock WD. Aggressive management of lung donors classified as unaccept: excellent recipient survival one year after transplantation. *J Thorac Cardiovasc Surg*. 2002;124(2):250-258.

16. Ganesh JS, Rogers CA, Banner NR, Bonser RS; Steering Group of the UK Cardiothoracic Transplant Audit. Donor cause of death and mid-term survival in lung transplantation. *J Heart Lung Transplant*. 2005;24(10):1544-1549.
17. Ciccone AM, Stewart KC, Meyers BF, et al. Does donor cause of death affect the outcome of lung transplantation? *J Thorac Cardiovasc Surg*. 2002;123(3):429-434; discussion 434-436.