One-lung ventilation: For how long?

Celal Tekinbas, MD,[a] Hulya Ulusoy, MD,[b] Esin Yulug, MD, PhD,[c] Mehmet Muharrem Erol, MD,[a] Ahmet Alver, PhD,[d] Engin Yenilmez, PhD,[c] Sukran Geze, MD,[b] and Murat Topbas, MD[e]

Objective: Lung injury induced by one-lung ventilation is rare, but it is a condition that may result in high mortality. This study evaluates the effects of one-lung ventilation and occlusion time on collapsed and contralateral lungs.

Methods: Sprague–Dawley rats were allocated randomly into 7 groups consisting of 6 animals each: sham; O1, 1 hour of occlusion/2 hours of re-expansion; C1, 3 hours of mechanical ventilation control; O2, 2 hours of occlusion/2 hours of re-expansion; C2, 4 hours of mechanical ventilation control; O3, 3 hours of occlusion/2 hours of re-expansion; and C3, 5 hours of mechanical ventilation control groups. In the occlusion groups, the left lung was collapsed by bronchial occlusion. Malondialdehyde activity was determined in the blood, and myeloperoxidase and malondialdehyde activity was determined in the collapsed and contralateral lungs. Lung tissues were also examined histopathologically.

Results: Malondialdehyde and myeloperoxidase levels rose as occlusion duration increased. This increase was greater in the occlusion groups than that in their own control groups. Increases were significant in the O2 compared with the O1 groups ($P < .005$). Histologically, tissue damage increased as occlusion time rose injury in collapsed and contralateral lungs. Injury was greater in the occlusion groups than injury in their own control groups ($P < .005$).

Conclusions: Our findings show that biochemical and histopathologic injury occur in collapsed and contralateral lungs in one-lung ventilation, and this injury increases as occlusion time rises. We believe that occlusion and occlusion time–related injury should be borne in mind in the clinic under conditions requiring the application of one-lung ventilation.

From the Departments of Thoracic Surgery,[a] Anesthesiology and Critical Care,[b] Histology and Embryology,[c] Biochemistry,[d] and Public Health,[e] Karadeniz Technical University, Faculty of Medicine, Trabzon, Turkey.

Received for publication Jan 30, 2007; revisions received March 22, 2007; accepted for publication April 12, 2007.

Address for reprints: Celal Tekinbas, MD, Department of Thoracic Surgery, Karadeniz Teknik Üniversitesi, Tıp Fakültesi, Göğüs Cerrahisi AD, 61080, Trabzon, Turkey (E-mail: celaltekinbas3@hotmail.com).

J Thorac Cardiovasc Surg 2007;134:405-10

0022-5223/$32.00

doi:10.1016/j.jtcvs.2007.05.003

Lung injury after thoracic surgery with one-lung ventilation (OLV) is a relatively uncommon condition, but it may result in major complications with high mortality.[1,2] There is still a lack of evidence concerning the role of oxidative stress on minor or major complications that develop after lung resection with OLV.[1,3]

During OLV the operated lung remains completely atelectatic for a period of time. The nonventilated lung is known to be hypoperfused owing to hypoxic vasoconstriction, and when the bronchial block is ended, the ensuing immediate lung re-expansion along with tissue reperfusion may generate oxygen-derived free radicals through a relatively well-described mechanism.[4,5] The single ventilated lung may be subjected to a combination of hyperoxia, volutrauma, and hyperinflation. Hyperoxia represents a potential source of oxidative stress.[2] Free radicals caused by reactions of oxidative stress are known to exert deleterious effects on cellular function, resulting in oxidative tissue damage and then in organ dysfunction.[6-9] At this point, the length of time at which the lung can be safely left atelectatic becomes a matter of importance.

Abbreviations and Acronyms
MDA = malondialdehyde
MPO = myeloperoxidase
OLV = one-lung ventilation

Our aim in the study was the experimental determination of the effects of OLV and OLV duration, as well as the biochemical and histopathologic examination of the collapsed and contralateral lung injury caused by OLV and the duration thereof.

Materials and Methods

The experiments were approved by the Karadeniz Technical University, Faculty of Medicine, Animal Care and Ethics Committee (Approval No. 2005/21). Adult male Sprague–Dawley rats weighing between 300 and 350 g were used. Animals were allocated randomly into 7 groups including 6 rats each: group 1 (sham), no OLV and mechanic ventilation; group 2 (O1), 1 hour of occlusion/2 hours of re-expansion; group 3 (C1), 3 hours of mechanical ventilation (control group); group 4 (O2), 2 hours of occlusion/2 hours of re-expansion; group 5 (C2), 4 hours of mechanical ventilation (control group); group 6 (O3), 3 hours of occlusion/2 hours of re-expansion; and group 7 (C3), 5 hours of mechanical ventilation (control group).

Surgical Procedure

The rats were anesthetized by intramuscular injection of 10 mg/kg of xylazine (Rompun; Bayer, Leverkusen, Germany) and 60 mg/kg of ketamine hydrochloride (Ketalar; Parke Davis, Berlin, Germany). Apart from the sham, rats were cannulated with a 16-gauge cannula (Nova Cath IV cannula; Medipro, Turkey) after tracheotomy. The right femoral vein was cannulated with a 24-gauge cannula (Nova Cath IV cannula). The rats were placed on the operating table in a supine position, and muscle relaxation was subsequently attained and maintained by hourly intramuscular injections of 2.0 mg/kg of pancuronium bromide (Pavulon; Organon Technika, Boxtel, The Netherlands). Next, the animals were connected to a ventilator in a pressure-controlled mode. Anesthesia was maintained by hourly intramuscular injections of 10 mg/kg of xylazine. For continuous intravenous infusion, the total volume of infused fluid was 4 mL/h, consisting of normal saline. The body temperature of the animals was kept at 38°C with a heating blanket.

With the exception of the sham group, all rats were placed in the right lateral decubitus position. The animals' lungs were ventilated at a peak inspiratory pressure of 12 cm H_2O, a rate of 60 beats/min, a tidal volume of 10 mL/kg, an inspired oxygen fraction of 1.0, a positive end-expiratory pressure of 6 cm H_2O, and an inspiratory/expiratory ratio of 1:2. This protocol was named "protocol A." At the semisterile condition, left posterolateral thoracotomy from the fifth intercostal space was performed on the occlusion groups (O1, O2, and O3). Since the bronchial artery could not be separated atraumatically, it was occluded together with the left main bronchus with a micro bulldog clamp. The pulmonary artery and vein were separated and maintained atraumatically to constitute the closest model to the OLV applied in humans. At 1, 2 and 3 hours of atelectasis, a different ventilatory protocol (protocol B) was used. This was a peak inspiratory pressure of 12 cm H_2O, a rate of 80 beats/min, a tidal volume of 5 mL/kg, an inspired oxygen fraction of 1.0, a positive end-expiratory pressure of 6 cm H_2O, and an inspiratory/expiratory ratio of 1:1.4. After atelectasis, the clamp was removed and lung re-expansion was observed and established within 2 hours by protocol A ventilation strategy. The control groups (C1, C2, and C3) were ventilated for 3, 4, and 5 hours, respectively, via protocol A. During all these procedures, functional oxygen saturation and heart rate were monitored from the left leg. At the end of re-expansion and mechanical ventilation, blood samples were obtained from the aorta and the lungs were then separated from mediastinal tissues just before the animals were put to death, which was done by blood aspiration via the thoracic aorta. In the sham group, immediately after general anesthesia blood and tissue samples were collected and the animals were then put to death.

Biochemical Assays

Blood samples were centrifuged at 2000*g* for 10 minutes to collect serum and plasma and were stored at −20°C for malondialdehyde (MDA) measurement. After this, tissue samples were obtained from the lower lobes of the collapsed and contralateral lungs for tissue MDA and myeloperoxidase (MPO) activity assays and were stored at −80°C until used for analysis.

Blood MDA assay. Lipid peroxidation in plasma (nanomoles per milliliter) and lung tissue (nanomoles per gram wet tissue) were determined as MDA concentration by the methods described by Yagi[10] and by Yoshida and associates,[11] respectively. Tetramethoxypropane was used as a standard.

Lung MPO activity. Lung samples (2.5%) were homogenized in 50 mmol/L potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyltrimethylammonium bromide and were freeze-thawed 3 times. The homogenate was centrifuged at 18,000*g* for 20 minutes. The upper layer was incubated for 2 hours at 60°C and centrifuged at 2000*g* for 15 minutes. The clear supernatant was used for analysis, and MPO activity in tissues was measured by the method described by Bradley and colleagues.[12] Enzyme activity was expressed as micromoles per milligram of tissue by the molar absorbency index (1.13×10^4) of oxidized *o*-dianisidine.

Histopathologic Examination

Lung tissues were fixed, dehydrated, cleared and embedded in paraffin, stained with hematoxylin and eosin, and investigated with a light microscope (Olympus BX51; Olympus Optical Co, Ltd, Tokyo, Japan). All tissue slides were examined at high magnification by a histologist who was blind to the animal groups; 5 high-power fields were randomly sampled, and the semiquantification of lung injuries was performed according to the defined criteria. Histopathologic semiquantitative grading criteria were as follows: grade 0, normal morphology; grade 1, slight intra-alveolar edema and inflamatory cell infiltration; grade 2, moderate alveolar edema and inflammatory cell infiltration; grade 3, severe alveolar edema and inflammatory cell infiltration plus focal hemorrhage; and grade 4, massive inflammatory cell infiltration and destruction of alveolar structure.

The tissues were fixed with 4% phosphate-buffered glutaraldehyde solution (24 hours/pH 7.4) at 4°C for electron microscopic examination. The specimens were postfixed in 1% osmium tetroxide for 2 hours at 4°C. Tissues were then dehydrated in ethanol, cleared in propylene oxide, and embedded in Epon for 18 hours at 60°C. All sections were cut on a Leica Ultracut ultramicrotome (Leica Microsystems GmbH, Wetzlar, Germany) and stained with uranyl acetate and lead citrate. We viewed and photographed each section with a JEOL 1010 C transmission electron microscope (JEOL Ltd, Tokyo, Japan).

Statistical Analysis

The groups were compared by Kruskal–Wallis analysis of variance (Mann–Whitney *U* test with Bonferroni correction as post hoc test). The results were expressed as mean ± standard deviation.

Results

We made no change in inspiratory oxygen percentage throughout the study procedure. There were no significant differences between the groups in terms of body weight or time between induction of anesthesia and start of the procedure.

Oxygen Saturation

No major alteration in oxygen saturation was determined in any rats throughout the course of the study procedure.

Blood MDA Levels

Compared with the sham, rat plasma MDA levels were observed to be significantly higher in all the study groups ($P = .01$). Plasma MDA levels were significantly higher in occlusion groups than in the control groups ($P < .005$). As occlusion duration rose, we also observed an increase in MDA levels. This rise was statistically significant between the O1 and O2 groups (Figure 1).

Lung MDA Levels

In collapsed and contralateral lungs, all groups' tissue MDA levels were significantly higher in both lungs compared with those of the sham groups ($P = .003$). In all occlusion groups, MDA levels were higher than those of the control groups at a comparable time. MDA levels were observed to increase as occlusion duration rose. The highest MDA level was in O3. The increase between the O1 and O2 groups was statistically significant ($P = .003$). Lower tissue MDA values were obtained in the contralateral lung in all groups compared with the collapsed lung (Figure 2).

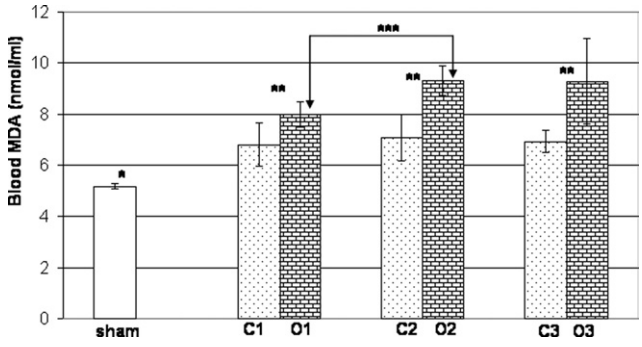

Figure 1. Blood MDA assay. Data are expressed as mean ± standard deviation. *$P < .010$ versus other groups; **$P < .05$ control group versus occlusion group; ***$P < .005$ O1 versus O2.

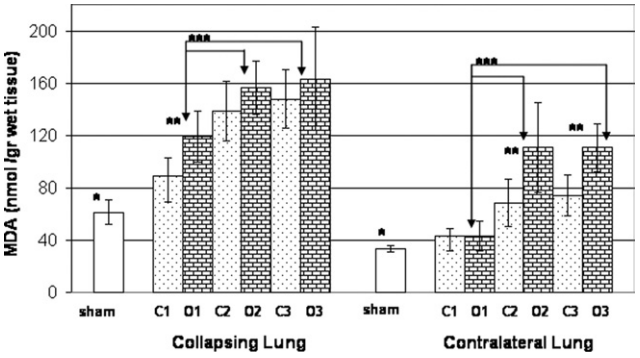

Figure 2. MDA levels in collapsing and contralateral lungs. Data are expressed as mean ± standard deviation. *$P < .010$ sham versus other groups; **$P < .05$ Cx versus Ox; ***$P < .005$ O1 versus O2 and O3.

Tissue MPO Activity

In the collapsed and contralateral lungs, tissue MPO activities were significantly higher in both lungs in all the study groups ($P < .005$) compared with the sham**.** We determined that MPO levels increased as occlusion times rose. Although the highest MPO activity was in the O3 in both lungs, the difference between the O1 with O2 and O3 in the collapsed and contralateral lungs was statistically significant ($P = .001$) (Figure 3).

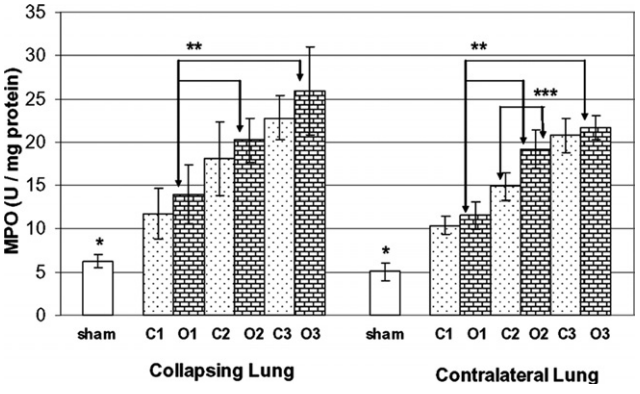

Figure 3. MPO activity in collapsing and contralateral lungs. *$P < .010$ sham versus other groups; **$P < .005$ O1 versus O2 and O3; ***$P < .01$ C2 versus O2.

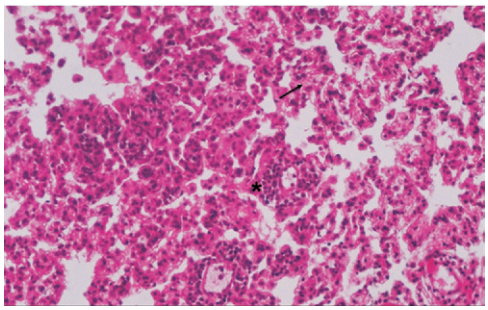

Figure 4. Light microscopic photomicrograph showed interstitial edema *(arrow),* inflammatory cell infiltration*(*),* and alveolar structure damage in the collapsed lung in the O3. (Hematoxylin and eosin, original magnification ×100.)

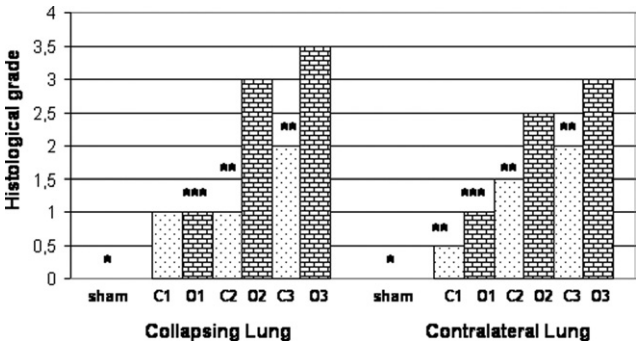

Figure 6. Histologic findings in collapsed and contralateral lung. *$P < .010$ sham versus other groups; **$P < .05$ Cx versus Ox; ***$P < .005$ O1 versus O2 and O3.

GTS

Histopathologic Evaluation

A light microscopic photomicrograph showed severe interstitial edema in the alveoli and prominent leukocyte infiltration and intra-alveolar hemorrhage in the O3 indicated a high degree of lung injury (Figure 4). In the contralateral lung, although the greatest injury was observed in the O3, alveolar edema and inflammatory cell infiltration were lighter than those in the collapsed lung (Figure 5). In experimental groups, an increasing histopathologic grade was observed with increasing collapse duration in the groups subjected to different collapse times (Figure 6).

Using electron microscopy, in the occlusion groups the greatest degeneration in the collapsed and contralateral lungs was in the O3. The increased vacuolization in type 2 pneumocytes, loss in lamellar bodies, and the increase in interstitial edema and collagen fibers in the alveolar septum were greater in the O3 than the other groups (Figure 7). In the contralateral lung, a thickening in the basal membrane of type 2 pneumocytes and dispersed vacuolization were observed in the O3. The loss in lamellar bodies was not as evident as in the collapsed lung (Figure 8).

Discussion

In the study, we identified increasing biochemical and histopathologic injury as occlusion time increased in the collapsed and contralateral lungs of the rats constituting the OLV model.

Long-term OLV with saturation and blood gas monitoring is frequently performed in thoracic surgery. The lung is a critical organ in the oxidative stress process of either systematic or pulmonary origin.[13] Oxygen deprivation from ischemia or nonventilation leads to cellular hypoxic injury.[10] The amount of generated oxygen-derived free radicals has been associated with the duration of OLV.[4]

Several methods have been used to define the ischemia/reperfusion tissue injury,[8,14] such as biochemical changes (plasma MDA, tissue MDA, and MPO), specific organ function tests, and the histopathologic analysis.

Lipid peroxidation, as a free radical–generating system, has been suggested to be closely related to ischemia/reperfusion–induced tissue damage, and MDA is a good

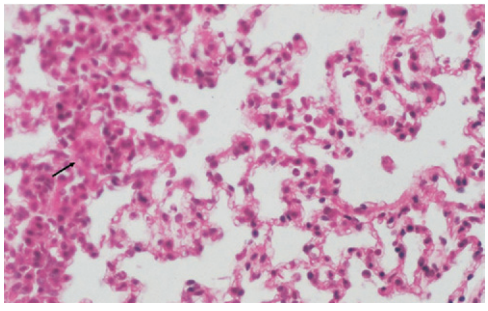

Figure 5. Light microscopic photomicrograph showed lighter inflammatory cell infiltration and alveolar structure damage *(arrow)* in the contralateral lung in the O3. (Hematoxylin and eosin, original magnification ×200.)

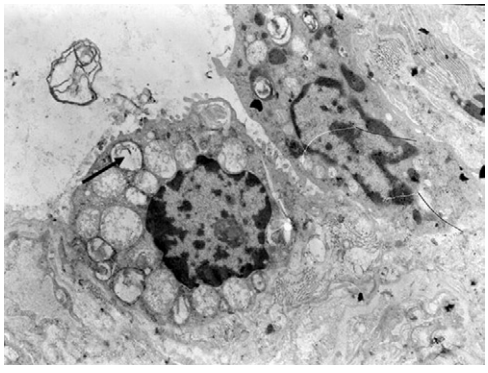

Figure 7. Electron micrograph showed localized significant vacuolation, and loss in lamellar bodies *(arrow)* was observed in type 2 pneumocytes in collapsed lungs in the O3 (original magnification ×5000).

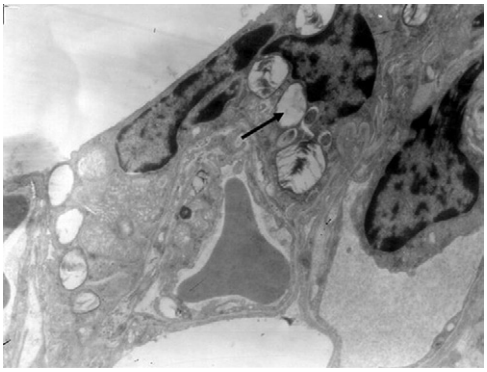

Figure 8. Decrease of vacuolation and lamellar bodies *(arrow)* were observed in type 2 pneumocytes in the contralateral lung in the O3 (original magnification ×10,000).

indicator of the rate of lipid peroxidation.[11,15] Lipid peroxidation leads to autocatalytic mechanism and cell membrane damage, and as a result of membrane damage, toxic substances lead to cell death by leading to the formation of reactive metabolites.[15] When the collapsed and contralateral lungs were compared, we determined that MDA levels were higher in the collapsed lung in all groups. Plasma and tissue MDA levels in both collapsed lung and contralateral lung increased with occlusion duration. Whereas the increase between the O1 and O2 was statistically significant, that between the O2 and O3 was not. We think that this may be related to the level of glutathione and other antioxidants that comprise cellular defense against oxidative injury, reaching maximum plasma concentration after the second hour.[16,17] We believe that this needs to be supported by both experimental and clinical studies.

MPO plays a fundamental role in oxidant production by neutrophils and has long been a marker of lung injury.[18] Pulmonary parenchyma is one of the largest reservoirs for neutrophils, monocytes, and macrophages. Neutrophils are a potential source of free oxygen radicals and are considered to be a major effector cell in tissue damage.[3,11] According to our findings, the MPO levels were higher in the collapsed lung in all groups than those in the contralateral lung. We ascribe this to the manipulated lung causing the inflammation. In addition, MPO levels were higher in occlusion groups than in their own control groups. MPO level was significantly higher in both lungs in the O2 compared with the O1, although there was no significant difference with regard to the O3. Yin and colleagues[19] showed that there was no increase in pulmonary MPO activity and serum tumor necrosis factor levels in a pig model 30 minutes and 1 hour after re-expansion in pigs subjected to 1 hour of OLV. We think that decreasing the trends of increased MPO levels after the second hour may be related to the release of endogen anti-inflammatory substances and maximal tissue concentration being attained after the second hour.

Histologically, the initial cellular response to lung injury involves endothelial cell dysfunction without obvious morphologic cell injury.[20] Morphologic alterations that accompany reperfusion after prolonged ischemia include alveolar capillary interstitial edema, hyaline membranization along alveolar ducts, polymorphonuclear cell infiltration, as well as the detachment of endothelial cells and type I pneumocytes from the basement membrane.[8,11,21,22] At histologic analysis of tissues, we determined that tissue injury increased as occlusion duration rose. Injury was greater in the collapsed lungs than in the contralateral lungs.

We identified a statistically insignificant difference in injury between the right and left lungs in the control group. We ascribe this to the gravity-related decrease in perfusion in the top, left lung, in the right lateral decubitus position, and to an increase in perfusion and the bottom, right lung.

In conclusion, our findings show that injury in collapsed and contralateral lungs in OLV increased as occlusion duration rose. We think that this injury may lead to negative changes in the lung and respiratory function impairments in the postoperative period. We therefore believe that the duration-related effects of OLV should be made the subject of clinical studies.

GTS

References

1. Gothard J. Lung injury after thoracic surgery and one-lung ventilation. *Curr Opin Anaesthesiol.* 2006;19:5-10.
2. Jordan S, Mitchell JA, Quinlan GJ, Goldstraw P, Evans TW. The pathogenesis of lung injury following pulmonary resection. *Eur Respir J.* 2000;15:790-9.
3. Williams EA, Evans TW, Goldstraw P. Acute lung injury following lung resection: is one lung anaesthesia to blame? *Thorax.* 1996;51:114-6.
4. Sylvester JT. Hypoxic pulmonary vasoconstriction: a radical view. *Circ Res.* 2001;88:1228-30.
5. Tarver RD, Broderick LS, Conces DJ Jr. Reexpansion pulmonary edema. *J Thorac Imaging.* 1996;11:198-209.
6. Misthos P, Katsaragakis S, Milingos N, Kakaris S, Sepsas E, Athanassiadi K, et al. Postresectional pulmonary oxidative stress in lung cancer patients. The role of one-lung ventilation. *Eur J Cardiothorac Surg.* 2005;27:379-82.
7. Misthos P, Katsaragakis S, Theodorou D, Milingos N, Skottis I. The degree of oxidative stress is associated with major adverse effects after lung resection: a prospective study. *Eur J Cardiothorac Surg.* 2006;29:591-5.
8. Esme H, Fidan H, Koken T, Solak O. Effect of lung ischemia-reperfusion on oxidative stress parameters of remote tissues. *Eur J Cardiothorac Surg.* 2006;29:294-8.
9. Grace PA. Ischaemia-reperfusion injury. *Br J Surg.* 1994;81:637-47.
10. Yagi K. Lipid peroxides and related radicals in clinical medicine. In: Armstrong D, editor. Free radicals in diagnostic medicine. New York: Plenum Press; 1994. p. 1-15.
11. Yoshida S, Busto R, Watson BD, Santiso M, Giensberg MD. Post-ischemic cerebral lipid peroxidation in vitro: modification by dietary vitamin E. *J Neurochem.* 1985;44:1593-601.
12. Bradley PP, Priebat DA, Christensen RD, Rothstein G. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *J Inves Dermatol.* 1982;78:206-9.

13. de Perrot M, Liu M, Waddell TK, Keshavjee S. Ischemia-reperfusion–induced lung injury. *Am J Respir Crit Care Med.* 2003;167:490-511.
14. Saito S, Ogawa J, Minamiya Y. Pulmonary reexpansion causes xanthine oxidase–induced apoptosis in rat lung. *Am J Physiol Lung Cell Mol Physiol*. 2005;289:400-6.
15. Omar R, Nomikos I, Piccorrelli G, Savino J, Agarwal N. Prevention of postischemic lipid peroxidation and liver cell injury by iron chelation. *Gut*. 1989;30:510-4.
16. Ross D. Glutathione, free radicals and chemotherapeutic agents. Mechanisms of free-radical induced toxicity and glutathione-dependent protection. *Pharmacol Ther.* 1988;37:231-49.
17. Hammond CL, Lee TK, Ballatori N. Novel roles for glutathione in gene expression, cell death, and membrane transport of organic solutes. *J Hepatol.* 2001;34:946-54.
18. Ng CS, Wan S, Arifi AA, Yim AP. Inflammatory response to pulmonary ischemia-reperfusion injury. *Surg Today*. 2006;36:205-14.
19. Yin K, Gribbin E, Emanuel S, Orndorff R, Walker J, Weese J, et al. Histochemical alterations in one lung ventilation. *J Surg Res*. 2007; 137:16-20.
20. Carden DL, Granger DN. Pathophysiology of ischaemia-reperfusion injury. *J Pathol.* 2000;190:255-66.
21. Her C, Mandy S. Acute respiratory distress syndrome of the contralateral lung after reexpansion pulmonary edema of a collapsed lung. *J Clin Anesth.* 2004;16:244-50.
22. Welbourn R, Goldman G, O'Riordan M, Lindsay TF, Paterson IS, Kobzik L, et al. Role for tumor necrosis factor as mediator of lung injury following lower torso ischemia. *J Appl Physiol.* 1991;70: 2645-9.