The role of tissue reperfusion in the reexpansion injury of the lungs

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Abstract

Objective: The aim was to discuss the balance between free radical damage and body defense mechanisms that occurred in reexpanded pulmonary tissue and to evaluate the relationship between the changes in the pulmonary circulation and the mentioned balance.

Methods: Twenty male Wistar Albino rats were used for these study results. Pneumothorax was created in the left hemithorax by percutaneous route in all the rats. After 7 days, the first group (n = 10) had a sternotomy under ketamine anesthesia. Following invasive measurement of pulmonary artery pressure, tissue samples were obtained from the lower lobes of the right and left lungs before reexpansion occurred. Tracheotomies were opened in the second group (n = 10) with a 16 gauge cannula. Following sternotomy, invasive mean pulmonary artery pressure measurements were obtained by the support of non-invasive cardiac monitorization. The lungs were aerated with 4 cmH2O oxygen and fixed volume support and 1 h of reexpansion was obtained. Invasive mean pulmonary artery pressure measurements were repeated after reexpansion and tissue samples were obtained from the lower lobes of left and right lungs. Nitric oxide (NO), malondialdehyde (MDA) and superoxide dismutase (SOD) levels were measured in tissue samples, surfactant staining and light microscopic evaluations were performed.

Results: At the end of the reexpansion, there was a decrease in mean pulmonary artery pressure (P < 0.01), MDA (P < 0.01) and SOD (P < 0.05) levels and an increase in NO (P < 0.05) levels. Under the light microscopic examination, in the samples that were provided with reexpansion, the alveolo-capillary membrane was thickened due to increasing edema, increase in the number of lymphocytes and return of the neutrophil leukocytes to the area. There was no significant difference between the groups in terms of surfactant staining.

Conclusion: The tissue reperfusion that is achieved with the restoration of blood flow during the reexpansion of collapsed lungs, can be the initial pathology in the chain of events that result in reexpansion injury.

Keywords: Lung; Reexpansion injury; Reperfusion

1. Introduction

Although the reexpansion injury of the lungs is a rarely encountered pathology, it might have a fatal course. It is generally unilateral. Even though the pathogenesis has not been clearly understood, entrance of the inflammatory cells to the tissue [1], changes in pulmonary artery pressure [2], secretion of biochemical mediators [1,3], increase in pulmonary vascular permeability [1,4,5], loss of surfactant [6] have all been held responsible as etiological factors in different studies. Although there are studies about oxygen-free radicals (ROS) in reexpansion injury, the information that is provided is limited. The aim was to discuss the balance between free radical damage and body defense mechanisms that occurred in reexpanded pulmonary tissue and to evaluate the relationship between the changes in the pulmonary circulation and the mentioned balance.

2. Materials and methods

2.1. Animals

Twenty male Wistar Albino rats with an average body weight of 246 ± 27.2 g were utilized for the study. 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.
2.2. Experimental design

In both groups, pneumothorax was induced by injecting 4 ml of air into the thorax via percutaneous route with a 22 gauge cannula that was placed in the left hemithorax. The adequacy of the pneumothorax was confirmed with control X-rays. For avoiding the possible reabsorption that might occur, 1–1.5 ml of additional air was injected into the thorax on day 3 and the continuation of the pneumothorax was achieved. Anesthetic substance (30 mg/kg i.m ketamin) was administered at a low dose in order to eliminate the risk of respiratory depression. X-rays were obtained from all the subjects on day 7 for confirming the stability of the pneumothorax (Fig. 1). During the pneumothorax inducing procedures, non-invasive systemic pressure measurements (from the tail) and continuous electrocardiographic monitorizations were performed on all the subjects. Major hemodynamic changes were not found. During this period one subject died due to pulmonary laceration and another due to respiratory depression. The respiratory depression was corrected by ventilating the subject via a mask.

The first group that was under ketamin anesthesia (60 mg/kg i.m. ketamin), underwent sternotomy \( n = 10 \). The adequacy of pneumothorax was confirmed during sternotomy as well. Pericardium was rapidly elevated for the exposure of the pulmonary artery and right ventricle outflow tract. Before having the reexpansion, the tip of the cannula (22 gauge cannula was used) was invasively located into the pulmonary artery through right ventricle outflow tract, mean pulmonary artery pressures were measured and recorded. During all these procedures, continuous non-invasive systemic blood pressure monitorization (from the tail) and electrocardiographic monitorizations were made. In all the samples, simultaneously with the pulmonary artery pressure readings, systemic blood pressure and electrocardiographic recordings were also obtained. Following this, tissue samples were obtained from the lower lobes of the right and left lungs. No subjects died in the first group.

Second group \( n = 10 \) underwent a tracheotomy with a 16 gauge cannula and then a sternotomy. The sufficiency of pneumothorax was confirmed during sternotomy. Before having the reexpansion, the tip of the cannula (22 gauge cannula was used) was invasively located into the pulmonary artery through right ventricle outflow tract, mean pulmonary artery pressures were measured and recorded. After taking the cannula out, hemostasis was achieved by applying gentle compression on right ventricle. Afterwards, reexpansion was established within 1 h by having fixed oxygen pressure of 4 cmH\(_2\)O and fixed volume support. At the end of reexpansion, invasive pulmonary artery pressure readings were obtained by the same route. During all these procedures, continuous non-invasive systemic blood pressure monitorization (from the tail) and electrocardiographic monitorizations were made. In all the samples, simultaneously with the pulmonary artery pressure readings, systemic blood pressure and electrocardiographic recordings were also obtained. Following this, tissue samples were obtained from the lower lobes of the right and left lungs. The obtained samples were kept at \(-80^\circ C\). In this group, due to hemodynamic problems (hemorrhage), hypotension occurred in two subjects and we excluded these subjects from the study group considering the possibility that the deterioration of the tissue perfusion might create ischemic changes in their lungs (Fig. 2).

2.3. Biochemical protocols

Nitric oxide (NO) (nmol/g) was quantified by the measurement of the NO metabolite, nitrite, using the Griess reagent as described before [7–9]. In short, serum (250 \( \mu l \)) was incubated at room temperature with 250 \( \mu l \) of substrate buffer (imidazole 0.1 mol/l, NADPH 210 \( \mu l \), flavinadenine dinucleotide 3.8 \( \mu l \); pH 7.6) in the presence of nitrate-reductase (Aspergillus niger, Sigma) for 45 min to

![Fig. 1. The radiographic appearance of the obtained pneumothorax on day 7.](image-url)
convert nitrate to nitrite. Excess reduced nicotinamide adenine dinucleotide phosphate, which interferes with the chemical detection of nitrite, was oxidized by continuation of the incubation of 5 µg (1 ml) of LDH (Sigma), 0.2 mmol/l (120 µl) pyruvate (Sigma) and 79 ml of water. Total nitrite was then analyzed by combining the samples with Griess reagent (1% sulfinilamide, 0.1% naphthalene-ethylene diamine dihydrochloride in 5% H₃PO₄ spectroquant: Merck, Darmstadt, Germany). Reacted samples were treated with 500 µl of trichloroacetic acid (20%), centrifugated for 15 min at 8000 g and the absorbances at 548 nm were compared with the standard graph of NaNO₂ which was obtained from the reduction of NaNO₃ (0–100 µmol/l).

Thiobarbituric acid (TBA) reacts with lipoperoxidation aldehydes, such as malondialdehyde (MDA) (nmol/g), as the most common method to assess lipid peroxidation in biological samples. The procedure was modified from Buege and Aust [10]. Briefly, 0.5 ml of plasma was added to a reaction mixture (1.0 ml) formed by equal parts of 15% trichloroacetic acid, 0.25 N HCl, and 0.375% TBA, plus 2.5 mM BHT and 0.1 ml of 8.1% SDS, followed by 30 min heating at 95°C; pH value of the analytical reaction mixture was about 0.9. BTH was used to prevent lipid peroxidation during heating. After cooling each incubation, the chromogen was extracted with n-butanol and read spectrophotometrically at 532 nm against a reaction mixture ‘blank’ lacking plasma but subjected to the entire procedure and extracted with n-butanol. To correct for background absorption, absorbance values at 572 nm were subtracted from those at 532 nm, the latter representing the absorption maximum of the 2:1 TBA:MDA adduct [11]. A molar extinction coefficient of 154,000 was used.

Superoxide dismutase (SOD) (U/mg prot.), was assayed with a Hitachi 902 autoanalyzer with test kits from Randox (Ransod) Cat No: SD 125.

2.4. Pathologic protocols

Light microscopic samples for analyses of alveolo-capillary membrane were stained with Haematoxyline–Eosine (H.E.).

Five micron-thick cross sections were taken from the paraffin blocks containing tissue. Before staining process, they were heated for 5 min; three times inside the antigen retrieval (DAKO, S2031) solution. Avidine-Biotin HRP (Detection system with 3,3′-diaminobenzidene as the chromogen) was used for immunohistochemical staining with surfactant-B (Neomarkers, MS-1300).

2.5. Data analysis

Pulmonary artery pressures (mmHg) were measured with a patient monitor (Petas TM 150 monitor; Petas corp.; TR) and a pressure monitoring kit (Viggo-Spectramed Inc.; Oxnard, CA, USA). Systemic pressure was performed with an animal monitor (Letica LE 5002 storage pressure mater; Spain). Electrocardiographic samples were recorded by an animal monitor. (Kenz-Cardioco 302 (5505) Suzuken Co. Ltd; Japan)

Pressure and biochemical results are presented as mean (SEM±). The overall significance of differences between
the groups was determined by Wilcoxon test. Pathological results were evaluated semiquantitatively by means of (negative) and three positive values (+ mild, rare patchy lymphocyte density; ++ moderate, significant amount of lymphocyte density in every field; +++ severe, formation of follicles due to the density of the lymphocytes).

3. Results

Non-invasive systemic pressure was measured as 70 ± 11.2 mmHg in all the subjects. There was a significant decrease in the mean pulmonary artery pressure at the end of the reexpansion (P < 0.01) (Table 1 and Fig. 3). At the end of the reexpansion, there was a significant increase in tissue NO levels (P < 0.05) (Table 1 and Fig. 3) together with a significant decrease in tissue MDA and SOD levels (P < 0.01 and P < 0.05, respectively) (Table 1 and Fig. 3). As for the biochemical parameters, the comparison of right lung tissue samples with the end-pneumothorax and end-reexpansion tissue samples did not yield any difference of statistical significance (Table 1).

Under light microscopic examinations, edema of the alveolo-capillary membrane was less significant on the tissue samples obtained from the right lung samples at the end of the pneumothorax and at the end of the reexpansion (Figs. 4 and 5, respectively). However, it increased in the left lung samples at the end of pneumothorax and reached highest levels in the left lung samples obtained at the end of the reexpansion (Figs. 6 and 7, respectively). Tissue samples from the right lung at the end of pneumothorax, from the right lung at the end of reexpansion and from left lung at the end of the pneumothorax had ++ lymphocyte content (Figs. 4, 5 and 6, respectively). In the left lung samples at the end of the reexpansion, the lymphocytes started to form islets and there was a +++ lymphocyte dominance (Fig. 7). Together with reexpansion, polymorphonuclear leukocytes (PNL) started to return to the tissue.

Table 1
Findings in the reexpansion injury of the lungs

<table>
<thead>
<tr>
<th></th>
<th>End of the pneumothorax</th>
<th>End of the reexpansion</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean pulmonary artery pressure (mmHg)</td>
<td>16.2 ± 3.0</td>
<td>10.6 ± 2.3</td>
<td>&lt; 0.01</td>
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<tr>
<td>Levels of tissue nitric oxide (NO) (nmol/g)</td>
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<tr>
<td>Left lung</td>
<td>20.7 ± 2.9</td>
<td>27.8 ± 3.6</td>
<td>&lt; 0.05</td>
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<tr>
<td>Right lung</td>
<td>22.9 ± 3.8</td>
<td>24.6 ± 2.5</td>
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<tr>
<td>Levels of tissue malondialdehyde (MDA) (nmol/g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left lung</td>
<td>87 ± 12.6</td>
<td>54 ± 7.2</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Right lung</td>
<td>72.5 ± 14.1</td>
<td>67.4 ± 13.1</td>
<td></td>
</tr>
<tr>
<td>Levels of tissue superoxide dismutase (SOD) (U/mg prot.)</td>
<td></td>
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<tr>
<td>Left lung</td>
<td>44.9 ± 5.1</td>
<td>30.3 ± 3.7</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>Right lung</td>
<td>38.2 ± 6.2</td>
<td>36.8 ± 5.5</td>
<td></td>
</tr>
</tbody>
</table>

* Values are given in mean ± SEM.
There was not any difference between the groups in terms of surfactant staining.

4. Discussion

Experimental and clinical studies have been performed in an attempt to identify the causes of reexpansion injury. Lung is a tissue that is resistant to hypoxia due to its dual blood flow and due to its ability to use the oxygen reserves in the alveolar spaces [12]. However, the studies conducted on unilateral pneumothorax lung have shown that significant hypoxia developed in the collapsed lung and lung blood flow was reduced to half because of hypoxic vasoconstriction [13,14].

In our study, we observed an increase in the mean pulmonary artery pressure. Pulmonary artery pressure is an indirect indicator of the increased vascular resistance which develops due to hypoxic vasoconstriction of the collapsed lung. In parallel to the increased pulmonary artery pressure, we think that blood flow decreased in the collapsed lung. Oxygen entrance to the tissue during reexpansion is directly through the alveolar route. Nakamura et al. [15] reported that the entrance of oxygen into the tissue by alveolar route increased as a result of reexpansion. We think that another important route of oxygen entrance into lung tissue is the reperfusion that follows the restoration of blood flow.

As a result of hypoxic stimulus and increase in the entrance of the oxygen through the alveolar route to the reexpanded tissue, there are significant increases in tissue NO levels. NO, which is increased during expansion, decreases hypoxic vasoconstriction and corrects pulmonary hypoperfusion by managing the restoration of tissue blood flow. Next, reperfusion period starts in lung tissue.

Although utilization of oxygen is indispensable for aerobic metabolisms, high amounts of oxygen supply to the tissue and inappropriate metabolism of oxygen results in an important tissue injury because of reactive oxygen species (ROS) [16,17]. Together with increased oxygen supply, there is an increase in the levels of ROS including the superoxide radical [18].

The ischemia-reperfusion studies that were performed revealed important information about the effects of lipid mediators, polypeptide mediators and immune complexes that increase during the reperfusion period. Together with tissue reperfusion, these mediators increase and cause functional dysfunction in endothelial cells, thus increasing the influx of monocytes, PNL and macrophages to the alveolo-capillary membrane. These blood cells that arrive in the tissue initiate a chain of reactions which result in the production of oxygen-free radicals including superoxide radicals [16,19,20].

Lung is the largest reservoir for monocytes, macrophages and PNLs. In our study, in the samples obtained at the end of the pneumothorax, + + lymphocyte dominance was found semi-quantitatively. Together with reexpansion, lymphocyte levels were +++ and PNLs started to remigrate to the area. We think that the reperfusion achieved during reexpansion increases the production of free radicals by...
activating the inflammatory cells in addition to increasing the oxygen supply to the tissue.

ROSs are extremely reactive due to the unpaired electrons that they have on their outer orbits. Their most important destructive effect is the lipid peroxidation that they initiate [21,22]. Under normal conditions, this is compensated by antioxidant mechanisms. However, with hypoperfusion and the following reperfusion period, production of ROSs increase and endogenous defense mechanisms become insufficient [16]. In tissue damage, the balance between the generation of free radicals and endogenous defense mechanisms is of utmost importance. This balance is achieved by several enzymes including SOD [23]. As a result of MDA consumption by SOD, we think that the anticipated differences between MDA and SOD parameters might have been masked. The microscopic findings obtained from the left lung samples at the end of the reexpansion (thick arrow) (H.E. × 100).

At the end of the pneumothorax period, there were high levels of MDA and SOD. These significantly decreased at the end of the reexpansion. We think that this decrease was due to the utilization of endogenous SOD which is a tissue defense mechanism that primarily affects MDA levels.

The activation of free radicals increases pulmonary microvascular permeability through lipid peroxidation [18]. The increase in the pulmonary microvascular permeability is accepted to be the endpoint in the reexpansion injury.

In our study, in parallel to the increase in free radical activity, there was an edema due to increase in pulmonary vascular permeability in the alveolo-capillary membranes of tissue samples of the reexpansion period.

We think that the increase in the edema is related to the rate of lipid peroxidation, which is under the control of endogenous defense mechanisms.

In other studies, it has been emphasized that the loss of surfactant might be responsible for the etiology [6]. In our study, we could not find a difference between the surfactant staining patterns of the groups.

In conclusion, the tissue reperfusion which is achieved by the restoration of blood flow during the reexpansion of collapsed lungs, can be the initial pathology in the chain of events resulting in reexpansion injury. We think that clinical course is determined by the resistance of endogenous defense mechanisms that are compensating the free radical activation which starts as a result of the biochemical process triggered by reperfusion.

References

[18] Jackson RM, Veal CF, Alexander CB, Brannen AL, Fulmen JD. Re-


