Effects on inflammatory cytokines and oxidative stress markers of pneumoperitoneum performed after thoracotomy in rats

Sıçanlarda torakotomi sonrası yapılan pnömoperiton işleminin inflamatuvar sitokinler ve oksidatif stres belirteçleri üzerindeki etkisi

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Background: This study aims to investigate the effects of pneumoperitoneum on inflammation after thoracotomy.

Methods: Thirty-two male Wistar type rats weighing 280-320 g were divided into four groups of eight rats each. In the control group rats were only anaesthetized. In thoracotomy group an automatic retractor was inserted for a 30-minute period after left thoracotomy. In the pneumoperitoneum group, 5 ml of room air was injected intraperitoneally. In the thoracotomy + pneumoperitoneum group, thoracotomy was performed and 5 ml of room air was injected intraperitoneally. Blood and lung tissue samples were taken at the 24th hour in all groups and were stored at -80 °C.

Results: In the thorocotomy group, the tumor necrosis factor-alfa, interleukin-6 and protein carbonyl levels were significantly higher than in the control group but no difference was found between the pneumoperitoneum and the thoracotomy + pneumoperitoneum groups.

Conclusion: Pneumoperitoneum performed after thoracotomy does not increase the inflammatory reaction and oxidative stress caused by thoracotomy. Pneumoperitoneum is an effective and easily performed method.

Key words: Cytokines; oxidative stress markers; pneumoperitoneum; thoracotomy.

Amaç: Bu çalışmada pnömoperiton işleminin torakotomi sonrası inflamasyon üzerine etkileri araştırıldı.

Çalışma planı: Otuz iki adet 280-320 g ağırlığında Wistar cinsi erkek sıçan her birinde sekiz sıçan olan dört gruba ayrıldı. Kontrol grubunda sıçanlara sadece anestezi uygulandı. Torakotomi grubunda sol torakotomi sonrası 30 dakika periyodlu otomatik retraktör yerleştirildi. Pnömoperiton grubunda batın içerisine 5 ml oda havası enjekte edildi. Torakotomi + pnömoperiton grubunda ise torakotomi uygulandı ve batın içerisine 5 ml oda havası enjekte edildi. Yirmi dört saat sonra tüm gruplardan kan ve akciğer örnekleri alındı ve -80 °C'de saklandı.

Bulgular: Torakotomi grubunda tümör nekroz faktörü-alpha, interlökin-6 ve protein karbonil düzeyleri kontrol grubundan anlamlı olarak daha yüksek idi fakat pnömoperiton ve torakotomi + pnömoperiton grupları arasında fark yok idi.

Sonuç: Torakotomi sonrasında yapılan pnömoperiton işlemi torakotominin neden olduğu inflamatuvar reaksiyonu ve oksidatif stresi artırıcı bir etki yapmamaktadır. Pnömoperiton efektif ve kolay uygulanan bir yöntemdir.

Anahtar sözcükler: Sitokinler; oksidatif stres belirteçleri; pnömoperiton; torakotomi.

After major lung surgery, the occurrence of residual pleural space, continuous alveolar air leak, and prolonged chest tube drainage results in pleuropulmonary infections or a prolonged hospital stay.[1,2] Prolonged chest tube drainage after resections increases the cost of hospitalization by using antibiotics, extending the hospital stay, and, in some cases, mandating reoperations.[2]

Residual pleural spaces are more common after upper lobe resections.[2] The presence of residual space without air leak is not a serious problem because of the high percentage of spontaneous remission.[2] The application of glues, patches, stapling devices for large incomplete fissures, peeling of restrictive pleura, pleural tenting, decortication of restrictive pleural peel, sectioning of the lower pulmonary ligament,

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thoracoplasty, and crushing of the phrenic nerve are some useful maneuvers.[1-3] Physiotherapy, pleurodesis, and the use of the Heimlich valve are all possibilities as conservative approaches.[1] Pneumoperitoneum is also an effective maneuver as has been reported by many authors.[1,2] Artificial pneumoperitoneum to elevate the diaphragm is an effective method for terminating a prolonged air leak and for reducing the pleural space.[1]

Cerfolio et al.[4] first described pneumoperitoneum for patients with emphysema, and he observed an increase in respiratory volume and a decrease in dyspnea. Predisposing factors are a persistent, large air leak, restrictive lung disease, fixed mediastinum secondary to radiation or other diseases, previous thoracic operations, and the effects of induction therapy.[1-3]

In normal metabolic processes, reactive oxygen species generation and antioxidant defense mechanisms are in balance. Any disturbance in the prooxidant antioxidant systems is considered to be oxidative stress, which can cause damage to lipids, proteins, carbohydrates, and nucleic acids.[5] It is well known that surgical trauma is a source of stress and causes an inflammatory response.

Proinflammatory activation and surgical trauma like thoracotomy can trigger an anti-inflammatory reflex reaction associated with immune depression and late infectious complications.[6] They mediate a further postoperative immune response and hyperinflammatory-induced organ injury.[7]

The inflammatory response to surgery such as thoracotomy is a complex and dynamic phenomenon. Pneumoperitoneum also affects inflammation.[7,8] The purpose of this study was to investigate the effects of pneumoperitoneum on inflammation after thoracotomy.

MATERIALS AND METHODS

Animals

Thirty-two male Wistar albino rats weighing 280-320 g were used. The study was approved by the local ethics committee and financially supported by the scientific research board. 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.[9] The 32 male rats were randomly divided into four groups (n=8).

Procedure

The rats were anesthetized with intraperitoneal (ip) administration of 50 mg/kg thiopentone (pental sodium, İE Ulagay, İstanbul, Turkey), and the left hemithorax was shaved in the thorocotomy groups. The shaved skin was then repeatedly swabbed with sterile betadine wipes to sterilize the area. The animals were intubated by an endotracheal catheter (14 gauge, PTFE intravenous catheter; Mediflon, Eastern Medikit, India) placed using tongue retraction. The catheter was connected to a rodent ventilator (Harvard Apparatus, Advance Safety Ventilator Pressure, 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).

The rats were randomly assigned to four groups:

- *Control group (n=8):* The rats were only anesthetized. Blood and lung tissue samples were taken at the 24th hour and were stored at -80 °C.

- *Thoracotomy group (n=8):* A left thoracotomy was performed under anesthesia, an automatic retractor was inserted for 30 minutes, and the incision was closed. Blood and lung tissue samples were taken at the 24th hour of thoracotomy and were stored at -80 °C.

- *Pneumoperitoneum group (n=8):* Only 5 ml of room air was injected intraperitoneally. Blood and lung tissue samples were taken at the 24th hour of pneumoperitoneum and were stored at -80 °C.

- *Thoracotomy + pneumoperitoneum group (n=8):* Thoracotomy was performed and after a 30 minute retraction period, 5 ml of room air was injected intraperitoneally. Blood and lung tissue samples were taken at the 24th hour of thoracotomy and were stored at -80 °C.

All animals were maintained in the supine position for the duration of the experiment. In the thoracotomy group, a 3 cm incision was made in the skin of the lateral chest wall between the right forth and fifth ribs. The deep and superficial muscles covering the ribs were retracted to expose the intercostal muscle, and this muscle and the pleura was incised 1.5 cm above the fifth rib. An automatic retractor was carefully placed and opened 1 cm. The retractor was inserted for a 30 minute period, and the wound opening was covered during this time by gauze soaked in normal saline. After the retraction period, the retractor was removed. The deep muscles covering the ribs were sutured with 2-0 silk, and the skin was closed with 4-0 nylon sutures. The endotracheal catheter was removed reassuring that, the animal was spontaneously moving. Tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), oxidative stress [malondialdehyde (MDA)] and antioxidant [superoxide dismutase (SOD)] levels, catalase (CAT), and protein carbonyl (PC)] were measured in the tissue and serum.

Antioxidant enzyme and oxidative stress activities

Total copper, zinc, and manganese SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al.[10] The SOD activity was expressed as Umg^{-1} protein.

The MDA level, which indicates lipid peroxidation, was determined using a method based on reaction with thiobarbituric acid (TBA) at 90-100 °C.[11] Results were expressed as nmol g^{-1} of wet tissue according to the standard graphic prepared from measurements with a standard solution (1,1,3,3-tetramethoxypropane).

Catalase activity was determined according to the method of Aebi.[12] The principle of that method is based on the determination of the rate constant k [s-1] of the hydrogen peroxide decomposition at 240 nm. The results are expressed as kg^{-1} protein. All samples were assayed in duplicate.

The oxidative stress to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine. Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in dinitrophenylhydrazine. Then the absorbance was read at 370 nm. The values are expressed as nmoles of carbonyl formed per mg.

Cytokine levels

Interleukin-6 and TNF-α levels were measured in serum and lung tissue homogenate. They were also measured with an enzyme-linked immunosorbent assay (ELISA) method. Data was expressed as pictogram per milliliter.

Statistics analysis

Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS Inc, Chicago, IL, USA) version 15.0 software for Windows. Data was expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used for nonparametric and parametric comparisons among the groups. Post hoc multiple comparisons were calculated with Tukey tests. A *p* value of less than 0.05 was accepted as statistically significant.

RESULTS

Systemic effects: In the thoracotomy group, TNF-α, IL-6 and PC levels were significantly higher than in the control group ($p<0.05$). There was no difference between the thoracotomy and thoracotomy + pneumoperitoneum groups ($p>0.05$). Serum and tissue levels and statistical analyses of the parameters between the groups are shown in Table 1 and *p* values of intergroup comparison are shown in Table 2.

Local effects: In the thoracotomy group, PC levels were significantly higher than in the control group ($p<0.05$). There was no difference between the thoracotomy and thoracotomy + pneumoperitoneum groups ($p>0.05$).

DISCUSSION

In our study, the TNF-α, IL-6, and PC levels in the thorocotomoy group were significantly higher than in the controls, but there was no difference between the groups where pneumoperitoneum was performed. Like us, some authors found that pneumoperitoneum does not increase cytokine levels.[13] The increase in these levels may be explained by surgical trauma alone and not by pneumoperitoneum.

Atmospheric air was used in our study, and contrary to Tung and Smith[14] exposure to air did not cause an increase in serum IL-6 levels. Other studies have also proposed the idea that circulating air may trigger

Table 1. Serum and tissue levels and statistical analyses of the parameters between groups

	Control	Thoracotomy	Pneumoperitoneum	TT+PP	*p*
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Serum TNF-α¶	15.2±3.2	52.5±7.1	22.7±5.0	58.8±4.5	<0.001*
Serum IL-6¶	2.5±0.6	3.7±0.5	2.5±0.5	3.5±0.7	0.001*
Serum PC†	2.1±0.3	3.4±0.5	2.7±0.4	3.4±0.3	<0.001*
Serum CAT‡	0.51±0.08	0.54±0.06	0.53±0.07	0.53±0.07	0.847
Serum SOD§	12.5±0.3	12.7±0.7	12.7±0.5	12.7±0.5	0.939
Serum MDAμ	14.4±1.6	15.2±1.2	15.0±1.0	16.0±1.2	0.116
Tissue PC	1.9±0.5	2.8±0.4	2.5±0.5	2.9±0.4	0.001*
Tissue CAT	0.50±0.09	0.50±0.10	0.52±0.08	0.49±0.09	0.934
Tissue SOD	10.7±1.3	11. 0±1.5	10.3±1.1	12.0±1.1	0.084
Tissue MDA	21.2±4.3	26.1±6.1	21.5±4.1	23.4±4.3	0.168

SD: Standard deviation; TNF-α : Tumor necrosis factor-alpha; IL-6: Interleukin-6; PC: Protein carbonyl; CAT: Catalase; SOD: Superoxide dismutaz; MDA: Malondialdehyde; ¶ Picogram/mililiter † Nanomol/mililiter; ‡ k/gram protein; § Unite/mililiter; μ Nanomol/gram wet tissue; * Statistically significant.

Table 2. *P* values of intergroup comparison

	Control and TT	Control and PP	Control and TT+PP	TT and PP	TT and TT+PP	PP and TT+PP
Serum TNF-α	<0.001*	0.068	<0.001*	<0.001*	0.091	<0.001*
Serum IL-6	0.007*	1.000	0.034*	0.008*	0.904	0.043*
Serum PC	<0.001*	0.063	<0.001*	0.031*	0.976	0.012*
Serum CAT	0.817	0.957	0.929	0.982	1.000	0.966
Serum SOD	0.947	0.999	0.966	0.978	1.000	0.988
Serum MDA	0.580	0.987	0.079	0.775	0.614	0.418
Tissue PC	0.003*	0.063	0.002*	0.554	0.998	0.446
Tissue CAT	0.999	0.961	0.999	0.987	0.992	0.925
Tissue SOD	0.979	0.925	0.220	0.743	0.403	0.067
Tissue MDA	0.189	0.999	0.783	0.235	0.677	0.847

TT: Thoracotomy; PP: Pneumoperitoneum; TNF-α : Tumor necrosis factor-alpha; IL-6: Interleukin-6; PC: Protein carbonyl; CAT: Catalase; SOD: Superoxide dismutaz; MDA: Malondialdehyde; * Statistically significant.

immune responses after surgery and that room air alone has an impact on stress instead of surgical trauma.[14]

The most important proinflammatory cytokines are IL-6 and TNF-α, and they also have been used as a marker of trauma and reflex organic response.[6,7] Serum IL-6 concentration is an early marker. It can rise one hour after surgery, peaking between two to four hours postoperatively, and can remain elevated for several days.[15] Tumor necrosis factor-α is produced mainly by macrophages, and the primary role of TNF is in the regulation of immune cells. Tumor necrosis factor is able to induce apoptotic cell death, induce inflammation, and inhibit tumorigenesis and viral replication. Tumor necrosis factor-α is a marker of cytokine production and activates the synthesis of several cytokines (IL-1, IL-6, GM-CSF), leukotrienes, and prostaglandin E2. Polymorphonuclear leukocytes are stimulated, and lung injury is produced.[16]

Superoxide dismutase, which causes superoxide breakdown and the subsequent production of hydrogen peroxide, has a central role in regulating reactive oxygen species levels.[17] Yang and Block[18] reported that vascular smooth muscle cells, pulmonary endothelial cells, and lung macrophages have been shown to generate superoxide under basal and stimulated conditions. Three isoforms of SOD have been found in the lung: copper-zinc SOD, manganese SOD, and extracellular SOD. Copper-zinc SOD, which is particularly associated with pulmonary endothelial and vascular smooth muscle cells, is found in the cytoplasm.[19] In this study, we measured copper-zinc SOD enzyme activity. In hypoxic conditions, SOD activation initially decreases.[20] After 48 hours of hypoxia, it increases by encouraging xanthine oxidase activity in the pulmonary artery endothelial cells.[20]

Catalase is a homotetrameric, heme-containing enzyme that catalyzes the conversion of hydrogen peroxide into water and oxygen with one of the highest turnover rates known in enzymology.[12] In the lung, CAT is found primarily in alveolar macrophages and the alveolar epithelium.[21] This enzyme is relatively constitutive, and no major induction of CAT by cytokines or oxidants in the lung has to our knowledge been reported.[22] In our study, there was no significant differences between the groups.

Malondialdehyde is a good indicator of the degree of lipid peroxidation.[16] Oxygen radicals react with polyunsaturated fatty acid residues in phospholipids, and MDA is one of the final products of that reaction in the cells. An increase in free radicals causes the overproduction of MDA and indicates the presence of oxidative damage in lung tissues.[11,16]

The carbonyl content of proteins is a useful marker of reactive oxygen species (ROS)-mediated damage during oxidative stress.[23] Protein oxidation is a complex process and involves many different amino acids with reversible and irreversible changes in many mechanisms.[24] Carbonylation is an irreversible process. Clinical and experimental studies suggest that the oxidative stress induced by ROS can cause lipid peroxidation that produces cell injury and exacerbates the inflammatory response. The endothelium and marginated neutrophils constitute the predominant sources of lung oxidants. Our study found that in the thoracotomy group, systemic and local levels of protein carbonyl were higher than in the controls, and this led to a significant increase in the oxidative damage parameters through the carbonylation of proteins and peroxidation of membrane lipids.[23,24]

Air space problems are frequent after thoracic surgery and cause several serious problems. Pneumoperitoneum is one of the useful methods that protects against these problems. Inflammation that occurs after surgery causes some other

complications with oxidative damage. We inquired whether pneumoperitoneum brings additional stress to lung tissue and designed a study to show the effects of pneumoperitoneum on inflammation after thoracotomy. Our results did not show a significant difference between the groups.

Pneumoperitoneum is a fundamental part of treatment that may influence the organic changes in a crucial way for patients and trigger a series of specific organic reactions, such as inflammation. These reactions especially affect the immune and metabolic responses. The intensity of the immune response has been documented in experimental studies and clinical trials. Pneumoperitoneum can damage the peritoneum by exposing its basement membrane, and mesenchymal cells show edema and retraction with spaces between them. Other kinds of organic aggression are associated with intense inflammatory responses and immunological changes. After an injury stimulus, the peritoneum rapidly accumulates polymorphonuclear cells and macrophages, interferon is activated, class II major histocompability complex is expressed, and pathogenic microorganisms are triggered. The cellular immune response may be evaluated by measuring cytokines in the blood. The interaction between proinflammatory and anti-inflammatory mediators is seen as a battle among opposite forces that often results in imbalance. This imbalance may cause a massive inflammatory response or immune suppression, and when the proinflammatory response prevails, anti-inlammatory therapeutics may be used.[6-8,13]

In conclusion, pneumoperitoneum performed after thoracotomy does not increase the inflammatory reaction and oxidative stress caused by thoracotomy and is an effective and easily performed method.

Declaration of conflicting interests

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