ABSTRACT

Background: This study aims to evaluate the efficacy and safety of ozoned autologous blood used for pleurodesis as well as histopathological changes forming on pleural surface.

Methods: This experimental study included 30 male albino Wistar rats (260 to 320 g, 6 to 8 months old). Rats were randomly divided into three groups of 10. In group 1, 2 mL/kg intrapleural autologous blood was administered. In group 2, 2 mL/kg intrapleural ozoned autologous blood (1 mL/kg of autologous blood and 1 mL/kg of ozone mixture) was administered. In group 3, 2 mL/kg intrapleural saline was administered. Pleural surfaces were graded via macroscopic and microscopic examination on the 30th day.

Results: Macroscopically, pleurodesis creating mild adhesion developed in group 1. General adhesion was seen in pleural space in group 2. No adhesion developed in group 3. Microscopically, ozoned blood caused moderate fibrosis and inflammatory response on visceral pleural surface (p<0.05). No significant change was seen in pleural surface macroscopically in groups 1 and 3.

Conclusion: In this study, we detected that ozone increases the pleurodesis efficacy of autologous blood in rats. However, further studies with human populations are required to validate our results.

Keywords: Autologous blood; ozone; pleurodesis.

ÖZ

Amaç: Bu çalışmada, plörodezis amacı ile kullanılan ozonlanmış otolog kanın etkinliği ve güvenilirliği ile plevral yüzeyde oluşan histopatolojik değişiklikler değerlendirildi.

Çalışma planı: Bu deneysel çalışmaya 30 erkek albino Wistar sıçan (260-320 g, 6-8 aylık) dahil edildi. Sıçanlar rastgele 10'arlı üç gruba ayrıldı. Grup 1'e plevra içine 2 mL/kg otolog kan uygulandı. Grup 2'ye plevra içine 2 mL/kg ozonlanmış otolog kan (1 mL/kg otolog kan ile 1 mL/kg ozon karışımı) uygulandı. Grup 3'te 2 mL/kg plevra içine serum fizyolojik uygulandı. Plevral yüzeyler 30. günde makroskopik ve mikroskopik incelemeye uygulandı.

Bulgular: Makroskopik olarak grup 1'de az yapışıklık yaratan plörodezis gelişti. Grup 2'de tüm plevral alandaki genel yapışıklık görüldü. Grup 3'te ise plevral yüzeyde mikroskopik olarak anlamlı bir değişiklik görülmedi. Mikrobiyolojik olarak ozonlanmış kan visseral plevral yüzeyde orta derecede fibrois ve inflamatur yanıt gösterdi. Etkisi plevral yüzeydeformingi (p<0.05). Grup 1 ve 3'te plevral yüzeyde mikroskopik olarak anlamlı bir değişiklik görülmedi.

Sonuç: Bu çalışmada sıçanlarda ozonun otolog kanın plörodezis etkinliğini artırduğu tespit edildi. Öte yandan, sonuçlarımızı doğrulamak için insan populasyonu ile ileri çalışmalar gerekmektedir.

Anahtar sözcükler: Otolog kan; ozon; plörodezis.

Pleurodesis is defined as the obliteration of pleural space to prevent persistent air leakage and massive pleural effusion. Pleural inflammation, fibrotic changes, neovascularization and collagen storage are needed for an efficient pleurodesis.[1] Various chemical agents -mostly talc - and autologous blood can be used for pleurodesis.[2] Success of pleurodesis with autologous blood is not sufficient despite its safety and inexpensiveness.[3] Some researchers define autologous blood pleurodesis as a ‘last resort’ or a procedure worth
trying for the treatment of persistent air leakages.[1,3]
A limited number of studies have been carried out regarding improving the effectiveness of autologous blood pleurodesis to this day.

Ozone-therapy (OT) is the application process of ozoned autologous blood to the circulatory system (intravenous) or body cavities (intraarticular, intraperitoneal, intrapleural).[4] Ozone is a toxic gas as its pure form, yet its blood mixture is successfully used in infected wounds, chronic skin ulcers, burns, and ischemic pathologies.[4,5] Ozone increases oxygenation by improving oxygen access to tissues and has an effect on platelets which causes increased growth factor synthesis.[6] Thus, in this study, we aimed to evaluate the efficacy and safety of ozoned autologous blood used for pleurodesis as well as histopathological changes forming on pleural surface.

MATERIALS AND METHODS
The study was conducted at GMMA Haydarpaşa Training Hospital between January 2015 and May 2016. Thirty male albino Wistar rats (260 to 320 g, 6 to 8 months old) were provided by the research center of GATA Haydarpaşa Training Hospital. All animals received humane care in compliance with the European Convention on Animal Care and the study protocol was approved by the Animal Ethics Committee of GATA Haydarpaşa Training Hospital (32/2014). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Animals were divided into three groups, each consisting of 10 rats. Groups 1 and 2 were the study groups and group 3 was the control group. In group 1, 2 mL/kg intrapleural autologous blood was administered. In group 2, 2 mg/kg intrapleural ozone and autologous blood mix (derived from mixing 1 mL/kg autologous blood and 1 mL/kg ozone) was administered. In group 3, only 2 mL/kg intrapleural saline was administered.

Rats were anesthetized with an intramuscular injection of xylazine hydrochloride (5 mg/kg) and ketamine hydrochloride (35 mg/kg). Thoracic cavity was reached via 22 gauge polytetrafluoroethylene catheter after 3-5 mm skin incision at the level of fifth intercostal space on right hemithorax under sterile conditions. The catheter was removed and skin was sutured after the administration of autologous blood and other agents to the pleural space. The animals were rotated to assure distribution of agents to the entire pleural surface. No complications or death occurred during these procedures.

Rats were sacrificed on postoperative 30th day under general anesthesia. Right hemithoracic ribs were cut from sternal junction and full exposure of the pleural space was obtained. Macroscopic scoring of pleurodesis was performed by a surgeon and a pathologist who were blinded to the groups, according to the method described by Hurewitz et al.[7] Scoring was as follows: grade 0= normal pleura; grade 1= few scattered adhesions; grade 2= generalized scattered adhesions; and grade 3= complete obliteration of the pleural space by adhesions.

Specimens were placed in formaldehyde filled storage container and sent to pathological examination. Alveolar inflammation fibrosis scoring was performed microscopically. Microscopic analysis was conducted by a pathologist, blinded to the groups. Degree of inflammation and fibrosis were graded as grade 0= absence of inflammation and fibrosis; grade 1= mild inflammation and fibrosis; grade 2= moderate inflammation and fibrosis; and grade 3= severe inflammation and fibrosis. Surrounding tissues including the diaphragm, liver, and chest wall were also examined.

Statistical analysis
Statistical analysis was performed using SPSS version 11.5 software (SPSS Inc., Chicago, IL, USA). Data were shown as mean ± standard deviation. Differences among groups for macroscopic and microscopic scores were evaluated by Kruskal-Wallis variance analysis, and Mann-Whitney U test was used for pairwise comparison of the groups. When the p value from the Kruskal-Wallis and Mann-Whitney U test statistics was statistically significant, multiple comparison test was used to determine which groups differed from others. A p value less than 0.05 was considered statistically significant.

RESULTS
Thorax cavities of the animals were opened and pleurodesis scoring was made. Macroscopic scores of adhesion formation for groups 1, 2, and 3 were 0.7±0.7, 1.9±0.7, and 0±0, respectively (Table 1). Ozone and autologous blood mix (group 2) generalized adhesions between visceral, parietal, and mediastinal pleura (grade 2). Pleurodesis of the autologous blood group (group 1) was weaker (grade 1). No adhesions were seen in group 3 (grade 0). Statistically significant differences in macroscopical scores were detected between all groups (p<0.05). No adhesions or inflammation were observed in the contralateral pleural surfaces of any group.

Scores of microscopic examination of hematoxylin and eosin stained lung and pleural surfaces of all
animals are shown in Table 1. Histopathological examination of group 1 revealed no inflammatory reaction on visceral pleural surface and alveolar interstitial distance appeared to be normal (grade 0), (Figure 1). In group 2, visceral pleural thickening with mild infiltration of inflammatory cells and interstitial thickening of parenchyma beneath were determined (grade 2), (Figure 2). In group 3, normal histological appearance on pleural surface and parenchyma beneath was determined (grade 0), (Figure 3).

A statistically significant difference was detected in microscopical scores between all groups (p<0.05). Contralateral pleural surface, liver, and diaphragm of animals did not show any inflammation.

**DISCUSSION**

Pleurodesis with autologous blood is a procedure mostly used in patients with persistent air leakage and has scarcely any side effects compared to chemical pleurodesis.[1] It was first used in 1987 by Robinson[8] in 25 chronic spontaneous pneumothorax patients and its success rate was 85% (n=21). Dumire at al.[3] were used for autologous blood pleurodesis successfully for air leakage in two patients who underwent lung resection in 1992 and this treatment was called as 'last resort'.[3]

Mitchem et al.[9] compared autologous blood with talc and doxycycline in terms of effectiveness of pleurodesis and determined that autologous blood was less effective in comparison to the other two agents in their rabbit model. Clot and fibrogenic activity of blood create pleurodesis via pleural irritation and inflammation, but tetracycline and talc create a quicker pleurodesis via direct pleural inflammation without patch effect.[9-10] Autologous blood’s success rate in pleurodesis is between 59% and 100%, so it is an improvable procedure.[10] Although autologous blood is a preferable procedure for pleurodesis, some researchers stated that it is not a treatment option worth trying. Yokomise et al.[11] performed pleurodesis on 10 patients, who had persistent air leakage after lung resection, with OK-432 (picibanil) mixed autologous blood for increased efficacy of pleurodesis and determined that air leakage stopped in all patients in two days. They reported the effectiveness of pleurodesis with OK-432, which determined to have an acceptable side effect.
range, and autologous blood mix as successful.\textsuperscript{[11]} Takeda et al.\textsuperscript{[12]} attempted to use autologous blood and OK-432 mix for pleurodesis on three spontaneous pneumothorax patients; however, the treatment was ended due to its serious side effects.

Biedunkiewicz et al.\textsuperscript{[13]} have administered intravenous OT to hemodialysis patients to prevent thrombotic complications which are common amongst hemodialysis patients and investigated any changes on the levels of coagulation product concentrations in plasma. They have determined no changes in antithrombin III, activated partial thromboplastin time, prothrombin time or fibrinogen plasma concentrations and no decrease in complication rates.\textsuperscript{[13,14]} Turczynski et al.\textsuperscript{[15]} have applied OT on 53 patients with arterial occlusive disease in lower extremities and determined improved intermittent claudication complaints. They have also determined decreased blood cholesterol levels in biochemical blood tests after OT.

We have determined that pleurodesis efficacy of existing blood components is increased via OT application to the pleural space. Autologous blood administration dosage in pleurodesis varies from 50 mL/kg to 250 mL/kg, but risk of empyema might increase in case of dosages over 120 mL/kg.\textsuperscript{[1]} Ozpolat et al.\textsuperscript{[10]} stated that 2-3 mL/kg dosage is convenient for an adequate pleurodesis in their pleurodesis study on rats.\textsuperscript{[1]} We have also acquired an adequate pleurodesis in rats with 2 mL/kg autologous blood. Furthermore, some experimental researches on the effects of ozone on immune system showed that ozone prevents tissue injury and bacterial translocation via reducing the oxidative stress. Sepsis model on rats also showed that OT helps containing infection on tissues.\textsuperscript{[5]} In our study, we have not encountered any evidence of infection in any group, particularly in ozone group. Kaldirim et al.\textsuperscript{[16]} reported that OT prevented lung damage, inflammation and necrosis ceased, and parenchymal damage was prevented in rats with paraquat induced lung damage after OT. Visceral pleural thickening with mild inflammatory cell infiltration and interstitial thickening in parenchyma were determined histopathologically in autologous bloods in our study. No signs of alveolar destruction were found.

In conclusion, ozoned autologous blood, an inexpensive and readily available agent, is a safe and effective alternative for pleurodesis. However, further studies with human populations are required to validate our results.

\textbf{Declaration of conflicting interests}

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\begin{figure}
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\includegraphics[width=\textwidth]{Figure_3.jpg}
\caption{Microscopic section of lung parenchyma in group 3. Normal histological appearance on pleural surface and parenchyma, (H-E x 100).}
\end{figure}


