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An Evaluation of Potent Radioprotective Effect of Dimethyl Sulfoxide for Acute Radiation-Induced Lung Injury: Tc99m-DTPA Transalveolar Clearance Scintigraphy Correlated by Histopathologic Findings

Akut Radyasyona Bağlı Akciğer Hasarlanmalarında Dimetil Sülfoksitin Radyokoruyucu Etkisinin Tc99m-DTPA Transalveolar Klirens Sintigrafisi ve Histopatolojik Bulgularla Araştırılması

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Öz

Bu çalışma, bir tavşan modelinde teknesyum-99m-dietilentriaminpentaasetik asit transalveolar klirens sintigrafisi ile radyasyona bağlı akciğer hasarının akut fazında dimetil sülfoksitin radyoprotektif etkilerini araştırmayı amaçlamaktadır. Yirmi beyaz Yeni Zelanda tavşanı (1) kontrol, (2) ışınlama, (3) ışınlama artı dimetil sülfoksit ve (4) tek başına dimetil sülfoksit şeklinde gruplandırıldı. Işınlama ve ışınlama artı dimetil sülfoksit gruplarındaki tavşanların sağ hemitoraks bölgeleri Cobalt60 tedavi ünitesi ile tek doz 20 Gy ışınlandı. Işınlamadan 30 dakika önce intraperitoneal yolla dimetil sülfoksit (4.5 gr/kg) uygulandı. Teknesyum-99m-dietilentriaminpentaasetik asit transalveolar klirens sintigrafisi ışınlamadan sonraki 14. günde yapıldı. Tavşanlar 15. günde sakrifiye edildi ve histopatolojik değerlendirme için her iki akciğer çıkarıldı. Işınlamadan önce dimetil sülfoksitin uygulanması, histopatolojik değerlendirmede dietilentriaminpentaasetik asidin alveolokapiller membrandan transalveolar klirens hızında belirgin bir uzamaya neden oldu (p=0.028), akciğer parankiminin anatomik yapısını korudu, alveollerdeki eksüda şiddetini belirgin şekilde azalttı (p=0.042). Çalışma sonuçlarımız, dimetil sülfoksitin, normal akciğer dokusunun yapısında olumsuz bir değişikliğe neden olmadan ve özellikle akut fazda alveolokapiller membranın bütünlüğünü koruyarak, ışınlamaya bağlı inflamatuar yanıtı güvenli bir şekilde ortadan kaldırdığını göstermiştir. Dimetil sülfoksit, RILI'nin önlenmesi için güvenli ve iyi tolere edilen bir koruyucu ajan gibi görünmektedir. Teknesyum-99m-dietilentriaminpentaasetik asit transalveolar klirens sintigrafisi, akut RILI'de radyasyon toksisitesini izlemek için ucuz, kolay tekrarlanabilir, hassas bir test olarak kabul edilmektedir.

Anahtar Kelimeler: Dimetil Sülfoksit, Radyasyona Bağlı Akut Akciğer Hasarı, Radyoprotektif Ajan, Radyoprotektör, Transalveolar Klirens Sintigrafisi

Abstract

This study aims to investigate the radioprotective effects of dimethyl sulfoxide in the acute phase of radiation-induced lung injury by technetium-99m-diethylenetriaminepentaacetic acid transalveolar clearance scintigraphy in a rabbit model. Twenty white New Zealand rabbits were grouped as (1) control, (2) sham irradiation, (3) irradiation plus dimethyl sulfoxide, and (4) dimethyl sulfoxide alone. Right hemithorax regions of the rabbits in the sham irradiation and irradiation plus dimethyl sulfoxide groups were irradiated with a single dose of 20 Gy by a Cobalt60 treatment unit. Dimethyl sulfoxide (4.5 gr/kg) was administered intraperitoneally, 30 minutes before irradiation. The technetium-99m-diethylenetriaminepentaacetic acid transalveolar clearance scintigraphy was performed on the 14th day after irradiation. The rabbits were sacrificed on the 15th day, and both lungs were removed for histopathologic evaluation. Administration of dimethyl sulfoxide before irradiation caused a marked prolongation in the transalveolar clearance rate of diethylenetriaminepentaacetic acid through the alveolocapillary membrane (p=0.028), protected the anatomic ultrastructure of the lung parenchyma, markedly decreased the severity of exudate in the alveoli in histopathologic evaluation. Our study results showed that dimethyl sulfoxide has safely eliminated inflammatory response induced by irradiation while not causing any negative alterations in the structure of the normal lung tissue and preserving the integrity of the alveolocapillary membrane, especially in the acute phase. Dimethyl sulfoxide appears to be a safe and well-tolerated protective agent for the prevention of RILI. Technetium-99m-diethylenetriaminepentaacetic acid transalveolar clearance scintigraphy is accepted as a cheap, easily reproducible, sensitive assay to monitor radiation toxicity reactions in acute RILI.

Keywords: Dimethyl Sulfoxide, Radiation-Induced Acute Lung Injury, Radioprotective Agent, Radioprotector, Transalveolar Clearance Scintigraphy

Introduction

The lung is a distinguished organ with little regeneration capacity, and it is particularly susceptible to irradiation. Its sensitivity is the major dose-limiting factor in thoracic radiotherapy because the lung is not a uniform organ. Several animal and human studies showed the probability of pulmonary irradiation that causes radiation-induced acute lung injury (RILI) has a threshold dose of 20-22.5 Gy, and it is followed by a sharply rising sigmoid dose-response curve with increasing morbidity and mortality over a minimal dose range (1-2). In the early 1990s, a consensus for pulmonary tolerance doses (5 and 50% rates of RILI at five years) regarding one-third, two-thirds, and whole single lung were visited by the Photon Treatment Planning Collaborative Working Group (3). Unfortunately, a consensus for complications of the paired lung was not achieved.

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RILI is treatment-related toxicity that can be severe in thoracic applications of radiotherapy, and it depicts both radiation pneumonitis (RP) and radiation fibrosis (4). In a systematic clinical review, its prevalence rate is reported ranging between 13-40% (5).

Multiple parameters (dosimetric and clinical) have been suggested related to RILI. The volume of irradiated lung, total dose, the percentage of total lung volume minus the planning target volume exceeding 20Gy, fractionation, and radiation quality are essential dosimetric parameters for RILI development (6,7,8). Increased tumor size, location, pulmonary functions, comorbid pulmonary diseases, smoking history, concomitant treatment modalities are suggested clinical parameters (9).

To date, various studies have demonstrated that RILI is a diverse and persistent process: Early-stage RILI is generally reported short-term, which may last up to 6 months after completion of thoracic irradiation. The pathological findings are alveolar fluid exudation, hyperemia and edema in the alveolar wall, inflammatory cell exudation, infiltration of megakaryocytic substances within the cell, and most importantly, alveolocapillary membrane damage, which is displayed as ground-glass opacification. Late-stage RILI manifests itself from the sixth month after completion up to 2 years following thoracic irradiation. Fibrosis of the alveolar wall is the trademark. Additionally, the thickening of the alveolar septum precludes and swipes the alveoli and capillaries (10).

Even though the entire process of pathophysiologic mechanisms underlying the RILI is yet to be understood, RILI has been accepted as an upfront problem with decreased local control of cancer and impaired patients' quality of life. To date, various technical and biological approaches have been investigated to prevent or attenuate normal lung tissue complications. Details for technical approaches are beyond the scope of this article; however, biologic approaches via radioprotector compounds constitute the point of interest for this study. These compounds, such as amifostine (11,12), genistein (13-15), pentoxifylline (15,16), are applied before irradiation, and they exert their potential radioprotective activity at a maximum extent on normal tissue and minimal or none in tumoral mass (17,18).

Dimethyl sulfoxide (DMSO) is a dipolar aprotic organic molecule. Its radioprotective, antiapoptotic, and anti-inflammatory features are widely studied (19-22). Even though the DMSO concentrations used in studies often remained unreported but instead accepted as nontoxic under 10%, side effects were practically assumed negligible (23). Although extensive literature is available on the protective capacity of DMSO against acute external irradiation, very little is known about its ability to radioprotection in the lungs (24).

Injury in the lung is broadly characterized by diffuse alveolar damage affecting the alveolocapillary membrane (25,26). A technetium-99m-diethylenetriaminepentaacetic acid (Tc99m-DTPA) clearance scintigraphy is sensitive for detecting postirradiation damage observed at 1-14 days. It seems helpful for clarifying the association changes of epithelial integrity and lung surfactant in radiation lung injury in an animal model (27-30).

This study aims to investigate the radioprotective effect of DMSO in radiation-induced lung injury in the early phase through Tc99m -DTPA transalveolar clearance scintigraphy and histopathologic assessment.

Material and Method

The study was carried out at the 'Laboratory for Experimental Studies of Trakya University Medical Faculty under the guidelines for the care and use of the laboratory animals established by the Animal Ethics Committee of Trakya University following the approval of the design by the Institutional Ethical Committee on Animal Trials (2007/83). Twenty tumor-free female New Zealand rabbits with an initial mean body weight of 2.9±0.1 kg were randomly divided into four groups: (1) control (CONT), (2) radiation alone (RT), (3) radiation plus DMSO (RT+DMSO), and (4) DMSO alone (DMSO). All rabbits were housed individually in standard cages and received food and tap water ad libitum.

The rabbits in the DMSO and RT+DMSO groups received purified DMSO (Dimethyl sulfoxide, Merck KGaA, Darmstadt, Germany) at a dose of 4.5gr/kg using an intraperitoneal injection 30 min before irradiation or sham irradiation.

The rabbits in the RT and RT+DMSO groups received anesthesia induced by intramuscular administration of 35-50 mg/kg ketamine (Ketalar, Pfizer Pharmaceutical Ltd. Istanbul-Turkey) and 5-10 mg/kg xylazine (Rompun, Bayer Pharma Ltd. Istanbul-Turkey). The rabbits were immobilized supine on a rough surface by taping the extremities. Irradiation was done with a single dose of 20 Gy, using a Cobalt60 treatment unit (Cirrus, cis-Bio Int., Gif Sur Yvette, France) to the right hemithorax, i.e., to the entire right lung from the apex to the diaphragm. AP/PA was designed two opposite-parallel fields, and a single dose of 20 Gy gamma irradiation was given at 80 cm SSD, defined at a depth of 2.5 cm through the anterior portal, without exposing the contralateral left lung. Cardiac protection was provided via standard block. For the rabbits in the DMSO group, sham irradiation was done on the same Cobalt60treatment unit over the same duration of the fraction. The dose rate was 184.86 cGy/min. Correct positioning of the fields was controlled for each rabbit using a therapy Simulator (Mecaserto-Simics, Paris, France).

Following irradiation and sham radiation therapy, animals were closely observed until their recovery from anesthesia.

All the rabbits in four experimental groups were subjected to a Tc99m-DTPA transalveolar clearance scintigraphy study on day 14 after irradiation. The Tc99m-DTPA (CIS, France) was chelated by introducing 30 mCi (1110 MBq) of sodium Tc99mO4– into 2-3 mL of saline. The Tc99m - DTPA was placed in the nebulizer reservoir of a commercially available system (Venticis II, CIS, France). Aerosols with a mass median diameter of 0.8 µ were produced with an oxygen inflow of 6.L/min. Rabbits were made to inhale from the radioaerosol using facemasks until a count rate of more than 250 cpm, duration time 3-6 min, and then were disconnected from the system. They were placed over a gamma camera (Orbiter, Siemens Corp., Iselin, NJ) with a low-energy, all-purpose collimator, and lung fields were imaged in posterior projection, acquired on a 64x64 matrix, 1.55 zoom factor. Clearance from the lugs was measured for 10 min. (15 s/frame) after the termination of inhalation. The first-minute image drew regions of interest (ROIs) around the lungs' periphery and the central airways. To obtain a pure alveolar ROI and exclude the entire bronchial activity, the outer one-third of each lung was used as the peripheral lung region. The inner two-thirds of the lungs were defined as the central lung region (Figure 1).

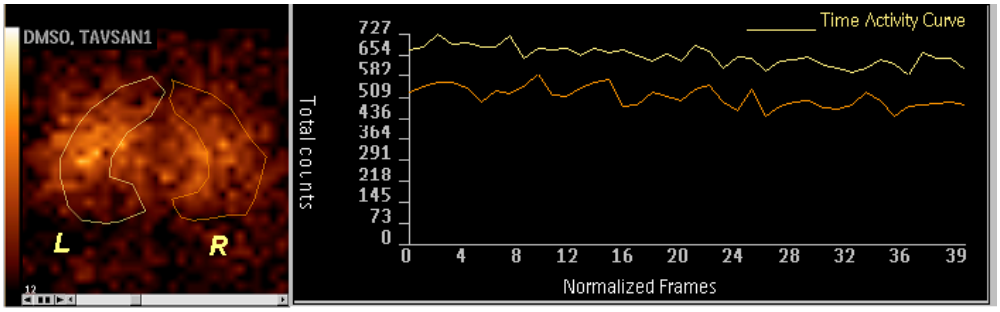

Figure 1. This figure shows regions of interest from a subject in the DMSO group, posterior view of both lungs in ^{99m}Tc-DTPA transalveolar scintigraphy

Radioactivity was corrected for Tc99m decay and plotted as a logarithmic function of time. An exponential line of best fit was determined by regression analysis. Pulmonary half-life (t½) was calculated from the slope of the line using the formula N=N0 e-kt (where N0 is an initial activity in the lung, N is the activity at time t, k is the slope) and used as an indicator of lung epithelial permeability for right lung.

The rabbits underwent euthanasia on the fifteenth day following irradiation and sham radiation therapy. Before euthanasia, rabbits were given intravenous propofol (Propofol, Abbott Laboratories, Istanbul-Turkey) at a 20 mg/kg dose. Euthanasia was performed by decapitation.

The lung specimens were immersed in 10% formalin and embedded in paraffin. Sections of 5 µm were obtained, deparaffinized, and stained with hematoxylin and eosin (H&E). The lung tissues of rabbits were blindfolded evaluated in random order with standard light microscopy.

Data representing the scintigraphic findings were expressed as mean±standard error (SEM), and the data of histopathological findings were expressed as median (min-max). Since the results did not show a normal distribution, they were analyzed by Kruskal-Wallis analysis of variance (ANOVA) and Mann Whitney-U tests. Statistical meanings between the groups were compared by Pos-hoc Bonferroni tests. All p values <0.05 were regarded as statistically significant. Statistical analysis software StatSoft Statistica 10.0 64 Bit Version (StatSoft, Tulsa, OK-USA) was used.

Results

The scintigraphic findings are presented in Table 1. The mean clearance rate of Tc99m-DPTA among all groups is 154.8±28.9 (p=0.023). When the RT+DMSO group was compared to the RT group, the increase in t½ clearance time was statistically significant (293.6±83.5 and 71.8±27.3; p<0.026). However, intergroup comparisons between CONT, RT, and DMSO groups were not statistically significant (p>0.05).

Histopathologic evaluation revealed that anatomic ultrastructure was entirely well preserved in the DMSO group as it is in the CONT group. Intraperitoneal administration of DMSO before the irradiation caused a statistically significant exudate decrease in the alveoli in the RT+DMSO group compared to the RT group (Figures 2a and 2b). A prominent decrease in the severity of inflammatory response, congestion, and hemorrhage in the alveoli was observed with the application of dimethyl sulfoxide; however, it did not reach statistical significance (Figures 3 a and b).

Table 1. 99mTc- diethylenetriaminepentaacetic acid clearance distribution in each group and p values

Scintigraphic Evaluation	KONT (n=5)	RT (n=5)	DMSO+RT (n=5)	DMSO (n=5)		*p* value	
$t_{½}$ clearance (min)§	142.4±26.2	71.8±27.3	293.6±83.5	111.6±22.9	II-IV II- I II-III III-IV III-I I-IV	1.000* 1.000* **0.026***¥ 0.090* 0.228* 1.000*	**0.023**¥$

§: Values are expressed as mean±standard error because they fit the normal distribution. *: ANOVA, $: Bonferroni t-test. ¥: p<0.05

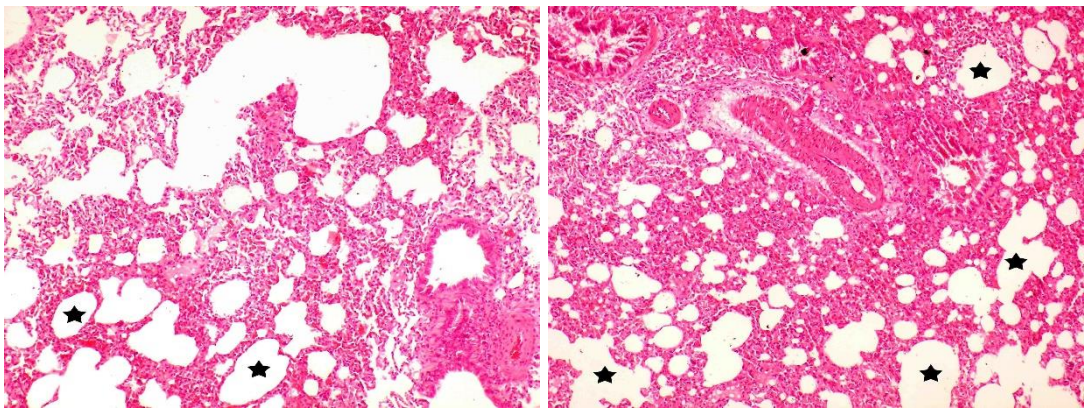

Figure 2a and 2b. Intraperitoneal DMSO administration before the irradiation caused a significant exudate decrease in the alveoli in the RT+DMSO group compared to the RT group (H&E, X200).

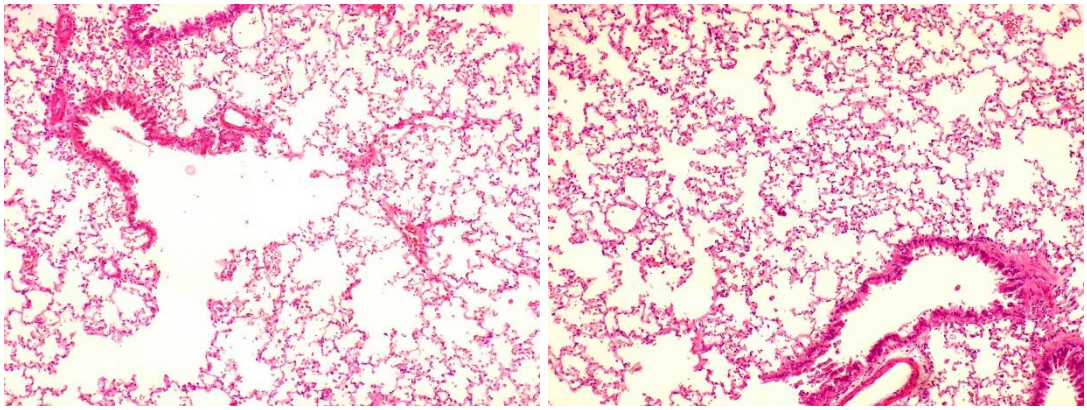

Figure 3a and 3b. DMSO application significantly decreased the severity of inflammatory response, congestion, and hemorrhage in the alveoli. (H&E, X200)

Discussion

Early prediction of radiation-induced organ injury would allow physicians to determine a personalized treatment regimen for each patient and deliver a radiation dose tailored to that individual's normal tissue sensitivity rather than the average population's tolerance dose (23). Adding a protective agent to the radiation therapy, which would not affect the organ's cellular structure and not interfere with the treatment outcome, would reduce the injury induced by radiation, increase the efficacy of the treatment, and reduce its cost.

Radiation-induced cell death involves creating free radicals that reach critical molecules like DNA, RNA, and membrane proteins, causing cell dysfunction and, ultimately, death. These reactions happen both in tumors and normal cells. The radiation injury is potentiated and mitigated via several factors that impact such radicals' biochemical properties like oxygen or sulfhydryl compounds (24, 25).

In the literature, various diagnostic procedures like gallium scintigraphy, pulmonary function tests, and bronchoalveolar lavage have been used to clarify the action mechanism of radioprotective agents, which are thought to reduce radiation-induced acute lung injury effectively (16,18,30,31). However, as a contemporary procedure, Tc99m-DTPA transalveolar clearance scintigraphy can be considered an attractive and superior method because it is a non-invasive, easily reproducible, and more specific method. Also, fewer radiation dose deposits in the subject's lungs (32,33).

The clearance of the Tc99m-DTPA complex throughout the alveolar surface depends on the concentration gradient across the alveolocapillary membrane and the diffusion distance or epithelial wall thickness (34). The rate of Tc99m-DTPA changes positively or negatively according to the phase of inflammatory changes in alveolar injury (32,35,36). In the early phase of RILI, alveolar epithelial permeability increases, and DTPA clearance becomes faster. The biological clearance half-time, t1/2, is reduced (35). Alterations in the DTPA lung clearance are accepted as the earliest indicators of RILI. The clinical importance of the Tc99m-DTPA transalveoler clearance scintigraphy lies in the method's sensitivity for detecting exceedingly early regional changes in and out of the irradiated areas (radiotherapy portal and shielded structures), enlightening the effect of irradiated lung volume and post-irradiation time dependency (32). The earliest regional changes in the pulmonary tissue due to irradiation with DTPA scintigraphy are observable from the first day to the following two weeks (27). These early changes in the murine models can represent the anticipated response in the human body (36). The DTPA scintigraphy procedure was performed two weeks following the irradiation in the presented study. The shortest t1/2 was observed in the RT group compared to the other groups. Four out of five rabbits had developed radiation pneumonitis. DTPA scintigraphy method had exclusively detected the RILI in the rabbits of the RT group.

The current study showed that the t1/2 value of Tc99m-DTPA of the DMSO+RT group was significantly longer than for the RT group ($p<0.028$). DMSO administration prolonged the Tc99m-DTPA clearance time compared to the RT group (293min vs. 71.8 min). Although intergroup analyses between CONT, RT, and DMSO groups were not statistically significant ($p>0.05$), the t1/2 value of Tc99m-DTPA of CONT and DMSO groups were similar (142 min vs. 116 min). This finding indicates that DMSO did not change the integrity of the alveolocapillary membrane.

Characteristically, in acute RILI, epithelial cells lining the alveolar walls and endothelial cells lining the vascular walls are involved (37). During this phase, the fundamental histological findings in man and animals are inflammation, edema in the alveoli, and interstitium. Inflammatory cell infiltration in the alveoli plus fibrin and protein-rich exudate accumulation become apparent. Vessel thrombosis may also occur with focal necrosis and subsequent organization. Interalveolar hemorrhage may also be present (38). Our study's histologic findings of acute RILI were inflammatory cell infiltration in the periphery of the small airways and alveoli, plus alveolar edema and exudate. H&E staining of lung tissues in CONT and DMSO groups supported the Tc99m-DTPA scintigraphic results; the basic structure was preserved in both groups, inflammatory cell infiltration in the peribronchial regions, septal infiltration in the alveolar wall, edema or exudate in the alveoli were not encountered (Figure 4a and 4b). When the RT+DMSO group was compared to the RT group, only the alveolar exudate difference was statistically significant ($p=0.042$). The presented study demonstrated that DMSO has a strong anti-inflammatory effect which explains the results of the histopathologic evaluation and Tc99m-DTPA scintigraphic results. Previous reviews in the literature described that DMSO would inhibit the inflammatory cell migration, participate in the modulation of cytokine response, prevent antibody production, and inhibit fibroblast proliferation under chronic circumstances (21,39). Intergroup comparisons in histopathologic evaluation other than the alveolar exudate did not reach statistical significance due to the small sample size, concentration, or dosage of DMSO and application route.

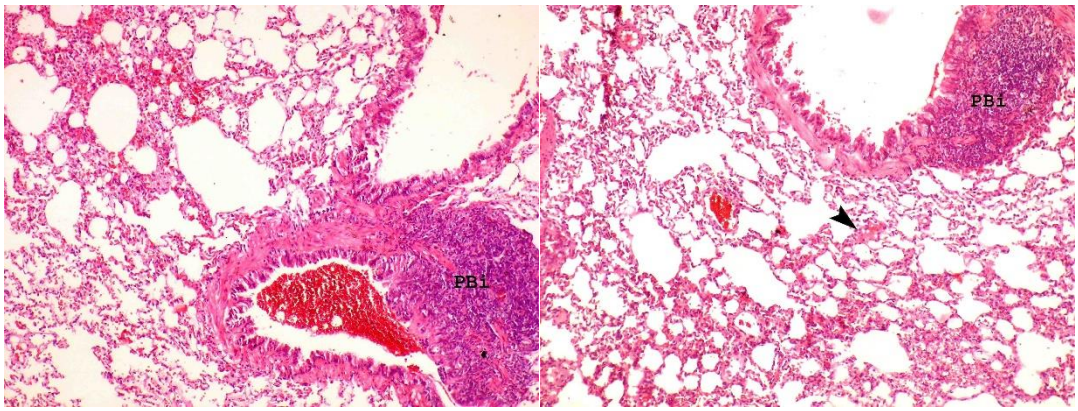

Figure 4. DMSO application preserved the basic structure of the lung parenchyma. As it is in CONT group, inflammatory cell infiltration in the peribronchial regions, septal infiltration in the alveolar wall, edema or exudate in the alveoli were not encountered in DMSO group (H&E, X200).

It has been reported in the literature that DMSO exerted its radioprotective activity by scavenging hydroxyl radical and aqueous electrons, which would come from radiohydrolysis of water

molecules in the medium by the hydrogen and sulfur atoms in its structure. These scavengers minimize the DNA damage induced by free radicals (21,39). DMSO application before gamma irradiation has caused fewer DNA double-strand breaks (24). Until today, the radioprotective effect of DMSO was studied with various organs such as skin, oral mucosa, testis, spleen, intestine, lymph nodes, bone marrow, kidney, fibroblast, and reproductive cell cultures of animals, and human renal, breast, and lung cancer cell cultures (19,21,24,39-42).

On the other hand, there are limited studies in the clinical setting; Zharinov et al. and Neklasova et al. studied the radioprotective effect of DMSO in cervical cancer patients. The expression of early reactions and late injuries of the rectum and urinary bladder were significantly lower in the DMSO treated group before radiation therapy. In these studies, the increased preventive effect was observed with the increased concentration of DMSO. Besides, the agent was found to provide no protective effect on tumor cells (43,44). Vinokurov et al. described a higher incidence of local control in radiotherapy of patients' corpus uteri when locally treated with DMSO before radiation therapy. A reduced incidence and degree of radiation injury to organs adjacent to the uterus were observed in their study (45).

DMSO has been proven effective in various pulmonary diseases like bronchiolitis, asthma, and obstructive chronic diseases (46,47). It is successfully combined with other medications (e.g., antibiotics, anti-inflammatory drugs). In children with bronchiolitis, DMSO application is reported to minimize the inflammatory process and viscosity of the pulmonary secretions, therefore, facilitating their expectoration (46). The abovementioned data supports the presented study results; decreased alveolar exudate and inflammatory deposits in the alveoli.

The study presented here evaluates the radioprotective effect of DMSO in acute RILI via Tc99m-DTPA alveolar clearance scintigraphy and histopathologic assessment. The results obtained showed that DMSO protects the integrity of the alveolocapillary membrane and the ultrastructure of the lung tissue without any harmful side effects. The literature review did not reveal any other study that assesses the protective effect of DMSO on acute RILI by Tc99m-DTPA transalveolar clearance scintigraphy.

However, this study may be criticized for several points: the small number of subjects and an absolute concentration, a fixed dose of dimethyl sulfoxide, and the intraperitoneal administration route are used herein. More studies with more significant numbers of normal and tumor-bearing subjects and different DMSO concentrations with different routes of administration are needed to evaluate the radioprotective effect of dimethyl sulfoxide in RILI because the early prediction of radiation-induced organ injury would allow physicians to plan a personalized treatment regimen for each patient to increase the efficacy of the treatment and reduce the related side effects.

Ethics Committee Approval: The protocol for the study is approved by Trakya University Ethical Committee on Animal Trials (Protocol Number: TUTFEK-2007/83).

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