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Short-Term Changes in Histopathological Markers of Irradiated Rat's Lung: Preliminary Study.

Y Tabarraei[1], Z Bakhshandeh[2], Z Behrouzkia[2], T Peirouvi[2], M Pourissa[3], M Asghari[4], MH Dehghan[5], S Refahi[6]*, and G Ghamami[7].

[1]Sabzevar University of Medical Sciences, Sabsevar, Iran.
[2]Urmia University of Medical Sciences, Urmia, Iran.
[3] Neurosciences research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[4]Tabriz University of Medical Sciences, Tabriz, Iran.
[5]Alborz University of Medical Sciences, Alborz, Iran.
[6]Ardebil University of Medical Sciences, Ardebil, Iran.
[7]Tehran University of Medical Sciences, Tehran, Iran.

ABSTRACT

To investigate early radiation-induced lung injury in cells of the rat's lung following whole thoracic radiation. Wistar rats were used in the experiments (weighing 170-210g). The animals were divided into 6 groups. One group received no radiation. Other groups were exposed to 9, 11, 13, 15, 17 Gy in a single dose of ^{60}Co gamma rays, respectively. The animals were sacrificed at 6 weeks after irradiation. The lungs were dissected and blinded histopathological evaluation was performed. When the lungs were removed at 6 weeks after whole thoracic irradiation, a mild level of inflammation, exudate, mononuclear cell infiltration, plasma cell, and thickened bronchial wall were observed in the lung cells to 9-11 Gy radiation groups. A small increase in histological damage of the previously described section plus hyperemia, thinned alveolar wall, and dilated alveolar space were seen in the irradiated rats to 13 Gy. In addition to the changes described in the last group, hemorrhage, necrosis occurred in the irradiated animals to 15-17 Gy. This study puts forward the histopathological evidence of radiation-induced acute lung injury to be evaluated further for lung protection during radiation therapy of thoracic cage. More investigation is needed to better clarify different aspects of radiation-induced lung injury. The specification of dose-dependent pathologic changes in our model will facilitate future investigations about the molecular mechanisms of radiation-induced lung injury using genetically modified Wistar rats.

Key words: Irradiation, Early lung injury, Histopathology, Rat

Corresponding author

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INTRODUCTION

Lung is one of the most radiosensitive organs of the body, and it is frequently irradiated as part of treatment programs for cancers of thoracic cage [19]. Radiation-induced pulmonary toxicity is a common and critical problem that limits the doses that can be delivered. Technological advances, such as three-dimensional treatment planning, gated radiotherapy and IMRT, help to minimise doses to normal structures, but physical manipulations can be limited by anatomy and the location of the tumor [2, 3, 9, 20].The risk, severity, and nature of early and late reactions in a patient depend on several factors. Radiation-related treatment factors include: total dose, dose per fraction, tolerance of the tissues and treatment schedule (ie, one versus two or three treatments per day). The tolerance of normal tissue may depend upon its functional reserve and its structural organization. The lung is able to tolerate high dose in a small volume, but is less able to tolerate a low dose to the whole lung [18]. Normal lung parenchyma is often unavoidably exposed to radiation during thoracic radiotherapy for bronchogenic and systemic neoplasms. It is well established that the absorption of ionizing radiation causes immediate biochemical, subcellular, and cellular damage, while its morphological expression in terms of gross tissue injury and organ dysfunction are often considerably delayed [4]. The cellular processes of radiation-induced injury begin within hours after irradiation, but the clinical and radiographic features may not be manifested for weeks to months after exposure [1]. Radiation damage to the lung can be described at all levels of organization from the molecular up to the organ level. The response of the lung has been assayed histopathologically, biochemically, physiologically, etc, using lung death as an endpoint. Experimentally we decided to study the early histopathologic changes in normal rat lung tissue that result from thoracic irradiation at different doses, using sensitizer, will be our next step.

MATERIALS AND METHODS

Thirty-six male Wistar rats, weighing 170-210 g, (8-10-week-old) were used for the experiments. Animals were obtained from the vivarian section of Department of Pharmacology, Tehran University of Medical sciences, and were housed 6 together in metal wire netting cages, with room temperature maintained at 20-22° C, relative humidity of 50-70%, an airflow rate of 15 exchange/h, to 12 h alternate light and dark cycle. Animals had free access to tap-water in glass bottles and standard rat chow. All the procedures in this study are in accordance with the guidelines for the care and use of laboratory animals, adopted by Ethics Committee of Tehran University of Medical Sciences (210/27686, Nov3, 2002). Prior to irradiation, the animals were anesthetized with an intraperitoneal (IP) injection of ketamine hydrochloride80mg/kg body weight, and xylazine 5mg/kg body weight (Alfasan, Woerden-Holland). Positioning was facilitated using a Lucite fixation setup, making it possible to irradiate 6 animals simultaneously. The rats were in a supine position and whole thoracic region irradiated by a Cobalt-60 unit (Theratron 780, AE Canada Ltd, Canada) at a depth of 2.5 cm, at a focuse-thoracic cage distance of 80 cm, and graded single doses of 9, 11, 13, 15, 17 Gy, with a dose rate of 99.84 cGy/min. The experiments comprise 6 animals per dose. The animals were held upright to allow recovery and were then observed during study. The rats underwent euthanasia at 6 weeks following radiation therapy. Prior to euthanasia, the rats received anesthesia using ketamine 50mg/kg administered using an IP

injection. Euthanasia was performed by way of transcardiac perfusion using 0.9% sodium chloride. The rats sacrificed and chests were opened immediately for the access and examination of the lungs. The lungs were dissected, instilled with 10% buffered formaldehyde, kept in 10% buffered formaldehyde for 24 h, embedded in paraffin, sliced into 5 µm thick sections and stained using hematoxylin and eosin (H&E). The histological examination was performed by a histologist, who was blinded to the experimental protocol, and viewed under the light microscope (LM), (BX50, Olympus Corporation, Tokyo, Japan) using a grid system. Lung injury was scored according to the following ten aspects: inflammation, exudate, mononuclear cell infiltration, plasma cell, thickened bronchial wall, hyperemia, thinned alveolar wall, dilated alveolar space, hemorrhage, and necrosis. The severity of histopathological lesions were graded on an arbitrary scale based on an approximate area of lesions denoted by different symbols as follows: (-), Nil; (+), mild: (++), moderate and (+++), severe.

RESULTS

Histopathological changes in lungs of rats are shown in Fig1-4 while lesions and severity of different histopathological lesions is summarized in Table1, 2.

Table 1: Renders the pathologic changes due to radiation in different dosage of lung.

Groups (9,11 Gy)	Groups (13 Gy)	Groups (15,17 Gy)
Inflammation, Exudate, Mononuclear cell infiltration, Plasma cell, Thickened bronchial wall	Inflammation, Exudate, Mononuclear cell infiltration, Plasma cell, Thickened bronchial wall, Hyperemia, Thinned alveolar wall, Dilated alveolar space	Inflammation, Exudate, Mononuclear cell infiltration, Plasma cell, Thickened bronchial wall, Hyperemia, Thinned alveolar wall, Dilated alveolar space, Hemorrhage, Necrosis

Table 2: Renders the severity of pathologic wastages of radiation in different dosage of lung.

Pathologic wastages	Groups					
	0Gy	9Gy	11Gy	13Gy	15Gy	17Gy
Inflammation	-	+	+	++	+++	+++
Exudate	-	+	+	++	+++	+++
Mononuclear infiltration	-	+	+	++	+++	+++
Plasma cell	-	+	+	++	+++	+++
Thickened bronchial wall	-	+	+	++	+++	+++
Hyperemia	-	-	-	++	+++	+++
Thinned alveolar wall	-	-	-	++	+++	+++
Dilated alveolar space	-	-	-	++	+++	+++
Hemorrhage	-	-	-	-	+++	+++
Necrosis	-	-	-	-	+++	+++

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The following changes were observed:

In Control group

Control animals sacrificed at 6 week showed normal histology (Fig. 1). The airspaces were separated by fine delicate interalveolar septa, normal vasculature with scant perivascular connective tissue (Fig. 1).

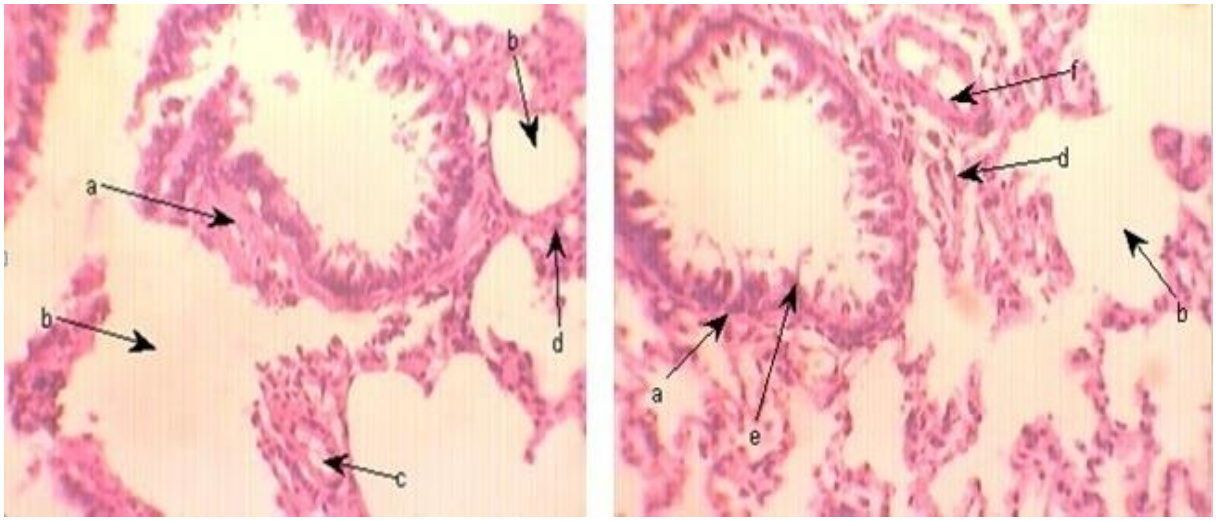

Fig 1 shows the histological lung tissue section of the rats receiving no radiation therapy. In these cases the lung tissues showed normal light microscopic structure and no histological alteration was observed. (a) lamina propria, (b) alveoli, (c) capillary, (d) intrestitum, (e) bronchiol, (f) arteriol (H&E, original magnification×400).

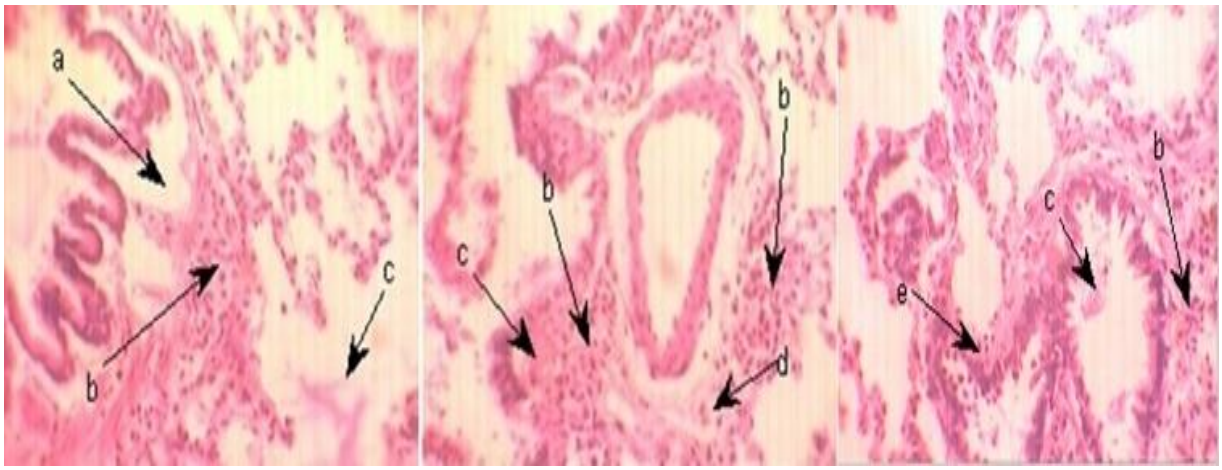

Fig 2 demonstrate the lung damage following a single dose to thoracic cage irradiation with 9, 11 Gy. A slight tissue changes were (a) inflammation, (b) mononuclear cell infiltration, (c) exudate, (d)plasma cell and (e)thickened bronchial wall. These changes were named as mild wastages (H&E, original magnification×400).

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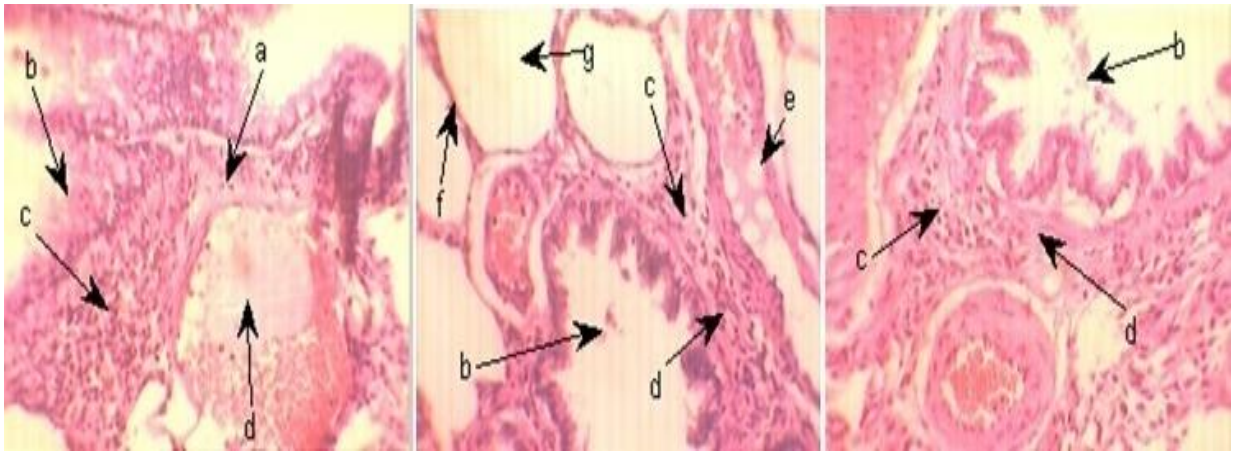

Fig3 shows tissue damage after a single dose to thoracic cage with 13Gy. A small increasing in histological damage (a) inflammation, (b) exudate, (c)mononuclear cell infiltration, plasma cell and (d)thickened bronchial wall) were apparent in animals undergoing 13 Gy radiation therapy. Also, (e) hyperemia, (f) thinned alveolar wall, (g) dilated alveolar space were evident. These changes considered as moderate (H&E, original magnification×400).

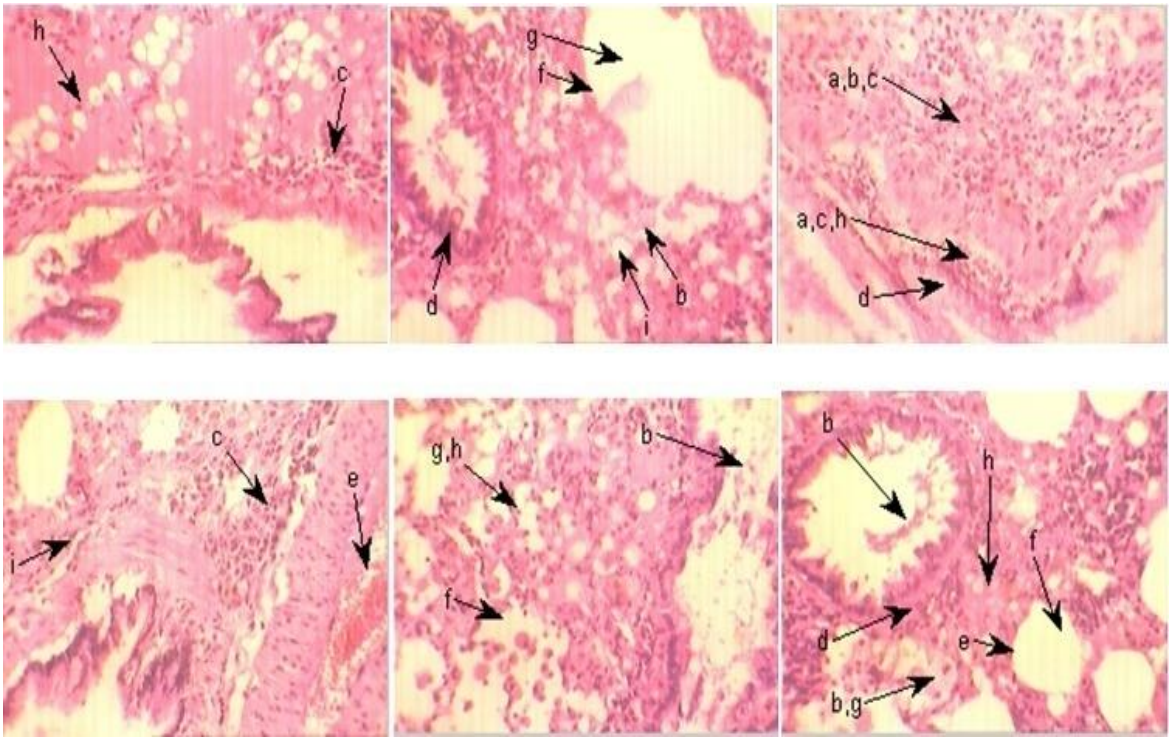

Fig4 illustration of changes after a single dose of whole thoracic region with 15-17Gy. A severe tissue changes were (a) inflammation, (b) exudate, (c) mononuclear cell infiltration, plasma cell, (d) thickened bronchial wall, hyperemia, (e) thinned alveolar wall and (f) dilated alveolar space were evident. Also, (g) hemorrhage, (h) necrosis appeared in this group. In comparison, the general damage was more pronounced in the rats that irradiated with high dose of 15-17 Gy(H&E, original magnification×400).

In 9, 11Gy-single dose group

Light microscopy at 6 week following single dose irradiation showed a mild interstitial inflammation. Slight exudative lesions consisting of eosinophilic material were seen in the interalveolar septa and the alveoli and bronchiol. There were slight mononuclear infiltrate in the interstitium consisting of lymphocytes and monocytes or occasional foci of macrophages. The plasma cells were present but in small numbers. There was a mild thickening of the bronchial wall due to collagenization.

In13Gy-single dose group

The microscopic appearance of lungs from animals sacrificed 6 week after 13 Gy single dose irradiation showed a moderate interstitial inflammation. Exudative lesions were increased. There were slight increased plasma cells in compare with that of previously described section. There was a moderate mononuclear infiltrate in the interstitium consisting of lymphocytes and monocytes or occasional foci of macrophages. There was a moderate thickening of the bronchial wall due to collagen deposits. The congestion of the dilated vessels was present, as was the interstitial lymphocytic infiltrate. Decreasing thickness of the alveolar septa and dilatation in the alveolar space due to edema were appearing in this dose.

In15, 17Gy-single dose group

The characteristic changes of severe inflammation and mononuclear infiltrate in the interstitium were present at 6 week after irradiation. The plasma cells were increased from the previously described sections. The severe exudative lesions and thickening of the bronchial wall due to collagenization were also present. The congestion was much more prominent than that seen with a single 13 Gy dose. A more decrease in alveolar wall thickness with an increasing alveolar sac due to edema was noticed when compared to the single dose of 13 Gy. Sections taken 6 week post irradiation continued to show persistent severe intra-alveolar hemorrhages and necrosis. There was no fibrosis in the three sections.

DISCUSSION

The pathological processes of radiation injury begin immediately after radiation exposure, but the clinical features may not become apparent for weeks, months, or even years after treatment. In the lung, changes detected 6 weeks after irradiation are mild even after a high dose but by 6 months there is widespread fibrosis [18]. In all radiation doses, we observed radiation-induced histopathologic changes including inflammation, exudate, mononuclear cell infiltration, plasma cell and thickened bronchial wall. These lesions more prominent in the high doses, demonstrating induced lung toxicity at higher radiation dose. Lung tissue of irradiated mice displays inflammation-associated histological changes during the pneumonitic phase [10]. Thus, dose dependency of such changes, including edema, interstitial and peribronchial inflammation, alveolar and bronchial wall thickness were studies in our model. We observed dose dependent alterations, indicative of tissue inflammation and with considerable differences between the irradiated rats. Inflammation

was observed at mild levels in 9-11 Gy irradiated group while the lesions were moderate and severe in 13, 15-17 Gy groups, respectively.

Thickening of bronchial wall were observed in a mild degree in 9-11 Gy radiation exposed group while in13, 15-17 Gy group the degree of these above mentioned lesions were moderate and severe respectively. Our approach reflects higher levels of thickened bronchial wall because of large superimposed collagen. The similar study by Elha on C57BL/6J mice indicated that first signs of collagen deposition were observed 84 days post irradiation with 22.5 Gy. These alterations largely reflect mouse strain and genetic background [6]. Vergara et al conducted a quantitative ultrastructural study in animal model following lung irradiation, they found that the numerical densities of monocyte-macrophages and plasma cells were increased to 7-fold and 6-fold compared with the value of control group, respectively [21]. Consistent with Vergara's results, our findings indicate that plasma cells were increased with increasing of radiation dose. The increased number of mast cells probably plays an important and complex role in the inflammatory response and in the development of lung fibrosis and remodeling of connective tissue [12]. The acute changes to lung radiation are characterized by intra-and interalveolar edema, macrophage and mononuclear cell infiltration [8]. Edema alveolar space as presence of fluid within the alveolar sac together with thinned alveolar wall due to stretching, dependent on radiation dose and were evident in our present study, all consistent with that reported by others [8, 17]. Previous studies have demonstrated that the alveolar type II and the endothelial cells are the initial cells affected by thoracic radiation. Within 2 hours, alveolar capillaries demonstrate endothelial disruption and increased permeability. The endothelial cells detach and obstruct capillary lumens. The lung parenchyma thickens as a result of local edema and congested capillary beds. Damaged alveolar type I cells detach, causing a denuded alveolar basement membrane. Alveoli fill with proteinaceous edema fluid, desquamated alveolar epithelial cells, neutrophils, macrophages, and plasma cells. Alveolar surface tension increases as a result of intra-alveolar edema and inadequate surfactant production by injured alveolar type II cells. Hyaline membranes form within the alveoli and distal conducting airways. Diffuse alveolar damage is the acute histopathologic pattern caused by radiation exposure [1, 15]. Another study by Marks, histologically, showed exudation of proteinaceous material into the alveoli, desquamation of epithelial cells from the alveolar walls, alveolar edema, and infiltration of inflammatory cells, leading to impairment of gas exchange and reduced lung compliance [14]. Concerning the histopathological evaluation of acute lung injury, the rat model provides the ideal setting since the proportion of the epithelial cells lining the alveolar wall is similar to that of the human lung and the delivery of radiation therapy to the rat lung using a single fraction of 10-25 Gy reproducibly induces acute injury [16]. These histological data are in conformity with congestion, exudates, edema, and hemorrhage in our study. The present data demonstrate that in our model, the early radiation damage to lung is histologically detectable within 6 weeks after irradiation. The severity of mentioned early lesions in our study varies in the different doses of radiation [18]. Changes involve edema of the air spaces and alveolar septa [4]. Findings of Coggle's study implicates that desquamative changes occur in epithelial and endothelial cells, with increasing numbers of mononuclear, inflammatory cells in the septa and air spaces which is consistent with our results. Another study by Khan indicated the degenerative changes characterized by leakage of proteins into the alveolar septa, thickening of alveolar septa, and edema of the interstitum [11]. In the first few weeks following thoracic irradiation,

degenerative changes to Type I and type II alveolar cells, showed noticeable increased capillary permeability, and swelling of the basement membrane. These changes are quite pronounced after single doses of 10-20 Gy [13]. Tissue histology during the early phase shows an increase in type II pneumocytes and a decrease in paranchymal cells and surfactant concentrations. Edema and inflammatory cells are present in the tissues and alveolar macrophages are prominent. Haematogenous exudates fill the alveoli, and hyaline membranes composed of fibrin develop [7]. The radiation-induced fibro-atrophic process describes both tissue atrophy due to parenchymal cell damage or loss, and replacement fibrosis. Although these pathological features may co-exist, fibrosis and atrophy can be considered as two distinct clinical entities, with differing underlying genetic and pathological mechanisms. Fibrosis results in reduced tissue elasticity and in soft tissues may cause symptoms of hardening, distortion and pain, whereas atrophy contributes to tissue shrinkage and loss of organ function. The clinical picture may vary according to the underlying dominant pathology or the involved anatomical site [22]. Radiation-induced fibrosis (RIF) and radionecrosis (RN) are late complications that are usually considered irreversible [5]. In present approach necrosis were found at radiation doses of 15-17 Gy. In our study, no fibrosis is evident at this time point and with γ ray radiation doses.

CONCLUSION

This study puts forward the histopathological evidence of radiation-induced acute lung injury to be evaluated further for lung protection during radiation therapy of thoracic cage. More investigation is needed and is underway to better clarify different aspects of radiation-induced lung injury. The specification of dose-dependent pathologic changes in our model will facilitate future investigations about the molecular mechanisms of radiation-induced lung injury using genetically modified Wistar rats.

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