Late histopathological findings in the thoracic irradiation: A preliminary study in the animal model

A. Takavar[1], B. Minaei[1], GH. Hadadi[2], S. Khoei[1], S. Refahi[1,3*], Z. Behrouzkia[4], M. Pourissa[5], G. Ghamami[1]

[1]Tehran University of Medical Sciences, Tehran, Iran.
[2]Fasa University of Medical Sciences, Fasa, Iran.
[3]Ardebil University of Medical Sciences, Ardebil, Iran.
[4]Urmia University of Medical Sciences, Urmia, Iran.
[5]Neurosciences research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
Corresponding Author: refahi@razi.tums.ac.ir

Abstract: To investigate late histopathological alterations in rat lung cells following single-dose of irradiation. **Methods and materials:** The thoracic cage of entire lung of Wistar rats was exposed to 17 Gy ^{60}Co gamma rays. The animals were sacrificed at 32 weeks after irradiation. The lungs were dissected and blinded histopathological evaluation was performed. **Results:** When the lungs were removed at 32 weeks after whole thoracic irradiation, histopathologically inflammation and mononuclear infiltrate in the interstitium, Intraalveolar hemorrhages, dilatation in the alveolar space with alterations of the alveolar wall, congestion of the dilated vessels, foam and dust cells and superimposed collagen were noted in all animals. **Conclusion:** At the end of the histological examination, it was seen that all of the animals had severe superimposed collagen and a large fibrous area. We conclude that, in the rat lung model with single dose of 17 Gy, the most interesting finding in this study was in the expression of late fibrosis.

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Introduction

The lung is one of the most radiosensitive organs, and it is frequently irradiated as part of treatment of programmes for cancers of the thoracic cage[1]. Within a few months of radiation, increases in tissue density associated with late fibrosis are typically seen on the X-ray imaging[2-3].Studies have shown that radiation-induced pulmonary fibrosis is a dynamic process characterized by a constant remodeling of fibrous tissue and long-term fibroblast activation[4-7]. The response of the lung has been assayed histopathologically, biochemically, physiologically, etc, using lung death as an endpoint. Experimentally and qualitatively we decided to study late histopathologic changes in normal rat lung tissue that result from thoracic irradiation by 17 Gy single doses, using herbal radioprotector, will be our next step.

Materials and methods

Fifteen 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 5 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 hydrochloride 80mg/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 3 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 single doses of 17 Gy, with a dose rate of 99.84 cGy/min. The animals were held upright to allow recovery and were then observed during study. The rats underwent euthanasia at 32 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. Fibrosis was defined as the thickened alveolar wall with superimposed collagen, qualitatively[1].

Results

We recorded histopathological changes in rats irradiated with 17 Gy over a period of 32 weeks. The histopathological appearances at 32 weeks by17 Gy irradiation were identical. Late histopathological changes in lungs of rats are shown in Fig1.

Light microscopy at 32 weeks following single dose irradiation showed severe inflammation and mononuclear infiltrate in the interstitium. Intraalveolar hemorrhages, alteration of the alveolar wall, dilatation in the alveolar space due to edema were observed in rats irradiated. Congestion of the dilated vessels, foam and dust cells were noted in large numbers. At the end of the histological examination, it was seen that all of the animals had severe superimposed collagen and a large fibrous area.

Discussion

Radiotherapy is an important treatment modality in the curative management of cancer. Despite technological developments in radiation delivery, normal tissues are inevitably included in the high dose treatment volume, leading to a risk of complications. Radiation fibrosis is an important component of the spectrum of radiation injury and at the present time treatment for this condition is limited[8]. Radiation response of the lung consists of three chronological stages: early, intermediate and late. The late phase of pathological organization and collagen deposits is irreversible. The pathogenesis of radiation-induced fibrosis is still a matter of dispute. Some authors consider damage to the vascular endothelium as principle lesion responsible for fibrosis[9]. Giri and etal in their study indicated animals that received a single 15 Gy dose continued to show edema and foam cells with thickening of the interalveolar septa due to collagen deposits at 6 months. Also they showed the extent of fibrosis in single dose reduced with the fractionated doses.

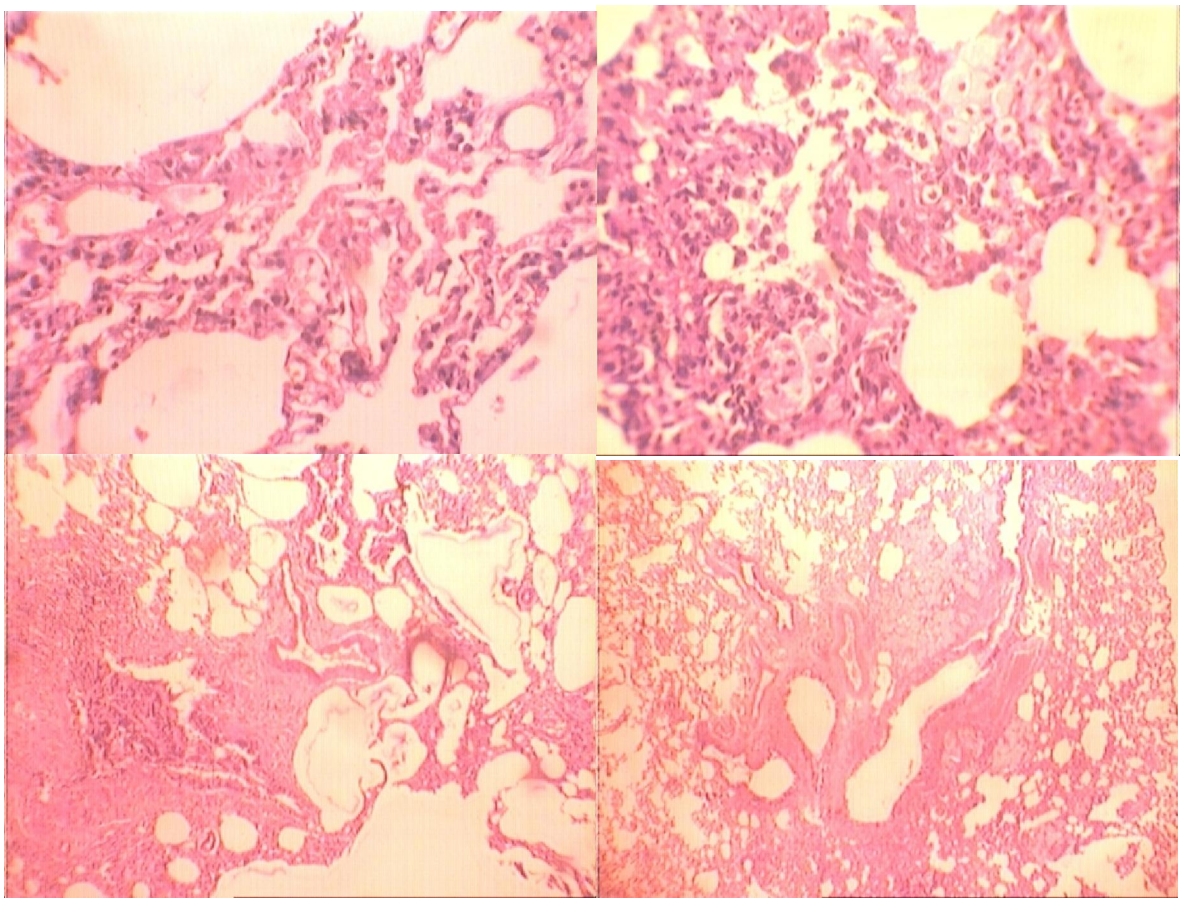

Fig1. Radiation- induced pulmonary histological alteration at 32 weeks after radiation exposure (H & E, ×400).

Pathogenesis of acute and late radiation damages may be related to the damage to different target cells[9]. This is in keeping with dissociation of late radiation damage observed in lung and in other tissues[10-11]. In another study by Elda and etal interstitial edema persisted in the irradiated lungs of the 22.5Gy group. The first signs of collagen deposition were observed 84 days post-irradiation with 22.5 Gy. In contrast, no increased collagen deposition could be detected at day 210 post-irradiation with 12.5 Gy[12]. Collagen production within the interstitium is increased in the report of Ataya[13]. It is known from earlier investigations that sensitivity of animals to radiation-induced fibrosis largely depends on the animal strain [14-16]. Kaliner and etal demonstrated that 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[17]. 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[18].

We conclude that, in the rat lung model with single dose of 17 Gy, the most interesting finding in this study was in the expression of late fibrosis.

Acknowledgments

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