

Protective effect of vitamin D on radiation-induced lung injury: Experimental evidence

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ABSTRACT

Background: Vitamin D, especially its most active metabolite 1,25-dihydroxyvitamin D₃ (Vit D) is essential in regulating a wide variety of biologic processes, such as regulating mesangial cell activation. The objective of this study was to assess the histopathological changes of effectiveness of Vit D as a protective agent against radiation induced lung injury. **Materials and Methods:** Eighteen Wistar rats were divided into three groups: control group (group 1:4 rats), irradiation alone group (group 2:7 rats) and irradiation+vit D (group 3:7 rats). Rats in group 2 and 3 were exposed to 20 Gy radiations to the right lung in a Co⁶⁰ radiotherapy machine under general anesthesia. Additionally, rats in group 3 received Vit D at a single dose of 0.2 mcg injected IM 2 hours before exposure to irradiation. Rats were sacrificed and lungs were dissected fifty days after post-irradiation. Myofibroblasts and vitamin D₃ receptors (VDR) in extracted lungs were stained by immunohistochemistry using alpha-smooth muscle actin (SMA) and VDR antibodies. Blinded histological evaluation was performed to assess lung injury. Lung injury was assessed by the acute lung injury score and myofibroblastic differentiation score. **Results:** Acute lung injury scores and myofibroblastic differentiation scores were significantly lower in the radiation+vit D group compared to irradiation alone group (p=0.001 and p=0.001, respectively). **Conclusion:** This study indicates that administration of vit D plays a protective role against acute lung injury through blocking myofibroblastic differentiation.

Keywords: Radiation, lung injury, vitamin D, VDR, calcitriol, fibrosis.

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INTRODUCTION

Radiation therapy is one of the most important therapeutic modalities for lung, esophagus, breast and lymphatic system malignancies. Radiation pneumonitis is a well-known complication of radiation therapy for thoracic malignancies which usually occurs in few weeks, followed by fibrosis a few months after the completion of the radiation therapy (1-5).

Pulmonary fibrosis is characterized by

mesenchymal cell proliferation and transdifferentiation of some of these cells to myofibroblasts (α smooth muscle actin (SMA) expressing fibroblasts), leading to excessive collagen accumulation in the alveolar and interstitial compartments of the lung. An inflammatory response as a result of injury may sometimes initiate these fibroproliferative events. Recruitment of inflammatory cells and fibroblasts and auto-induction of transforming growth factor- β (TGF- β) in these cells is involved in induction of radiation induced pulmonary

fibrosis^(1-5,6).

Calcitriol known as 1,25-dihydroxyvitamin D₃ (Vit D) is a steroid hormone best known for its activity in regulating calcium and bone metabolism. Beside this function of Vit D, it regulates many cellular processes and modulates the activity of various defense and immune cells including monocytes, macrophages, lymphocytes, or epithelial cells⁽⁷⁾.

The effect of Vit D on pulmonary cell biology is complex. Ramirez *et al.* demonstrated Vitamin D Receptors (VDR) expression in lung fibroblasts and revealed that Vit D inhibits TGF- β 1 induced fibroblast proliferation. In addition, they showed that TGF- β 1 stimulation of SMA expression and polymerization was inhibited by vit D and suggested that vit D inhibits the pro-fibrotic phenotype of lung fibroblast and epithelial cells under TGF- β 1 stimulation⁽⁸⁾.

The goal of the present study was to evaluate whether histopathological changes and immunohistochemical (I) myofibroblastic expression in lung tissue in response to radiation exposure are affected by Vit D.

MATERIALS AND METHODS

Animals

The Animal Studies Ethic Committee at Pamukkale University approved this study. Animal received human care in compliance with the Guide for the Care and Use of Laboratory Animal Resources Commission on Life Sciences, National Research Council, published by the National Academy Press, Washington, 1996.

The study included 18 female Wistar albino rats with average weight of 250-300gr. Eighteen rats were divided randomly into three groups: no radiation control group (group 1: 4 rats), irradiation alone group (group 2: 7 rats) and irradiation+vit D (group 3:7 rats).

Animals were anesthetized with 10 mg/kg xylazinehydrochlorate and 15 mg/kg ketamine hydrochloride diluted in sodium chloride administered intramuscularly.

Irradiation was delivered by Theratron ⁶⁰Co

teletherapy unit. Rats were irradiated in supine position individually using a right anterior 3×2 cm with a single 20 Gy fraction dose. Radiation field was shielded with lead blocks to reduce the dose to the left hemithorax. Vit D 0.2 μ gr (Calcijexamp, Abbot, (Lot# 13021NJ)) was given to the animals intramuscularly 2 hours before irradiation exposure in group 3.

All rats were sacrificed 50 days after irradiation.

Histopathological and immunohistochemical analysis

Lungs dissected from all groups were fixed in 10% neutral buffered formalin in 24 hours. After routine process, samples were embedded in paraffin. Five micron thick sections were stained with hematoxylin and eosin (H&E) to assess morphological alterations in lung tissue and stained with Masson trichrome to examine fibrosis. Slides were systematically scanned in a microscope using X10 objective. Acute lung injury scores were determined according to Serin *et al.*⁽⁹⁾ by using parameters like alveolar wall thickness, intra-alveolar edema, intra-alveolar neutrophils, intra-alveolar erythrocytes, activated macrophage accumulation, alveolar fibrosis, hyaline arteriosclerosis, and collapse and assessed semi-quantitatively on a scale of 0 (not increased), 1 (moderately increased), and 2 (significantly increased). The assessments and grading were performed by two pathologist blinded to the treatment groups. The extend of radiation induced lung injury total score was graded on a scale 0-3= score 0 (low), 4-7= score 1 (intermediate), 8-16= score 2 (high). Sections from parafin blocks of all groups were performed for immunostainVDR (Lot# 1039, sc 1009 polyclonal antibody, Santa Cruz Biotechnology) and α -SMA (Monoclonal mouse antibody, clone 1A4, Dako). Slides were examined for VDR and α -SMA by an experienced histologist and two pathologists blinded to the groups. The extent of immune-peroxidase reaction was classified semi-quantitatively as follows: negative and slightly positive (0); moderately positive (1); strongly positive (2).

Statistical analysis

Statistical analysis was performed on the basis of Kruskal-Wallis and Mann-Whitney U test (SPSS 16.0 for Windows) and p values ≤ 0.05 were considered as statistically significant.

RESULTS

Hematoxylin and eosin staining of lung sections showed increased alveolar wall thickness, intra-alveolar neutrophil accumulation, intraalveolar erythrocytes, activated macrophages accumulation, alveolar fibrosis, hyaline arteriosclerosis and collapse in group 2 while severity of lung damage varied between rats in group 3. Total score of radiation induced acute lung injury were low in both group 1 and group 3, and high in group 2 and a statistically significant difference was found between group 2 and 3 ($p=0.001$). P values of total scores shown in table 1. Histopathological appearances of group 2 and 3 are shown in figure 1.

Immunohistochemical cytoplasmic expression of SMA was determined in inter alveolar interstitial myofibroblastic cells in all groups (figure 2). Myofibroblastic differentiation score was significantly lower in group 3 than group 2 ($p=0.001$).

Immunohistochemical expression of VDR was seen throughout the full epithelial layer. A majority of staining pattern was cytoplasmic. P values of myofibroblastic differentiation scores and VDR scores are shown in also table 1.

DISCUSSION

Radiation pneumonitis after 4-6 weeks following radiation therapy is a major problem in some patients that is specified by profound structural damage in lung parenchyma (1, 3-5). Histopathological analysis is the most common technique used to quantify the extent of lung injury of radiation in rodent models. The rat models delivery of radiation therapy to the rat

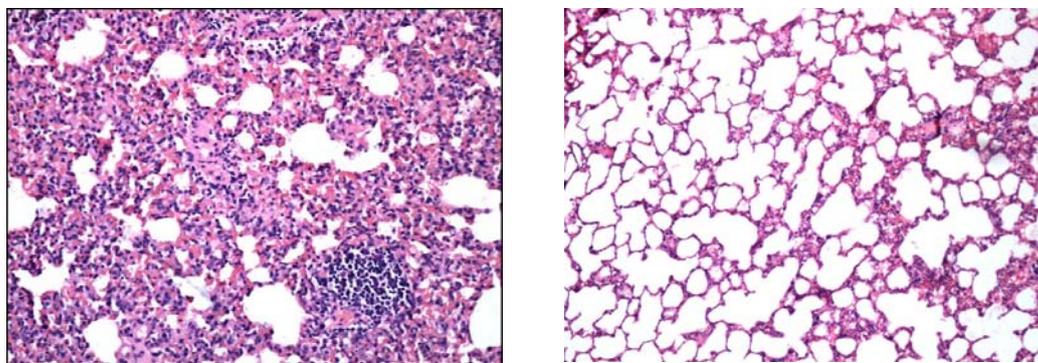


Figure 1. a) Thickening of the alveolar septae due to presence of inflammatory cells and exudative material in irradiation alone group. b) Minimal thickening of the alveolar septa in D vit+irradiation group (H&E X200).

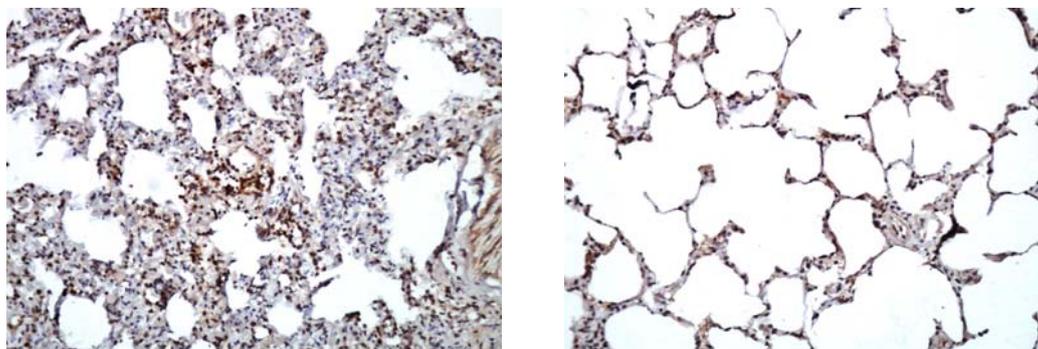


Figure 2. (a) High myofibroblastic expression in radiotherapy alone group (b) Low myofibroblastic expression in vit D+radiotherapy group (SMA immunohistochemistry, X200).

Table 1. Comparison of the histopathological and immunohistochemical parameters for acute radiation injury in rats.

Mean Rank	Group 1	Group 2	Group 3	p value
Alveoler wall thickness	4.00	14.50	7.64	0.004
Intraalveolar edema	9.00	10.29	9.00	0.710
Intraalveolar neutrophils	6.75	15.00	5.57	0.001
Intraalveolar erythrocytes	8.25	13.71	6.00	0.007
Activated macrophages	5.50	14.71	6.57	0.001
Alveolar fibrosis	6.00	15.00	6.00	0.001
Hyaline arteriosclerosis	6.50	13.00	7.71	0.073
Collapse	4.50	13.50	8.36	0.038
Total score	4.50	14.50	7.36	0.004
Myofibroblastic differentiation	5.25	15.00	6.43	0.001
VDR expression	7.50	13.21	6.93	0.030

lung using a single fraction of 10-25 Gy reproducibly induces acute lung injury ⁽¹¹⁾. Histopathological major signs of this destruction are interstitial thickness, alveolar complete obliteration and macrophage infiltration at the alveolar space and interalveolar septum ^(1, 3-5). In our study, we observed immunohistochemical staining of VDR in lung epithelial tissues in all groups.

Acute lung injury was evident for the rats that underwent radiation therapy using a single fraction of 20 Gy based on the presence of histopathological changes of acute lung injury. We compared radiation alone group with radiation + Vit D group and noticed significant difference in the latter group such as meaningful decreases in inter-alveolar wall thickness, intra-alveolar erythrocytes, activated macrophage accumulation, alveolar fibrosis, and collapse rates and myofibroblastic differentiation parameters.

Ionizing radiation results in free hydroxyl radical release which in turn targets interstitial capillary endothelium in lung. Vascular damage ends with the migration of plasma proteins and inflammatory cells like macrophages and lymphocytes. TGF- β 1, which is released by macrophages, plays an important role in radiation induced lung damage ^(1, 2-5, 10).

Vitamin D plays an essential role in vertebrate evolution not only for bone health, but also for overall health and well-being. While the role of Vit D in calcium and bone homeostasis has been well described, its

activities on other physiological and pathophysiological processes have been recognized only in the last years. Last step of Vit D metabolism is mainly localized to the proximal kidney tubule, however, many other cell types, including lung epithelial cells, are capable to perform this reaction. It is believed that this local production of Vit D is responsible for non calcemic health benefit of vitamin D ⁽⁷⁾.

Vit D has complex effect on pulmonary cell biology and immunity with impact on inflammation, host defense, wound healing, repair, and other processes ^(8, 12). A study by Takano *et al.* determined that 1,25 dihydroxyvitamin D is not inhibiting monocyte recruitment but neutrophil recruitment and suppresses IL-8 production in a hamster model of lipopolysaccharide-induced acute lung injury ⁽¹³⁾. Initial efforts to reveal the protective action of vitamin D in early post radiation lung damage were reported by Graham et al in 1990. Effects of dexamethasone, indomethacin, cromolyn, cyproheptadine, theophylline and diethylcarbazine were investigated by measuring vascular permeability-surface area product (PS) as an indicator of post radiation injury. All components, including Vit D were found to be effective in some degrees at reducing lung injury on the irradiated side and the authors concluded that prostaglandin, leukotriene and histamine release from macrophages and mast cells are involved in post radiation lung injury pathways ⁽¹¹⁾.

Recently, in another article by Yazıcı *et al.* it was suggested that Vit D may have a protective role in radiation induced lung injury. In that study, rats were administered 0.25µgr/kg/day vitamin D for 8 and 12 weeks and radiated with the same dose as in our study were compared with control groups and changes were examined with electron microscopy. The researchers suggest that vitamin D inhibits collagen gel construction, induces type II pneumocyte proliferation and surfactant synthesis in the lungs and decreases vascular permeability caused by radiation in addition to protecting alveolar structure and the cells lining the alveolar walls⁽¹⁴⁾.

In this study, we showed VDR expression of epithelial cells in rat lung tissue before and after radiation. We induced acute lung injury after 20Gy irradiation, characterized by increased histopathological parameters of alveolar wall thickening in the lung tissues, accumulation of neutrophils, intraalveolar erythrocytes, activated macrophages, alveolar fibrosis, hyaline arteriosclerosis and the collapse of the lung. We also showed decreased myofibroblastic activity, and lung injury in vitamin D group. In conclusion, we suggest that calcitriol may have a protective effect against radiation induced acute lung injury, maybe through inhibition to myofibroblastic differentiation. The significance of our data, however, needs to be further investigations before its clinical impact can be determined.

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