

Establishment of a rat model of radiation-induced lung injury

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ABSTRACT

► Original article

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Background: Radiation-induced lung injury is a refractory side effect in lung cancer radiotherapy, the mechanism still remains unclear, hence an appropriate animal mode may become useful to investigate it. **Materials and Methods:** 50 female Wistar rats were randomly divided into 5 groups, average 10 rats/cage: A. control group; B. 3Gy×10f; C. 6Gy×5f; D. 12.5Gy×1f; E.15.3Gy×1f. By different doses of radiation to the right lung of rats under American Varian linear accelerator, all the rats were executed at 30days after irradiation, lung tissues were collected to observe the pathological changes by HE staining, and additional lung tissues were extracted mRNA to measure gene expression of TNF- α and IL-18 by qRT-PCR. **Results:** In contrast to the control group, congestion, gore, edema, broadening alveolar interval and the bulla can be observed after irradiation. 3Gy×10f radiation led to most rat death because of frequent anaesthesia. 12.5Gy×1f radiation induced mild lung injury. 6Gy×5f and 15.3Gy×1f radiation induced remarkable lung injury. However, 15.3Gy×1f was seldom adopted in clinical settings. The measurement of cytokine variation by qRT-PCR also indicated that 6Gy×5f radiation stimulated the most obvious elevation of IL-18, while 15.3Gy×1f radiation elevated TNF- α most. **Conclusion:** A rat model of radiation-induced lung injury was appropriately established, 6Gy×5f radiation was the most suitable radiation method for establishing this rat model.

Keywords: Rat, radiation, lung injury, model.

INTRODUCTION

Lung cancer is among the leading causes of patient death in the world, especially in developing countries ⁽¹⁾. Radiotherapy is a mainstay of treating neoplasm in lungs, however, fibrosis is the main chronic side effect of radiotherapy that potentially prevents to deliver the necessary dose to benefit cancer patients ⁽²⁾. Radiation pneumonitis and subsequent lung fibrosis are widely recognized to be a critical, dose-limiting event of normal tissue damage occurring during radiotherapy of thoracic malignant tumors. So far, generally accepted animal models to study the efficacy of potential anti-fibrotic drugs caused by radiation are still deficient. Several animal models simulating radiation-induced interstitial

pulmonary fibrosis have been described, such as mice model ⁽³⁾, yet mice are too small to stabilize or precisely evaluate radiation efficacy. Hence we adopt rats to simulate radiation-induced lung injury, which is more suitable.

The aim of current study was to specify irradiation-induced lung changes occurring as a short term sequel of irradiation. The non-irradiated normal left lung served as an 'internal' control for the irradiated right lung. By comparing the changes of radiated rats with internal control and group A, we attempt to establish a rat model of radiation-induced lung injury. Meanwhile, we also aim to investigate the most suitable radiation dose and method for establishing this model, so that further pharmaceutical study can be achieved on this model.

MATERIALS AND METHODS

All the experiments below were accomplished in Cancer Center lab of Renmin hospital of Wuhan university, China in 2013.

Animals

50 Wistar rats from the experimental animal center of Hubei province were used for this study. Temperature was monitored around 25°C and relative humidity were continually monitored 40-60%, respectively. The experiments and procedures were performed in accord with GLP-requirements. At the time of irradiation, the rats were weighing about 200-250g.

Ethical consideration

Permissions were obtained from local authorities and Medical Ethical Committee of Wuhan University, which ethically approved to test on laboratory rats.

Animals preparation and groups

Standard feed was adopted with 10 rats per cage. All of the rats were randomly divided into 5 groups: A. control group; B. 3Gy×10f; C. 6Gy×5f; D. 12.5Gy×1f; E. 15.3Gy×1f.

3Gy×10f was biologically equivalent to 12.5Gy×1f, and physically equivalent to 6Gy×5f. Likewise, 6Gy×5f was biologically equivalent to 15.3Gy×1f. Before radiation, all rats were anaesthetized by isoflurane gas.

Irradiation

Rats were immobilized using experimental procedures. Correct positioning of the fields were adjusted for each individual rat using a therapy simulator. 1.5×2.5cm area was selected as the radiation field for the right lung. The rats were irradiated under 6 MV X-ray (Varian Linear accelerator) to the right hemithorax. The dose rate was approximately 6Gy/min. The radiation methods were: A. control group; B. 3Gy×10f; C. 6Gy×5f; D. 12.5Gy×1f; E. 15.3Gy×1f, respectively, SSD=100cm. Figure 1 displayed the radiation field of 2 rats.

Pathological examination

Lung tissues were fixed in 4%



Figure 1. This picture displayed the radiation field of rats which were anaesthetized by isoflurane gas. The right lungs were irradiated, while left lungs were protected.

paraformaldehyde solution, followed by overnight fixation, paraffin embedded, sliced at 5µm and then HE stained. Then they were observed under 400 times magnification of microscopic analysis.

Cytokine measurement

Relative gene expression of TNF-α and IL-18 was detected by qRT-PCR, according to the manufacturers' protocols. The results were then compared among different groups.

Statistical analysis

The data was analyzed by SPSS 19.0, and was expressed as $\bar{x} \pm SD$. Data in different groups were tested by homogeneity of variance. t-test was applied when sample size are equal, *t'*-test was applied when sample size are unequal. The difference would be considered significant if $P < 0.05$, which has statistical significance.

RESULTS

Apparent feature changes of the rats

Group A: rats were gradually putting on weight, with bright hair and vigorous body, they remained good appetite and active behavior.

Group B: 6 rats died, the rest became irritable, with fast breath and losing weight.

Group C: rats were remarkably losing weight, with retarded activities, hair of radiated area falling off, the whole body of hair weakened.

Group D: rats were mildly losing weight, with reduced activities, becoming retarded.

Group E: rats were remarkably losing weight, with retarded activities, trichomadesis, gaunt.

Pathological observation

Gross observation

Compared with group A, by naked eyes, we could observe swelling, congestion, and edema of lungs. The higher radiation dose induced the more severe lung injury. Some specimens showed bullous emphysema and pulmonary injury. Some bleeding spots appeared at the surface of the lung. At 30 days of group D, lungs became dark-red, atrophy and even cirrhosis.

Figure 2 displayed some typical lungs.

Histopathological examination

Normal lung tissue was coated with simple columnar epithelium, and the alveolar septum was clear. Figure 3 indicates that 30 days after irradiation induced acute inflammatory response, leading to exudation, interstitial tissue edema and lung capillary hyperemia. Interstitial edema led to thickening alveolar wall, with some pulmonary bulla and focal necrosis. 30 days after irradiation, the lungs showed congestion and edema. Alveolar corruption occurred in the right lung irradiation group.

The gene expression variation of cytokine by qRT-PCR

Radiation injury to tissues results in the recruitment of inflammatory cells and the production of endogenous mediators of

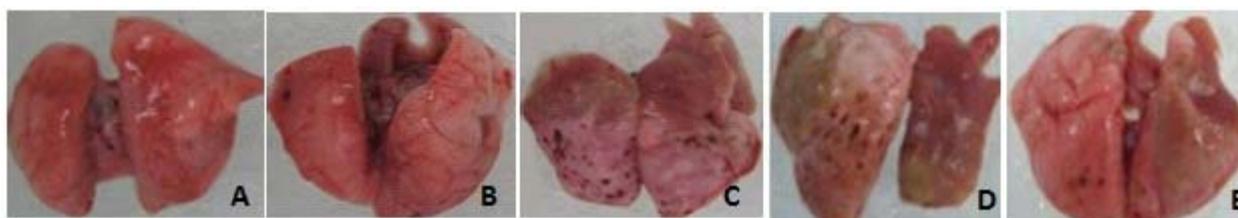


Figure 2. A represents a normal rat lung, with fresh, vividly red color, smooth surface. B represents 3Gy×10f radiated lungs. C represents 6Gy×5f radiated lungs. D represents 12.5Gy×1f radiated lungs. E represents 15.3Gy×1f radiated lungs.

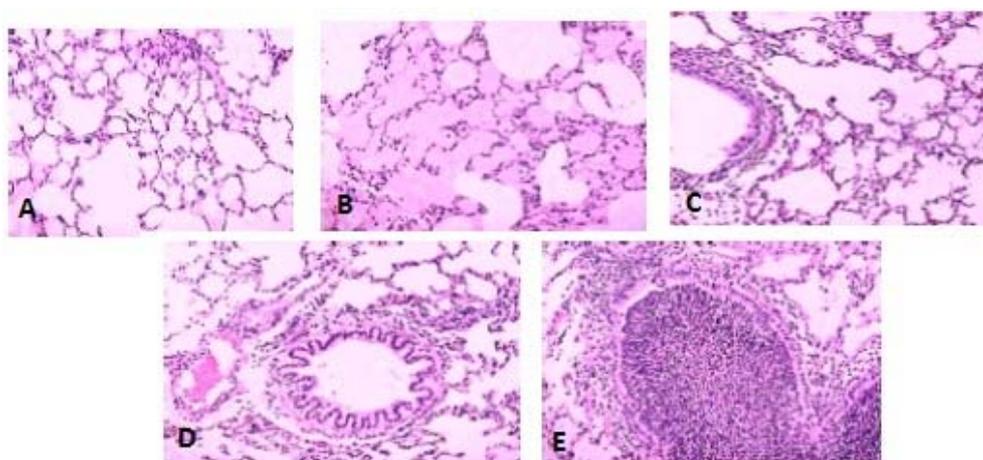


Figure 3. Illustrated the histological response of rat lungs irradiated after a post-exposure period of approximately 30 days. (A) Normal lung (non-irradiated) from a rat. Normal alveolar structure. (B) 3Gy×10f radiation dose of the right lung: edema, exudation, and inflammatory cell infiltration. (C) 6Gy×5f radiation dose of the right lung: alveolar septal thickening, inflammation, and injury, including alveolar recruitment of inflammatory cells. (D) 12.5Gy×1f radiation led to aggregation of more cells in an area of inflammation, with typical lung injury, corruption of alveolar structures. (E) 15.3Gy×1f radiation induced the most severe inflammatory cell infiltration, a large number of alveolar structure corrupted, with obvious exudation.

inflammation, including cytokines and chemokines, and eicosanoids.

TNF- α and IL-18 were selected as 2 representative cytokines which tightly associated with radiation-induced inflammation. Figure 4 reflected that TNF- α elevation was positively related to radiation dose, while figure 5 indicated that 6Gy \times 5f radiation stimulated the highest level of IL-18. All results are presented as the mean $\bar{x} \pm SD$ of five independent experiments and analysed by Student's *t* test.

DISCUSSION

Despite advances in radiobiology, precise mechanisms by which radiation induces lung injury remain controversial (4). A better understanding of the pathogenesis of radiation-related normal tissue injury and

molecular mechanisms is necessary (5). The establishment of animal models plays a vital role in the investigation of pathogenesis of radiation-induced lung injury. Recently, mice have been adopted to establish animal models of radiation-induced lung injury (6), yet they are too small to standardize or observe, which can't genuinely reflect the pathological changes of radiation-induced lung injury. By contrast, rats often behave obedient and are easy to manipulate, which is superior to mice.

Paul R. Graves *et al.* (7) assumed that radiation-induced lung injury was the result of an abnormal healing response. The occurrences of radiation-induced injury are related to the radiation field, radiation dose, measurement rate, division, irradiated sites, the primary disease or chemotherapy (8). Machtay *et al.* (9) adopted a single dose of 13.5Gy to irradiate mice, followed by successive observation for 24 weeks. Meng lingling *et al.* (10) also adopted 6MV X-ray, a single fraction irradiation of 30Gy to right lung of mice, observing 2 months. Although they eventually induced typical lung injury and fibrosis, their results still indicated that a single fraction of high irradiation dose was liable to increase the death rate of mice. Hence we chose multiple low dose fractionated irradiation for rats.

In our study, we have found that, compared to the non-irradiated group, 3Gy \times 10f radiation led to much rat death, hence such a method is not suitable. 12.5Gy \times 1f radiation has induced mild lung injury, yet the inflammation was not so remarkable as expected. Despite the fact that 15.3Gy \times 1f induced remarkable lung injury, a large amount of alveolar structures were severely damaged, which was unsuitable for clinical settings. With respect to 6Gy \times 5f radiation, not only obvious lung injury and pneumonitis were aptly induced, but also animal safety was guaranteed, conforming to clinical reality. The pathological changes were just in accord with Szabo S's report (11).

Figures 4 and 5 demonstrated an increase of mRNA levels for TNF α and IL-18 after radiation, which involved in fibrogenesis and extracellular matrix remodelling (12). The activation of fibroblasts to myofibroblasts is a key step in

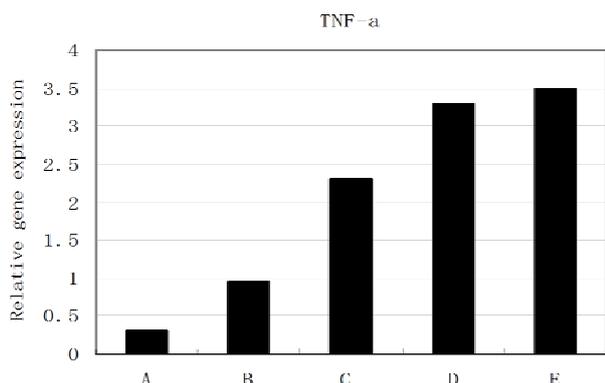


Figure 4. represents the relative mRNA expression of TNF- α after different irradiation methods, compared with control group A, $p < 0.05$.

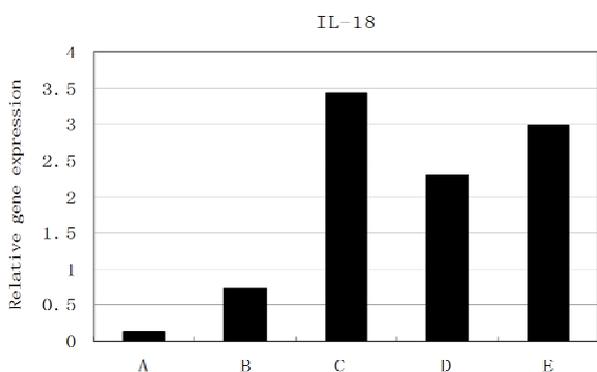


Figure 5. represents the relative mRNA expression of IL-18 after different irradiation methods, compared with control group A, $p < 0.05$. 6Gy \times 5f radiation elevated IL-18 most.

radiation fibrosis⁽¹³⁾. Our results indicated that 6Gy×5f stimulated the increase of IL-18 remarkably. The use of cytokine measurements may become a diagnostic tool for radiation pneumonitis. Our rat model of radiation-induced lung injury may become useful for future pharmaceutical intervention of this refractory disease.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Song QB, Hu WG, Wang P, Yao Y, Zeng HZ (2011) Identification of serum biomarkers for lung cancer using magnetic bead-based SELDI-TOF-MS. *Acta Pharmacol Sin*, **32**: 1537-1542.
2. Li M, Abdollahi A, Grone HJ, Lipson KE, Belka C, Huber PE (2009) Late treatment with imatinib mesylate ameliorates radiation-induced lung fibrosis in a mouse model. *Radiat Oncol*, **4**: 66.
3. Zhang J, Zhang WJ, Huang CF (2009) Establishment and identification of mice model of radiation induced lung injury. *Chin J Cancer Prev Treatm*, **16**: 1455-1457.
4. Zhou S, Nissao E, Jackson IL, Leong W, Dancy L, Cuttitta, F, Vujaskovic, Z, Sunday, ME (2013) Radiation-induced lung injury is mitigated by blockade of gastrin-releasing peptide. *Am J Pathol*, **182**: 1248-1254.
5. Westbury CB and Yarnold JR (2012) Radiation fibrosis--current clinical and therapeutic perspectives. *Clin Oncol*, **24**: 657-672.
6. Wang ZD, Qiao YL, Tian XF, Zhang XQ, Zhou SX (2012) Toll-like receptor 5 agonism protects mice from radiation pneumonitis and pulmonary fibrosis. *Asian Pac J Cancer Prev*, **13**: 4763-4767.
7. Graves PR, Siddiqui F, Anscher MS, Movsas B (2010) Radiation pulmonary toxicity: from mechanisms to management. *Semin Radiat Oncol*, **20**: 201-207.
8. Du ZZ, Ren H, Song JF, Zhang LF, Lin F (2013) Rabbit model of radiation-induced lung injury. *Asian Pac J Trop Med*, **6**: 237-241.
9. Machtay M, Scherpereel A, Santiago J, Lee J, McDonough J (2006) Systemic polyethylene glycol-modified (PEGylated) superoxide dismutase and catalase mixture attenuates radiation pulmonary fibrosis in the C57/bl6 mouse. *Radiation Oncol*, **81**: 196-205.
10. Meng Ling-ling, Feng Lin-chun, Shi Huai-yin, Ma Lin, Chen Hua, Yang Dong, OU Guang-ming, Chen Xin-jing (2008) *Academic Journal of PLA Postgraduate Medical School*, **29**:134.
11. Szabo S, Ghosh SN, Fish BL, Bodiga S, Tomic R, Kumar G, Morrow NV, Moulder JE, Jacobs ER, Medhora M (2010) Cellular inflammatory infiltrate in pneumonitis induced by a single moderate dose of thoracic x radiation in rats. *Radiat Res*, **173**: 545-556.
12. Westbury CB, Reis-Filho JS, Dexter T, Mahler-Araujo B, Fenwick K (2009) Genome-wide transcriptomic profiling of microdissected human breast tissue reveals differential expression of KIT (c-Kit, CD117) and oestrogen receptor-alpha (ERalpha) in response to therapeutic radiation. *J Pathol*, **219**: 131-140.
13. Westbury CB and Yarnold JR (2012) Radiation fibrosis-current clinical and therapeutic perspectives. *Clin Oncol*, **24**: 657-672.

