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Rabbit model of radiation-induced lung injury

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ABSTRACT

Objective: To explore the feasibility of establishing an animal model of chronic radiation-induced lung injury. **Methods:** Twenty-eight New Zealand white rabbits were randomly divided into 3 groups (the right lung irradiation group, the whole lung irradiation group and the control group). Animal model of radiation-induced lung injury was established by high-doses radiotherapy in the irradiation groups, then all rabbits underwent CT and pathological examinations at 1, 2, 4, 8, 12, 16 weeks, respectively after radiation. **Results:** Within 4 weeks of irradiation, some rabbits in the right lung irradiation group and whole lung irradiation group died. CT and pathological examinations all showed acute radiation pneumonitis. At 8–12 weeks after irradiation, CT scanning showed ground glass samples signs, patchy shadows and fibrotic stripes. Pathological examination showed the fibrosis pulmonary alveolar wall thickened obviously. **Conclusions:** The clinical animal model of chronic radiation-induced lung injury which corresponds to practical conditions in clinic can be successfully established.

1. Introduction

The radiotherapy is a common method to treat chest tumors. Lung tissue is a moderately sensitive organ to the radiotherapy. Radiation-induced lung injury may occur when the radiation dose exceeds the threshold of the lung tissue, which include early radiation pneumonitis and late radiation pulmonary fibrosis. Currently, animal models for the study of radiation-induced lung injury are mainly acute radiation-induced lung injury models^[1,2], which are not conducive to study chronic lung injury. This experiment took New Zealand white rabbits as experimental subjects, to establish chronic radiation-induced lung injury model with high-doses radiotherapy, an to study the imaging and pathology changes in different injury phases.

2. Material and methods

2.1. Experimental animal grouping

Twenty-eight well developed rabbits in female and male (aged 3–4 months, weighing 2.5–3 kg) were provided by the Experimental Animal Center of Guilin Medical College. The animals were randomly divided into three groups, the right lung irradiation group ($n = 12$), the whole lung irradiation group ($n = 12$) and the control group ($n = 4$).

2.2. Animal model establishment

For right lung irradiation group and whole lung irradiation group, the model was established with Siemens linear accelerator type TH MEVATRON PRIMUS to produce 6MV-X line and irradiate unilateral right lung area. The rabbits were injected with phenobarbital sodium (30 mg/kg) through ear vein. They were located under simulator guide (type TH SIMVIEW NT) to determine the radiation field

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(Figure 1). TMR table was checked according to the radiation fields to obtain dose of each irradiation. The irradiation was performed every other day on work days (the right lung irradiation group with a total dose 40 Gy, the whole lung irradiation group with a total dose 30 Gy). After irradiation, the animals were sent to animal room after recovered from anesthesia. No experimental animals died during the irradiation.

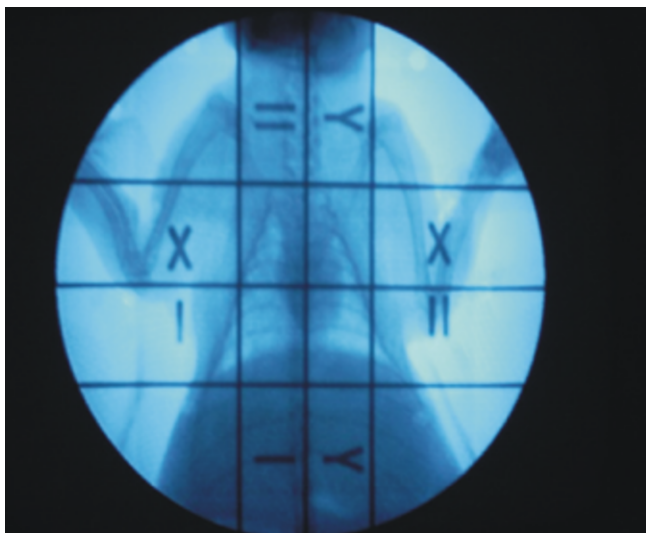


Figure 1. Animal location.

2.3. Processing of the animal model

Every 2 rabbits were randomly selected respectively in each group at the 1st, 2nd, 4th, 8th, 12th, 16th weeks after irradiation and then underwent CT examination. Every 3 rabbits were randomly selected respectively at 4th, 8th, 12th, 16th weeks after irradiation. These rabbits were sacrificed and the lungs were removed. Ten fields of each section (magnification 400 time) was selected. The thickness of alveolar wall was measured after pathological examination.

2.4. Pathological examination

Lung tissues were fixed with 10% formalin, paraffin embedded, sectioned and then HE stained. It was observed under low magnification and high magnification, with the magnification of 100 times and 400 times, respectively.

2.5. Statistical analysis

The data was analyzed by SPSS 12.0, and was expressed as Mean \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 had statistical significance.

3. Results

3.1. Pathological observation

It showed that lung injury beyond the radiation field often happened in the right lung radiation groups. At 4th week, slight swelling of the lung tissue and congestion and edema could be observed directly with the naked eye, some specimens showed bullous emphysema and pulmonary fibrosis. At 8th weeks bleeding spots appeared at the surface of the specimen; At 12th weeks, it revealed dark-red specimen and volume atrophy; and there was no significant change from the 12 weeks (Figure 2).

Pathological observation: normal lung tissue epidermal was coated with simple columnar epithelium, and the alveolar septum was clear. Four weeks after irradiation, the main changes in acute inflammatory response were exudation interstitial tissue edema and lungs capillary hyperemia. Interstitial edema led to thickening alveolar wall, with some pulmonary bulla (Figure 3) and focal purulent necrosis. Eight weeks after irradiation, the congestion and edema of pulmonary could be observed. Alveolar proliferation occurred in the right lung irradiation group, with no obvious increased thickness of the alveolar wall, while there was significantly increased thickness of the alveolar wall in the whole lung irradiation group. There was minimizing alveolar spaces. Red blood cells and hyaline membrane appeared at some in the alveolar space. Inflammatory cells were observed on the lung tissue section. Twelve weeks after irradiation, the alveolar septum extended and alveolar spaces diminished. Fibroblasts and foam cells appeared, with fibrous tissue hyperplasia (Figure 4) and local cerebral consolidation of lung tissue. Sixteen weeks after irradiation, the alveolar wall was thinner compared with 12 weeks, especially in the right lung group. Fibroblasts appeared in the alveolar wall, with fibrous tissue hyperplasia. After 40 Gy dose-ray irradiation of the right lung, alveolar wall thickness changes were as shown in Table 1. After 30 Gy dose-ray irradiation of the right lung, alveolar wall thickness changes were as shown in Table 2.



Figure 2. General observation.

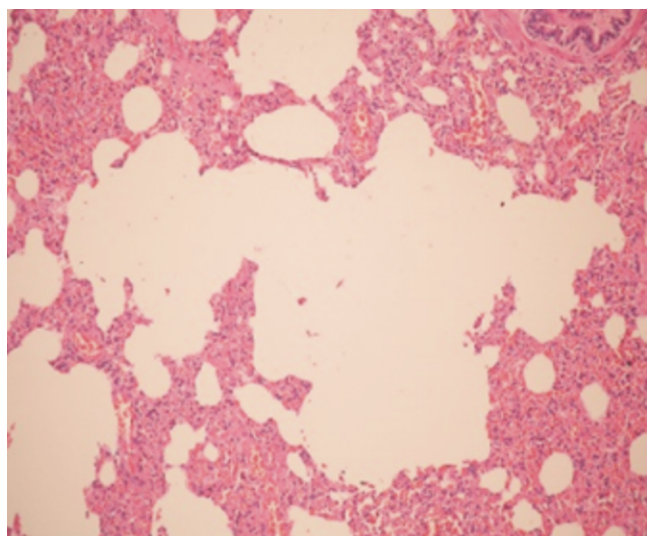


Figure 3. Acute lung injury change after 4 weeks, with bullae of lung.

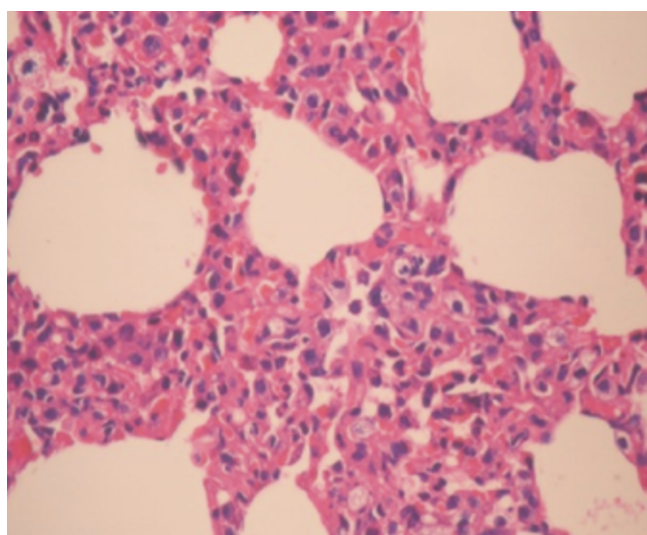


Figure 4. Thickening alveolar wall and fibrosis change.

Table 1

Comparison of rabbit alveolar wall thickness after 40 Gy irradiation (μm).

Groups	Time after irradiation			
	After 4 weeks	After 8 weeks	After 12 weeks	After 16 weeks
Control group	4.62±0.35	4.47±0.44	4.57±0.34	4.55±0.33
Right lung irradiation group	11.29±3.89	6.63±2.77	13.97±4.81	5.86±1.80
<i>t'</i> value	-5.869 9	-2.604 5	-6.719 6	-2.402 9
<i>P</i> value	<0.05	<0.05	<0.05	<0.05

Note: Data were tested with homogeneity of variance, *t'*-test was applied since sample size are unequal of the data.

Table 2

Comparison of rabbit alveolar wall thickness after 30 Gy irradiation (μm).

Groups	Time after irradiation			
	After 4 weeks	After 8 weeks	After 12 weeks	After 16 weeks
Control group	4.62±0.35	4.47±0.44	4.57±0.34	4.55±0.33
Right lung irradiation group	7.83±2.80	12.67±4.72	15.00±6.35	12.42±5.93
<i>t'</i> value	-3.881 4	-5.941 2	-5.665 5	-4.576 2
<i>P</i> value	<0.05	<0.05	<0.05	<0.05

Note: Data were tested with homogeneity of variance, *t'*-test was applied since sample size are unequal of the data.

3.2. CT Findings

After 14 days of irradiation, CT image of double lung showed no abnormal in the irradiation group; At 8 weeks there were ground glass samples signs and patchy shadows; After 8 weeks there were patchy shadows and fibrotic stripes (Figure 5). In 12 weeks and 16 weeks, CT image had no change.

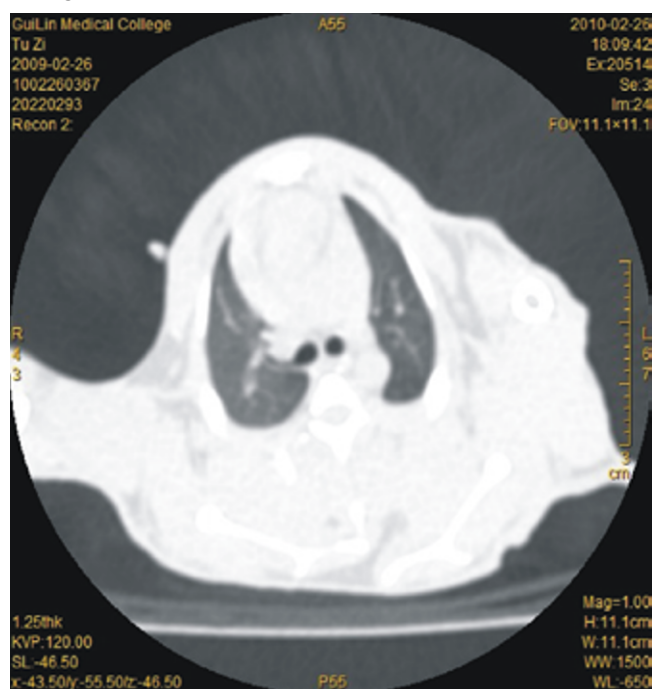


Figure 5. CT result showing patchy shadows and fibrotic stripes.

4. Discussion

4.1. Establishment of animal models with chronic radiation-induced lung injury

Lung injury is an acute or chronic injury caused by various internal and external pathogenic factors. The establishment

and application of animal models plays an important role in the study of pathogenesis and pathophysiology of the lung injury. At present, rats were usually used to establish animal models of radiation-induced lung injury^[3,4]. However, it has significant limitations for imaging and pathology observation because of its small size, which can't accurately reflect the changes in the radiation-induced lung injury. Rabbit has gentle temperament and is easy to operate with its larger size. It can be more accurate to simulate the pathological changes of the lung injury process, with more experimental superiority than rats^[5]. We have successfully established an animal model of chronic radiation-induced lung injury in this experiment. After high-dose irradiation, some of the experimental animals showed no obvious radiation-induced lung injury. We think there are two possible reasons: insufficient irradiation cumulative dose and some experimental animals insensitive to irradiation.

Radiobiology research found that under standard curing conditions, 100 cm² normal lung tissue radiation tolerance dose for TD5/5 is 30 Gy, TD50/5 is 35 Gy, for whole lung radiation tolerance for TD5/5 is 15 Gy, TD50/5 is 25 Gy. The occurrences of radiation-induced injury are related to the radiation field, radiation dose, measurement rate, division, irradiated sites, the primary disease before treatment and whether or not use of the chemotherapy drugs during the treatment. In the initial stage of this experiment, we have chosen 40 Gy dose for the whole lung irradiation, but when the cumulative radiation dose up to 35 Gy, some experimental animals died. Therefore, we changed to 30 Gy dose for the whole lung irradiation. In the latter part of the experiment, pathology and imaging found the radiation-induced lung injury. So it is safe and feasible to establish chronic lung injury model by applying high-doses radiotherapy alternately on weekdays, with the right lung irradiation dose 40 Gy and the whole lung irradiation dose 30 Gy.

In addition, we found that lung injury beyond the radiation field often happens in the right lung radiation groups. Therefore we think that during radiotherapy, the accurate estimation of radiation dose, the clear radiation field range and effectively shelter from irradiation of the non-irradiated tissue are very important to prevent damages beyond the radiation field.

4.2 Imaging findings of chronic radiation-induced lung injury

Early radiation pneumonia is difficult to find in the X-ray. Zhang *et al*^[6] checked X-ray of 28 cases whose CT showed early radiation reaction, only 10 cases were positive. In clinical practice, it has become a consensus that CT is the

most effective method as current diagnosis of radioactive lung injury. However, in this study CT scanning only showed ground glass samples signs, patchy shadows and fibrotic stripes. That is quite different from the CT finding of radiation-induced lung injury in human. We think the reasons is that although the rabbit lung tissue is larger, but it still could not reach the effective resolution of CT's extent. So how to found the radiation-induced lung injury more accurately is one of the challenges of the radiation-induced lung injury model in the future^[7,8].

4.3 Pathological manifestations of chronic radiation-induced lung injury

Radiation-induced lung injury is a common complication of chest radiotherapy. The basic pathological processes of it are early radiation pneumonitis and late pulmonary fibrosis, there is no strict boundary between them. The manifestations of the radiation pneumonitis are congestion, gore, edema, broadening alveolar interval and the bulla^[9,10]. The manifestations of the radiation-induced lung fibrosis are pleural thickening of the fat layer, lung volume reduction, thickening of the alveolar walls and a large number of fibroblasts deposition. Radiation fibrosis is a late manifestation of acute radiation pneumonitis. Four weeks after irradiation we can observe the phenomenon described above, mainly showed bulla and consolidation of the lower lobe fibrosis. While most of them had no significant radiation pneumonitis phase, directly showed the development of chronic pulmonary fibrosis^[11].

Some scholars believe that the radiation-induced lung fibrosis is a form of lung tissue reconstruction. In early radiation-induced lung injury, collagen type IV in lung tissue increased, with thickening of the alveolar septa. Meanwhile, the lung tissue produced a variety of matrix metalloproteinases (MMPs), degraded excessive collagen type IV. Then the degradation products stimulate the secretion of the MMPs, this vicious process eventually leading lung injury to the development of fibrosis^[12-14]. Fibroblasts are the major effector cells of the radiation-induced lung injury. In late radiation-induced lung injury, the proliferation of fibroblasts release collagen type I and type III which can lead to pulmonary fibrosis^[15-17]. In the study of lung tissue reconstruction of radiation-induced lung injury, Diao *et al*^[18] found that irradiation can directly stimulate lung fibroblast proliferation, but can not synthesize and release collagen type IV. The interaction of the collagen type IV synthesis and the macrophages and fibroblasts after irradiation can lead to the reconstruction of lung injury.

In short, the experiment studied the feasibility of establishing the chronic radiation-induced lung injury

in animal models. That is conducive to explore the pathophysiological features and pathogenesis of the chronic lung injury, and can be further improved for therapeutic intervention.

Conflict of interest statement

We declare that we have no conflict of interest.

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