

Experimental research of pulmonary injury on irradiation combined with pemetrexed

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Abstract Objective: The aim of our study was to examine whether irradiation combined with pemetrexed can exacerbate pulmonary injury. **Methods:** Two groups of male Wister Rats were subjected to bilateral apex of lungs irradiation (a single dose of 12 Gy), with or without pemetrexed (20 mg/kg) by intraperitoneal injection at the same time; a third group of weight- and age- matched animals were treated with pemetrexed alone, as the same dose scheme, time and route of injection. The fourth group served as control. The whole lung mounts were dissected to histological evaluation, while serum cytokine transforming growth factor- β 1 (TGF- β 1) analysis were compared at 1, 7, 21, 35, 49 days post-irradiation after irradiation. **Results:** Histological examination showed a thickening of alveolar septal, accumulation of inflammatory cells. The irradiation treatment group and the radiation-chemo treatment group showed a statistically significant higher level of TGF- β 1 ($P < 0.05$) than other two groups, but there were no differences between these two irradiation groups. **Conclusion:** These results demonstrated that pemetrexed can not aggravate pulmonary injury and it could be safely used in concurrent or sequential radio-chemo-therapy in lung adenocarcinoma.

Key words radiation-induced pulmonary injury; transforming growth factor- β 1 (TGF- β 1); pemetrexed

Radiotherapy has a potential role in all stages of non-small cell lung cancer, as either definitive or palliative therapy [1], also for treating breast cancer, esophagus cancer and various lymphomas. A major complication of thoracic radiotherapy, namely radiation-induced lung injury (RILI), including pneumonitis and lung fibrosis, results in patients not receive an adequate dose of radiation for tumor control. The risk of developing radiation-induced pneumonitis depends on the dose, volume of lung irradiated, dose fractionation scheme, and systemic therapies, e.g. chemotherapy, hormone therapy, or immunotherapy. Previous studies demonstrated that the potent fibrinogenic action of the cytokine transforming growth factor- β 1 (TGF- β 1) in damaged lung tissue after thoracic irradiation and showed that it led to lung fibrosis [2–5]. TGF- β 1 signaling promotes epithelial cell apoptosis, proliferation/transdifferentiation of myofibroblasts, induction of plasminogen activator inhibitor-1 (PAI-1), as well as collagen synthesis [6]. The role of TGF- β 1 in the development of RILI has been validated in experimental mouse [7], and Biswas S *et al* had shown that mouse lung contains significant amounts of activated TGF- β 1 following thoracic irradiation [8]. Pemetrexed is a pyrrolopyrimidine antifolate. When were approved by Food and Drug

Administration, pemetrexed is widely used in the treatment of advanced lung adenocarcinoma, which is effective, well-tolerable and can improve quality of life of the patients [9]. As National Comprehensive Cancer Network goes, the standard of care for patients with inoperable stage II and stage III is concurrent chemoradiotherapy. So we intent to study whether radiotherapy combined with pemetrexed can augment or attenuate pulmonary injury.

Materials and methods

Animals

Male SPF Wistar rats (aged 10 weeks, weighing 250–300 g) were obtained from the Center for Experimental Animals at Qingdao Medical College, with a National Animal Use license number of SCXK (LU) 20090007. All experiments were approved by the Animal Care and Use Committee at Qingdao Medical College, which complies with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). All efforts were made to minimize the number of animals used and their suffering. Five animals were housed per cage at an environmental temperature of (24 ± 1) °C and a 12/12 h light/dark cycles, were fed with food and water ad libitum, and allowed to acclimatize for one week prior to treatment. The animals were randomly as-

signed into four groups ($n = 20$ each), namely, the control group (group 1), the irradiation treatment group (group 2), the chemo-treatment group (group 3), and the radiation-chemo treatment group (group 4).

Irradiation and chemo-treatment

All of the animals were anesthetized with an intraperitoneal injection of 10% chloral hydrate at a dose of 0.3 mL/100 g prior to experiment. The bilateral apex of lungs (between 5 cm and 2 cm from xiphoid process) were irradiated at a dose of 12 Gy, which had been shown to result in lung injury by preliminary experiment. The radiation parameters were as follows: beam energy, 6 MV X-rays; dose rate, 300 cGy/min; source–surface distance, 100 cm; depth, 1.5 cm (0.5 cm tissue compensator) and the field size (3.000 × 20.000 cm) was set to provide adequate coverage of the bilateral apex of lungs. The chemo-treatment group received Pemetrexed 20 mg/kg by intraperitoneal injection [10]. On the same date, the radiation-chemo treatment group performed at the same dose scheme of irradiation and pemetrexed, and the control group animals received false irradiation and normal saline by intraperitoneal injection.

Sample collection

The animals were anesthetized, weighed and killed by cervical dislocation at 1, 7, 21, 35, 49 day after irradiation and chemo-treatment. These periods have been proven to be sufficient for the development of RILI in rats [3]. The lungs were dissected immediately, weighed and washed with physiological saline solution to prepare them for the subsequent experiments. In addition, the rat's angular vein blood were sampled and checked centrifugal serum -700C save standby.

Histological examination

The apex of lungs was excised in 0.5 cm thickness, fixed in 10% neutral-buffered formalin for 24 h, then embedded in paraffin for 9 h. Tissue sections with a thickness of 5 μ m were obtained, and were stained via Hematoxylin-Eosin (HE). The slides were examined via light microscopy to examine histological changes.

ELISA analysis

The levels of TGF- β 1 were detected via ELISA by using

a commercially available rat TGF- β 1 ELISA Kit.

Statistical analysis

Results were given as mean \pm SD. The expression levels of TGF- β 1 between groups were compared with the least significant difference test. $P < 0.05$ was considered to indicate a statistically significant difference. A SPSS17.0 statistical software was applied for statistical analysis.

Results

Gross morphological changes

From Fig. 1, we could see the normal rats had a pink, soft, and smooth surface, as well as fair elasticity upon touching. Compared with the irradiation group, the lungs of the rats were swollen with collapsed surfaces and poor elasticity, and exhibited limited fibrotic involvement, white mass and appeared edematous, with noticeable hemorrhaging.

Histopathological changes

From Fig. 2, significant histopathological changes and inflammatory reactions were observed in the irradiation group, including the accumulation of numerous inflammatory cells in the alveolar spaces, heavily thickened alveolar walls, extensive collagen deposition and collapsed alveolar spaces.

Expression of cytokines in the lung tissues

As demonstrated in Table 1 and Fig. 3, the mean TGF- β 1 level at the 7th day was (25.23 \pm 1.13) pg/mL for the control group, (76.80 \pm 1.13) pg/mL for the irradiation group, and (23.62 \pm 1.14) pg/mL for the chemo-treatment group, (89.40 \pm 1.37) pg/mL for the radiation-chemo treatment group. The rats that developed RILI (group 2 and group 4) showed a statistically significant higher level of TGF- β 1 than others ($P < 0.05$), and there was no difference between these two groups ($P = 0.23$, $P > 0.05$). At the 21, 35, 49 days, we obtain the same results, namely the mean TGF- β 1 level in group 2 and group 4 was higher than other groups ($P < 0.05$), and significant difference were not detected between the irradiation treatment group (group 2) and the radiation-chemo treatment group (group 4; $P = 0.07$, $P > 0.05$).

Table 1 Mean values of TGF- β 1 in each group

Group	TGF- β 1 (pg/mL)				
	1 d	7 d	21 d	35 d	49 d
1	28.69 \pm 1.14	25.23 \pm 1.13	25.89 \pm 1.16	23.72 \pm 1.35	22.56 \pm 1.71
2	27.69 \pm 1.24	76.80 \pm 1.13	80.20 \pm 1.43	93.20 \pm 1.26	95.50 \pm 1.76
3	26.53 \pm 1.23	23.62 \pm 1.14	27.65 \pm 1.56	24.23 \pm 1.33	25.21 \pm 1.25
4	27.20 \pm 1.12	79.40 \pm 1.37	83.26 \pm 2.89	94.29 \pm 2.45	95.37 \pm 2.67



Fig. 1 The gross appearance of irradiated lungs

Discussion

Accompany with incidence of cancer, the awareness of the risk of RILI is critical for treating patients especially now that concurrent chemoradiotherapy is being widely used for treating lung cancer. In past years, researchers have focused on physical and biological parameters, and most of dosimetric factors showed an association with RILI [11]. The role of TGF-β1 in the development of RILI

has been extensively studied. Persistent elevation of cytokines, such as TGF-β1, has been proven to initiate a cascade of signaling events and play an important role in the inhibition of epithelial cell proliferation, while it appears that TGF-β1 can mediate the cellular response of normal lung tissue to irradiation [12-13]. Bentel G and Jirtle RL evaluated that the risk of normal tissue injury after radiation therapy is not only increased by local production of TGF-β1 but also by exposure of tissues to elevated circulating levels of TGF-β1 [14]. Researchers also found that irradiation can induce epithelial-mesenchymal transition in esophageal cancer cells in a dose-dependent manner and the mechanism may be associated with activation of TGF-β1 [15]. Our finding suggested that TGF-β1 markedly increased after radiation, and its expression and activation was sustained throughout the course of disease progression. Further more, the level of TGF-β1 was approximately identical between the irradiation treatment group and radiation-chemo treatment group, while there was slightly higher in chemo-treatment group than that of control group.

Pemetrexed is a novel, multi-targeted antifolate chemotherapy agent that is active in various tumors including mesothelioma, non-small cell lung cancer (NSCLC), breast, colon and bladder carcinoma. It inhibits several

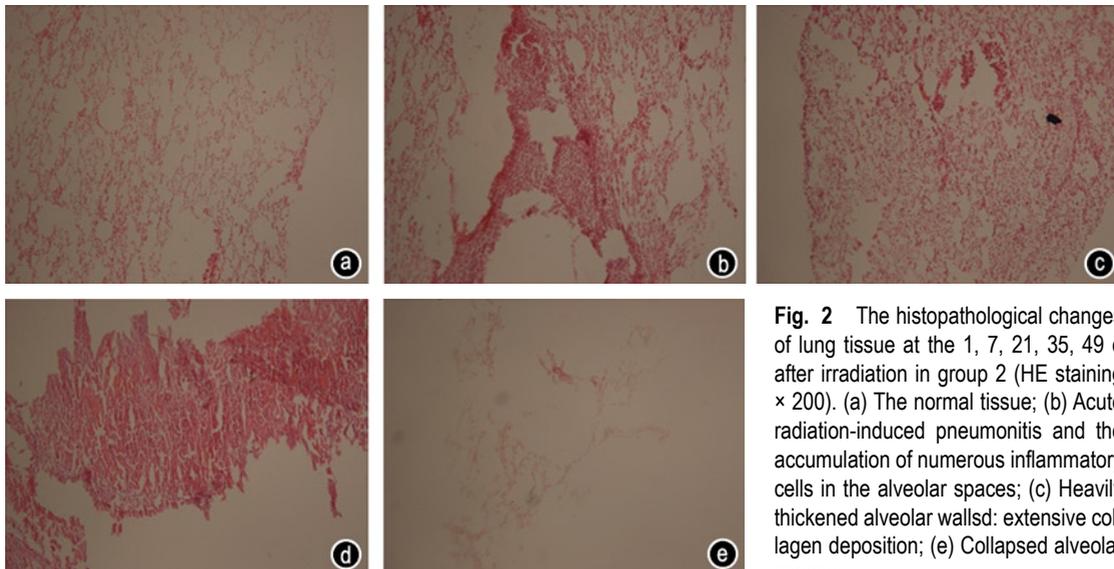


Fig. 2 The histopathological changes of lung tissue at the 1, 7, 21, 35, 49 d after irradiation in group 2 (HE staining × 200). (a) The normal tissue; (b) Acute radiation-induced pneumonitis and the accumulation of numerous inflammatory cells in the alveolar spaces; (c) Heavily thickened alveolar walls: extensive collagen deposition; (e) Collapsed alveolar spaces.

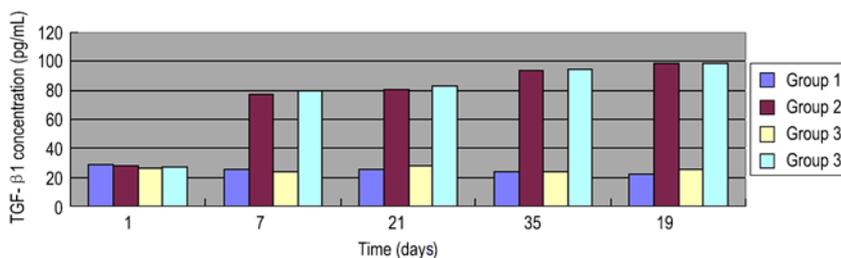


Fig. 3 The concentration of TGF-β1 in each group

enzymes in the folate pathway including thymidylate synthase (TYMS), dihydrofolate reductase, and glycylamide ribonucleotide formyltransferase [16]. Shimizu T *et al* specifically analyzed that TYMS messenger RNA (mRNA) expression affected the therapeutic efficacy of pemetrexed and progression-free survival was prolonged in advanced stage NSCLC patients with lower TYMS mRNA expression compared to those with higher TYMS mRNA expression [17]. In our study, we didn't find pemetrexed can induce lung injury and considered that an anti-metabolic agent induce less adverse effect. In conclusion, pemetrexed can not aggravate radiation-induced pulmonary injury and it could be safely used in concurrent or sequential radio-chemotherapy in lung adenocarcinoma.

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