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Tetrahydropalmatine attenuates irradiation induced lung injuries in rats

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ABSTRACT

Aims: The lung is a major organ targeted by irradiation (R) in cancer radiotherapy of the thoracic region. Irradiation induced lung injury (RILI) is a common major obstacle in thoracic cancer radiotherapy. Tetrahydropalmatine (THP) has been shown to have a protective effect against oxidative stress. This study was designed to investigate the potential radioprotective effect of THP against RILI and to elucidate the underlying mechanisms.

Materials and methods: Sprague–Dawley rats were treated with THP and R. THP was delivered 1 h before R. Using TUNEL staining to explore the effectiveness of THP displayed on R induced pulmonary cells apoptosis. Lung histopathologic findings, bronchoalveolar lavage fluid (BALF) levels of total cell counts, protein and inflammatory cytokines, fibrotic factors (hydroxyproline content), apoptotic mediators (caspase-3 and cytochrome c) and malondialdehyde (MDA) were also evaluated after R.

Key findings: THP significantly ameliorates the deleterious effects of R. Further studies showed that THP decreased lung injury by inhibiting the pulmonary cells apoptosis; reduced lung inflammation by decreasing BALF cells recruitment and lowering BALF protein levels; reduced pulmonary fibrosis by decreasing collagen content of lung tissues. THP also ameliorated oxidative modification of rat lungs as evidenced by levels of lipid peroxidation. BALF cytokine analysis, moreover, pointed to a mitigation of the chronic inflammatory profile of irradiated lungs as a result of the protective effect of THP treatment.

Significance: THP can effectively attenuate RILI through anti-apoptosis, anti-fibrosis and anti-inflammation mechanisms.

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1. Introduction

Ionizing irradiation (RT) is an important therapeutic modality in the treatment of thoracic tumors [1,2]. However, the lung is a very radiosensitive organ and its injury is a dose limiting factor in the utility of thoracic radiotherapy. Clinically significant radiation lung injury occurs in up to 30% of patients irradiated for lung cancer and about 10–15% of other thoracic oncology patients [3,4]. Thoracic irradiation (R) can induce both acute and late effects on the lung. R-induced lung injury (RILI) can occur in two phases. The early phase (<6 months) is called radiation pneumonitis as evidenced by alveolar edema, alveolar neutrophils, alveolar erythrocytes, and foamy macrophages according to histopathological evaluation [5,6]. Alveolar damage is considered as the first symptom of a lung injury [7]. The latent phase (>6 months) is characterized by pulmonary fibrosis months to years after radiotherapy. Vascular injury in the early phase of an inflammatory response is one of the prominent symptoms which progresses with extensive increase in collagen and extracellular matrix, and finally leads to tissue fibrosis [6,8].

However, the molecular mechanism of RILI remains controversial. The currently view is that RILI results from a sequence of biological changes, including increased generation of reactive oxygen and nitrogen species, early cell apoptosis that is mediated by direct radiation ionization [9], secretion of inflammatory cytokines, and inflammatory cell recruitment into the lung parenchyma. Currently the methods for prevention and cure of RILI are relatively restricted [10,11]. Amifostine (WR-2721) is the only Food and Drug Administration-approved radioprotector used clinically. However, the toxicity and worse selectivity of Amifostine (WR-2721) has greatly limited its use at the required protective doses [12,13]. The new radioprotective compounds with better efficacy are urgently needed.

Rhizoma corydalis is a Chinese herbal medicine of fundamental importance which has been traditionally used to help invigorate the blood, move qi, reinforce vital energy and alleviate pain [14]. L-tetrahydropalmatine (THP) is one of the major active components extracted from the corydalis yanhusuo plant has been found to possess anti-coagulant, anti-nociceptive, anti-hyperalgesic, anti-oxidant, anti-viral, and anti-inflammatory activities [15,16]. THP has also been reported to have anti-apoptotic and cardioprotective effects on myocardial ischemia reperfusion injury [16]. THP has also been indicated to possess anti-oxidative activity during liver injury induced by CCl4 [17], and have

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radio-protective effect on endothelial cells injury induced by irradiation [18]. This study is designed to explore whether THP could attenuate RILI, as indicated by R-induced cellular damage through biochemical and histological analysis, and explore the underlying mechanism.

2. Materials and methods

2.1. Animals and ethics

Sprague-Dawley rats (200–250 g, 7–8 weeks old) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). They were kept at a constant temperature (22 ± 1 °C) with 12 h light and dark cycles. The rats were fed with standard rat chow and water ad libitum. All the experimental protocols involving animals were approved by Guang An Men Hospital of Chinese Medical Science Research Institutional Animal Care Committee.

2.2. Irradiation

Rats were placed in holders under ketamine anesthesia and irradiated with a single dose of 0 Gy (sham) or 15 Gy over their right hemithorax, using a linear accelerator (Clinac DHX, Varian Medical Systems Inc., USA) producing 6 MV photons at a focus. The dose rate was 4.7 Gy/min. Non-irradiated parts of the body were shielded with 6 cm of lead. 1 h prior to R, rats were treated with either vehicle (same volume of saline) or THP (40 mg/kg b.w. Sigma-Aldrich Chemical Co. St. Louise, MO, USA) by intraperitoneally for thirty days. Irradiated rats were given the same volume of saline without THP. The standard doses of THP used for the rats in the present study were based on previous study [16,19,20]. Mice were sacrificed at 3 h, 6 h, 24 h, 4 weeks, 8 weeks, 12 weeks, and 16 weeks post R and bronchoalveolar lavage fluid (BALF) or lung tissue were collected for further analysis.

2.3. TUNEL assay

TUNEL assay was used to assess for DNA strand breaks with an apoptosis detection kit (Roche, Basel, Switzerland). Briefly, tissue slides were fixed in 4% paraformaldehyde for 10 min followed by washing in PBS and blocking in 3% H_2O_2 methanol for 10 min. Tissue sections were then permeabilized in solution containing 0.1% Triton X-100 and 0.1% sodium citrate. For TUNEL, sections were labeled with 25 μL of TUNEL reaction mixture containing 1:2 dilution of enzyme for 2 h at 37 °C in a humidified chamber. TUNEL-positive cells were quantified in five random ×400 images per group under light microscopy.

2.4. Immunohistochemical analysis

The expressions of some apoptosis-related markers on paraffin-embedded sections were analyzed by immunohistological staining. After quenching of endogenous peroxidase, antigen retrieval, and blocking of nonspecific binding, the sections were incubated with the primary antibodies to cleaved caspase-3 (dilution of 1:200; Cell signaling technology), cytochrome c (dilution of 1:75; Santa Cruz) [21,22], this antibody cannot penetrate the mitochondrial membrane, thus only detecting cytochrome c released from the mitochondria at 4 °C overnight. After washing three times, ChemMate™ EnVision™/HRP (DAKO) or sionate Esecondary anti-goat IgG (Sigma) was added, followed by incubation for 30 min at room temperature. Staining was developed in DAB solution, with counterstaining by hematoxylin. The sections in which the primary antibodies were omitted were used as negative controls.

2.5. Evaluation of oxidative lung injury

Malondialdehyde (MDA), that is produced after lipid peroxidation as a measure of oxidative damage in membranes was measured in homogenized lung tissues using a commercially available kit (Nanjing Institute of Jiancheng Bioengineering, Nanjing, China) according to manufacturer's protocol. The results were expressed as nmol MDA/mg of lung protein.

2.6. Histological and electron microscopy analysis

For histological examination, irradiated lung tissue was obtained 4 weeks, 8 weeks, 12 weeks, and 16 weeks after R and fixed in 10% neutral-buffered formalin for 48 h, paraffin-embedded and sectioned at an average thickness of 5 mm. The sections were stained with hematoxylin and eosin and Masson's trichrome, and observed under a light microscope [Olympus (BH-2), Nagano, Japan]. For electron microscopy, tissue was obtained 3 h after R and fixed with 2.5% glutaraldehyde in 0.1 M cacodylate, contrasted with O_sO_4, uranylacetate and lead citrate and investigated on a HITACHI H-7650. Images were acquired on a MegaViewII slow-scan-CCD camera using ITEM 5.0 software (Soft-imaging-systems, Münster, Germany).

2.7. Measurement of lung hydroxyproline

Collagen deposition was estimated by determining the hydroxyproline content of the left lung. Samples were hydrolyzed with 6 N HCl at 105 °C for 18 h. The samples were resuspended in 2 ml of deionized water and 1 ml of chloramine T dissolved in 5 mol/L sodium acetate/10% isopropanol. Next, 0.5 ml of Ehrlich's reagents were added, mixed, and incubated at 65 °C for 10 min. The

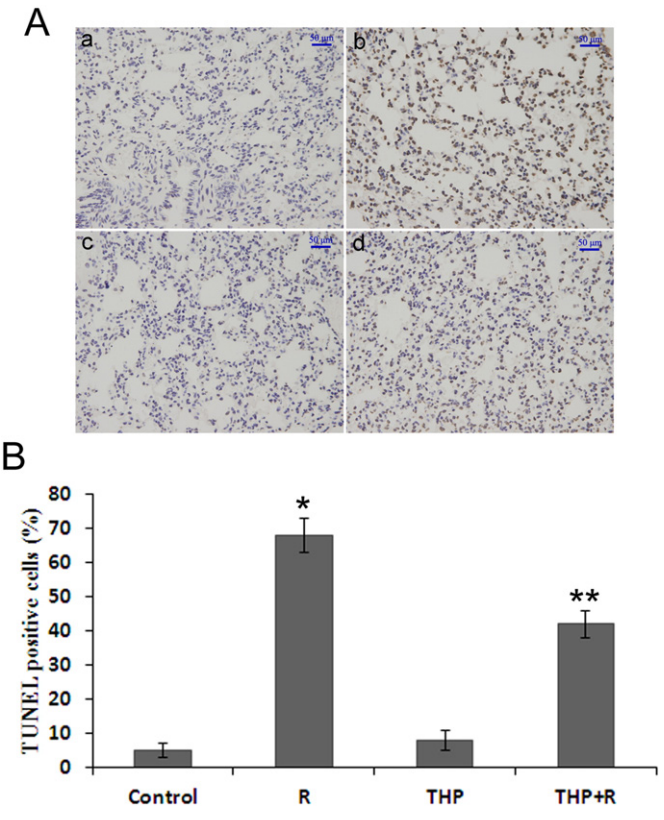

Fig. 1. Effect of Tetrahydropalmatine (THP) on irradiation (R)-induced apoptosis of pulmonary cells. Rats were sacrificed 3 h after 15 Gy thoracic R. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling (TUNEL) assayed the apoptotic cells which were stained in a color of brown. TUNEL-positive cells were defined as apoptotic cells. Magnification at 200× (Scale bar = 50 μm). A: Control; B: R; C: THP; D: THP + R. (Fig. 1A.) Statistical analysis of pulmonary cell apoptosis (Fig. 1B). (n = 5) *P < 0.001, compared with the control group and the THP only group; **P < 0.01, compared with the R only group. a: Control; b: R; c: THP; d: THP + R.

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