Role of Quercetin on Inhibiting TGF-β1 and TNF-α in Lung of Rats with Silicosis

Haibing Peng
Hebei United University, Hebei, China,
E-mail:leizi19760728@sina.com

***Abstract*--Silicosis, characterized by many cytokines expression, especially TNF-α and TGF-β1, is the most prevalent occupational disease world-wide. Here we examined whether quercetin can regulate expressios of TGF-β1 and TNF-α in lung of rats with silicosis. Totally 75 male Wistar rats, were randomly divided into three groups: group control, group model and group quercetin, (n=25). SiO_2 powders were douched in the trachea of rat to make the silicotic model. TGF-β1 and TNF-α were measured by immunohistochemica and ELISA. The results revealed that the contents of TNF-α and TGF-β1 in group model on all the time points were higher than the others, quercetin decreased the expressions of TNF-α and TGF-β1. The current study results further suggest that quercetin may have therapeutic efficacy in treatment of silicosis of the lung.**

Key words: quercetin; silicosis fibrosis; TGF-β1; TNF-α

I. INTRODUCTION

Silicosis, a common occupational respiratory disease, is a pathological condition of the lungs due to inhalation of particulate matter containing crystalline silica. Although advances in occupational safety and health make this disorder highly preventable, silicosis remains the most prevalent occupational disease world-wide. In China, the number of cases has increased rapidly due to the expansive growth of industry and the absence of available methods to prevent dust [1, 2]. By the end of 2010, China recorded 676,541 total cases of pneumonoconiosis (approximately 10,000new cases per year), with half of the cases being silicosis [3].Furthermore, there is no therapy for silicotic disease in general, largely because the underlying basis of fibrosis is unclear. Quercetin (3, 4, 5', 3', 4'- five hydroxy flavone), a typical representative of flavonols and the main flavonoids in the human diet, is the most widely distributed in plant community and has widely application value. The purpose of the current study was to explore the inhibition of quercetin on the expression of TGF-β1 and TNF-α in lung of rats with silicosis, which may have therapeutic efficacy in treatment of silicosis of the lung.

II. MATERIALS AND METHODS

A. In vivo Experimental Protocol and Disease Model

Male Wistar rats, weighting 180±10 g, were reviewed and approved by the Institutional Animal Care and Use Committee at the Hebei United University. Animals received food and water according to guidelines set by the National Institute of Health (NIH). The rats were anesthetized with isoflurane and received either silica solution (5 mg/kg, 1 ml) or 0.9% saline (vehicle) by trachea instillation. Prior to instillation, the 5 um silica particles (NIOHP, The National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention, Beijing, China) were baked at 180℃ for 6 hours. Quercetin was purchased from Sigma company, USA. Rats were divided into 3 groups: 1) control group (instilled with 0.9% saline and then intraperitoneal 0.9% saline for 2w); 2) silicotic model group (instilled of SiO_2, and then intraperitoneal 0.9% saline for 2w); 3) Quercetin group (instilled with SiO_2 and intraperitoneal quercetin solution 50 mg/(kg d) 0.5 ml for 2w). Each experimental group included 25 animals and all rats were raised for 4w. Finally rats were put to death on the third/seventh/fourteen /twenty-first/ twenty-eighth day after instilled of SiO_2, and every 5 rats in each group. Before they were dead, the whole blood samples were collected from abdominal aorta and then were centrifuged for 5 mins at 2300r/min. The supernatant was stored at -20 ℃. Middle lobe of right lung was immersed in 10% formalin for tissue fixation. And then some were stained with hematoxylin-eosin (H&E) and subjectively evaluated under light microscopy by a pulmonary pathologist who was blinded to the animal groups. Some were determined by immunohistochemistry. The other portions were frozen in liquid nitrogen and stored at –70 ℃ for later biochemical analysis.

B. ELISA

The plasma concentrations of TNF-α were determined by Enzyme-Linked ImmunoSorbent Assay kits (Rat TNF-α ELISA kit; Sigma, USA) following the suggested manufacturer's protocol.

C. Immunohistochemistry for TGF-β1

Paraffin-embedded sections were permeabilized with 0.2% Triton and blocked with 5% bovine plasma albumin (BSA) in 0.1 M phosphate-buffer saline (PBS) for 30 minutes to reduce nonspecific binding, then were incubated with primary antibodies anti-TGF-β1 (1:100, Santa Cruz Biotechnology, USA) followed by the biotinylated secondary antibody and finally Streptavidin-Peraxidase (Wuhan Boshide Biological Engineering Co. Ltd, China). Immunoreactivity was visualized with DAB. A brown color staining was considered a positive result.

D. Statistical Analysis

Values were expressed as mean±standard deviation. Comparisons between multiple independent groups were conducted using one-way ANOVA followed by post hoc analysis with the Bonferroni test. Group differences resulting in p-values of less than 0.05 were considered to be statistically significant.

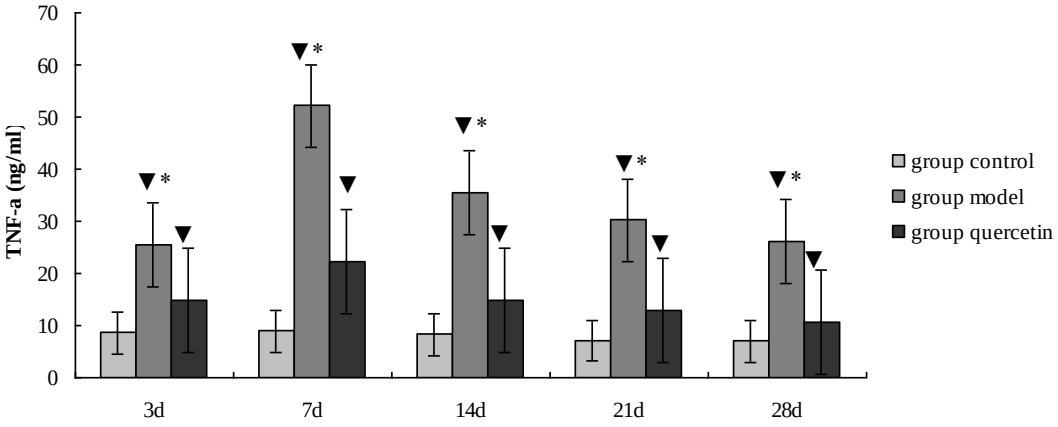
III. RESULTS

A. Morphological Changes of Lung Tissue under Light Microscope

Hematoxylin-eosin stain revealed that on the 7th day, in rats silicotic model, acute inflammation significantly was showed, and a large number of nuclear and mononuclear cells were invasive in Alveolar and interstitial lung with edema and capillary congestion, severely the normal structure of alveolar were destructed by inflammatory granulation tissue. On the 14th day, inflammation was reduced somewhat with less hemorrhage and edema, but fibroblasts and collagen fibers increased were found and lung interval was widened obviously. On the 28th day, inflammation of lung tissue was slightly less than before, along with the alveolar structure destruction and alveolar cavity significantly smaller. We observed obviously that alveolar septal and alveolar cavity were occupied by collagen and fibrin, and a large number of collagen deposition around the capillaries, then alveolar septa were thickened and interstitial lung increased with more leukomonocyte. Quercetin group and silicotic model group had the similar pathological changes, but the former was lighter than the latter.

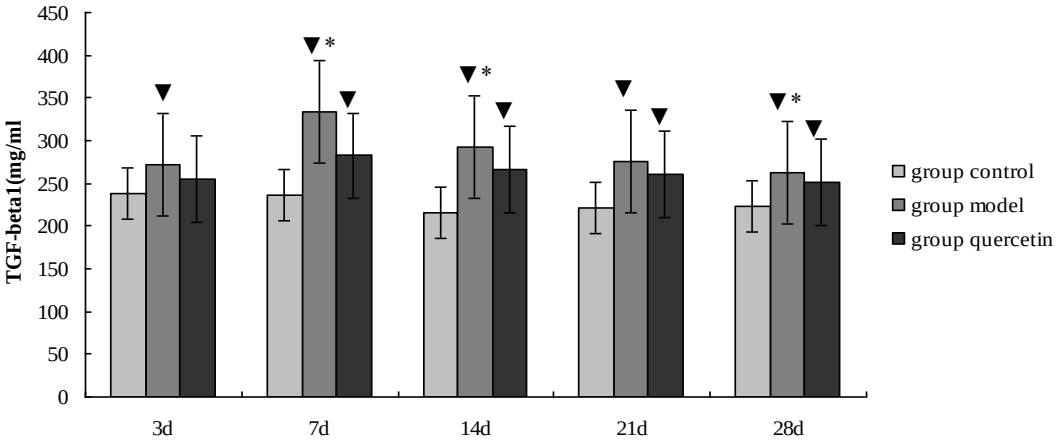
B. The Content of TNF-α in Blood Serum of Rats

As shown in Figure 1, the content of TNF- α in blood serum of rats in control group at each time point was not statistically significant ($P > 0.05$). Compared with control group, the expression of TNF- α in silicotic model group increased by 2.95, 5.86, 4.29, 4.24, 3.74 fold, respectively ($P<0.05$). However the up-regulation of TNF- α observed in silicotic model was significantly reversed by quercetin treatmen by 58.04%, 42.54%, 41.98%, 42.48% and 41.37% of silicotic model, respectively($P<0.05$). TNF- α levels were peak on the 7th day in both silicotic model group and quercetin group, which were higher than the 14th, 21st, 28th day significantly, respectively ($P<0.05$).



▼*P<0.05* compare with group control; **P<0.05* compare with group queretin

Figure 1 The content of TNF-α in blood serum of rats



▼*P<0.05* compare with group control; **P<0.05* compare with group queretin

Figure 2 The expression of TGF-β1 protein in lung tissue of rats

C. *The Expression of TGF-β1 Protein in Lung Tissue of Rats*

As shown in Figure 2, the expression of TGF-β1 protein in lung tissue of rats in control group at each time point was not statistically significant ($P > 0.05$), and TGF-β1 protein in silicotic model group was more than the others at each time point, respectively ($P<0.05$). Immunohistochemistry results revealed that expression of TGF-β1 was also observed in alveolar macrophage, fibroblast cells, bronchial epithelial cells, alveolar epithelial cells and vascular endothelial cells. Of significant interest, quercetin markedly reduced the appearance of TGF-β1 compared with silicotic model group, but still higher than control group at each time point, respectively ($P<0.05$). TGF-β1 protein was the peak on the 7th day in both silicotic model group and quercetin group, which was higher than the 14th, 21st, 28th day significantly, respectively ($P<0.05$).

IV. DISCUSSION

Silicosis is a process of inflammation and fibrosis of the interwoven. In the early period there was inflammatory response, including many cytokines, which formed a system of complex cytokine networks. When the fibrogenic cytokines were predominated in the system, it would lead to the formation of pulmonary fibrosis. Previous work has shown that TGF-β1 and TNF-α are key fibrogenic cytokines in the occurrence and development of lung silicosis [4]. In the current study, we observed that TGF-β1 was also observed in alveolar macrophage, fibroblast cells, bronchial epithelial cells, alveolar epithelial cells and vascular endothelial cells; TGF-β1 protein in silicotic model group was more than the others at each time point. TGF- β 1 is more powerful fibrogenic cytokine and can widely regulate proliferation and differentiation of cells and participate in repair and fibrosis in the organization [5, 6]. TNF- alpha, a proinflammatory cytokine, which was in alveolar macrophages (AM), a variety of mononuclear cells and alveolar epithelial cells and other cells, can be related to cell membrane receptors and then play an important role in the early, middle and late pulmonary fibrosis. The 17th National Academic Exchange occupation disease presented that TNF-α played a key role in pulmonary fibrosis induced by the dust particles especially silica in experimental animal, which nuclear factor-kappa B (NF-K B) might be activated, but the anti TNF antibody could particularly improve the pulmonary fibrosis with SiO_2-induced silicosis. In the current study, we observed that the content of TNF- α in blood serum of rats in silicotic model group was dramatically increased and further confirmed the important role of TNF- α in pulmonary fibrosis.

Quercetin, one of the most common flavonoids in the diet, belongs to flavonols. Quercetin is rich in onions, apples, tea. In vitro and in vivo, quercetin has many functions, such as antioxidation, anti-inflammatory, anti proliferative, anti atherosclerosis [7]. Quercetin could reduce the expression of TGF- β 1 in the HSC, which inhibited the development of hepatic fibrosis [8], and reduce the release of proinflammatory cytokines TNF-a. Previously, we have found that quercetin inhibits expressions of TGF- β 1 in human embryonic lung fibroblast in vitro[9]. The current study advances the field by demonstrating quercetin has an anti-fibrotic effect on silicosis in vivo, an effect that involves inhibition of TGF- β 1and TNF-α. In conclusion, the results further suggest that quercetin may have therapeutic efficacy in treatment of silicosis of the lung.

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