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The Effects of N-acetyl-seryl-aspartyl-lysyl-proline on Expression of NF-κb and MCP-1 in Rats with Silicosis

Qian Li, Fang Yang*, Lijuan Zhang, Hong Xu, Wenli Zhang
Medical research center Hebei United University, Thangshan, China
Email: liqian324@yahoo.com.cn

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ABSTRACT

In the present study, we developed silicosis of rat model by bronchial perfusion SiO_2 dust, and intervenes with AcSDKP, immunohisto chemistry was used to detect NF-κb and MCP-1 expression in lung tissue, and positive cells were counted. We found that compared with silicotic model group, the positive cells of NF-κb and MCP-1 were decreased significantly in anti-fibrosis treatment of AcSDKP group. The findings suggest that AcSDKP could inhibit the expression of NF-κb and MCP-1 in lung tissue of silicosos, this may be related to AcSDKP inhibit of macrophage infiltration in lung tissue and reduced the degree of dust alveolitis.

Keywords: N-acetyl-seryl-aspartyl-lysyl-proline; Silicosis; Monocyte Chemotactic Protein-1; Nuclear Factor-κb

1. Introduction

The main pathological change of silicosis is the fibroblast proliferation and collagen deposition in lung tissue. Recent studies have shown that cytokines play an important role in the development of silicosis, they constitute a complex signal transduction molecules network system and the chain reaction system, leading to infiltration of inflammatory cells, fibroblasts and mesenchymal connective tissue deposition is characterized by a chronic immune-mediated inflammation[1]. Rat silicosis model adopted by the bronchial perfusion SiO2 dust, and intervenes with AcSDKP, study the therapeutic effects of AcSDKP on silicotic fibrosis, provides new method for silicosis preventing and controlling.

2. Materials and Methods

2.1. Ethics Statement

Male Wistar rats, weighting 180 ± 10 g, were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the Hebei United University. Animals were given free access to food and water and were cared for according to guidelines set by the National Institute of Health (NIH)

*Corresponding author.

2.2. Induction of Silicosis and AcSDKP Treatment

Rats were anesthetized with Isoflurane and then received either silica solution (50 mg/rat, 1ml) or 0.9% saline (as a vehicle control) by trachea. Prior to instillation the 5 μm silica particles (Sigma , St. Louis, MO, USA) were baked at 180℃ for 6 hours. AcSDKP [800 μg/(kg d), Bachem AG company, USA] or control (0.9% saline) was given via a miniosmotic pump (Alzer 2 ml4, DURECT Co. Ltd, USA) planted into the abdominal cavity. Rats were divided into 3 groups (n = 10), 1) control group (instilled with 0.9% saline, and then treated with 0.9% saline for 4 w) ; 2) silicotic model group (instilled of SiO_2, and then treated with 0.9% saline for 4 w); 3) anti-fibrosis treatment of AcSDKP group (instilled with SiO_2, and treated with AcSDKP for 4 w) [2].

2.3. Immunohistochemistry for NF-κb and MCP-1

Take the lungs to organize 4% paraformaldehyde to be fixed, conventional paraffin wax embedding, 6 μm serial section, for immunohistochemical method dyeing. After incubation with 5% horse serum, sections were incubated with primary antibodies against NF-κb and MCP-1 (Santa cruz Biotechnology, USA) followed by the biotinylated secondary antibody and finally the ABC reagent (Wuhan Boshide Biological Engineering Co. Ltd, China). Immunoreactivity was visualized with DAB. A brown color staining was considered a positive result. Counts the

positive cells of NF-κb and MCP-1.

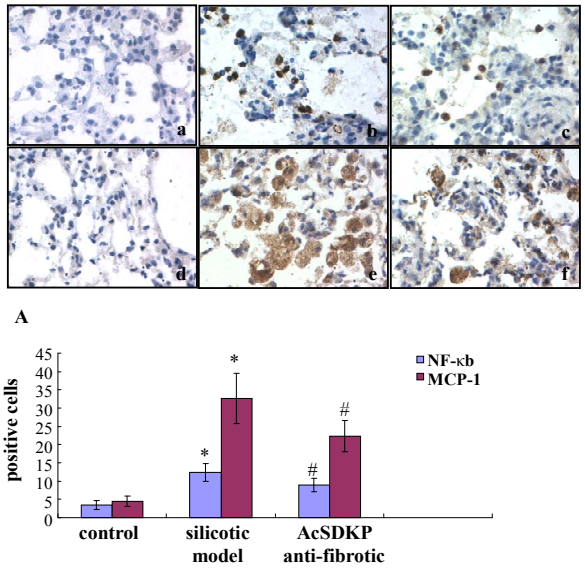
3. Results

3.1. Morphological Observation

Surfaces of lung are smooth and no nodule formation in control group. Canous nodules distribute sporadically on the surface and cutting surface in silicotic model group. Compared with silicotic mode group, the numbers of canous nodules decreased on the surface and cutting surface in the groups with AcSDKP treatment.

3.2. Expression of NF-κb and MCP-1 in Lung of Rat with Silicosis

Immunohistochemistry staining shows that NF-κb and MCP-1 only express in a small number of alveolar macrophages and fibroblasts in control group (**Figure 1**). NF-κb and MCP-1 expressions increase in silicotic nodules and interstitial fibrotic area. The expressions of NF-κb and MCP-1 in AcSDKP treatment are less than that in silicotic model. Positive cells counting shows that NF-κb and MCP-1 expressions in silicotic model group are increases by 2.76 fold and 5.40 fold compared with control group. AcSDKP treatment decreases the expressions of NF-κb and MCP-1. The positive cells counting of NF-κb and MCP-1 in anti-fibrotic treatment group is 70.12% and 65.37% of silicotic model group respectively. Variance analysis reveals that differences are significant ($p < 0.05$).



(a) control group for NF-κb; (b) silicotic model group for NF-κb; (c) AcSDKP anti-fibrotic group for NF-κb; (d) control group for MCP-1; (e) silicotic model group for MCP-1; (f) AcSDKP anti-fibrotic group for MCP-1. (A) The positive cells expression of NF-κb and MCP-1 in the lung of rat. $^{*}p < 0.05$ vs contro; $^{\#}p < 0.05$ vs silicotic model.

Figure 1. Effect of Ac-SDKP on NF-κb and MCP-1 analyzed by Immunohistochemistry in rats with silicosis. (× 400).

4. Discussion

N-acetyl-seryl-aspartyl-lysyl-proline(AcSDKP) is a the physiological hematopoietic growth inhibition factor, studies have shown that AcSDKP inhibit proliferation and collagen synthesis of cardiac fibroblasts [3,4]. In recent years NF-κB prepared in the silicosis fibrosis pathogenesis research is paid attention, NF-κb is a transcription activation function of proteins, with the wide range of promoter or Enhancer combining parts of κb site specificity and promote their transcription, NF-κB involved in many of the former transcriptional regulation of inflammatory mediators molecules promoting cytokines, adhesion molecules, chemokines, inflammatory factors, oxidative stress-related enzymes released in large quantities, so as to promote organ inflammation and fibrosis[5]. MCP-1 is a chemokine CC subfamily, its main function is the monocyte-macrophage cell chemotaxis, in addition, MCP-1 except took one kind of front inflammation cell factor participates in the adjustment white blood cell outside the chemotaxy, it also has stimulates the ciliary cell to multiply, the collagen synthesizes as well as induces certain presses the function which the fibrosis cell factor or the medium produce[6]. Our studies found that positive cells of NF-κb and MCP-1 expressions increase in silicotic model group compared with control group, it is shown that in the stimulation of SiO_2 dust, the production and expression of NF-κb and MCP-1 increased in silicosis lungs of rats. More important discoveries found that in silicosis lungs of rats, the positive cells of NF-κb and MCP-1 were significantly decreased after given AcSDKP intervention. This indicated that, AcSDKP can suppress the production and expression of NF-κb and MCP-1 in the lung with the SiO_2 dust stimulation, reduced the inflammatory response in the lungs of silicotic rats.

5. Conclusions

Our data indicate that AcSDKP possibly through inhibition of NF-κb and MCP-1 production and expression, reduce the synthesis and release of pro-fibrotic cytokines, thereby inhibiting or reducing extracellular matrix synthesis and deposition of the lungs, reducing the extent of silicosis fibrosis and inhibiting the progression of fibrosis.

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