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Effect of Atorvastatin on Lung Apoptosis-Associated Protein Caspase-12 in Chronic Obstructive Pulmonary Disease (COPD) Model Rats

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Abstract

Objective: To determine the effect of atorvastatin on the expression of lung apoptosis-associated protein caspase-12 in Chronic Obstructive Pulmonary Disease (COPD) model rats.

Methods: 30 rats were randomly divided into three groups: the normal group, the control group, and the treatment group. The control group and the treatment group were treated by being exposed to smoking and intratracheal instillation of lipopolysaccharide to establish the COPD model rats. 2 weeks later, the treatment group was treated with 2.5 mg/kg atorvastatin daily for 6 weeks. Nothing was done in the normal group. The rats were executed after 8 weeks. Lung tissue sections stained by Hematoxylin and Eosin (HE) were observed. The protein and gene expression of lung apoptosis-associated caspase-12 in each group was investigated by RT-PCR and immunohistochemistry.

Results: Pulmonary histopathology: emphysema and airway inflammation were observed in the control group, and the symptoms of the treatment group were lighter. RT-PCR: compared with the normal group, a higher level of caspase-12 mRNA was expressed in the control group and the treatment group ($p<0.05$); compared with the control group, lower level of caspase-12 mRNA was expressed in the treatment group ($p<0.05$). Immunohistochemistry: compared with the normal group, the expression of caspase-12 increased in the control group and the treatment group ($p<0.05$). Compared with the control group, the expression of caspase-12 decreased ($p<0.05$).

Conclusion: Atorvastatin has a protective effect on the COPD model rats by decreasing the protein and gene expression of caspase-12 which can reduce the apoptosis of COPD lung tissue.

Keywords: Chronic obstructive pulmonary disease (COPD); Caspase-12; Atorvastatin; Rats

Introduction

The Chronic Obstructive Pulmonary Disease (COPD) is characterized by airflow limitation that is not fully reversible. This kind of airflow limitation usually progresses progressively, associated with abnormal inflammatory reactions of harmful particles or gases in the lungs, but its pathogenesis is not yet clear. Researchers found that lung tissue apoptosis plays an important role in the development of COPD [1,2]. Caspase-12, an activator of apoptosis, has an inevitable effect on all kinds of apoptosis. Besides lipid regulation, statins can regulate apoptosis too. Yet the effect of statins on caspase-12 in COPD lung tissue and its mechanism is not clear. We apply atorvastatin to COPD rat models to determine the effect of atorvastatin on the expression of lung apoptosis-associated protein caspase-12 in COPD model rats by observing the pathological change in lung tissue, the transcription, and expression of apoptosis-associated protein caspase-12.

Materials and Methods

Materials

30 healthy male SPF grade Wistar rats offered by laboratory animal center of Central South University; Lipopolysaccharide (LPS) from Sigma in America; Atorvastatin(10 mg/tablet) from Jialin Pharmaceutical in Beijing; Furong cigarettes from China Tobacco in Hunan; SP kit and DAB from Zsbio Commerce Store in Beijing; Trizol, reverse transcription kit, PCR kit from TAKARA company; Rabbit anti-mouse caspase-12 polyclonal antibody from SANTA CRUZ company.

Methods

Animal grouping and modeling: According to a table of a random number, 30 rats were randomly divided into 3 groups with 10 in each group: the normal group, the control group, and the treatment group.

Nothing was done in the normal group except daily feeding. According to reference [1,3] after anesthetized with an intraperitoneal

injection of 10% chloral hydrate, the control group was treated with intratracheal instillation of LPS 200 μg/200 μL through the tracheal intubation then upright rotation for 10 to 20 seconds on the 1st day and the 14th day. Days 2 to 13 and days 15 to 28, the control group was treated by being exposed to smoking in a homemade plexiglass closed smoker box for 30 minutes every day, 15 cigarettes per day. The treatment group was treated by smoking and intratracheal instillation of LPS with the same condition and time. Differently, after being exposed to smoking for 2 weeks, the treatment group was treated with 2.5 mg/kg atorvastatin for intragastric administration daily for 6 weeks. After that, all three groups were executed.

Animal disposal and specimen collection: Modeling rats were anesthetized with an intraperitoneal injection of 10% chloral hydrate. Then blood collection of inferior vena cava was operated through splanchnocoele with a 10 mL injector. The blood collection should be as much as possible. Open the left ventricle with tissue scissors then perfuse it with saline through the pulmonary arteries in the right ventricle by an intravenous infusion set to clean the blood in the pulmonary vessel. Then the right lungs were take-out and were fixed in 4% paraformaldehyde and embedded in paraffin to prepare 5 μm paraffin sections for Hematoxylin and Eosin (HE) and immunohistochemistry. Another lung tissue was collected with liquid nitrogen for extraction of RNA.

Detecting the expression of caspase-12 by RT-PCR: Total RNA was eluted with Trizol. After the reverse transcription of cDNA, total RNA was expanded using PCR. Primer sequences are as follow: caspase-12,

Upstream primer 5'-CGTGCAGTCAACTGCCGCAAGA-3'

Downstream primer 5'-CGGGGTCACAGGCCAGGTATG-3'

β-actin upstream primer 5'-AATGCATCCTGCACCACCAA-3'

downstream primer 5'-GTAGCCTATTCATTGTCATA-3'

RNA was extracted from lung tissue by Trizol method. The purity, concentration, and integrity of the total RNA were detected. RNA reverse transcribes into cDNA. The amplified product was stained in 2% agarose gel electrophoresis and ethidium bromide then observed under Burdick lamp. The ratio of integral absorbance of caspase-12 to β-actin amplified product was used to express the relative value of mRNA for each specimen.

Detecting the expression of caspase-12 by immunohistochemistry staining: 3% hydrogen peroxide for 10 min at room temperature, microwave antigen retrieval for 20 min at moderate heat, normal serum for blocking drops and reaction at room temperature for 20 min were added to the paraffin sections orderly. Then, primary antibody at a concentration of 1:50 was added respectively, kept overnight at 4℃. On the second day, after reaction at room temperature for 30 min, reagent 1 for 60 min reaction at 37℃ and reagent 2 for 90 min reaction at 37℃ were added to the paraffin sections respectively. Then, the sections were treated with the DAB method, fully washed by water, restained with hematoxylin. Finally, the sections were dehydrated and sealed. The average optical density of the positive substances (light yellow, brownish-yellow and tan particles) was determined, and the relative amount of caspase-12 was shown in the average optical absorbance density.
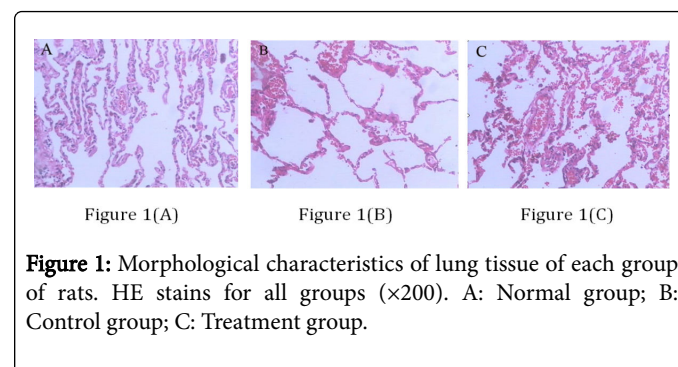
Statistical method: SPSS 18.0 Statistical Software was applied for data analysis. The results were measured by x ± s. Comparisons between groups were tested by ANOVA analysis. If p ≤ 0.05, differences were significant.

Results

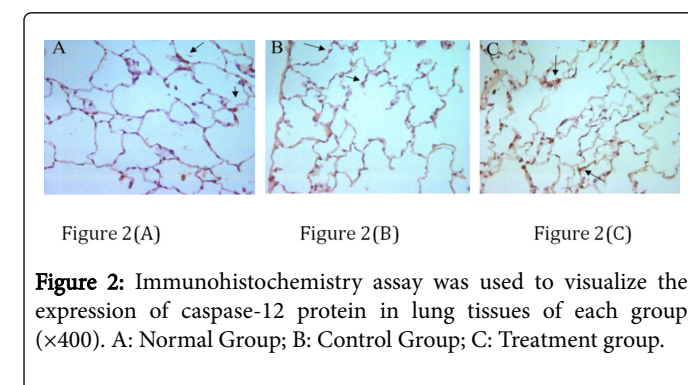### Comparison of pathological changes in each group

The normal group: Epithelial cells of the mucous membranes of windpipe were complete and the cilia neatly arranged. The construction of air passages is normal. No inflammatory cell infiltration was seen around the air passages and blood vessels. The size and construction of alveoli are normal. The control group: Pathological changes of emphysema were found. Cilia of mucous membranes of windpipe and bronchi adhered and flattened. Epithelial cells abscission was found in some air passages. And inflammatory cell infiltration was largely found in the submucosa. Blood capillary around the windpipe dilated and congested. The alveolar wall became thin, fractured, and fused.
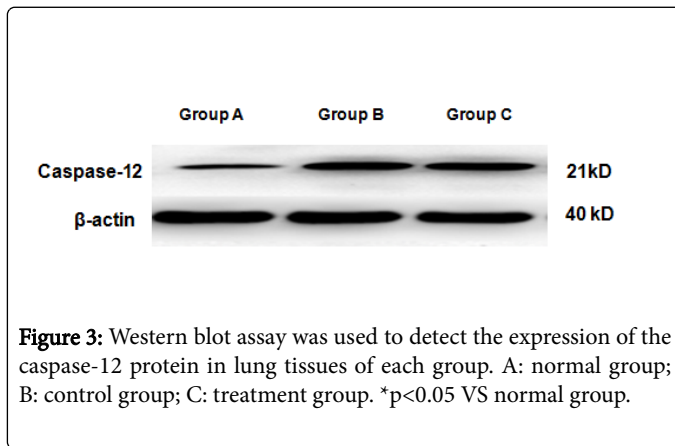
The treatment group: Symptom of emphysema was found. But compared with the control group, epithelial cells abscission and inflammatory cell infiltration lessened and fewer alveoli were destroyed (Figure 1).

Figure 1(A)   Figure 1(B)   Figure 1(C)

Figure 1: Morphological characteristics of lung tissue of each group of rats. HE stains for all groups (×200). A: Normal group; B: Control group; C: Treatment group.

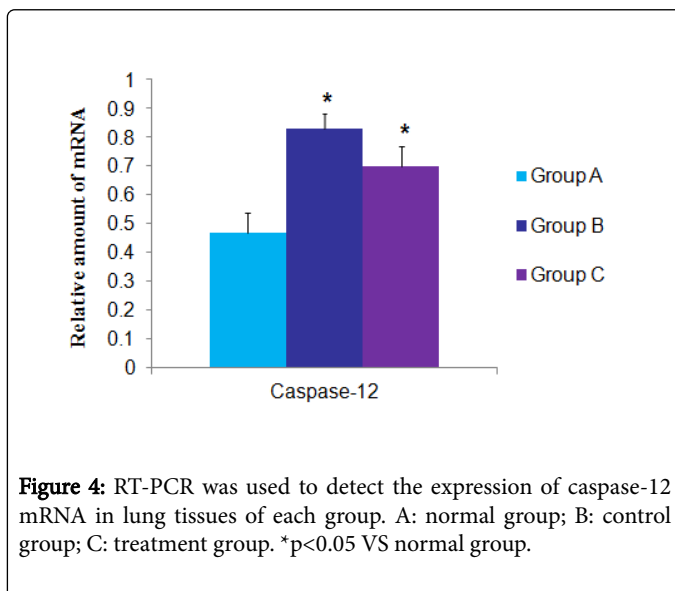### Comparison of immunohistochemistry results in each group

Caspase-12 was mainly expressed in the cytoplasm of airway epithelial cells, alveolar epithelial cells, and vascular smooth muscle cells. Compared with the normal group, the expression of caspase-12 increased in the control group and the treatment group (p<0.05). Compared with the control group, the expression of caspase-12 decreased in the treatment group (p<0.05) (Figures 2 and 3).

Figure 2(A)   Figure 2(B)   Figure 2(C)

Figure 2: Immunohistochemistry assay was used to visualize the expression of caspase-12 protein in lung tissues of each group (×400). A: Normal Group; B: Control Group; C: Treatment group.

Figure 3: Western blot assay was used to detect the expression of the caspase-12 protein in lung tissues of each group. A: normal group; B: control group; C: treatment group. *p<0.05 VS normal group.

Comparison of test results of caspase-12 mRNA of lung tissue in each group

Compared with the normal group, a higher level of caspase-12 mRNA was expressed in the control group and the treatment group (p<0.05); Compared with the control group, lower level of caspase-12 mRNA was expressed in the treatment group (p<0.05) (Figure 4).

Figure 4: RT-PCR was used to detect the expression of caspase-12 mRNA in lung tissues of each group. A: normal group; B: control group; C: treatment group. *p<0.05 VS normal group.

Discussion

In recent years, the number of COPD patients has been increasing constantly. The high mortality of COPD has caused serious social and economic burden and has become a serious public health problem [4]. In this study referred to reference [1-3], the control group and the treatment group were treated by being exposed to smoking and intratracheal instillation of lipopolysaccharide to establish the COPD rat models. In the control group, pathological changes of emphysema were found; cilia of mucous membranes of windpipe and bronchi adhered and flattened; epithelial cells abscission was found in some air passages; inflammatory cell infiltration was largely found in submucosa; blood capillary around the windpipe dilated and congested; and the alveolar wall became thin, fractured, and fused, which are in line with the pathological characteristics of COPD.

The pathogenesis of COPD is complex, mainly including four aspects: chronic inflammation of the air passages and lungs, imbalance of protease/antiprotease, imbalance of oxidation/antioxidation, and dysfunction of the autonomic nervous system. New research shows that apoptosis of lung tissue plays a role in the development of COPD [5,6]. Apoptosis is physiological cell death that is lured by virulence factors and abnormal signals. Under normal physiological conditions, apoptosis ensures that the normal development and life process of an organism can go smoothly. But in the pathological case, apoptosis is an important cause of some diseases. The gene regulation of apoptosis is complex and the caspase family is the core of apoptosis. Different apoptosis process induced by different stimulation will activate different caspase. But caspase-12, an activator of apoptosis, has an inevitable effect on all kinds of apoptosis. It is found that, after the infusion of caspase-12 activator into the respiratory tract, the lung parenchymal cells and lung interstitial cells would undergo apoptosis, followed by the emphysema, and the emphysema would worsen gradually. While under the same conditions, the addition of caspase-12 inhibitor significantly alleviated emphysema and reduced the apoptosis of lung interstitial cells. This suggests that lung tissue cells are involved in the formation of emphysema through the caspase pathway. Besides lipid regulation, statins can regulate apoptosis too [7,8]. Studies have shown that atorvastatin can inhibit the activity of apoptosis-associated protein caspase-12 which can reduce the apoptosis by increasing the stability of mRNA of endogenous Nitric Oxide Synthase (eNOS) and the biological activity of nitric oxide [9]. It is reported by Guerard [10] that, of Pulmonary Artery Hypertension (PAH) rats, the pulmonary artery endothelial cell apoptosis decreased, eNOS expression increased and caspase-12 expression decreased after statin treatment. This suggests that statin can inhibit the apoptosis of pulmonary artery endothelial cells. What's more, studies have shown that atorvastatin can induce and phagocyte apoptotic cells *in vitro* and *in vivo*, of which the mechanisms might work by inhibit the membrane localization of iso-amylene and RhoA. Also, statin can increase the level of peroxisome activated receptors, increase the expression of recognition receptor CD36 in apoptosis of alveolar macrophages, and increase the phagocytosis of apoptotic cells.

Our study finds that compared with the normal group, a higher level of caspase-12 mRNA and protein was expressed in the control group and the treatment group; while after atorvastatin treatment, compared with the control group, lower level of caspase-12 mRNA was expressed in the treatment group. This suggests that atorvastatin has a protective effect on the COPD model rats by decreasing the protein and gene expression of caspase-12 which can reduce the apoptosis of COPD lung tissue.

Atorvastatin can reduce the apoptosis of COPD lung tissue, and its mechanism may be related to the decrease of gene and protein expression of caspase-12, which provides a theoretical basis for the application of atorvastatin in COPD.

Conclusion

Atorvastatin has a protective effect on the COPD model rats by decreasing the protein and gene expression of caspase-12 which can reduce the apoptosis of COPD lung tissue.

Acknowledgment

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