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Salidroside attenuates LPS-induced inflammatory activation in young rats with acute lung injury via PI3K/Akt signaling pathway

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ABSTRACT

This experiment aimed to analyze the salidroside effect on lipopolysaccharide (LPS)-induced inflammatory activation in young rats with acute lung injury (ALI) via PI3K/Akt signaling pathway. In this study, sixty SD young rats were divided into 5 groups (control, model, salidroside low-dose, salidroside medium-dose and salidroside high-dose), with 12 rats in each group. ALI rat model was established. In the control and model group, rats were intraperitoneally injected with normal saline, while the salidroside low-, medium-, and high-dose groups were intraperitoneally injected with 5, 20, and 40 mg/kg salidroside, then the pathological changes of lung tissue, lung injury score, wet/dry lung weight ratio, neutrophils and TNF-α, MPO, MDA, NO, p-PI3K and p-AKT were detached and compared between these groups. Results showed that the ALI rat model was successfully established. The lung injury score, wet/dry lung weight ratio, neutrophils and TNF-α in alveolar lavage fluid, MPO, MDA, NO, p-PI3K and p-AKT in the lung tissue of the model group were increased than the control group. With the increase of salidroside dose, lung injury score, wet lung weight/dry lung weight ratio, neutrophils and TNF-α in alveolar lavage fluid, and the levels of MPO, MDA, NO, p-PI3K and p-AKT in lung tissues of the salidroside group were decreased then model group ($P < 0.05$). In conclusion, salidroside may reduce the activation of inflammatory cells in the lung tissue of young rats with LPS-induced ALI by activating PI3K/AKT signaling pathway, thereby exerting a certain protective effect on the lung tissue with LPS-induced ALI.

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Introduction

Acute lung injury (ALI) is a kind of progressive and acute refractory hypoxemia, which even develops into acute respiratory distress syndrome. Infection is the main cause of ALI, especially lipopolysaccharide (LPS)-induced endotoxemia (1). Studies have found that endotoxin could regulate inflammation and endothelial barrier dysfunction in ALI caused by bacteria (2). The pathogenesis of ALI is complex, which may be related to inflammatory mediators and oxidative stress (OS). The accumulation of reactive oxygen radicals can promote inflammatory factors and enhance inflammatory response. Inflammatory factors can not only remove harmful microorganisms but also cause lung tissue damage (3). At present, there is no effective treatment for ALI, mainly treated with sedation and respiratory support. Salidroside is the main active component of *Rhodiola rosea*, which has anti-hypoxia, anti-aging, hypoglycemic and anti-tumor effects. Studies have found that *R. rosea* can also improve blood flow resistance, lower alveolar-arterial oxygen partial pressure and reduce the degree of lung injury, which can significantly reduce the possibility of traumatic ALI (4). However, whether it can improve LPS-induced ALI is unknown. In this study, whether salidroside can reduce LPS-induced inflammatory activation of young rats with ALI and its related mechanism were discussed and analyzed.

Materials and Methods

Experimental animal

Sixty young SD rats (vitalriver Technology Co., Ltd.) were randomly selected, with the body weight of (55 ± 11) g, the age was 3 weeks. All rats were fed at (22±2) ℃ with humidity of (56±11) % in each day and night alternate for 12 h, with a free diet and water intake.

Main instruments and reagents

Biological microscope (Shanghai Shunyu Optical Technology (Group) Co., Ltd. Model: SOPTOP EX21); Paraffin slicer (Shenyang Hengsong Technology Co., Ltd., Model: HS-S7220); Real-time PCR instrument (Sun

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Yat-sen University Da'an Gene Co., Ltd., Model: ViiA 7); Salidroside (Shanghai Yaji Biotechnology Co., Ltd., Specification: 20mg/dose); TNF-α detection kit (Shanghai Jingkang Bioengineering Co., Ltd.); NO detection kit (Shanghai Kaifang Biotechnology Co., Ltd.); MDA detection kit (Hefei Laier Biotechnology Co., Ltd.); Rabbit anti-human AKT polyclonal antibody (Shanghai Zeye Biotechnology Co., Ltd.); LPS (Shanghai Gushen Biotechnology Co., Ltd.).

Experimental methods

Establishment of a rat model of ALI: The young rats were anesthetized, placed in the supine position, and an incision was made in the neck to expose the trachea. 3mg/kg LPS was injected from the trachea to the lung, and the liquid was evenly distributed in both lungs by rotation and sutured layer by layer. The young rats were randomly selected and divided into 5 groups (control, model, salidroside low-dose, salidroside medium-dose and salidroside high-dose), with 12 rats in each group. The control group and the model group were intraperitoneally injected with normal saline, and the salidroside low, medium and high dose groups were intraperitoneally injected with salidroside at 5, 20 and 40 mg/kg. All young mice were sacrificed after 8h of treatment.

Observational indicators

The lung tissue was taken after the young rats were sacrificed, and the upper lobe of the left lung was cut and fixed in formalin solution. 4μm paraffin sections were routinely prepared, dewaxed with xylene, dehydrated with gradient alcohol, stained with hematoxylin and eosin dye solution, rinsed with tap water, and dehydrated with alcohol. After xylene transparency and neutral gum sealing, the lung tissue changes of each group were observed using a microscope.

Lung injury score: The inferior lobe of the left lung was taken, and the Smith method was used to evaluate the lung injury score, including alveolar interstitial hemorrhage, alveolar fibrin exudation, neutrophil exudation from alveolar space and septum, and alveolar septal widening, with 0 to 4 points for each index. No lesion was scored 0 points, the lesion ranges less than 25% was scored 1 point, 26%-50% was scored 2 points, 51%-75% was scored 3 points, and more than 76% was scored 4 points. The sum of the above items is the lung injury score.

Wet/dry lung weight ratio: The inferior lobe of the right lung of young rats was taken, and the weight was recorded as wet weight. After weighing, the rats were put into an oven at 65℃ to dry for 48 h, and the weight was recorded as dry weight.

The alveolar lavage fluid was centrifuged, collected the supernatant for the determination of TNF-α level by ELISA. The sediment was resuspended and smeared on slides. The smear was covered with Wright's staining solution, rinsed with phosphate buffer, and dried naturally, and neutrophils in the alveolar lavage fluid were counted.

The superior lobe of the right lung was taken, homogenized, centrifuged, and the supernatant was taken. The levels of Myeloperoxidase (MPO) were measured by an MPO detection kit. The changes in malondialdehyde (MDA) levels were determined using an MDA detection kit. Nitric oxide (NO) kit was used to measure the changes in NO level.

p-PI3K and p-AKT expression levels in lung tissues of young rats were determined by WB. The protein from lung tissue was extracted, and the separating gel and stacking gel was prepared successively. The protein was denatured by boiling, and the sample was loaded for electrophoresis. The protein on the gel was electro-transferred to the PVDF membrane, then incubated at 4 ℃, overnight. The primary antibody was diluted and blocked at 37℃ for 1h. The membrane was rinsed 3 times, with 10min each time. The secondary antibody was diluted, the membrane was washed again, and the image was quantified by a chemiluminescence imager.

Statistical method

Variance analysis was used for the comparison of measurement data, and the SNK-q test was used for the pairwise comparison of sample means among multiple groups. A statistical result of $P < 0.05$ was considered statistically significant. The SPSS21.0 software package was used for statistical data analysis in this study. Compared with the control group, $^{a}P<0.05$; compared with the model group, $^{b}P<0.05$; compared with the salidroside low-dose group, $^{c}P<0.05$; compared with salidroside medium-dose group $^{d}P<0.05$.

Results

Morphological changes in lung tissue

In group control, the lung tissue structure and the alveolar cavity were clear, there was no edema in the alveolar septum, and no inflammatory cell (IC) infiltration. In the group model, the lung tissue structure was disordered, the alveolar cavity became smaller, the alveolar septum became wider and thicker, and IC infiltration was observed. With the increase in salidroside dose, the lung tissue pathological changes in the salidroside group were improved than the model group, and the improvement was obvious in the salidroside high-dose group. The results were shown in Figure 1.

Lung injury scores in each group

The lung injury score of the model group was obviously increased than the control group ($P < 0.05$), meanwhile, with the increase of salidroside dose in the salidroside group, it was decreased than the model group ($P < 0.05$) (Table 1).

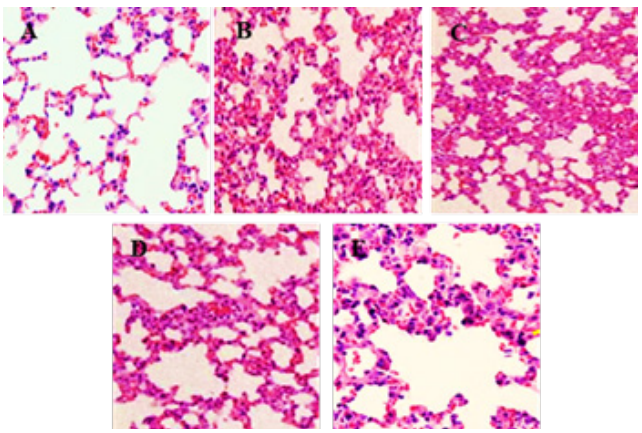

Figure 1. Morphological changes of lung tissue in each group. A. Control group; B. Model group; C. Salidroside low-dose group; D. Salidroside medium-dose group; E. Salidroside high-dose group.

Changes in lung wet/dry lung weight ratio in each group

The ratio of lung wet weight to dry weight of the model group was strikingly increased than the control group (P<0.05), meanwhile, with the increase of salidroside dose in the salidroside group it gradually decreased the model group(P<0.05) (Table 2).

Changes in neutrophils and TNF-α levels in alveolar lavage fluid in each group

The alveolar lavage fluid neutrophils and TNF-αlevels in the model group notably increased in the control group (P < 0.05), meanwhile, with the increase of salidroside dose in the salidroside group, they gradually decreased than the model group (P < 0.05) (Table 3).

Changes in MPO, MDA and NO levels in the lung tissue of each group

The lung tissue MPO, MDA and NO levels in the model group were remarkably increased in the control group (P < 0.05), meanwhile, with the increase of salidroside dose in the salidroside group, they gradually decreased in the model group(P < 0.05) (Table 4).

Expression levels of p-PI3K and p-AKT in lung tissue of young rats in each group

The lung tissue expression levels of p-PI3K and p-AKT in the model group were increased than the control group (P < 0.05), meanwhile, with the increase of salidroside dose in the salidroside group, they were gradually increased than model group (P < 0.05) (Figure 2).

Discussion

ALI is a critical manifestation of multiple organ dysfunction syndrome in the lungs, which can cause diffuse pulmonary fibrosis, reduced lung compliance, oxygen diffusion disorder, etc., mainly manifested as progressive

Table 1. Lung injury scores in each group ($\bar{x}\pm s$).

Groups	n	Lung injury score (points)
Control	12	0.33±0.14
Model	12	3.49±0.19[a]
Salidroside low-dose	12	3.18±0.15[ab]
Salidroside medium-dose	12	2.23±0.20[abc]
Salidroside high-dose	12	1.71±0.16[abcd]
F		663.60
P		<0.001

Table 2. Lung wet/dry weight ratio in each group ($\bar{x}\pm s$).

Groups	n	Wet lung weight / dry lung weight
Control	12	3.61±0.19
Model	12	5.55±0.13[a]
Salidroside low-dose	12	5.19±0.18[ab]
Salidroside medium-dose	12	4.85±0.17[abc]
Salidroside high-dose	12	3.96±0.21[abcd]
F		255.46
P		<0.001

Table 3. Changes of neutrophils and TNF-α levels in alveolar lavage fluid in each group ($\bar{x}\pm s$).

Groups	n	Neutrophils (×10⁶/mL)	TNF-α (pg/mL)
Control	12	0.43±0.26	23.39±3.53
Model		9.76±0.43[a]	96.40±6.89[a]
Salidroside low-dose	12	8.30±0.45[ab]	82.06±5.20[ab]
Salidroside medium-dose	12	6.23±0.40[abc]	66.14±4.29[abc]
Salidroside high-dose	12	3.18±0.20[abcd]	42.83±4.15[abcd]
F		1318.53	423.45
P		<0.001	<0.001

Table 4. Changes of MPO, MDA and NO levels in lung tissue of each group ($\bar{x}\pm s$).

Groups	n	MPO (U/mg)	MDA (nmol/mg)	NO (μmol/mg)
Control group	12	0.53±0.23	1.57±0.26	1.24±0.37
Model	12	2.78±0.20[a]	6.34±0.33[a]	13.49±0.62[a]
Salidroside low-dose	12	2.37±0.16[ab]	5.65±0.25[ab]	9.17±0.60[ab]
Salidroside medium-dose	12	1.63±0.19[abc]	4.26±0.30[abc]	6.84±0.38[abc]
Salidroside high-dose	12	1.08±0.15[abcd]	2.61±0.27[abcd]	3.58±0.21[abcd]
F		285.52	599.62	1284.86
P		<0.001	<0.001	<0.001

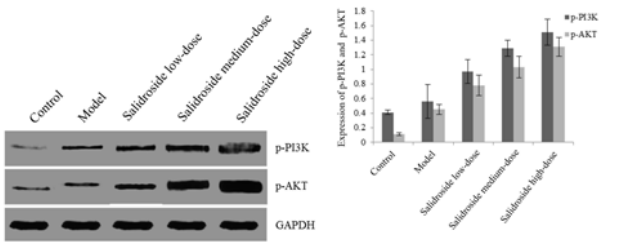

Figure 2. p-PI3K and p-AKT expression in lung tissue of young rats.

dyspnea and refractory hypoxemia, and eventually developing into respiratory failure (5). ALI develops rapidly and has a high mortality rate, which has become an important cause of death in critically ill patients and seriously threatens the life and health of patients. Studies have found that infection is the main cause of ALI, which is caused by Gram-negative bacilli account for most infectious diseases (6). LPS, as a major cell wall of Gram-negative bacilli, can specifically recognize and activate inflammatory mediators such as monocytes/macrophages and neutrophils, and then induce the occurrence of ALI. Salidroside is an important active component of *R. rosea*, and it has important protective effects on the cardiovascular, nervous, skeletal, etc. system. In addition, salidroside has an effective anti-inflammatory effect (7-8). Some scholars have found that salidroside can effectively remove and prevent the formation of free radicals and reduce the permeability of alveolar capillaries in ALI caused by oleic acid, thus playing a role in protecting lung tissue (9). In this study, the young rat was used as the research objects, and the young rat model of LPS-induced ALI was established. The mechanism of salidroside in improving LPS-induced ALI was investigated.

Studies have found that when ALI occurs, the balance between inflammatory factors and anti-inflammatory factors in the body is broken, which may be one of the material bases for the progression of ALI (10). TNF-α is a key factor in the early initiation of inflammation in the lung, which can induce the level of IL-6 and other inflammatory factors. TNF-α can also cause chemotaxis of neutrophil accumulation and activation in the lung, thereby promoting the production of many reactive oxygen species. Moreover, many reactive oxygen free radicals accumulation in the body can promote inflammatory factors, enhance the inflammatory response, and further aggravate the damage of lung tissue (11-12). MPO, MDA and NO are important indicators of OS, which are significantly related to the degree of OS response. Our study showed that salidroside had a protective effect on LPS-induced ALIlung tissue of young rats, and could effectively reduce the inflammatory activation of IC in ALIlung tissue of young rats, and reduce the oxidative reaction of lung tissue.

PI3K is a family of phosphatidylinositol kinases with serine/threonine protein kinase activity. Upon activation, PI3K can bind to the PH region of AKT to partially activate AKT and translocation it to the inner surface of the cell membrane (13). According to relevant reports, activation of the PI3K/AKT pathway can up-regulate the level of alveolar epithelial sodium channels in ALI, thereby expelling excess alveolar edema fluid and leading to the exudation of alveolar protein fluid, thus exerting a protective effect on lung tissue (14). In the study of the LPS-induced ALI model, Wang et al. (15) found that activation of the PI3K/AKT signaling pathway could significantly reduce lung microvascular endothelial injury caused by LPS, and thus play a role in protecting pulmonary vascular endothelial cells. The results were similar to those found in this study. The results of this study suggested that the salidroside could activate PI3K/AKT signaling pathway.

In summary, salidroside may reduce the ICactivation in LPS-induced ALIlung tissue of young rats by activating PI3K/AKT signaling pathway, and exerting a certain protective effect on the lung tissue with LPS-induced ALI.

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