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EXPERIMENTAL STUDY

Effect of Liuweibuqi capsule, a Chinese patent medicine, on the JAK1/STAT3 pathway and MMP9/TIMP1 in a chronic obstructive pulmonary disease rat model

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Abstract

OBJECTIVE: To observe effect of Liuweibuqi Capsule, a Traditional Chinese Medicine (TCM), on the janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathway and matrix metalloproteinases (MMPs) in a chronic obstructive pulmonary disease (COPD) rat model with lung deficiency in terms of TCM's pattern differentiation.

METHODS: Rats were randomly divided into a normal group, model group, Liuweibuqi group, Jinshuibao group, and spleen aminopeptidase group (n=10). Aside from the normal group, all rats were exposed to smoke plus lipopolysaccharide tracheal instillation to establish the COPD model with lung deficiency. Models were established after 28 days and then the normal and model groups were given normal saline (0.09 g/kg), Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg), Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. Changes in symptoms, signs, and lung histology were observed. Lung function was measured with a spirometer. Serum cytokines were detected using enzyme-linked immunosorbent assay, and changes in the JAK/STAT pathway, MMP-9, and MMPs inhibitor 1 (TIMP1) were detected by immunohistochemistry, RT-PCR, and western blotting, respectively.

RESULTS: Compared with the normal group, lung tissue was damaged, and lung function was reduced in the model control group. Additionally, the levels of interleukin (IL)-1β, γ interferon (IFN-γ), and IL-6 were higher, while IL-4 and IL-10 were lower in the model control group than those in the normal group. The expressions of JAK1, STAT3, p-STAT3, and MMP-9 mRNA and protein in lung tissue were higher, and TIMP1 mRNA and protein was lower in the model group compared with the normal group. After treatment, compared with the model group, the expression of inflammatory cytokines was lower in each treatment group, and expressions of JAK/STAT pathway, MMPs were lower. Compared with the positive control groups, the Jinshuibao and spleen aminopeptidase groups, lung function was better, and JAK1, STAT3, and p-STAT3 protein were lower and TIMP1 was higher in the Liuweibuqi group.

CONCLUSION: Liuweibuqi capsules can improve the symptoms of COPD possibly by regulating the expression of the JAK1/STAT3 pathway and MMP9/TIMP1.

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Key words: Pulmonary disease, chronic obstructive; Lung deficiency; Liuweibuqi capsules; Janus kinases; STAT Transcription Factors; Matrix metalloproteinases

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a respiratory condition that manifests as central airway mucus hypersecretion, and peripheral airway reconstruction and scar tissue formation.[1] The pathogenesis of COPD is complex, but the JAK/STAT signaling pathway and protease/anti-protease systems play an important role.[2,3] COPD is also associated with matrix metalloproteinases (MMPs), and tissue inhibitor of metalloproteinase (TIMPs). Studies have shown that the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) signaling pathway can lead to the accumulation of a large number of proinflammatory cytokines, and increase the inflammatory response.[4] The direct result of airway injury is lung tissue damage, which causes coughing, wheezing, and reduced lung function. MMP and TIMP expression imbalance causes degradation of extracellular matrix deposition disorder, which results in airway damage and emphysema. This study investigated the expression and relationship between the JAK/STAT signaling pathway and MMPs/TIMPs in a rat model of COPD. Preclinical findings show that Liuweibuqi capsules can not only reduce airway inflammation in patients with COPD, but also significantly improve cough, wheezing, shortness of breath, fatigue, and improve lung function in patients with COPD.[5,6] In this study, a rat COPD model was established by administration of lipopolysaccharide and smoke. Liuweibuqi capsules were administered after model establishment and lung function and lung tissue morphological changes, and changes in the STAT pathway and MMP system were observed.

MATERIALS AND METHODS

Experimental animals

Fifty male Sprague-Dawley (SD) rats (180-200 g) were purchased from the Experimental Animal Center of Anhui Province (Hefei, China). All animals were housed in specific pathogen free (SPF) conditions, and given access to water and food ad libitum. The study protocol was approved by the Ethics Committee of Anhui University of Chinese Medicine.

Model establishment and grouping

Rats were randomly divided into five groups by random number table method, with 10 rats in each: normal control (NC) group, model control (MC) group, Liuweibuqi group (LWBQ), Jinshuibao group (JSB), and spleen aminopeptidase group (PAT). Apart from the NC group, all rats were anesthetized with 10% chloral hydrate and their tracheas exposed to 200 μL of 1 mg/mL lipopolysaccharide (LPS, Sigma, St. Louis, MO, USA, No. 20110314574). The rats were placed in a chamber with smoke from ignition of a 50 g sawdust and 0.682 g cigarette tobacco mixture (Chuzhou, China, tar 13.5 mg/g, nicotine 0.48 mg/g). The rats were exposed to the smoke for 30 min per day for 28 days to establish the rat models.[7] The *Qi* deficiency models were established by changing smoking for three times per day in the first 22 days.[8] COPD model criteria were based on lung function and pathological changes of lung tissue. The *Qi* deficiency model criteria were based on biological signs of change, including activity, hair, body weight, food intake, and coughing.

Treatment administration

Treatments started from day 28. The NC and MC groups were given 0.9% (physiological) saline (10 mL/kg). The LWBQ, JSB, and PAT groups were respectively given Liuweibuqi capsules (0.35 g/kg), Jinshuibao capsules (0.495 g/kg), and spleen aminopeptidase (0.33 mg/kg) for 30 days.

Medicine and reagents

Liuweibuqi Capsules were from Anhui Medical University First Affiliated Hospital Pharmacy Center, batch number: 2011070501 (Hefei, China). Jinshuibao Capsules were from Jiangxi Jinshuibao pharmaceutical company (Jiangxi, China). Spleen aminopeptidase was from Dalian Baili pharmaceutical manufacturing (Dalian, China). LPS was from Sigma; cigarettes were from Chuzhou Cigarette Factory (Chuzhou, China); IFN (interferon)-γ, IL (interleukin)-4, IL-6, and IL-10 detection kits were purchased from R&D Systems (Emeryville, CA, USA); JAK1, p-JAK1, STAT3, p-STAT3, MMP9, and TIMP1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Observation of rats

Rat body weight, activity, reaction time, fur and feces, death rate, respiratory rate, coughing, respiratory secretions, sputum, and other COPD symptoms and signs were monitored.

Evaluation of pulmonary function

Pulmonary function was observed by forced vital capacity (FVC) and average expiratory flow, which was calculated by dividing FVC by the value for forced expiratory flow in 0.3 s ($FEV_{0.3}$) and multiplying by 100%. Additionally, peak expiratory flow (PEF) was assessed. These measurements were obtained using the pulmonary function test apparatus for small animals 30 days after administration.

Rat lung tissue morphology

Lung tissues were fixed in 10% formalin after 48 h. The right upper lobe and hilar organizations were routinely washing, dehydrated, embedded in paraffin, and cut into 5 μm serial sections. The sections were then dewaxed, dehydrated, stained with HE, and observed with light microscopy (Olympus Inc., Tokyo, Japan). Bronchus and lung tissue were analyzed for morphological changes (Jetta Company, Shanghai, China).

Detection of IFN-γ, IL-4, IL-6, and IL-10 in serum using enzyme linked immunosorbent assay(ELISA)

Detection was performed in accordance with the instructions of ELISA kit manufacturer (R&D Systems, Emeryville, CA, USA). In brief, the primary antibody was (interferon-γ, interleukin-4, interleukin-6, and interleukin-10) diluted (1-10 μg/mL) in coating solution. Then, 100 μL diluted antibody was added to appropriate wells, and incubated for 2 h at room temperature. The plate was emptied, residual liquid removed, and washed twice with 300 μL 0.05% Tween-20. Then, 300 μL blocking solution was added to each well and incubated for 1 h at room temperature. Then, the plate was washed twice with 300 μL wash solution. Next, 100 μL of diluted biotinylated detection antibody was added to each well and incubated for 1 h at 37 ℃, then the plate was washed three times. Then, 100 μL diluted AKP conjugated streptavidin was added to each well and incubated for 1 h at room temperature. The plate was emptied and washed three times for 5 min each. The plate was then washed five more times. Then, 200 μL of substrate was placed into each well and the color developed for 30 min at room temperature. Finally, 0.05 mL 2 M of H_2SO_4 was added to each well, and immediately read with a plate reader at 405-410 nm.

Immunohistochemical detection of lung tissue MMP-9, TIMP1, JAK1, STAT3

Immunohistochemical staining of MMP-9, TIMP1, JAK1, and STAT3 in lung tissue was detected by the streptavidin-perosidase (SP) method according to the manufacturer's instructions. The negative control used phosphate buffered saline (PBS) instead of an anti-carry. Positive staining was indicated by brown particles. Five high-power fields were randomly selected on each section for analysis by an image analysis system. Immunoreactive cells were analyzed, and the average optical density was measured.

Detection of MMP-9, TIMP1, JAK1, STAT3 mRNA in lung tissue by RT-PCR

Total RNA of lung tissue was extracted using TRIzol (Invitrogen, Diego, CA, USA, No. 1259673). Reverse transcriptase was from Invitrogen (Diego, CA, USA, No. 696045), thermal cycler was from Biometra (Goettingen, Germany, model: T1-Thermoblock), electrophoresis apparatus was from Bio-Rad (Hercules, CA, USA, model: Power PAC 300), and the gel image analysis instrument was from Bio-Rad (Hercules, CA, USA, model: Gel Doc XR). Band brightness of MMP-9, TIMP1, JAK1, STAT3 changes were observed, and the optical density of each band was analyzed. The levels of MMP-9, TIMP1, JAK1, and STAT3 are given relative to glyceraldehyde-3-phosphate Dehydrogease (GAPDH) levels as control.

Detection of JAK1/STAT3 pathway, MMP-9, TIMP1 protein of lung tissue by western blot analysis

Protein samples of lung tissue were prepared and subjected to protein gel electrophoresis (Bio-Rad). The gel was transferred to a membrane, blocked, and incubated with a primary antibody: the JAK1 (Santa-Cruz, CA, USA, sc-376996), p-JAK1 (Santa-Cruz, CA, USA, sc-377043), STAT3 (Santa-Cruz, CA, USA, sc-482), p-STAT3 (Santa-Cruz, CA, USA, sc-8001-R), MMP-9 (Santa-Cruz, CA, USA, sc-21733), or TIMP1 (Santa-Cruz, CA, USA, sc-21734) diluted 1 : 1000. The diluted primary antibody was incubated at room temperature for 1-2 h. Secondary antibody was incubated for 30-60 min. Membranes were visualized with enhanced chemiluminescence. Software was used for analysis and processing of protein bands. The relative density of each band in each group was calculated to beta-actin as the control.

Statistical analysis

Continuous variables are the mean ± standard deviation ($\bar{x} \pm s$). All samples were tested to ascertain if they followed a normal distribution. Data comparison among groups was performed using analysis of variance. Comparison between groups were tested by One-Way analysis of variance and least significant difference test. SPSS Version 17.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses. $P < 0.05$ was considered significant.

RESULTS

Biological observation

In the NC group, the food intake and body weight gradually increased. Rat respiratory rate was steady, there were no respiratory secretions, and no smell or sputum. The MC rats showed edge off appetite, brown hair and shedding. Rat body weight was less than that of the NC group ($P < 0.05$, Table 1). The MC group had shortness of breath, and the respiratory rate of the MC group (2.12 ± 0.69) was significantly higher than that of the NC group (1.42 ± 0.39). Additionally, respiratory secretions spilled over from the nose and mouth in the MC group. There were symptoms of cough and airway sputum, indicating that the MC group had lower lung function, and decreased exercise tolerance. These are the characteristics of *Qi* deficiency in TCM.

After treatment, the symptoms of rats were better in comparison with the MC group. Body weight and respiratory frequency were significantly better than that in the MC group, and the LWBQ group was better than those in the other treatment groups ($P < 0.05$, Table 1).

Table 1 Comparison of body weight and respiratory frequency in rats ($n = 10$, $\bar{x} \pm s$)

Group	Body weight (g)	Respiratory rate (Hz)
NC	76.6±16.6	1.4±0.4
MC	51.2±15.5[a]	2.1±0.3[d]
LWBQ	63.2±14.9[b]	1.6±0.4[b]
JSB	55.6±14.4[c]	1.7±0.5
PAT	57.8±12.7	1.9±0.4[c]

Notes: the normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase. Compared with NC group, [a]$P < 0.01$, [d]$P < 0.05$; compared with MC group, [b]$P < 0.05$; compared with LWBQ group, [c]$P < 0.05$.

Changes in lung function

Compared with the NC group, lung function parameters such as $FEV_{0.3}$, FVC, $FEV_{0.3}$/FVC were lower in the MC rats ($P < 0.05$). Compared with the MC group, $FEV_{0.3}$, FVC, and $FEV_{0.3}$/FVC were higher in the treatment group ($P < 0.05$). Compared with the LWBQ group, $FEV_{0.3}$, $FEV_{0.3}$/FVC, and PEF were lower in the PAT group ($P < 0.05$, Table 2).

Morphological changes in lung tissue

In the NC group there were tracheal mucociliary regular columnar epithelial cells, the cilia were arranged in neat rows, trachea and bronchi were seen at all levels of goblet cells, and there was no gland hyperplasia or inflammatory cell infiltration (Figure 1A). In the MC group, there was tracheal epithelial shedding, goblet cell and glandular hypertrophy, and there was wall and inflammatory cell infiltration. Additionally, the bronchial mucosal folds increased in variable length, there were many neutrophils in small endobronchial tissue, there were narrow distal terminal bronchioles, the respiratory bronchioles and alveolar ducts had cystic dilatation, alveolar enlargement, bubble wall thinning, and there was centrilobular emphysema. Some alveolar expansion was observed to be alveolar emphysema (Figure 1B). In the LWBQ group, there was little inflammatory cell infiltration, less destruction of alveolar septa, less tracheal mucociliary damage, and more complete columnar epithelial cells. The cilia were also arranged in a more structured pattern (Figure 1C). The JSB group (Figure 1D) and PAT group (Figure 1E) had moderate infiltration of inflammatory cells in the bronchial alveolar lumen, epithelial necrosis, fewer cilia, and some alveolar expansion into vesicles of varying sizes.

Changes in serum cytokines

Compared with the NC group, the expression of IL-4 and IL-10 was lower, and IFN-γ and IL-6 expression was higher in the MC group ($P < 0.05$). Compared with the MC group, IL-4 and IL -10 expression were elevated, while IFN-γ and IL-6 were lower in the LW-

Table 2 Comparison of lung function parameters in rats ($n = 10$, $\bar{x} \pm s$)

Group	$FEV_{0.3}$ (mL)	FVC (mL)	$FEV_{0.3}$/FVC (%)	PEF (mL/s)
NC	6.0±1.5	7.7±1.9	93.8±2.7	42.6±6.8
MC	4.9±1.1[a]	5.5±2.0[d]	80.4±2.8[d]	34.8±7.2[d]
LWBQ	6.0±1.0[b]	7.1±2.2[b]	92.9±3.0[e]	29.5±4.9[e]
JSB	5.2±1.0	6.9±1.8[b]	91.4±2.8[e]	40.3±4.5[e]
PAT	4.6±0.9[c]	6.9±1.7[b]	88.9±2.3[bc]	35.8±8.6[c]

Notes: the normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase; $FEV_{0.3}$: forced expiratory flow in 0.3 s; FVC: forced vital capacity; PEF: peak expiratory flow. Compared with NC group, [a]$P < 0.05$, [d]$P < 0.01$; compared with MC group, [b]$P < 0.05$, [e]$P < 0.01$; compared with LWBQ group, [c]$P < 0.05$.

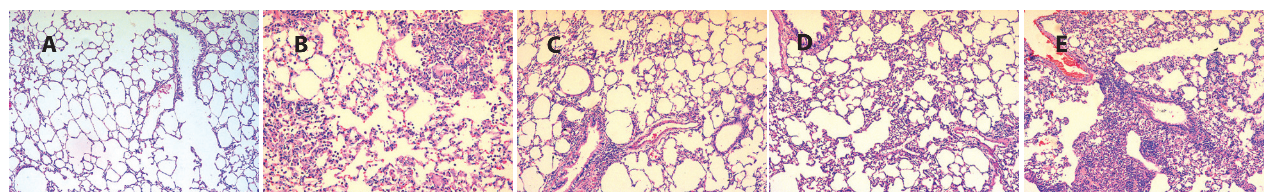

Figure 1 The morphological changes in lung tissue (HE, ×200)

A: NC group; B: MC group; C: LWBQ group; D: JSB group; E: PAT group. The normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase; HE: hematoxylin and eosin.

BQ group (P < 0.05). Compared with the LWBQ group, IL-6 expression was higher in the PAT group, and IL-6 and IFN-γ expression was higher in the JSB group (P < 0.05, Figure 2).

Comparison of MMP-9, TIMP1, JAK1, and STAT3 optical density value in lung tissue

Compared with the NC group, MMP-9, JAK1, and STAT3 optical density values of lung tissue were higher, while TIMP1 optical density values were lower in the MC group. MMP-9, JAK1, and STAT3 average optical density values in the LWBQ group were lower, and TIMP1 optical density was higher than that in the MC group. Compared with LWBQ, JAK1, and STAT3 optical density values were higher in the JSB group. MMP-9 optical density value was higher, and TIMP1 lower in the PAT group compared with the LWBQ (Figure 3).

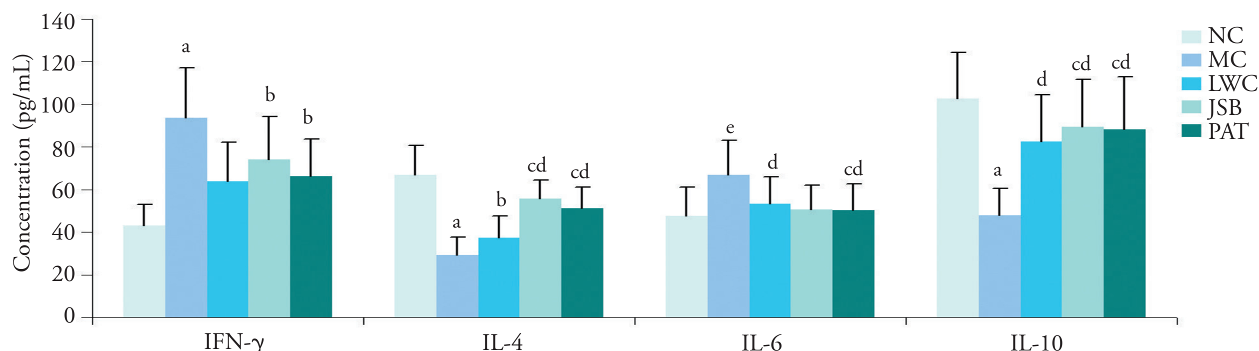

Figure 2 Expression of serum IFN-γ, IL-4, IL-6, and IL-10 in rats

The normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control group; MC: model control group; LWBQ: Liuweibuqi capsule group; JSB: Jinshuibao capsule group; PAT: spleen aminopeptidase group; IFN-γ: interferon-γ; IL: interleukin. Compared with NC group, [a]P < 0.01, [e]P < 0.05; compared with MC group, [d]P < 0.05, [b]P < 0.01; compared with LWBQ group, [c]P < 0.05.

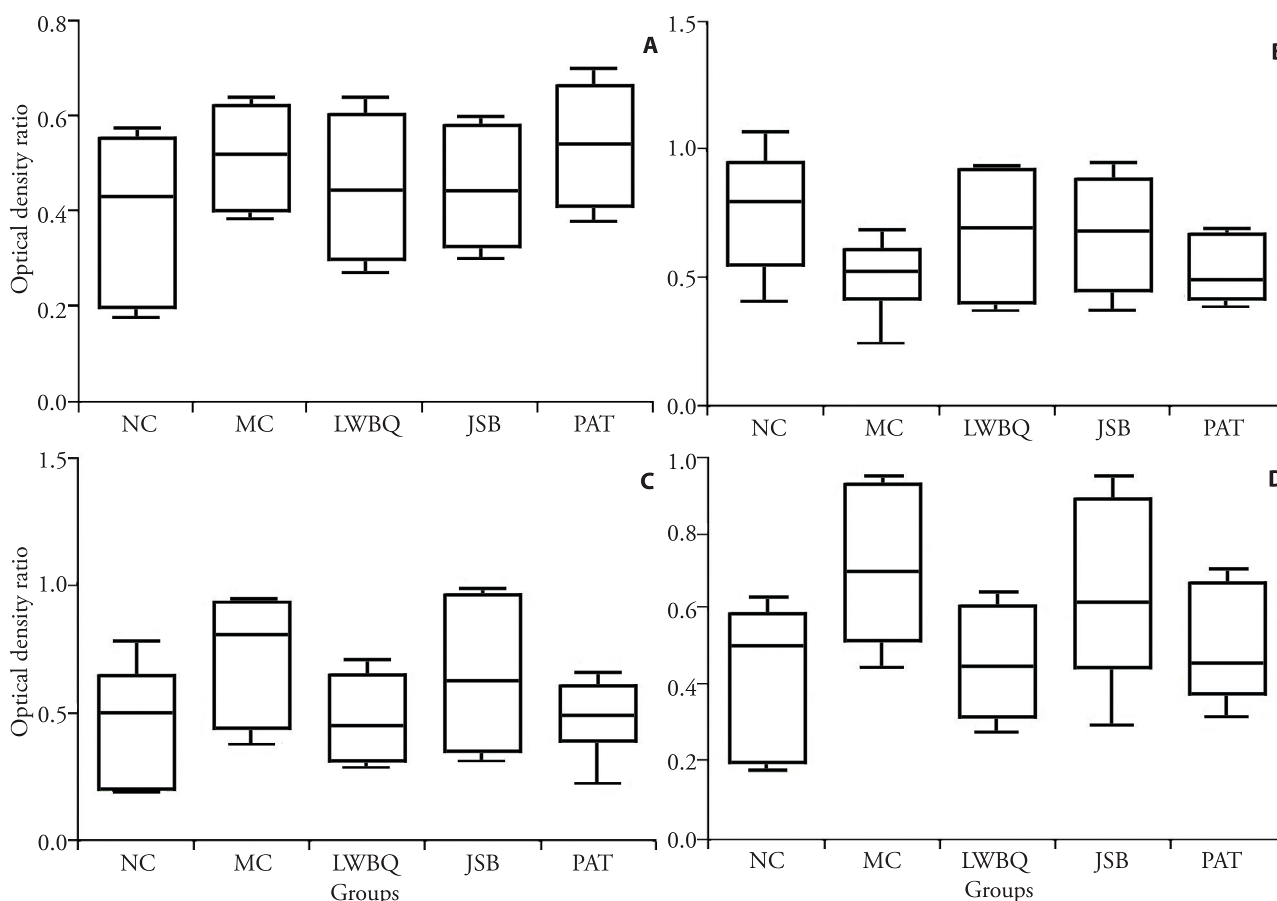

Figure 3 Comparison of optical density values for MMP-9, TIMP1, JAK1, and STAT3 in lung tissue

A: MMP9, B: TIMP1, C: JAK1, D: STAT3. The normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase; JAK: janus kinase; STAT: signal transducer and activator of transcription; NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase.

Expression of JAK1/STAT3 signaling pathway, MMP-9, TIMP1 gene and protein in lung tissue

RT-PCR and western blot results showed that compared with the NC group, JAK1, STAT3, and MMP-9 mRNA expression in lung tissue were higher (P<0.05, Table 3, Figure 4), and the expression of MMP-9, JAK1, p-JAK1, STAT3, and p-STAT3 protein were higher (P < 0.05, Table 4, Figure 5), but TIMP1 mRNA and protein expression were lower in the MC group (P < 0.05, Table 3, 4; Figures 4, 5). Compared with the MC group, JAK1 and MMP-9 mRNA expression and JAK1, p-JAK1, MMP-9, and p-STAT3 protein expression were lower, but TIMP1 mRNA and protein expression were higher in the LWBQ group (P < 0.05, Table 3, 4; Figure 4, 5). Compared with the LWBQ group, MMP-9 mRNA and JAK1, p-JAK1, and MMP-9 protein were higher, but TIMP1 protein was lower in the JSB group (P < 0.05, Table 3, 4; Figure 4, 5). JAK1 mRNA and JAK1, p-STAT3, and MMP-9 protein were higher, while TIMP1 protein was lower in the PAT group compared with the LWBQ group (P < 0.05, Table 3, 4; Figure 4, 5).

Correlation analysis

JAK1 mRNA, STAT3 protein were positively correlated with IL-6. MMP-9 mRNA was negatively correlated with $FEV_{0.3}$ and IL-10. p-STAT3 protein was positively correlated with IFN-γ. TIMP1 protein was positively correlated with PEF. STAT3 protein was negatively correlated with $FEV_{0.3}$. MMP-9 protein was negatively correlated with PEF (P < 0.05). JAK1, STAT3, and MMP-9 were positively correlated with p-STAT3. STAT3 was positively correlated with p-JAK1. MMP-9 was negatively correlated with TIMP1 (P < 0.05, Tables 5, 6).

DISCUSSION

This study created an intratracheal instillation of lipopolysaccharide COPD model. This model can simulate the COPD clinical pathology of airway inflammatory cell infiltration resulting in mucus hypersecretion. The model also induces TCM *Qi* deficiency by passive smoking, which causes airway remodeling, emphysema, and other structural changes. The results showed that shortness of breath and respiratory frequency significantly accelerated the outflow of respiratory secretions. Moreover, cough and airway sputum could be heard in the model rats. Therefore, this model is consistent with COPD and lung deficiency clinical pathological features.

Modeled rats with COPD have airway infiltration of inflammatory cells, pathological changes in lung tissue, decreased pulmonary function. LPS can directly or indirectly stimulate lung macrophages and neutrophils, thereby inducing JAK1 phosphorylation in the JAK1/

Table 3 Comparison of MMP-9, TIMP1, JAK1, and STAT3 mRNA in lung tissue (n = 10, $\bar{x} \pm s$)

Group	MMP-9	TIMP1	JAK1	STAT3
NC	0.69±0.16	1.40±0.21	0.36±0.08	0.42±0.09
MC	1.34±0.26^{a}	0.59±0.17^{a}	0.68±0.15^{a}	0.57±0.17^{e}
LWBQ	0.92±0.17^{b}	1.15±0.22^{b}	0.47±0.12^{b}	0.47±0.15
JSB	1.14±0.24^{c}	0.92±0.21^{d}	0.55±0.10^{d}	0.51±0.09
PAT	0.93±0.20^{b}	0.79±0.23^{d}	0.59±0.08^{c}	0.53±0.11

Notes: the normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase; JAK: janus kinase; STAT: signal transducer and activator of transcription. Compared with NC group $^{a}P < 0.01$, $^{e}P < 0.05$; compared with MC group $^{d}P < 0.05$, $^{b}P < 0.01$; compared with LWBQ group, $^{c}P < 0.05$.

Table 4 Comparison of MMP-9, TIMP1, JAK1, and STAT3 protein in lung tissue (n = 10, $\bar{x} \pm s$)

Group	JAK1	p-JAK1	STAT3	p-STAT3	MMP-9	TIMP1
NC	0.85±0.12	0.51±0.09	0.68±0.18	0.55±0.06	0.78±0.11	0.98±0.18
MC	1.02±0.21^{a}	0.97±0.17^{e}	0.93±0.17^{e}	0.79±0.14^{e}	1.26±0.27^{e}	0.66±0.14^{e}
LWBQ	0.64±0.15^{b}	0.48±0.05^{b}	0.85±0.19	0.51±0.12	0.85±0.18^{b}	0.85±0.12^{b}
JSB	0.89±0.17^{bd}	0.79±0.11^{cf}	0.82±0.15	0.58±0.13	0.98±0.21^{cf}	0.71±0.15^{c}
PAT	0.86±0.16^{c}	0.49±0.08^{f}	0.81±0.15	0.64±0.19^{c}	1.02±0.23^{cf}	0.72±0.13^{cf}

Notes: the normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase; JAK: janus kinase; STAT: signal transducer and activator of transcription. Compared with NC group, $^{a}P < 0.05$, $^{e}P < 0.01$; compared with MC group, $^{c}P < 0.05$, $^{b}P < 0.01$; compared with LWBQ group, $^{c}P < 0.05$, $^{d}P < 0.01$.

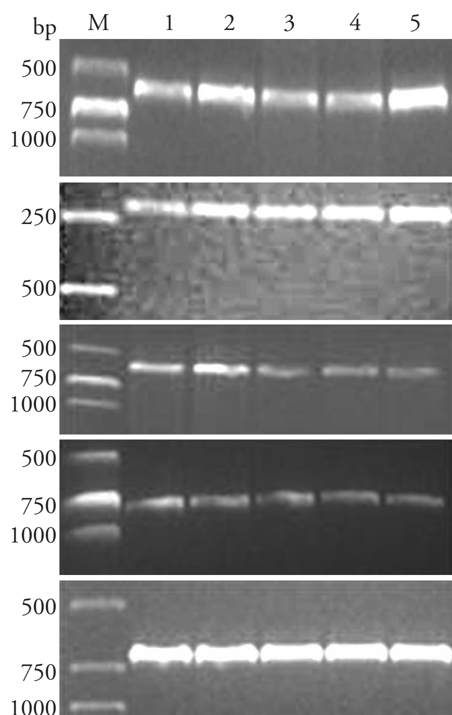

Figure 4 Expression of JAK1/STAT3 signaling pathway, and MMP-9, TIMP1 mRNA in each group
M: marker; 1: NC group; 2: MC group; 3: LWBQ group; 4: JSB group; 5: PAT group. The normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase; JAK: janus kinase; STAT: signal transducer and activator of transcription.

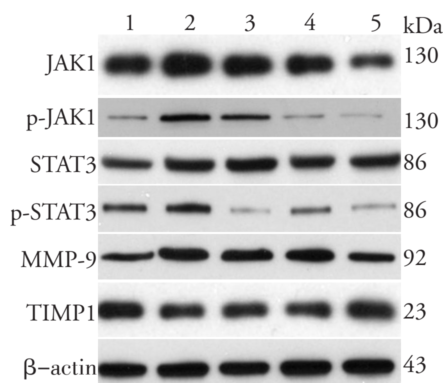

Figure 5 Expression of JAK1/STAT3 signaling pathway, and MMP-9, TIMP1 protein in each group
1: NC group; 2:MC group; 3: JBS group; 4: PAT group; 5: LWBQ group. The normal and model groups were given normal saline (0.09 g/kg); Liuweibuqi group was given Liuweibuqi capsule (0.35 g/kg); Jinshuibao group was given Jinshuibao capsules (0.495 g/kg), and the Spleen group was given spleen aminopeptidase (0.33 mg/kg), once a day for 30 days. NC: normal control; MC: model control; LWBQ: Liuweibuqi capsule; JSB: Jinshuibao capsule; PAT: spleen aminopeptidase; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase; JAK: janus kinase; STAT: signal transducer and activator of transcription.

STAT3 signaling pathway. JAK1 activation can rapidly activate STAT3 phosphorylation. Activation of the JAK1/STAT3 pathway was involved in cell proliferation, differentiation, and apoptosis. The STAT pathway transduction process occurs *via* cytokine receptors on the cell membrane in which liganded receptors dimerize causing a complex aggregation of receptors. JAK1 becomes phosphorylated, and then STAT3 becomes phosphorylated. p-STAT moves to the nucleus and interacts with other transcription factors to regulate gene transcription.[9,10] p-STAT3 can also contribute to increased inflammatory response, thereby increasing the severity of COPD. IL-6 is a major regulator of JAK1/STAT3 activation in COPD lung epithelial and endothelial cells. IL-6 can activate STAT3 directly, and is accompanied by the activation of STAT3.

Study has shown that expression of MMP-9 is higher, and TIMP-1 levels are lower in COPD patients. We found that MMP-9 and inflammatory cytokines are positively correlated. Further correlation analysis showed that JAK1, STAT3, and MMP-9 were positively correlated with p-STAT3, and STAT3 was positively correlated with p-JAK1, while MMP-9 was negatively

Table 5 Relationships between the JAK1/STAT3 pathway, MMP-9, and TIMP1 in COPD (*r* value)

Index		FVC	$FEV_{0.3}$	PEF	IFN-γ	IL-4	IL-6	IL-10
mRNA	JAK1	- 0.145	- 0.095	- 0.089	- 0.001	- 0.283	0.479^a	0.074
	STAT3	- 0.095	- 0.114	- 0.047	- 0.078	- 0.463^a	- 0.067	- 0.265
	MMP-9	0.245	- 0.476^a	- 0.217	- 0.085	- 0.221	- 0.064	- 0.486^a
	TIMP1	- 0.154	- 0.405	0.174	- 0.142	- 0.164	- 0.065	- 0.145
Protein	JAK1	- 0.117	- 0.096	0.106	0.064	- 0.066	- 0.201	- 0.391
p-JAK1	STAT3	0.074	- 0.475^a	- 0.095	- 0.026	- 0.206	0.454^a	0.067
	p-STAT3	- 0.296	- 0.024	0.283	0.515^b	- 0.165	0.266	- 0.195
	MMP-9	- 0.135	- 0.093	- 0.494^a	- 0.213	- 0.045	- 0.042	- 0.309
	TIMP1	0.108	0.186	0.468^a	0.017	0.024	0.307	0.305

Notes: *r* is relevance coefficient. MMP: matrix metalloproteinase; TIMP: tissue inhibitor of matrix metalloproteinase; JAK: Janus kinase; STAT: signal transducer and activator of transcription; IFN-γ: interferon-γ; IL: interleukin; $FEV_{0.3}$: forced expiratory flow in 0.3 s; FVC: forced vital capacity; PEF: peak expiratory flow. a, b is the *P* value range, between the abscissa and ordinate index, $^aP < 0.05$, $^bP < 0.01$.

Table 6 Correlation between JAK1/STAT3 pathway and MMP-9, TIMP1 protein (*r* value)

Index	JAK1	p-JAK1	STAT3	p-STAT3	MMP-9	TIMP1
JAK1	-	0.196	0.328	0.487[a]	0.224	- 0.297
p-JAK1	0.196	-	0.472[a]	0.315	0.196	- 0.083
STAT3	0.328	0.472[a]	-	0.511[b]	0.258	- 0.279
p-STAT3	0.487[a]	0.315	0.511[b]	-	0.473[a]	- 0.302
MMP-9	0.224	0.196	0.258	0.473[a]	-	- 0.506[b]
TIMP1	- 0.297	0.083	- 0.279	- 0.302	- 0.506[b]	-

Notes: *r* is relevance coefficient, MMP: matrix metalloproteinase, TIMP: tissue inhibitor of matrix metalloproteinase, JAK: janus kinase, STAT: signal transducer and activator of transcription, IFN-γ: interferon-γ, IL: interleukin. a, b is the *P* value range, between the abscissa and ordinate index, [a]$P < 0.05$, [b]$P < 0.01$.

correlated with TIMP1. MMP-9 expression and MMP-9/TIMP-1 was higher in the model group compared with the normal group, indicating that there was airway inflammation in COPD. Excessive activation of the JAK1/STAT3 signaling pathways can reduce the expression of TIMP-1, which cannot effectively inhibit MMP-9 expression. MMP-9 over-expression degraded the enhanced role of the extracellular matrix, and increased airway inflammation. Study showed that the MMP/JAK/STAT3 signaling axis led to excessive activation of COX-2 expression, which causes airway and lung tissue damage.[12]

Lung deficiency is the primary factor of COPD, and is treated in TCM with supplementary *Qi*. This study showed that Liuweibuqi capsules significantly improved the signs and symptoms of COPD, improved tissue morphology and lung function, increased IL-4, IL-10, and TIMP1 expression, reduced JAK1, STAT3, p-STAT3, and MMP-9 expression, and inflammation in rats modeled with lung deficiency. Liuweibuqi capsules include Renshen (*Radix Ginseng*), Huangqi (*Radix Astragali Mongolici*), Yuzhu (*Rhizoma Polygonati odorati*), Yizhi (*Fructus Alpiniae Oxyphyllae*), Chenpi (*Pericarpium Citri Reticulatae*), Rougui (*Cortex Cinnamomi Cassiae*). Renshen (*Radix Ginseng*) and Huangqi (*Radix Astragali Mongolici*) may nourish vitality, according to TCM. Ginseng polysaccharides and ginseng saponins can regulate immune function and reduce inflammation caused by LPS.[13,14] Study found that astragalus polysaccharides can not only reduce the body's inflammatory response, but also adjust the lung *Qi* role of immunity in mice.[15,16] Animal studies found that astragalus has beneficial effects on allergic airway inflammation inhibition.[17] Moreover, Huangqi (*Radix Astragali Mongolici*) can regulate the JAK/STAT pathway expression, and astragalus saponins can reduce JAK, STAT1, and STAT3 protein expression. Astragalus polysaccharides and saponins can inhibit MMP-9 and TIMP-1 expression in damaged lung tissue and improve lung function. Further study has found that shenqi combination can reduce MMP-9 protein expression in rat lungs with COPD lung deficiency and inhibits extracellular matrix collagen deposition, which prevents COPD airway remodeling.[18] Citrus essential oil can relax the tracheal smooth muscle. Moreover, citrus has effects on asthma, and can promote humoral and cellular immunity.[19] Finally, cinnamon water extract can inhibit NO production, has anti-inflammatory effects, and can inhibit reticuloendothelial phagocytosis and antibody formation, thereby regulating the body's immune function.[20]

In summary, Liuweibuqi capsules improved COPD symptoms. The mechanism behind it is possibly related to the regulation of JAK1/STAT3 signal transduction, the balance of MMP-9/TIMP-1, and inhibition of the inflammatory response.

REFERENCES

1 National Pharmacopoeia Committee. Chinese Pharmacopoeia 2010 edition: Part I. Beijing: China Medical Science Press, 2010: 707.

2 **Guo T**, Li YY. Progresses on pharmacological and toxicological effects of Dengzhanxixin injection. Zhong Guo Zhong Yao Za Zhi 2012; 37(18): 2820-2823.

3 **Wei X**, Ye XQ, Xie YM, et al. Post-marketed re-evaluation of fleabane injection and Dengzhan Shengmai capsule study on treatment in patients with ischemic stroke. Zhong Guo Zhong Yao Za Zhi 2011; 36(20): 2789-2792.

4 **Li YY**, Xiang YY, Xie YM, Shen H. Study of analysis 1390 adverse drug reaction cases of parenterally administered Dengzhanxixin based on China spontaneous response system. Zhong Guo Zhong Yao Za Zhi 2013; 38(18): 2998-3002.

5 **Yang W**, Cheng H, Xie YM, Yang H, Zhuang Y. DZI using character analysis in clinical based on real world HIS database. Zhong Guo Zhong Yao Za Zhi 2012; 37(18): 2718-2722.

6 **Yang W**, li L, Xie YM, Zhuang Y. Treatment outcomes of parenterally administered Dengzhanxixin for treatment of cerebral infaction based on real world hospital injection system data. Zhong Guo Zhong Yao Za Zhi 2013; 38(18): 3141-3146.

7 **Shen H**, Yang W, Xie YM, Zeng XB. Exploration of clinical outcomes on coronary heart disease of parenterally administered Dengzhanxixin using propensity score. Zhong Guo Zhong Yao Za Zhi 2013; 38(18): 3166-3171.

8 **Lu P**, Zeng XB, Xie YM, Yang W, Li YY, Wang ZG. Ex-

plore Dengzhanxixin injection effecting on outcome of coronary heart disease based on propensity score. Zhong Guo Zhong Yao Za Zhi 2013; 38(18): 3121-3126.

9 **Li YY**, Xie YM. Literature metrology analysis of adverse reactions reports of Denzhanxixin injection. Zhong Guo Zhong Yao Za Zhi 2012; 37(18): 2789-2791.

10 **Nie XL**, Shen H, Xie YM, Hu J, Zhang YL, Li YY. Meta-analysis of Dengzhanxixin injection treatment for unstable angina pectoris. Zhong Guo Zhong Yao Za Zhi 2012; 37(18): 2768-2773.

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