

# Jiawei Yuping Fengsan Granules Relieve Secondary Lung Injury in Ischemic Stroke

Yongquan Liu<sup>1,\*</sup>, Luchang Yin<sup>2</sup>

<sup>1</sup>Respiratory Department, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, 261041, China

<sup>2</sup>Internal Medicine-Cardiovascular Department, Affiliated Hospital of Weifang Medical University, Weifang, Shandong, 261041, China

\*Corresponding author: wyfyliuyongquan@126.com

**Abstract:** To observe whether pre-treatment with Jiawei Yuping Fengsan granules can alleviate secondary lung injury in ischemic stroke. 30 SPF grade male SD rats were randomly divided into three groups: sham surgery group (sham group), cerebral ischemia-reperfusion group (IR group), modified Yupingfeng powder particle pretreatment cerebral ischemia-reperfusion group (Y-IR group), sham surgery group: no intervention; IR group: equal amount of physiological saline; Y-IR group: Jiawei Yuping Fengsan granules 10mg/kg. After successful modeling, ischemia lasted for 3 hours and reperfusion lasted for 2 hours. After the experiment, the total cell count and neutrophil percentage in bronchoalveolar lavage fluid (BALF) were measured; Measure oxidative stress factors (MDA, SOD) in rat lung tissue and blood; The expression level of inflammatory factors (TNF- $\alpha$ , IL-1 $\beta$  and IL-6). Compared with the IR group, the expression levels of total cell count and neutrophil percentage in bronchoalveolar lavage fluid (BALF) of rats in the Y-IR group significantly decreased, with statistical significance ( $P < 0.05$ ). Compared with the IR group, the expression level of MDA in lung tissue and serum of rats in the Y-IR group significantly decreased, while SOD activity significantly increased, with statistical significance ( $P < 0.05$ ). Compared to the IR group, in the lung tissue and serum of rats in the Y-IR group, The expression level of TNF- $\alpha$ , IL-1 $\beta$ , IL-6 content significantly decreased, and the difference was statistically significant ( $P < 0.05$ ). Jiawei Yuping Fengsan Granules have a protective effect on secondary lung injury caused by ischemic stroke by inhibiting neutrophil aggregation, inhibiting inflammatory response, and alleviating oxidative stress.

**Keywords:** Jiawei Yuping Fengsan granules; Ischemic stroke with secondary lung injury; Inflammatory factors

## 1. Introduction

Stroke associated pneumonia (SAP) is one of the most common complications after stroke, increasing the mortality and disability risk of stroke, as well as the clinical hospitalization time. At present, there is a lack of preventive treatment methods for SAP in clinical practice. This study utilized a rat model of cerebral ischemia-reperfusion lung injury to compare histopathology and detect changes in the expression of inflammatory factors and signaling proteins in serum and lung tissue through techniques such as ELISA and Western Blot. From a molecular biology perspective, the study explored the mechanism of action of Jiawei Yuping Fengsan Granules in treating post-stroke pneumonia, providing new ideas and guidance for clinical prevention and treatment.

## 2. Materials and Methods

### 2.1 Experimental animals and grouping

30 SPF grade male SD rats, weighing 200-250g, aged 7 weeks. Strictly follow the animal feeding rules for feeding. According to the principle and method of random grouping, the patients were divided into three groups: sham surgery group (sham group), cerebral ischemia-reperfusion group (IR group), Jiawei Yuping Fengsan Granules pre-treatment cerebral ischemia-reperfusion group (Y-IR group), sham surgery group: no intervention was given; IR group: equal amount of physiological saline; Jiawei Yuping Fengsan Granules 10mg/kg, administered orally to pre-treatment rats 3 days before modeling,

twice a day, with the last dose administered 3 hours before modeling.

Preparation of Jiawei Yuping Fengsan Granules: Formula: 30g of raw astragalus, 10g of *Atractylodes macrocephala*, 10g of Fangfeng, 10g of *Pinellia ternata*, 15g of *Gastrodia elata*, 10g of *Poria cocos*, 10g of dried tangerine peel, 6g of roasted licorice. Soak in sufficient amount of ultrapure water for 30 minutes, then simmer over low heat until boiling, then simmer over medium heat for 30 minutes, filter out the medicinal liquid, and add the same amount of ultrapure water to the remaining medicinal residue. After simmering for 15 minutes, filter out the medicinal liquid; Merge the filtrate twice, concentrate it using a rotary evaporator to a concentration of 1 mg/ml of the medicine, and store it at 4 °C for later use.

## 2.2 Model Preparation

After one week of adaptive feeding, male SD rats were intervened according to their respective groups. Both the IR group and Y-IR group used an improved suture method combined with tracheal injection of *Pseudomonas aeruginosa* to establish a rat model of post-stroke pneumonia. 12 hours before modeling, rats fasted and couldn't help but water. Anesthetized by intraperitoneal injection of 10% chloral hydrate (3 mL/kg), the rats were fixed in a supine position on a sterile operating table. After skin preparation, disinfection and drape were routinely spread, and a 1.0-1.5 cm incision was made in the center of the neck. The right cervical fascia and muscle tissue were bluntly separated to fully expose the surgical field. The right common carotid artery, vagus nerve, external carotid artery, and internal carotid artery were separated, and the proximal end of the common carotid artery and the proximal end of the external carotid artery were ligated. After using silk thread to block the blood supply to the internal carotid artery, a small incision is made horizontally on the blood vessel wall about 5 mm away from the bifurcation of the common carotid artery. Using tweezers, the prepared thread plug is slowly and gently inserted parallel to the direction of the blood vessel. At the same time, the silk thread is slowly relaxed and gently pushed about 1.8-2.0 cm. The end of the thread plug reaches the starting point of the middle cerebral artery to reduce resistance. Then, the thread plug is fixed, excess thread is cut off, and disinfected. Separate the trachea of rats, inject 0.2 mL of *Pseudomonas aeruginosa* bacterial suspension into a 1 mL syringe, then slowly stand the rats upright for 30 seconds to evenly distribute the bacterial suspension in both lungs, and finally suture the surgical incision. The operation steps of the Sham group are the same as those of the IR group, but without inserting wire ties or injecting *Pseudomonas aeruginosa* solution.

Sham group: Only bilateral femoral veins and femoral arteries were separated from rats, without clipping, and exposed for 5 hours; IR group: Ischemia for 3 hours, followed by removal of arterial clamp and reperfusion for 2 hours. The subsequent operations of Y-IR group are the same as those of IR group

## 2.3 Collecting Data

- 1) Total cell count and neutrophil percentage in bronchoalveolar lavage fluid (BALF)
- 2) Determination of oxidative stress factors (MDA, SOD) in rat lung tissue and blood

According to the instructions of the testing kit, the changes in MDA and SOD activity in samples such as serum and tissue are detected by colorimetric method.

- 3) Detection of inflammatory factors in lung tissue and serum of ELISA rats (TNF- $\alpha$ , IL-1 $\beta$  and IL-6)

## 2.4 Statistical analysis

Statistical analysis was performed on the data using SPSS 22.0 software, with econometric data presented as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). Univariate analysis of variance was used for inter group comparisons, and pairwise comparisons were conducted  $\chi^2$  tests, with  $P < 0.05$  as the significant difference.

### 3. Result

#### 3.1 Total cell count and neutrophil percentage in bronchoalveolar lavage fluid (BALF)

Compared with the Sham group, the expression levels of total cell count and neutrophil percentage in alveolar lavage fluid (BALF) of rats in the IR and Y-IR groups were significantly increased, with statistical significance ( $P < 0.05$ ); Compared with the IR group, the expression levels of total cell count and neutrophil percentage in alveolar lavage fluid (BALF) of rats in the Y-IR group were significantly reduced, with statistical significance ( $P < 0.05$ ). See Table 1

Table 1: Total cell count and neutrophil percentage in BALF ( $\bar{x} \pm s$ )

Group	Total cell count	neutrophil percentage(%)
Sham	443524±945124	6.54±1.32
IR	1506324±296354 <sup>a</sup>	68.24±7.42 <sup>a</sup>
Y-IR	872365±14623 <sup>ab</sup>	30.45±2.17 <sup>ab</sup>

a: Compared with the Sham group,  $P < 0.05$ ; b :Compared with the IR group,  $P < 0.05$

#### 3.2 Determination of oxidative stress factors (MDA, SOD) in lung tissue and blood of rats

Compared with the Sham group, the expression levels of MDA in lung tissue and serum of rats in the IR group and Y-IR group were significantly increased, while SOD activity was significantly decreased, with statistical significance ( $P < 0.05$ ); Compared with the IR group, the expression level of MDA in lung tissue and serum of rats in the Y-IR group significantly decreased, while SOD activity significantly increased, with statistical significance ( $P < 0.05$ ). See Table 2

Table 2: Comparison of oxidative stress damage levels in rat lung tissue and serum ( $\bar{x} \pm s$ )

Group	MDA		SOD	
	Lung tissue(pg/mg)	Serum (pg/m L)	Lung tissue(pg/mg)	Serum (pg/m L)
Sham	1.07±0.49	2.14±0.51	73.68±11.14	113.93±11.57
IR	4.24±0.83 <sup>a</sup>	5.18±0.66 <sup>a</sup>	31.69±8.65 <sup>a</sup>	51.28±6.22 <sup>a</sup>
Y-IR	2.93±0.54 <sup>ab</sup>	3.45±0.48 <sup>ab</sup>	51.69±12.71 <sup>ab</sup>	73.51±7.99 <sup>ab</sup>

a: Compared with the Sham group,  $P < 0.05$ ; b: Compared with the IR group,  $P < 0.05$

#### 3.3 Detection of inflammatory factors in lung tissue and serum of 3 rats (TNF- $\alpha$ , IL-1 $\beta$ and IL-6)

Compared with the Sham group, in the lung tissue and serum of rats in the IR and Y-IR groups, The expression level of TNF - $\alpha$ , IL-1 $\beta$  and IL-6 content was significantly increased, and the difference was statistically significant ( $P < 0.05$ ); Compared to the IR group, in the lung tissue and serum of rats in the Y-IR group, the expression level of TNF - $\alpha$ , IL-1 $\beta$  and IL-6 content nt significantly decreased, and the difference was statistically significant ( $P < 0.05$ ). See Table 3

Table 3: Comparison of inflammatory factors in rat lung tissue and serum ( $\bar{x} \pm s$ )

Group	IL-1 $\beta$		IL-6		TNF- $\alpha$	
	Lung tissue(pg/mg)	Serum (pg/m L)	Lung tissue(pg/mg)	Serum (pg/m L)	Lung tissue(pg/mg)	Serum (pg/m L)
Sham	147.150±38.18	171.83±52.55	273.51±91.20	548.37±130.02	32.39±8.45	68.64±10.31
IR	385.81±103.53 <sup>a</sup>	495.88±101.47 <sup>a</sup>	1003.52±134.04 <sup>a</sup>	1430.64±139.27 <sup>a</sup>	76.27±13.97 <sup>a</sup>	129.96±12.37 <sup>a</sup>
Y-IR	268.32±79.91 <sup>ab</sup>	332.98±106.49 <sup>ab</sup>	782.22±190.10 <sup>ab</sup>	1016.18±168.62 <sup>ab</sup>	57.83±13.62 <sup>ab</sup>	108.28±8.75 <sup>ab</sup>

a: Compared with the Sham group,  $P < 0.05$ ; b: Compared with the IR group,  $P < 0.05$

### 4. Discuss

Cerebral infarction combined with pneumonia can be considered as "stroke" combined with "pulmonary fever" and "cough". Research has found that the distribution of traditional Chinese medicine syndrome elements and types in stroke complicated with pneumonia focuses on phlegm dampness and blood fatigue. At present, many research treatments are based on common principles such as calming the liver and suppressing wind, clearing heat and phlegm, resolving phlegm and

unlocking the organs, promoting blood circulation and removing blood stasis, and awakening the mind and opening the orifices. The spleen is the source of qi, blood, and biochemistry, nourishing the essence of vital energy, and serving as the central immune regulatory center of the body. The origin of the Yuping Fensan Granules comes from the "Study of the Origin Formula", which can nourish qi, solidify the surface, and stop sweating. Huangqi in the formula is sweet and warm. It can greatly nourish the qi of the spleen and lungs inside, and can solidify the surface and stop sweating outside. It is a medicinal herb for the emperor. Baizhu can strengthen the spleen and qi, and help Huangqi strengthen the power of nourishing qi and consolidating the surface. It is a medicinal herb for the minister<sup>[1]</sup>. When two medicines are used together, if the qi is strong and the surface is solid, sweat will not flow out, and evil energy will be difficult to invade. Assisted in preventing wind and dispelling pathogenic factors, combined with *Astragalus membranaceus* and *Atractylodes macrocephala* to strengthen the body, and also used to dispel pathogenic factors. On the basis of Yuping Fensan Granules, it is combined with *Qingbanxia* to eliminate dampness and phlegm, *Poria cocos* to invigorate the spleen and absorb dampness, *Chenpi* to regulate phlegm, *Tianma* to calm the liver and extinguish wind, and licorice to harmonize various herbs, tonifying qi and harmonizing the middle. The above medicines collectively have the effects of tonifying qi, invigorating the spleen, consolidating the exterior, stopping sweating, resolving phlegm, and suppressing wind.

The percentage of neutrophils counted in bronchoalveolar lavage fluid (BALF) can intuitively reflect the severity of acute lung injury. Neutrophils are an important component of innate immunity and play an important role in maintaining respiratory homeostasis<sup>[2]</sup>. Neutrophils can penetrate from endothelial cells to the inflammatory site and activate, releasing cytotoxic substances. However, excessive activation can lead to host cell damage and inflammation related diseases. From the experiment, it can be seen that compared to the IR group, the expression levels of total cell count and neutrophil percentage in alveolar lavage fluid (BALF) of rats in the Y-IR group were significantly reduced, with statistical significance ( $P < 0.05$ ). This indicates that the pre-treatment of Jiawei Yuping Fensan granules has a certain degree of anti neutrophil aggregation and protective effect on lung injury caused by reperfusion.

MDA is a product of lipid peroxidation, produced through the action of oxygen free radicals on unsaturated fatty acids in biofilms. Its main function is to undergo oxidation reactions with deoxyribonucleic acid and proteins in the body, and its content can represent changes in the content of oxygen free radicals in the body. The clearance of oxygen free radicals in the body mainly relies on the antioxidant enzyme system, and SOD is one of the important antioxidant enzymes in the body. Its determination can reflect the ability of tissues to resist lipid peroxidation<sup>[3]</sup>. Oxygen free radicals play a crucial role in lung injury caused by cerebral ischemia-reperfusion. Lipid peroxidation metabolites start to act when a body experiences cerebral ischemia, and during cerebral ischemia-reperfusion, they can act as electron acceptors and enter the ischemic tissue of the body, leading to a continuous increase in oxygen free radicals and accompanying blood circulation into the human lungs. This exacerbates the lipid peroxidation reaction in the lungs, causing structural damage to the lung tissue and a significant increase in the molecular weight of apoptosis in the lung tissue, resulting in abnormal lung function. Our experimental results showed that compared to the IR group, the expression level of MDA in lung tissue and serum of rats in the Y-IR group significantly decreased, while SOD activity significantly increased, with statistical significance ( $P < 0.05$ ). It indicates that the organization may have suffered lipid peroxide damage and there may be obstacles in clearing oxygen free radicals in the body. At the same time, it also indicates that Jiawei Yuping Fensan granules have a certain degree of anti free radical damage, which in turn plays a protective role on the distal organs of ischemia-reperfusion.

Studies have shown that during cerebral ischemia-reperfusion, The expression of the inflammatory cytokine TNF- $\alpha$ , IL-6 and IL-1 $\beta$  is significantly increased and significantly higher than the expression of TNF- $\alpha$  in lung tissue. This suggests that brain tissue is one of the important early organs involved in inflammation mediators such as IL-6 and TNF- $\alpha$ . So how do these inflammatory cytokines cause lung damage? Among these cytokines, TNF- $\alpha$  May play a leading role as TNF- $\alpha$  It has multiple biological effects and can trigger a series of chain reactions in the body, including the regulation of immune function and inflammatory lesions. Through a series of related studies, it is suggested that TNF- $\alpha$  Lung injury is caused by the following pathways<sup>[4-5]</sup>: (1) Inflammatory mediators such as IL-6 and TNF- $\alpha$  enter lung tissue via veins, leading to activation and release of large amounts of TNF- $\alpha$  by pulmonary macrophages. TNF- $\alpha$  activates the corresponding signaling pathway and the transcription factor NF- $\kappa$ B, leading to a cascade reaction of monocyte macrophage secretion of IL-6, IL-1 $\beta$  and IL-8 which can cause lung injury; (2) TNF- $\alpha$  released into the blood stream can also activate the Caspase family enzymes, induce cell apoptosis and cause lung injury; (3) TNF- $\alpha$  released into the bloodstream can also activate PMN, which can release free radicals and proteolytic enzymes leading to lung injury. In

our study, we found that compared with the Sham group, the expression level of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 content in the lung tissue and serum of rats in the IR and Y-IR groups was significantly increased, and the difference was statistically significant ( $P < 0.05$ ); Compared to the IR group, the expression level of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 in the lung tissue and serum of rats in the Y-IR group significantly decreased, and the difference was statistically significant ( $P < 0.05$ ). It can be seen that Jiawei Yuping Fengsan granules can protect ischemia-reperfusion organs by inhibiting the expression of inflammatory factors during the anti-inflammatory process.

## 5. Conclusion

In summary, Jiawei Yuping Fengsan granules have a protective effect on secondary lung injury caused by ischemic stroke by inhibiting neutrophil aggregation, inhibiting inflammatory response, and alleviating oxidative stress.

## Acknowledgments

This paper was funded by Shandong Traditional Chinese Medicine Technology Development Project (M-2023030);

## References

- [1] Feng Feng, Meng Yufeng, Dai Zhong, Guo Fang, Feng Cuiling Comparison of the regulatory effects and mechanisms of modified allergic decoction and Yupingfeng powder on Th17/Treg cell balance in PM2.5 induced lung injury rats [J] *Journal of Beijing University of Traditional Chinese Medicine*, 2020, 43 (11): 910-917
- [2] Lin Chuyue, He Jinbo, Wang Xiaoting, et al. Dexmedetomidine affects NF -  $\kappa$  B expression alleviates ischemia-reperfusion lung injury in rats [J]. *Strait Pharmacology*, 2022, 34 (09): 8-12
- [3] Chen Tingting, Tuerxun Ay Maimaiti, Chen Siyu, etc. Resveratrol regulates Sirt1 to alleviate endoplasmic reticulum stress associated with acute lung injury after myocardial ischemia/reperfusion in diabetes [J]. *Shaanxi Traditional Chinese Medicine*, 2022, 43 (04): 437-441
- [4] Huang Yi, Yuan Dezhi, Li Jinfang. Mechanisms and treatment progress of acute lung injury secondary to cerebral ischemia/reperfusion [J]. *Chinese Journal of Clinical Neuroscience*, 2021, 29 (06): 666-670
- [5] Lu Guoneng, Lan Shengkai, Hu Chunjiang, et al. Study on the protective effect of ginkgo biloba extract injection pre administered on lung injury caused by lower limb ischemia-reperfusion [J]. *Jilin Medical Journal*, 2020, 41 (11): 2607-2610