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Mechanism of curcumin inhibiting pyroptosis in infectious acute lung injury through NLRP3 inflammatory pathway

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Abstract

Purpose of this study the mechanism of curcumin inhibiting pyroptosis in infectious acute lung injury through NLRP3 inflammatory pathway. In this study, SPF SD male rats were randomly divided into blank control group, curcumin group, lipopolysaccharide (LPS) group, LPS + curcumin group, LPS + Nigerin treatment group, LPS + Nigerin + curcumin treatment group. Three in each group. Animal models were established according to the experimental design. Blood samples were collected after anesthesia, and the lung wet/dry weight ratio was calculated. TUNEL was used to detect pyroptosis of alveolar macrophages in left lower lobe, and Elisa was used to detect the expression levels of inflammatory factors IL-1β and IL-18 in left lung tissue. Western Blot was used to detect the expression of pyroptosis-related proteins such as NLRP3, Caspase1 and GSDMD in right lung alveolar lavage fluid. Compared with the blank control group, the lung wet weight and lung dry weight of the curcumin group were significantly increased ($P < 0.01$, $P < 0.05$). Compared with the LPS group, the lung wet weight and lung dry weight of the curcumin group There was a statistical difference in weight increase ($P < 0.01$). Compared with the blank control group, the apoptosis rate of lung tissue in the curcumin group, LPS group and LPS+ nigericin group was significantly increased ($P < 0.01$). Compared with the LPS + nigericin group, the lung tissue apoptosis rate in the LPS + curcumin + nigericin group was decreased, but there was no statistical difference ($P > 0.05$). LPS and LPS combined with Nigerian bacteriocin can cause pyroptosis in lung tissue. Curcumin can inhibit pyroptosis of lung tissue macrophages caused by LPS and LPS combined with Nigerian bacteriocin, and reduce inflammatory response.

Keywords: curcumin; nLRP3; cell pyroptosis; acute lung injury.

Practical Application: Curcumin can inhibit pyroptosis of lung tissue macrophages caused by LPS and LPS combined with Nigerian bacteriocin, and reduce inflammatory response.

1 Introduction

Acute lung injury (ALI) is an acute respiratory failure induced by infection, trauma, acute pancreatitis, burn and aspiration. The morbidity and mortality are very high. It is characterized by refractory hypoxia, impaired gas exchange, excessive inflammatory response and acute respiratory failure (Matthay et al., 2020). Acute lung injury is common in ICU patients, and the main cause is sepsis (Johnson et al., 2018). Studies have found that pyroptosis related genes Caspase-1 and NLRP3 can affect LPS-induced lung injury in mice, indicating that pyroptosis plays an important role in the pathogenesis of sepsis and ALI (Wang, 2019). Cell pyroptosis, as a programmed cell death pathway, is a form of cell death caused by activated inflammatory cells, in which the inflammatory cells are represented by nucleotide-binding oligomeric domain-like receptor (NLR) family (Li et al., 2021). Studies have shown that curcumin has a wide range of pharmacological activities, including antioxidant, anti-inflammatory and antibacterial properties, which can inhibit NLRP3 inflammatory bodies involved in intracellular signal transduction pathways in inflammation and reduce their expression levels (Saeedi-Boroujeni et al., 2021). However, the specific mechanism of pyroptosis in acute lung injury remains to be reported (Xiao et al., 2020). In order to further investigate the mechanism of curcumin in pyroptosis of acute lung injury cells, the NLRP3 pathway closely related to pyroptosis was used in this study to explore the effect of curcumin on pyroptosis of macrophages in mice with acute lung injury.

2 Materials and methods

2.1 Material

Experimental animals

Eighteen SPF SD male rats, weighing 180-220g, were provided by Zhuhai Baishitong Biotechnology Co., Ltd., production permit SCXK (Guangdong) 2020-0051, adaptive feeding for 3 days, feeding temperature and humidity: 20 ~ 26 °C, 40 ~ 70%, during

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the free drinking water and diet, using 12 h: 12 h day and night intermittent lighting.

Awarded by the institutional ethics committee of The Fourth Affiliated Hospital of Guangzhou Medical University under the institutional guidelines on animal care and use.

Main reagent

curcumin (Production Batch No.: C10901808, Shanghai McLin Biotech Co., Ltd.); lipopolysaccharide (Biosharp white shark); trypan blue (Production Batch No.: 20180612 Beijing Mengyimei Biotechnology Co., Ltd.); f12K medium (Beijing Solebo Biotechnology Co., Ltd.); fetal bovine serum (production batch number: SA201126, Wuhan Punuosai Life Science and Technology Co., Ltd.); double antibody (streptomycin + penicillin) (production batch number: J200049, Situofan Biotechnology Co., Ltd.); eLISA kit (Wuhan Elite Biotechnology Co., Ltd.); nigerian bacteriocin (production batch number: C13127938, McLin Company) ; phosphatase inhibitor, BCA protein content detection kit (Nanjing Kaiji Biological Development Co., Ltd); pageRμLer Prestained Protein Ladder (Production Batch No. 26617, Thermo); internal reference antibody information, dilution multiple: 1: 10000.

Main instruments

Carbon Dioxide incubator, Semerfel Technology Company ; low speed centrifuge, Hunan Xiangyi Laboratory Instrument Development Co., Ltd.; high speed centrifuge, Pearl River Black Horse; ultrasonic cell broken instrument, Ningbo Xinzhi Biotechnology Co., Ltd.; electrophoresis instrument, Beijing Baijing Biotechnology Co., Ltd.; -80 °C ultra low temperature refrigerator, Qingdao Haier Group; 4 °C, -20 °C refrigerator, Hefei Meiling Co., Ltd.

2.2 Method

Animal grouping and model establishment

After adaptive feeding for 3 days, 18 rats were quarantined. The rats were randomly divided into 6 groups: blank control group, curcumin group, LPS group, LPS + curcumin group, LPS + Nigerin treatment group, LPS + Nigerin + curcumin treatment group, 3 rats/group. The combination of LPS and LPS + Nigerin group was used to induce acute lung injury animal model. The specific operation of all experimental groups was as follows:

Blank control group: intraperitoneal injection of 5% DMSO ; normal diet, 1/24 h, continuous 5 times.

Curcumin group: Rats were intraperitoneally injected with curcumin solution (100 mg/kg), normal diet, once/24 h, for five consecutive times.

LPS group (model group): Before modeling, the rats were fasted for 12 h without water, and LPS (2 mg/kg) was injected intraperitoneally. After modeling, the rats were given feed for half an hour, and then fasted for three times after 12 h.

LPS + curcumin group: fasting 12 h before modeling, then intraperitoneal injection of LPS (2 mg/kg) + curcumin solution (100 mg/kg). After half an hour of administration, the rats were fed with feed. After 12 hours, the rats were fasted. The LPS model was established three times, and the curcumin was given five times.

LPS + Nigeria bacteriocin treatment group (model group): rats were fasted without water for 12 hours before modeling, LPS (2 mg/kg) + Nigeria bacteriocin (1 mg/kg) was injected intraperitoneally, and feed was given half an hour after modeling. After 12 hours, rats were fasted for 3 times.

LPS + Nigeria colistin + curcumin treatment group: fasting 12 h before modeling, intraperitoneal injection of LPS (2 mg/kg) + Nigeria colistin (1 mg/kg) + curcumin solution (100 mg/kg), half an hour after administration to feed, 12 h after fasting, LPS + Nigeria colistin was given three times, curcumin was given five times (see Table 1 for the dosing scale).

Samples

There were collected according to the experimental design, weighed, and collected blood from abdominal aorta after isoflurane inhalation anesthesia. After 24 h of storage at 4 – 8 °C, the samples were centrifuged at 1 500 r/min for 5 min, and the serum was separated and stored at – 80 °C for further use. The left upper lobe of the lung was dried in a 65 °C thermostat and weighed after 24 hours. The lung wet/dry weight ratio was calculated. Part of the left lower lobe of the lung was preserved in 4% paraformaldehyde for TUNEL detection. The pyroptosis of alveolar macrophages in all rats was observed. The other part was preserved at -80 °C. The levels of inflammatory factors such as IL-1β and IL-18 in lung tissue were detected by Elisa method. Bronchoalveolar lavage was performed on the right lung. Bronchoalveolar lavage was performed three times with pre-cooling (4 degrees) sterile saline 1 mL, a total of 3 mL. Bronchoalveolar lavage fluid (BALF) was recovered to the sterile centrifuge tube, 1500 r/min, centrifuged for 10 min, and the supernatant was collected and stored at -80 °C.

Paraffin section

There were immersed in xylene for 15 min at room temperature and then replaced with xylene for 15 min; soak in anhydrous ethanol for 5 min, 95% ethanol for 5 min, 90% ethanol for 5 min, 80% ethanol for5min, 70% ethanol for5min, distilled water for5min. Sealing with 3% H2O2 for 15 min at room temperature and washing with distilled water for 5 min each. Put the tissue slices into 0.01M sodium citrate buffer solution (pH 6.0), heated in microwave oven for 4 min to boiling, powered off, washed three times with PBS for 5 min at room temperature. Add 5% BSA blocking solution, room temperature 20 min, remove more liquid. Add TUNNEL reaction mixture (reaction mixture: every 45 μL Lable Solution, add 5 μL Solution) to cover the enzyme

Table 1. Dose design.

Group	Dosage	Concentration	Dosage
Curcumin	100 mg/kg	20 mg/mL	5 mL/kg
LPS	2 mg/kg	0.4 mg/mL	5 mL/kg
Nigerian bacteriocin	1 mg/kg	0.2 mg/mL	5 mL/kg

film, 37 °C 1 h. The coating film was removed and immersed in PBS at room temperature for 3 times, 5 min each time. Add Converter - POD reaction solution, cover the membrane, 37 °C incubation 30 min. The coating film was removed and immersed in PBS at room temperature for 3 times, 5 min each time. Add DAB color solution, under the microscope to grasp the degree of color (apoptotic cells were brown, normal cells blue). Wash with deionized water to terminate the reaction, dehydrate, seal transparently, microscopically.

Under light microscope, the magnification of all slices was 400 ×. The expression of Tunel was observed in 4 fields per slice and the apoptosis rate was counted (note: (apoptosis rate = (number of apoptotic cells/total cells) * 100%); the apoptosis rate in the table is actually the average of the apoptosis rate in four fields of vision)

ELISA

Determination of IL-6, IL-1β, TNF-α and MCP-1 levels in serum and BALF was performed in strict accordance with the instructions of ELISA kits

Western blot

Detection of NLRP3 and GSDMD protein expression in lung tissue.

Total protein extraction

10 μL PMSF (100 mM) and 10 μL Cocktail were added into 1 mL lysis buffer and shaken on ice. Each tube sample was added with 100 μL lysis solution containing PMSF and lysed on ice for 30 min. In order to make the cells fully lysed, the cells were often shaken back and forth. After pyrolysis, centrifuged at 14000 rpm for 5 min at 4 °C. The supernatant after centrifugation was transferred to a clean centrifuge tube and stored at -80 °C. The extracted protein solution and 5 × buffer were mixed at 4:1, boiled for 10 min, slowly restored to room temperature, slightly centrifuged, stored at -20 °C.

Preliminary quantification of protein samples

Dilution test sample: 2 μL of each sample was diluted 20 times with 38 μL H2O. They are arranged in order. Preparation of BCA reagent concentration determination working fluid: take 50 volume A liquid, add 1 volume B liquid, fully mixed, now available. Preparation of 96-well plate, take 20 μL diluted standard samples and experimental samples in 96-well plate. 200 μL working fluid was added to each well and incubated at 37 °C for 30 min. The absorbance (OD value) was read in the microplate reader, and the wavelength was 560 nm.

SDS-PAGE electrophoresis

Before electrophoresis, each well was washed with 1 × electrophoresis buffer. 1 × electrophoresis buffer was added to the upper and lower electrophoresis tanks, and the buffer level in the upper tank should exceed the top of the sample hole. According to sample loading order and sample loading volume in order. Electrophoresis: 80V constant voltage electrophoresis to bromophenol blue to separation gel, 120V constant voltage electrophoresis to bromophenol blue just out of the gel bottom.

Protein transfer

The PVDF membrane was pretreated with methanol for 3 ~ 5 seconds and immersed in the transfer buffer for half an hour. Remove the gel, put it on the filter paper, forming the gel transfer deposition layer, filter paper, gel, PVDF membrane, filter paper, gel transfer deposition layer such a sandwich structure. This operation must completely remove the bubbles. Place the transfer clamp in the positive and negative directions. At low temperature, 100V constant pressure 116 minutes.

Immune imprinting

Remove hybrid membrane, TBST rinse 5 min, 1 time. 5% skim milk powder solution closed at room temperature for 1 hour. TBST membrane washing 10 min, 1 time. Suitable dilution concentration of primary antibody 4 °C overnight. TBST membrane washing 10 min, 4 times. The corresponding secondary antibody diluent was incubated at 37 °C for 1 h. TBST membrane washing 10 min, 4 times. Place the hybrid film on a transparent plastic plate, not to let the film dry. The chemiluminescent substrate was evenly added to the surface of the membrane with a clean liquid shifter, and the reaction lasted for 5 min. Use the filter paper provided by the kit to absorb the substrate solution on the membrane surface and put it into the dark box Photography.

2.3 Statistical method

SPSS22.0 software was used for data analysis and comparison. The measurement data were expressed as $\bar{x} \pm s$, and t test was used for comparison. The enumeration data were expressed as rate (%), and the comparison was performed by χ^2 test; $p < 0.05$ indicated that the difference was statistically significant, and $P < 0.01$ indicated that the difference was statistically significant.

3 Results

3.1 General observation

After animal modeling, mental retardation and slight weight loss occurred.

Effect of wet/dry lung weight (W/D) on mice in each group

As shown in Table 2, compared with the blank control group, the lung wet weight and lung dry weight in the curcumin group were significantly increased ($P < 0.05$). Compared with LPS group, the increase of lung wet weight and lung dry weight in curcumin group was statistically significant ($P < 0.05$) (Figure 1).

3.2 Apoptosis rate of lung tissue

As shown in Table 3, compared with the blank control group, the apoptosis rates of lung tissue in curcumin group and LPS group were significantly increased ($P < 0.05$).

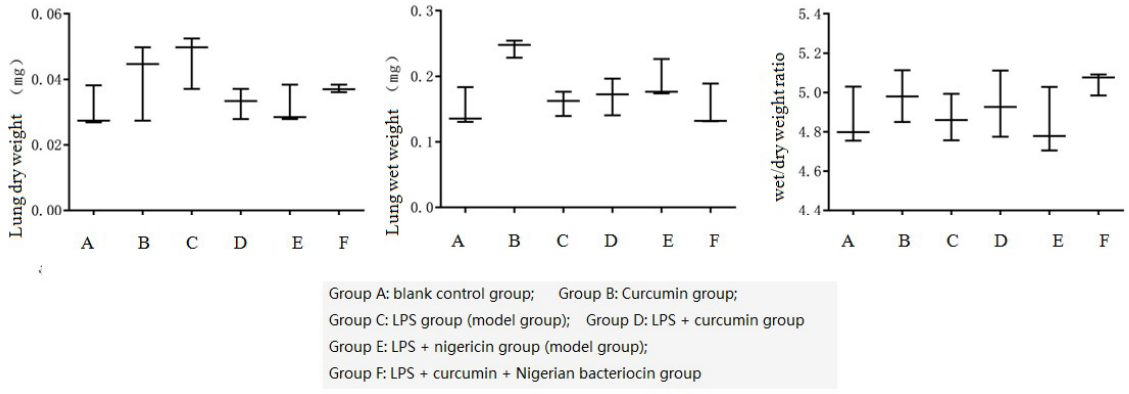

Figure 1. Wet/dry weight ratio of rat lung tissue.

Table 2. Wet/dry weight of animal lung ($\bar{x} \pm s$).

Group	N	Lung wet weight	Lung dry weight	Wet / dry weight
Blank control group	3	149.6 ± 29.3	30.7 ± 6.5	4.9 ± 0.1
Curcumin group	3	243.7 ± 13.5#**	49.0 ± 4.0#**	5.0 ± 0.1
LPS group (model group)	3	159.4 ± 18.8	32.8 ± 4.6	4.9 ± 0.1
LPS + curcumin group	3	169.7 ± 28	34.3 ± 5.2	4.9 ± 0.2
LPS + Nigerian bacteriocin group (model group)	3	192.4 ± 29.6	39.8 ± 6.6	4.8 ± 0.2
LPS + curcumin + Nigerian bacteriocin group	3	150.8 ± 33	29.8 ± 6.3	5.1 ± 0.1

Note: using one-way ANOVA and rank sum test, compared with the blank control group, "#" P < 0.05, "##" P < 0.01, compared with LPS group, "* *" P < 0.01.

As shown in Table 4, compared with the blank control group, the increase in the apoptosis rate of lung tissue in the LPS + Nigerian bacteriocin group was statistically significant ($P < 0.05$) (Figure 2 and 3).

3.3 Levels of IL-18 and IL-1β in lung tissue

As shown in Table 5, there was no significant difference in IL-18 and IL-1β between each group (P > 0.05). The levels of IL-18 and IL-1β in each group were higher than those in the blank control group, but there was no statistical difference ($P > 0.05$). IL-18 and IL-1β levels in curcumin group, LPS + curcumin group, LPS + Nigerian bacteriocin group and LPS + curcumin + Nigerian bacteriocin group were lower than those in LPS group, but there was no statistical difference ($P > 0.05$) (Figure 4).

3.4 Western blot to detect the expression of NLRP3 and GSDMD proteins induced by LPS and Nigerian bacteria in pyroptosis cells .

As shown in Table 6, compared with the blank control group, the Caspase-1 of curcumin group decreased slightly, NLRP3 and GSDMD showed an increasing trend, and the trend of three apoptotic proteins was inconsistent. Compared with the LPS group, the NLRP3 and Caspase-1 apoptosis proteins in the curcumin + LPS group were increased, and the GSDMD apoptosis proteins were decreased, and the trends of the three apoptosis proteins were inconsistent.

Compared with the LPS + Nigerian bacteriocin group, the NLRP3, Caspase-1 and GSDMD three apoptotic proteins in the LPS + Nigerian bacteriocin + curcumin group were all decreased consistently, and the decrease of Caspase-1 and GSDMD two regulatory proteins was large, indicating that under the conditions of this model (Figure 5).

Table 3. Comparison of apoptosis rate in lung tissue of rats in each group ($\bar{x} \pm s$, n = 3).

Group	N	Apoptosis rate (%)	*P*
Blank control group	3	14.9 ± 7.6	
Curcumin group	3	46.5 ± 4.1##	$P_1 = 0.003$
LPS group	3	53.9 ± 3.3##	$P_1 = 0.001$
LPS + curcumin group	3	45.8 ± 5.9	$P_2 = 0.107$

Note: using one-way ANOVA and rank sum test, compared with the blank control group, "#" P1 < 0.05 and "##" P1 < 0.01; Compared with LPS group, "*" P2 < 0.05.

Table 4. Comparison of apoptosis rate in lung tissue of rats in each group ($\bar{x} \pm s$, n = 3).

group	N	Apoptosis rate (%)	*P*
Blank control group	3	14.9 ± 7.6	
LPS + Nigerian bacteriocin group	3	62.5 ± 5.5##	$P_1 = 0.001$
LPS + curcumin + Nigerian bacteriocin group	3	50.2 ± 8.1	$P_2 = 0.117$

Note: using one-way ANOVA and rank sum test, compared with the blank control group, "##" P1 < 0.01; Compared with LPS group, "*" P2 < 0.05.

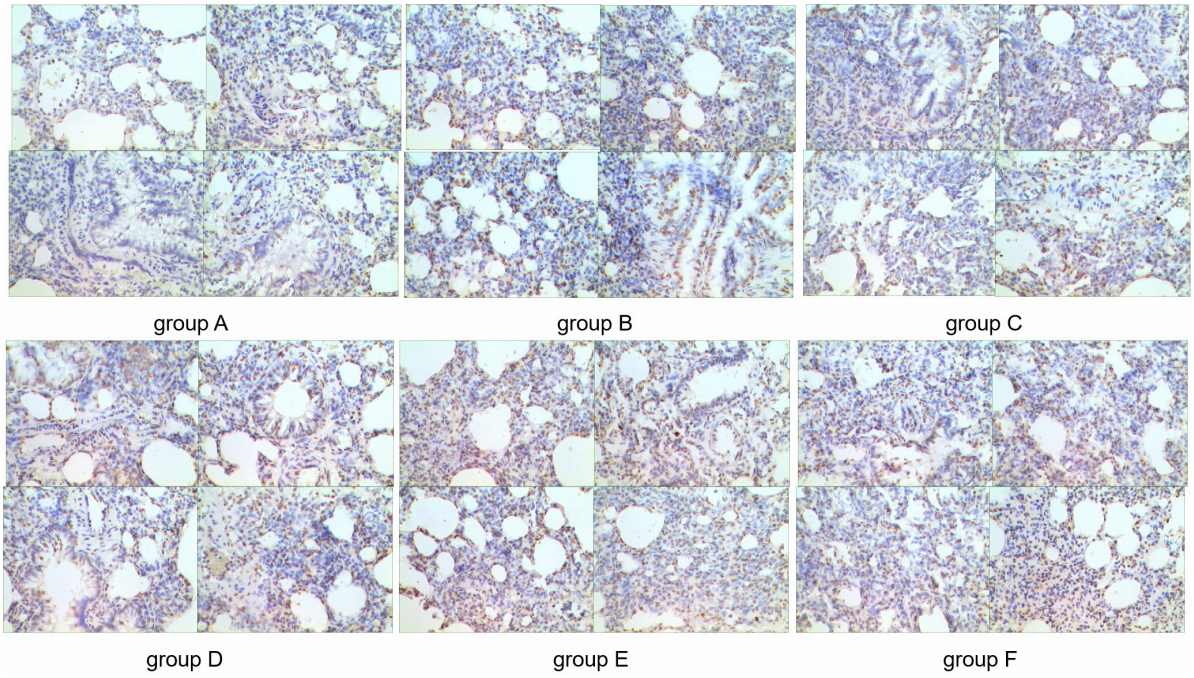

Figure 2. Comparison of apoptosis rate of lung tissue cells by TUNEL staining.

Table 5. IL-18 and IL-1 in lung tissue of rats in each group β Level comparison ($\bar{x} \pm s$).

group	N	IL-18 (pg/mL)	IL-1β (pg/mL)
Blank control group	3	14.7 ± 4.3	5.11 ± 0.94
Curcumin group	3	21.6 ± 5.5	6.64 ± 1.21
LPS group	3	23.2 ± 19.3	9.20 ± 4.25
LPS + curcumin group	3	16.0 ± 2.3	5.40 ± 0.51
LPS + Nigerian bacteriocin group	3	17.9 ± 4.5	5.83 ± 0.99
LPS + curcumin + Nigerian bacteriocin group	3	15.3 ± 3.0	5.24 ± 0.67

Note: using one-way ANOVA and rank sum test, compared with the blank control group; "#" $P < 0.05$; Compared with LPS group, "*" $P < 0.05$.

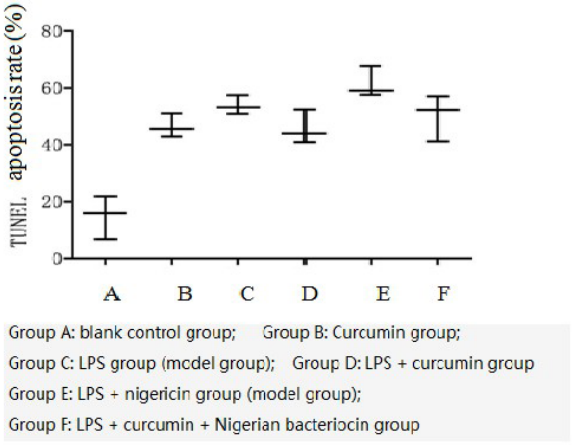

Figure 3. Comparison of apoptosis rate in each group.

4 Discussion

Acute lung injury is a common life-threatening respiratory system disease in clinical practice. It is characterized by refractory hypoxemia and lung compliance reduction, and the imbalance of the ratio of severe ventilation to blood flow. It is characterized by increased pulmonary capillary permeability and a large number of inflammatory cells in the lungs (Zeng et al., 2019). More and more evidences show that curcumin has strong antioxidant and anti-inflammatory properties, and has little side effects on human body (Wan, 2020). It is worth noting that the inhibitory effect of curcumin on NLRP3 - mediated inflammatory pathway has been reported ; for example, Qu et al. (2021) proved that curcumin effectively improved mouse colitis induced by sodium dextran sulfate (dSS) by inhibiting the activation of NLRP3 inflammasome ; this study also reported that the use of specific NLRP3 inhibitors to inhibit NLRP3 inflammatory bodies in vivo could significantly eliminate the additional inhibitory effect of curcumin on DSS-induced inflammatory bowel disease. Since NLRP3 activation plays a central role in LSP-induced ALI (Zhou et al., 2020) . This study aims to investigate whether curcumin can regulate NLRP3 inflammasome activation to improve LPS and LPS + Nigeria colistin-induced acute lung injury.

Alveolar macrophages are closely related to the occurrence of acute lung injury (Hu et al., 2017). When the lung is stimulated by exogenous pathogenic factors, pulmonary macrophages will recognize by pattern recognition receptors (including toll like receptor, NR4A1 receptor, nod like receptor and classical inflammatory body composed of pyhin like protein family, adapter protein and caspase-1), open the body's first immune defense and release a large number of inflammatory mediators, Cause diffuse inflammatory changes in lung tissue, infiltration

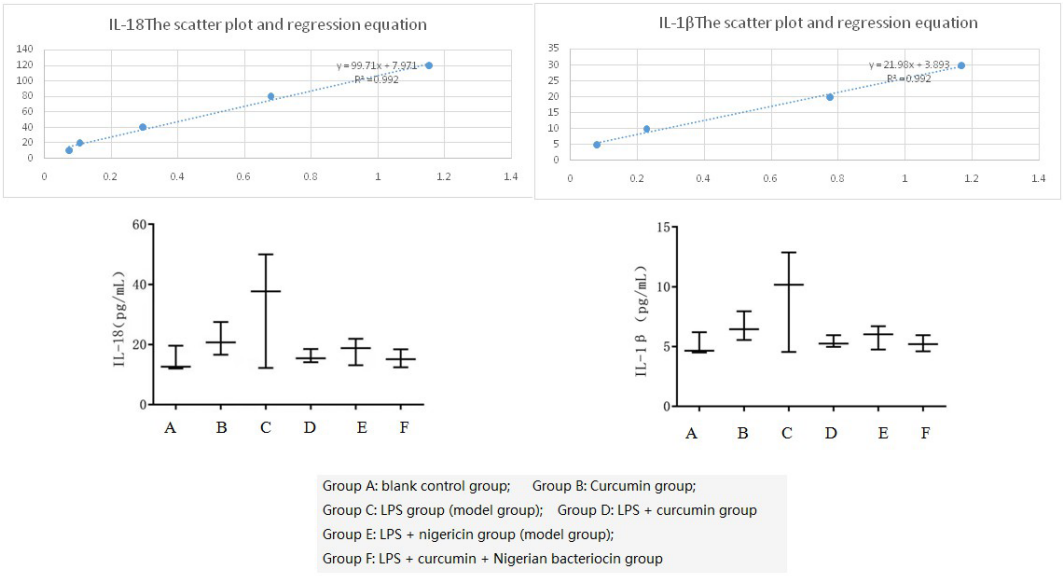

Figure 4. IL-18 and IL-1 in lung tissue homogenate of rats in each group β level.

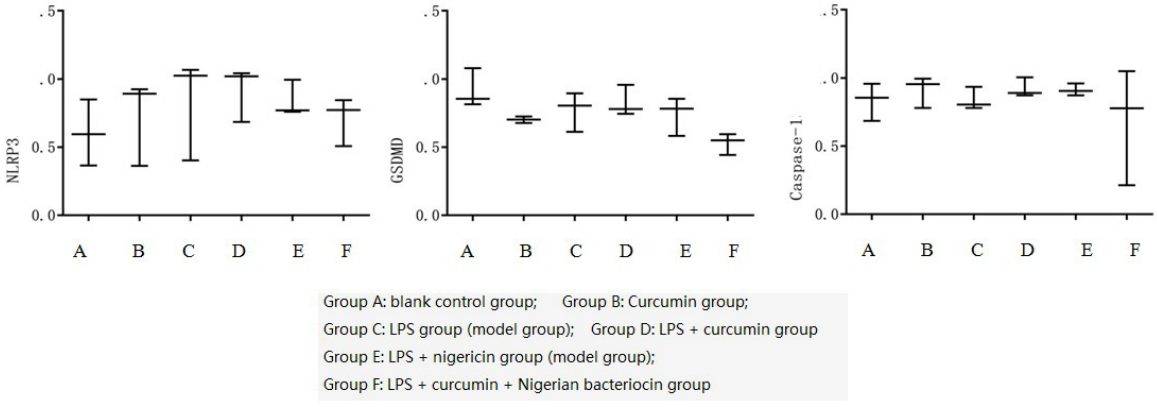

Figure 5. Expression of NLRP3, gsdmd and caspase-1 protein in lung tissue of rats in each group.

Table 6. Expression of NLRP3 and gsdmd proteins in cells detected by Western blot ($\bar{x} \pm s$, g).

Group Blank control group	N	Ratio of gray value to internal parameter		
		NLRP3	Caspase-1	GSDMD
Curcumin group	1	0.324 ± 0.088	0.600 ± 0.191	0.706 ± 0.178
LPS group	1	0.411 ± 0.099	0.593 ± 0.150	0.935 ± 0.241
LPS + curcumin group	1	0.592 ± 0.066*	0.704 ± 0.336	1.017 ± 0.176
LPS + Nigerian bacteriocin group	1	0.622 ± 0.072	0.828 ± 0.406	0.877 ± 0.228
LPS + curcumin + Nigerian bacteriocin group	1	0.522 ± 0.065*	0.688 ± 0.218	0.691 ± 0.152
group	1	0.345 ± 0.092	0.675 ± 0.163	0.546 ± 0.142

Note: using independent sample t-test analysis, compared with the blank control group, "*" $P < 0.05$.

of inflammatory cells in alveoli and alveolar edema (Freire & Conneely, 2018; Koppenol-Raab et al., 2017). The results of comparing the lung wet weight/dry weight (w/D) of mice in each group showed that the lung dry wet ratio of LPS + curcumin + nigericin group was higher than that of the blank group ($P < 0.01$, $P < 0.05$). The lung dry wet ratio of curcumin group was significantly different from that of LPS group ($P < 0.01$), indicating that curcumin group, LPS group, LPS + curcumin group and LPS + nigericin group (model group) may induce inflammatory reaction.

When the classical inflammatory NLRP3 is activated in acute lung injury, it will promote the activation of Caspase-1 precursor. Activated caspase-1 can promote inactive interleukin proteins (such as proil-1) by shearing β、 Proil-6, etc.) becomes active IL-1 β、 IL-6 induces inflammatory response (McRae et al., 2018). Pyrosis is different from other forms of programmed cell death. It mainly depends on caspase-1, 4, 5 and 11. Its basic characteristics are cell swelling, formation of plasma membrane pores (dissolution) and release of proinflammatory cytokine interleukin-1 β (IL-1 β) And IL-18 (Wang et al., 2021; Lu et al., 2020), but the essence is still a new way of cell death represented by NLR family. Compared with the blank group, macrophages and apoptosis regulating cells in curcumin group, LPS group and LPS + nigericin group increased significantly ($P < 0.01$); The apoptotic cells of lung tissue in LPS + curcumin group were less than those in LPS group, but there was no significant difference ($P > 0.05$); The apoptotic cells of lung tissue in LPS + curcumin + nigericin group were less than those in LPS + nigericin group, but there was no significant difference ($P > 0.05$). This suggests that LPS and the combination of LPS and nigericin can cause apoptosis in lung tissue, and curcumin may promote LPS induced apoptosis.

In recent years, more and more studies have found that cell scorch death in the state of infection is closely related to the inflammatory response of the body. In LPS and LPS / Nigerian bacteriocin induced cell death, LPS can bind to toll like receptor 4 (TLR4) and promote the transcription of inflammatory mediators. Nigerian bacteriocin can activate inflammatory bodies by activating inflammatory bodies, recruiting caspase-1 and making it shear itself, and then activate. Activated caspase-1 can shear IL-1 β And IL-18, making it an activated form. At the same time, gsdmd, a component of inflammatory bodies, shears and participates in the formation of cell membrane pores. On the one hand, inflammatory mediator IL-1 β、 IL-18 can be released to the outside of cells through small holes (Songane et al., 2018); On the other hand, the formation of small holes leads to the change of osmotic pressure inside and outside the cells, which leads to swelling and eventually cell rupture. It can be seen that cell scorch death can induce the death of infected cells, eliminate pathogens in cells and limit their growth. However, a large number of cell death can lead to excessive inflammatory response, IL-1 β、 The release of IL-18 can activate other immune cells to accumulate a large number of inflammatory cell infiltration to the infected site, and aggravate the release of inflammatory factors again, resulting in fever, hypotension and other manifestations (İsmi et al., 2017). In this study, the mouse models were established by curcumin, LPS, LPS + curcumin, LPS + nigericin and LPS + curcumin + nigericin. It was found that the expression of NLRP3 in lung tissue and IL-18 and IL-1 in serum of mice in each group β The levels of IL-18 and IL-1 in curcumin group, LPS + curcumin group, LPS + nigericin group and LPS + curcumin + nigericin group were higher than those in blank group ($P > 0.05$) β The levels were lower than those in LPS group, but the difference was not significant ($P > 0.05$). According to the research reports of domestic and foreign scholars (Ning et al., 2020; Niu et al., 2021), LPS can activate NLRP3 inflammatory body and induce IL-6 and IL-1 β The release of proinflammatory substances makes the body produce inflammatory reaction rapidly, leading to acute lung injury. Western blot showed that compared with the blank control group, the expression of caspase-1 in curcumin group decreased slightly, and NLRP3 and gsdmd increased; Compared with LPS group, NLRP3 and caspase-1 of curcumin + LPS group cells increased and gsdmd pyroprotein decreased, which may be related to the dose of curcumin in the study. In the study of Chen et al. (2018), curcumin can inhibit the release of a large number of inflammatory factors induced by LPS and reduce the level of oxidative stress of alveolar macrophages within a certain concentration range. Compared with LPS + nigericin group, NLRP3, caspase-1 and gsdmd of cells in LPS + nigericin + curcumin group decreased consistently, and the decrease range was still large, suggesting that curcumin can inhibit cell death under the condition of LPS + nigericin + curcumin group model, indicating that curcumin can inhibit the induction of macrophages by LPS + nigericin through interfering with NLRP3 pathway and affect cell scorch, Improve the inflammatory response, so as to achieve the effect of treating acute lung injury, which is consistent with the research of other scholars (Zhang et al., 2019).

This study has certain limitations. The number of experimental samples is small, and many differences are not statistically significant, which affects the preciseness of the experiment and still needs further verification. However, it explores the mechanism of curcumin in treating infectious acute lung injury through NLRP3 inflammatory pathway, which provides a theoretical basis for curcumin in treating acute lung injury and other diseases.

5 Conclusion

In conclusion, the combination of LPS and curcumin can cause the apoptosis of lung cells. Based on the apoptosis rate and the results of Western blot, curcumin can improve cell viability, promote cell proliferation and promote LPS induced apoptosis, but it can inhibit the apoptosis of pulmonary macrophages caused by LPS and the combination of LPS and Nigerian bacteriocin, reduce the inflammatory response, and improve inflammation related diseases.

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