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Mechanism of Action of Berberine in Attenuating LPS-induced Acute Lung Injury in Mouse Cells

Anqing Guo[1], Hui Ding[2,*]

[1]Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
[2]Children's Hospital, The Second Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang 712046, Shaanxi, China
Correspondence Author

Abstract: ***Objective:*** ***To explore the mechanism of berberine in alleviating acute lung injury and related pathway targets.*** ***Methods:*** ***Lipopolysaccharide was used to construct a model of acute lung injury, and the specific binding proteins were screened by berberine molecular probes, and the binding proteins were analyzed by bioinformatics.*** ***Results:*** ***Berberine and its probes significantly reduced the content of inflammatory factors (TNF-α), and GO analysis showed that the biochemical process of target protein enrichment was the response to estrogen stimulation and the connection of unfolded proteins in molecular function. Involved in protein processing in the endoplasmic reticulum in the KEGG analysis pathway.*** ***Conclusion:*** ***Berberine can alleviate acute lung injury by stimulating estrogen to participate in protein processing in the endoplasmic reticulum.***

Keywords: Berberine, Acute lung injury, Chemical proteomics**.**

1. Introduction

Acute lung injury (ALI) is an acute respiratory insufficiency or failure resulting from diffuse interstitial and alveolar oedema caused by damage to pulmonary capillary endothelial cells and alveolar epithelial cells during non-cardiac diseases such as severe infections, shock, trauma and burns [1]. Acute respiratory distress syndrome is a severe manifestation of acute lung injury and is a high mortality respiratory disease that often presents with acute hypoxic respiratory failure, increased alveolar permeability, alveolar oedema and cardiac filling abnormality, and is pathophysiologically characterised by decreased lung volume, decreased lung compliance, and severe ventilation/blood flow ratio imbalance, and clinically presents with progressive hypoxaemia and respiratory distress [2]. Many studies have shown that acute lung injury can be induced by indirect or direct lung infections caused by a variety of injury-causing factors, including various cell types including vascular endothelial cells, macrophages, neutrophils, pro-inflammatory factors (interleukin-1β, interleukin-18, and tumour necrosis factor α) and a variety of cell types, including vascular endothelial cells and macrophages, and the production of various immune mediators are key pathogenic mechanisms [3]. The viruses that cause ALI/ARDS often stimulate the release of high levels of pro-inflammatory factors TNF-αand IL-6 from respiratory epithelial cells, dendritic cells, and macrophages, resulting in cytokine storms, and therefore acute lung injury can be attenuated by attenuating cytokine storms [4]. So far, there is no specific treatment for acute lung injury, and mechanical ventilation and drugs are often used for clinical treatment [5]. The current use of a large number of anti-inflammatory drugs, including those that inhibit inflammatory factors or receptor or pathway signaling, effectively alleviates acute lung injury, but their side effects and resistance limit their clinical application, so it is important to find safe and effective drugs to alleviate acute lung injury [6].

Berberine (BBR), a natural isoquinoline alkaloid, is widely found in traditional Chinese medicines (TCM) such as Huanglian, Phellodendron Bark, and Xizi Cao, etc. It possesses anumber of biological functions, such as anti-inflammatory, anti-free radical oxidation, antimicrobial, and immunomodulation, and it has an important value in the treatment of diseases related to the immune, respiratory, and endocrine systems [7]. In recent years, some studies have shown that berberine can attenuate acute lung injury through its anti-inflammatory properties. XU et al [8] found that berberine attenuated TLR4/NF-κB signaling, inhibited inflammatory factors such as IL-1, TNF-αand IL-6, and reduced the release of pro-inflammatory cytokines, thus attenuating acute lung injury. WANG et al. found that berberine could reduce acute lung injury in macrophages and hepatocytes via the endoplasmic reticulum stress response to inhibit LPS-induced inflammatory response. Yao et al. found that berberine inhibited the NLRP3 inflammatory vesicle pathway in MDA-MB-231 cells. The study of YU et al [9] demonstrated that berberine inhibited NF-κB and suppressed LPS-induced acute pneumonia. We used LPS-induced mouse bronchial epithelial cells in our study, aiming to elucidate the mechanism of berberine's action on attenuating acute lung injury in mouse models through proteomics combined with the analysis of biological information, and to provide theoretical basis for its clinical application.

2. Materials and Methods

2.1 Cell Lines

Human bronchial epithelial cells beas-2b (batch number: TCH-C132) were purchased from Starfish Biotechnology Co.beas-2b cells were cultured in DMEM medium with supplemented 10%FBS, 0.5 ml penicillin/streptomycin and incubated in a 37°C cell culture incubator containing 5%CO_2.

2.2 Experimental Materials

Berberine (batch no.21379) was purchased from Shanghai Yuanye Biotechnology Co, Ltd; Trypsin (batch no.25200-56), Streptavidin magnetic beads Beads(batch no.20353)were purchased from Life Technologies Corporation; Dimethyl Sulfoxide (DMSO) (batch no.D8370), Caulmers Brilliant Blue Staining Set (batch no.P1305) were purchased from Solarbio; TCEP (batch no.C4706), copper sulphate(batch no.461657), TBTA (batch no.678937) were purchased from Sigma-Aldrich; Biotin-Azide (batch no.HY129832) was purchased from MCE; Human Tumour Necrosis FactorαKit (Biotin-Azide (batch no.HY129832) was purchased from MCE; Human Tumour Necrosis FactorαKit (batch no.A-H00115A) was purchased from Jiangsu Henfeng Biotechnology Co.

2.3 Experimental Instruments and Equipment

Enzyme labeller (Thermo Fisher Scientific), CO_2 constant temperature cell incubator (Panasonic), biological safety cabinet (Suzhou Antai), inverted microscope (BM), metal bath (SCILOGEXHB120-S), chemiluminescence imaging analysis system (Shanghai Qinxiang Scientific Instrument Co.Ltd.), freezing centrifuge (Eppendorf), constant temperature Homogeniser (Hangzhou Yuning Instrument), Ultrapure Water System (Millipore Corporation).

2.4 Methods

2.4.1 Design and synthesis of small molecule probes

Reactive molecular probes generally consist of three parts, the reactive moiety, the linker moiety reporter moiety. A photoaffinity marker (bis-acridine) and an alkyne group were added to the easily modified functional group of berberine. The carbene active site of the photoaffinity tag after activation under UV illumination is covalently cross-linked to the C-H bond on nearby proteins, thus facilitating better capture of the target protein. The alkyne group 1 is designed to click chemically with azidobiotin, thus coupling on the biotin tag.

2.4.2 Cell viability determination

Cell viability was assessed using a cell counting data kit (CCK-8) according to the instructions of the data vendor.

2.4.3 Determination of TNF-αby ELISA

Determine the level of cellular inflammatory factor TNF-αby ELISA kit, according to the instruction of the kit.

2.4.4 Experimental grouping

Three groups were set up for the experiment: berberine probe group, berberine group and competition group (berberine+probe). Three biological replicates were set up in each group, totalling 9 samples.

2.4.5 Probe labelling concentration screening

Discard the cell culture medium, add PBS wash and collect the cells with a cell scraper, transfer to a 1. 5mL EP tube and centrifuge at 3000rpm for 3min, discard the supernatant. Add 300μL of cell lysate to each tube, sonicate with an ultrasonic breaker, and transfer the supernatant to a new 1. 5mL EP tube. A 96-well plate was taken and protein quantification was performed using the BCA kit according to the instructions. A certain concentration of small molecule probe was added to both competitive and non-competitive groups and incubated in a shaker for 60 min. the reacted proteins were transferred to a 96-well plate, irradiated at 365 nm for 10 min, and then transferred to a centrifuge tube. TCEP (1mM), TBTA (0. 1mM), CuSO4 (1mM) were added to the above samples, and 0. 3μL of rhodamine (N3) was added to the samples, protected from light, and the samples were incubated in a shaker for 60min. 12. 5uL of 5x loading buffer was added to the samples after completion of the above reactions, and the gels were prepared a 10%separator gel and a 5%concentrate gel. A 10uL sample was applied to each well and the gel was pressed using 130V and electrophoresed at 190V. The gel was carefully removed from the glass plate, placed under water and rinsed twice to wash all surrounding residue, and photographed using fluorescence imaging. Add the Caulmers Brilliant Blue staining solution to cover the gel surface and incubate on a shaking bed for 10 min, add the decolourising solution to cover the gel surface and place on a shaking bed for 10 min, discard the decolourising solution, add water and place on a shaking bed for overnight incubation, and take pictures on the next day.

2.4.6 Competition concentration screening

Take 96-well plate, use BCA kit for protein quantification according to the instructions, add competitor to the competition group, and incubate in a shaker for 30 min. subsequent steps are the same as 2. 4. 5.

2.4.7 Proteomics experiments

The protein concentration of the cell extracts was determined using the BCA protein assay kit. Competitors were added to the competition group and incubated for 30 min in a shaker (800 rpm 25°C). Small molecule probes were added to both the competition and non-competition groups and incubated for 60 min in a shaker (800 rpm 25°C). The reacted proteins were transferred to a 96-well plate and irradiated at 365 nm for 10min, and then transferred to centrifuge tubes. TCEP (1mM), TBTA (0. 1mM), CuSO4 (1mM), Biotin-Azide (1mM) were added to the above samples and incubated at room temperature for 2h. Streptavidin magnetic beads were removed to mix all the beads required for the experiment and placed in 15mL centrifuge tubes, 5mL of room temperature PBS was added directly and centrifuged for 4 min at 25°C (1400 g), remove the supernatant and repeat the wash 5 times, and wash with water 5 times in the same way. Beads were resuspended with 100μL of PBS and added to the sample and incubated overnight at 4°C with vortex shaking. Proteins were denatured for 10 min after heating in a metal bath to 95°C. Gels were pressed at 80 V for 10 min, and electrophoresis was performed at 120 V. The samples were incubated at 4°C for 5 min.

2.5 Mass Spectrometry

The above nine samples were subjected to mass spectrometry

detection, comparative analysis, and small molecule binding proteins were obtained, from which candidate target proteins were screened for subsequent functional verification experiments.

2.6 GO, KEGG Analysis

The subcellular structure prediction software CELLO was used to analyse the subcellular localisation of the quantitative proteins, and the functional enrichment of the quantitative proteins was analysed by gene function GO and KEGG pathway enrichment.

2.7 Statistical Analysis

The experimental data were statistically analysed using SPSS26. 0 software, and the data results were expressed in terms of, if they conformed to normal distribution, one-way ANOVA was used for comparison between groups; if they did not conform to normal distribution, non-parametric tests were used. Histograms were produced using Graphpad prism software. $p<0.05$ was considered statistically significant.

3. Results

3.1 Small Molecule Probe Labelling Experiments

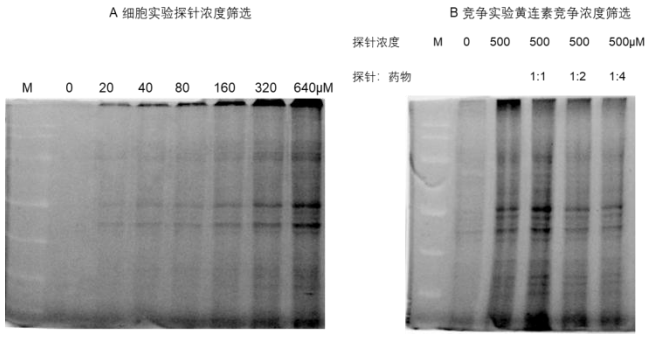


Figure 1: probe concentration labelling and competitive concentration screening results

As shown in Figure 1, the fluorescence intensity of the protein labelled by the probe gradually increased with the increase of the probe concentration, indicating that the probe can enter the cell and can effectively label the intracellular proteins. When the probe concentration of 640μM has moderate protein fluorescence labelling intensity, due to the non-saturated concentration can be used for competitive labelling experiments, at the same time enough signal intensity to ensure the effective identification of the subsequent binding protein, so the 640μM concentration of the probe was selected for the subsequent competitive photolabelling experiments as well as target fishing experiments. Compared with the probe group, the fluorescence intensity of some protein bands was significantly reduced when 1-fold, 2-fold or 4-fold concentration of the original drug berberine was added after preincubation, indicating that the binding site of the probe and the target protein could be competitively occupied by berberine, and that the probe and the berberine were bound to the same target of action, and that the target proteins labelled by the probe had high credibility. Considering that both berberine and probe have certain toxicity to cells, and the pretreatment concentration of berberine at 500μM can show strong competitive binding effect to the probe, a 4-fold competitive concentration of the original drug was chosen as the next target angling experiment.

3.2 Effects of Berberine and Its Probes on Cell Proliferation

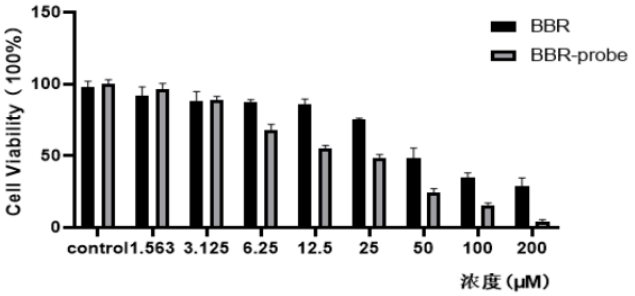


Figure 2: Effect of berberine and its probes on cell proliferation

In order to investigate the effect of berberine and its probe on cell proliferation at different concentrations, we examined the proliferation of beas-2b cells under eight concentration conditions using CCK8 reagent. As shown in Figure 2, different concentrations of berberine and its probe inhibited the proliferation of beas-2b cells to different degrees compared with the blank group. With the increase of concentration, the proliferative activity of berberine probe gradually decreased, especially at the concentration of 200μmolL-1, the proliferative activity of cells was the worst. Combined with the observation of cell growth status and the results of CCK8 assay, berberine and its probe culture concentrations of 1. 563, 3. 125, 6. 25, and 12. 5μmol-L-1 were chosen to culture beas-2b cells in the subsequent experiments, and the samples were collected at 48 h. The results showed that berberine was effective for the proliferation of beas-2b cells in the subsequent experiments, and the results showed that berberine was effective for the proliferation of beas-2b cells. The IC50 value of berberine for beas-2b cell proliferation inhibition was 59. 93μM, while the IC50 value of the probe was 17.82μM, which was comparable to the two activities, and the anti-inflammatory activity of the berberine probe was retained, which can be used for the subsequent screening of targets.

3.3 Effect of Berberine and Its Probe on TNF-αexpression

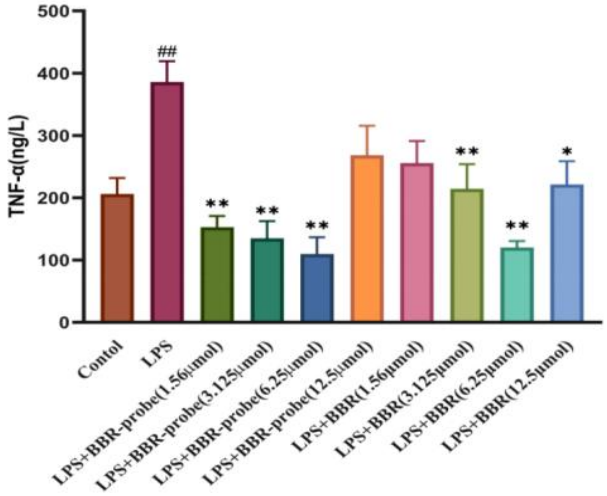


Figure 3: Effect of berberine and its probe on TNF-α expression

Notes: Compared with the blank group, the #P<was 0.05, ##P<0.01.

Compared with the model group, *P<0.05 and **P<0.01.

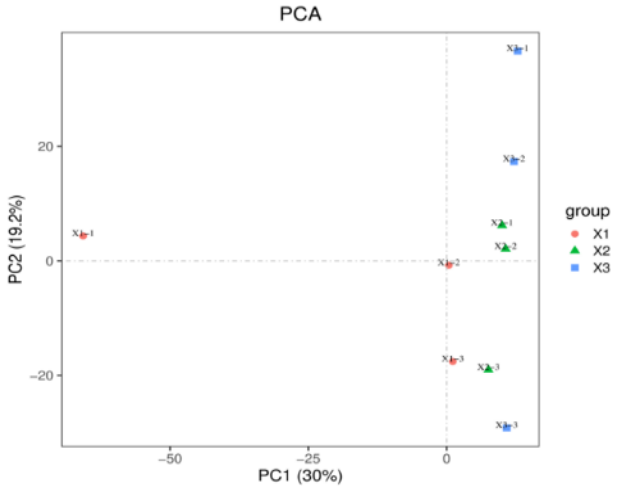
As shown in Figure 3, LPS was used to induce acute lung injury in mouse cells, and TNF-αcontent was significantly increased in the control group compared with the blank group(P<0.01=0.004), and LPS successfully induced the inflammatory response in the ALI cell model; in comparison with the control group, the LPS-BBR-P concentrations of 1.56, 3.125, and 6.25 all significantly reduced the TNF-αcontent (P<0.01), which was significantly different from the model control group, and berberine and its probe could inhibit the occurrence of inflammatory response to LPS-induced lung injury. From the results of TNF-αand CKK8 assays, it is evident that the synthesised berberine probe has similar biological activities with berberine, and the probe can be used in subsequent target screening experiments.

3.4 Proteomic Analyses

Although berberine has been validated to ameliorate LPS-induced inflammatory responses, the underlying molecular mechanisms are unclear. Therefore, MS-based quantitative proteomics was applied to systematically analyse or elucidate the differential expression levels of proteins in berberine and its probe-treated ALI lung tissues. The three sample groups identified 4980, 5591, and 4679 proteins, respectively, of which 235 were filtered to be able to reproduce plausible proteins containing quantitative information.

Subcellular localisation analysis showed that 80 were located in figure B, 63 in the nucleus, 34 in the mitochondria, 24 in the extracellular matrix, 17 in the cytoplasmic plasma membrane, 8 in the cytoplasm, 4 in the cytoskeleton, and 4 in the endoplasmic reticulum suggesting that the cytoplasm contains the highest number of plausible proteins. Clustering of PCA samples proved a high degree of stability of the chromatographic separation, consistency and reproducibility in figure A.

A



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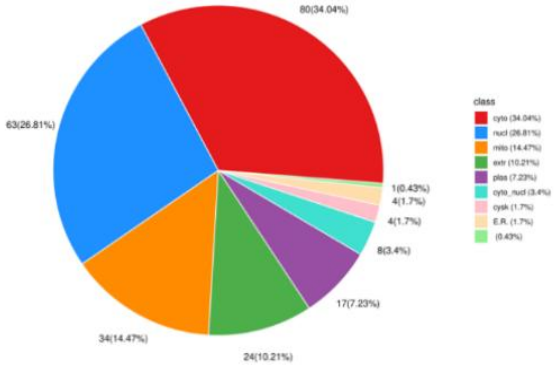
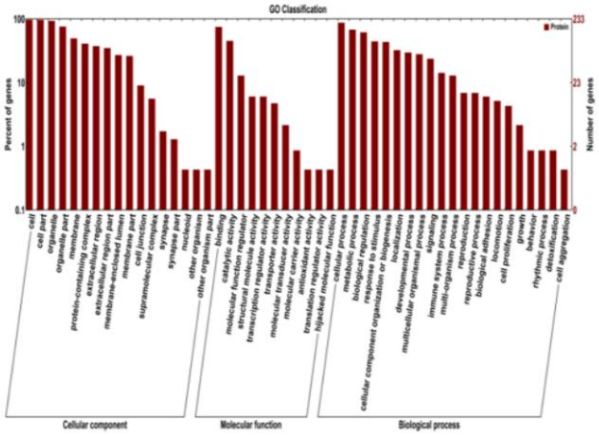


Figure 4: GO functional and KEGG pathway enrichment analysis

As shown in Figure 5, A total of 235 plausible proteins containing quantitative information were identified by mass spectrometry and protein, and the 235 proteins screened were subjected to functional annotation enrichment analyses in GO databases, in which proteins were enriched to the cellular localization (CC) in which proteins were mainly originated from cells and organelles. At the level of participation in molecular function (MF), proteins mainly play molecular binding, catalytic activity, and molecular function regulation. The top 3 in significance of proteins involved in biological processes (BP) are cellular, metabolic, and biochemical processes.

Enrichment analysis of the screened plausible proteins in the KEGG database involved KEGG pathways mainly involved in cancer, endocrine and metabolic diseases, virus-associated infectious diseases, and neurodegenerative lesions in the disease category. Involved in metabolic part of energy metabolism. Endocrine and neurological in the organismal system aspect. Signalling in terms of environmental information processing. Transcription in gene information transduction, growth, death, transport and heterotaxy in cellular processes.



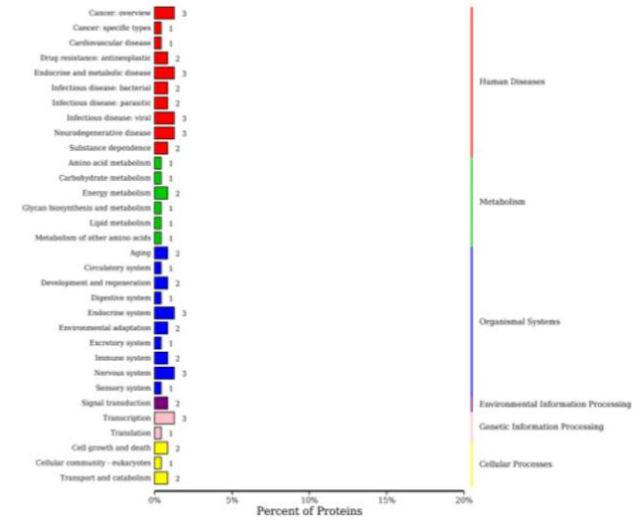


Figure 5: Protein-protein interaction network analysis

As shown in Figure 6, to investigate the potential targets of berberine for ALI, through network pharmacological analysis, we identified common targets between the disease and berberine, demonstrating a multicentric interaction network that emphasises the importance of core target proteins with more node connections. GO analysis of 53 of these common targets revealed that the targets are involved in a variety of biochemical processes, including RNA polymerase II transcription, cellular response to oestrogen stimulation, positive regulation of transcriptional elongation, retinal development in phasic eyes, positive regulation of protein ubiquitination, macroautophagy, ribosomal small-subunit biogenesis, and positive regulation of proteasomal ubiquitin, enriched in cellular components often located in lysosomal membrane, and molecular functions are mainly oxidoreductase activity acting on CH-OH groups, unfolded protein binding, and iron ion binding. These targets are often involved in protein processing in the endoplasmic reticulum, neurodegenerative lesions.

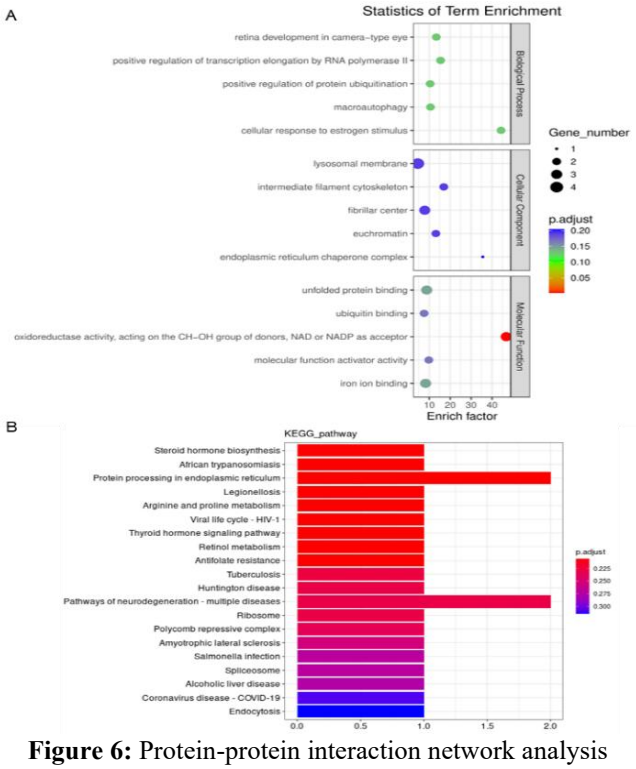


Figure 6: Protein-protein interaction network analysis

4. Discussion

Traditional Chinese medicine (TCM) is a speciality medicine in China, and its evidence-based treatment, individualised treatment, and multi-component and multi-targeted treatment with TCM have been shown to be effective in anti-inflammation [10]. In recent years, some researchers have found that alkaloids that can be extracted from Rhizoma Coptidis, such as berberine, have good anti-inflammatory activity and can inhibit the expression of inflammatory proteins TNF-α, IL-1βand NO in vitro [11]. And berberine has been proved to be well tolerated with fewer adverse effects and has good application advantages [12]. It has been found that LPS can cause metabolic abnormalities and exacerbate oxidative stress and inflammatory responses by activating the TLR4/NF-κB signaling pathway and releasing pro-inflammatory cytokines [13]. LPS is one of the potent inflammatory activators, which is an important cause of ALI important reason for the occurrence of ALI [14]. TNF-αis one of the earliest and most important endogenous mediators of pro-inflammatory factors, which mediates neutrophil recruitment and stimulates the release of pro-inflammatory factors from adventitial cells. TNF-αis an indicator of the severity of lung injury [15].

In order to explore the anti-inflammatory mechanism of berberine in LPS-induced acute lung injury, human bronchial epithelial beas-2b cell line was used in the experiment, and LPS-induced ALI model was constructed, and the ELLSA assay verified that the pro-inflammatory factors were significantly increased after LPS-induced beas-2b cells, and at the same time, different concentrations of berberine and its probe had a strong inhibitory effect on pro-inflammatory factors, such as TNF-α. In order to better screen the specific binding proteins, we used different concentrations (1. 563, 3. 125, 6. 25, 12. 5, 25, 50, 100, 200) of berberine and its probes with different drug competition ratios to test the cell viability, to explore the appropriate concentration, to make the molecular probes, and finally to determine the optimal concentration of 500μm, and the optimal drug competition ratio of 1: 4.

In order to elucidate the material basis and molecular mechanisms of berberine treatment of ALI, we performed a combined analysis using proteomics and network pharmacology. GO analysis showed that target proteins were enriched to biochemical processes in response to estrogen stimulation, and that unfolded proteins were connected in molecular functions. In KEGG analysis pathways involved in protein processing in the endoplasmic reticulum. This suggests that berberine may attenuate acute lung injury by participating in protein processing in the endoplasmic reticulum in response to estrogen stimulation. It was shown that GPR30, an estrogen-binding receptor, is present in the endoplasmic reticulum and is anti-inflammatory by inhibiting the p38MAPK/NF-κB pathway [16]. It can be concluded that the anti-inflammatory function of oestrogen in target tissues may be related to specific receptor binding mediated by the endoplasmic reticulum. Estrogen deficiency activates the expression of IL-7 causing chronic low production of TNF-αand causing an inflammatory response [17]. 17βEstrogen inhibits the production of the pro-inflammatory factor PGE2 in LPS-activated microglial cells, which

regulates microglial activation and inflammatory responses [18]. Endoplasmic reticulum stress is often caused by an imbalance between the need for protein folding and the ability to fold proteins, and oestrogen reduces oxidative stress and thus endoplasmic reticulum stress, leading to a reduction in the unfolded protein response in the cell. Alternatively, oestrogen may favour the reduction of oxidative stress and endoplasmic reticulum stress [19]. Recent studies have shown that natural herbs have strong advantages as anti-inflammatory agents, for example, berberine extracted from Rhizoma Coptidis may be effective in the treatment of metabolic inflammatory diseases through the down-regulation of NF-κB, cytokines, and inflammation-related pathways. Berberine may reduce pro-inflammatory factor release by inhibiting endoplasmic reticulum stress [20].

In conclusion, our experimental results demonstrate that berberine inhibits pro-inflammatory factor release and attenuates LPS-induced acute lung injury through a mechanism that involves protein processing in the endoplasmic reticulum through stimulation of estrogen. Although in the current study, we explored that berberine may reduce the stress response of the endoplasmic reticulum to achieve anti-inflammatory effects by stimulating the secretion of estrogen and participating in the processing of proteins in the endoplasmic reticulum, there are still some limitations in the study, and we were unable to find the core target proteins and pathways, and the present study is rather one-sided, and more experiments are needed to verify it in the future.

5. Conclusion

In summary, the experiments used chemical proteomics to screen the specific binding proteins of berberine for the treatment of acute lung injury, it was concluded that berberine could significantly reduce the level of TNF-αexpression, and the subsequent analysis inferred that berberine's target proteins could be bound to the corresponding receptors on the endoplasmic reticulum to stimulate estrogenic response and participate in the processing of proteins on the endoplasmic reticulum, thus reducing the endoplasmic reticulum stress and achieving the anti-inflammatory effect for acute lung injury. This can reduce endoplasmic reticulum stress and achieve the anti-inflammatory effect on acute lung injury.

References

[1] Auriemma C L, Zhuo H, Delucchi K, et al. Acute respiratory distress syndrome-attributable mortality in critically ill patients with sepsis [J]. Intensive Care Med, 2020, 46(6): 1222-1231.

[2] He Y Q, Zhou C C, Yu L Y, et al. Natural product derived phytochemicals in managing acute lung injury by multiple mechanisms [J]. Pharmacol Res, 2021, 163: 105224.

[3] Wang Q, Wen W, Zhou L, et al. LL-37 improves sepsis-induced acute lung injury by suppressing pyroptosis in alveolar epithelial cells [J]. Int Immunopharmacol, 2024, 129: 111580.

[4] Su X, Jing X, Jiang W, et al. Polyphosphazene nanodrugs for targeting delivery and inflammation responsive release of curcumin to treat acute lung injury by effectively inhibiting cytokine storms [J]. Colloids Surf B Biointerfaces, 2023, 229: 113446.

[5] Ling Q, Fang J, Zhai C, et al. Berberine induces SOCS1 pathway to reprogram the M1 polarization of macrophages via miR-155-5p in colitis-associated colorectal cancer [J]. Eur J Pharmacol, 2023, 949: 175724.

[6] Chen S, Chen E, Guan X, et al. Magnetically controlled nanorobots induced oriented and rapid clearance of the cytokine storm for acute lung injury therapy [J]. Colloids Surf B Biointerfaces, 2024, 234: 113731.

[7] Zhao J V, Huang X, Zhang J, et al. Overall and Sex-Specific Effect of Berberine on Glycemic and Insulin-Related Traits: a Systematic Review and Meta-Analysis of Randomized Controlled Trials [J]. J Nutr, 2023, 153(10): 2939-2950.

[8] Lin P, Gao R, Fang Z, et al. Precise nanodrug delivery systems with cell-specific targeting for ALI/ARDS treatment [J]. Int J Pharm, 2023, 644: 123321.

[9] Xu G, Wan H, Yi L, et al. Berberine administrated with different routes attenuates inhaled LPS-induced acute respiratory distress syndrome through TLR4/NF - kappaB and JAK2/STAT3 inhibition [J]. Eur J Pharmacol, 2021, 908: 174349.

[10] Li S, Hou J, Wang Q, et al. Angong niuhuang wan attenuates LPS-induced acute lung injury by inhibiting PIK3CG/p65/MMP9 signaling in mice based on proteomics [J]. Heliyon, 2023, 9(9): e20149.

[11] Lee C H, Chen J C, Hsiang C Y, et al. Berberine suppresses inflammatory agents-induced interleukin - 1beta and tumor necrosis factor-alpha productions via the inhibition of IkappaB degradation in human lung cells [J]. Pharmacol Res, 2007, 56(3): 193-201.

[12] Xia Q, Wang W, Liu Z, et al. New insights into mechanisms of berberine in alleviating reproductive disorders of polycystic ovary syndrome: Anti-inflammatory properties [J]. Eur J Pharmacol, 2023, 939: 175433.

[13] Zhang F, Guo F, Zhang Y, et al. Huashibaidu formula attenuates sepsis-induced acute lung injury via suppressing cytokine storm: Implications for treatment of COVID-19 [J]. Phytomedicine, 2023, 109: 154549.

[14] Liu Y, Zhu Y, Wang L, et al. Acid-sensitive ion channel 1a regulates TNF-alpha expression in LPS-induced acute lung injury via ERS-CHOP-C/EBPalpha signaling pathway [J]. Mol Immunol, 2023, 153: 25-35.

[15] Zeng M, Zhang B, Ren Y, et al. A sesquiterpene isolated from the stems and leaves of Dioscorea opposita thunb. Transforms the composition of immune cells through ERbeta in a mouse model of LPS-induced lung injury [J]. Heliyon, 2022, 8(9): e10500.

[16] Gu L, Ke Y, Gan J, et al. Berberine suppresses bone loss and inflammation in ligature-induced periodontitis through promotion of the G protein-coupled estrogen receptor-mediated inactivation of the p38MAPK/ NF-kappaB pathway [J]. Arch Oral Biol, 2021, 122: 104992.

[17] Cui H, Li J, Li X, et al. TNF-alpha promotes osteocyte necroptosis by upregulating TLR4 in postmenopausal osteoporosis [J]. Bone, 2024, 182: 117050.

[18] Liu H, Huang Y, Yang J, et al. Involvement of estrogen receptor activation in kaempferol-3-O-glucoside's protection against aging-related cognition impairment

and microglial inflammation [J]. Exp Cell Res, 2023, 433(2): 113849.
[19] Haas M J, Raheja P, Jaimungal S, et al. Estrogen-dependent inhibition of dextrose-induced endoplasmic reticulum stress and superoxide generation in endothelial cells [J]. Free Radic Biol Med, 2012, 52(11-12): 2161-2167.
[20] Sajadimajd S, Deravi N, Forouhar K, et al. Endoplasmic reticulum as a therapeutic target in type 2 diabetes: Role of phytochemicals [J]. Int Immunopharmacol, 2023, 114: 109508.