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Huashibaidu formula attenuates sepsis-induced acute lung injury via suppressing cytokine storm: Implications for treatment of COVID-19

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ABSTRACT

Background: Acute lung injury (ALI) is a common complication of sepsis with poor effective interventions. Huashibaidu formula (HSBD) showed good therapeutic effects in treating coronavirus disease 2019 (COVID-19) patients.

Purpose: This study was designed to investigate the therapeutic potential and precise mechanism of HSBD against sepsis-induced ALI based on network pharmacology and animal experiments.

Materials and methods: Network pharmacology was used to predict the possible mechanism of HSBD against sepsis. Next, a sepsis-induced ALI rat model via intraperitoneal lipopolysaccharide (LPS) was constructed to evaluate the level of inflammatory cytokines and the degree of lung injury. The expression of inflammation-related signaling pathways, including TLR4/NF-κB and PI3K/Akt was determined by western blot.

Results: Network pharmacology analysis indicated that HSBD might have a therapeutic effect on sepsis mainly by affecting inflammatory and immune responses. Animal experiments demonstrated that HSBD protected the lung tissue from LPS-induced injury, and inhibited the levels of inflammatory cytokines such as interleukin (IL)-1β, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-γ and tumor necrosis factor (TNF)-α in the serum and IL-1β, IL-5, IL-6, IL-18, GM-CSF, IFN-γ and TNF-α in the lung tissue. Western blot results revealed that HSBD downregulated the expression of TLR4/NF-κB and upregulated the expression of PI3K/Akt.

Conclusion: The therapeutic mechanism of HSBD against sepsis-induced ALI mainly involved suppressing cytokine storms and relieving inflammatory symptoms by regulating the expression of TLR4/NF-κB and PI3K/Akt. Our study provides a scientific basis for the mechanistic investigation and clinical application of HSBD in the treatment of sepsis and COVID-19.

Introduction

Sepsis is a life-threatening clinical syndrome related to a dysregulated host response to infection. Among the injured organs, the lung is highly vulnerable, and is also the most frequent organ to fail (Sadowitz et al., 2011). The respiratory failure during sepsis may sequentially develop into acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), leading to an inevitable risk of multiorgan dysfunction syndrome (Thompson et al., 2017). The severity of sepsis is attributed to an activated and amplified inflammatory cascade called cytokine storm (Chousterman et al., 2017). Specifically, a cytokine storm refers to an excessively exaggerated immune response leading to dysregulation and even exhaustion, triggered by microorganism infections, autoimmune conditions, and monogenic disorders (Oldstone

Abbreviations: ALI, acute lung injury; ARDS, acute respiratory distress syndrome; AD, average degree; ASPL, average shortest path length; CC, closeness centrality; CSF, colony-stimulating factor; COVID-19, coronavirus disease 2019; DXMS, dexamethasone; GO, Gene Ontology; GM-CSF, granulocyte-macrophage colony stimulating factor; HE, hematoxylin and eosin; HSBD, huashibaidu formula; IFN, interferon; IL, interleukin; KEGG, Kyoto Encyclopedia of Genes and Genomes; LPS, lipopolysaccharide; SD, standard deviation; TLR, toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

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Cytokine storms are characterized by elevated levels of circulating inflammatory cytokines including interleukin (IL), interferon (IFN), chemokine, tumor necrosis factor (TNF), growth factor, colony-stimulating factor (CSF), and TGF-β family members (Gupta et al., 2020), which are considered one of the major pathological features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, and an important cause of death among patients with severe coronavirus disease 2019 (COVID-19) (Fajengebaum and June 2020).

At present, there are no efficient drugs or therapies to treat sepsis-induced ALI. The main treatments include noninvasive ventilation, invasive mechanical ventilation, prone positioning, neuromuscular blockade, extracorporeal membrane oxygenation, anti-inflammatory therapy and supportive care (Kim and Hong, 2016). Anti-inflammatory therapy is an important and effective treatment. The role of corticosteroids in the treatment of sepsis-induced ALI remains inconclusive (Wang et al., 2019). Corticosteroids have the ability to improve pulmonary function, alleviate the inflammatory response, and enhance the survival rate of ALI. However, the clinical application of corticosteroids is usually accompanied by a variety of adverse effects, such as hyperglycemia, hypokalemia, dyslipidemia, osteoporosis, myopathy and immunosuppression (Schäcke et al., 2002; Walker, 2007).

Huashibaidu formula (HSBD) was developed by Chinese academicians Lai Huang and his medical team at the Chinese Academy of Medical Sciences, and was used to treat COVID-19 patients. This prescription consists of 14 kinds of traditional Chinese medicines (TCMs), such as Ephedra sinica Stapf (Mahuang), Prunus armeniaca L. (Kuxingren), Glycyrrhiza uralensis Fisch. (Gancao), Agastache rugosa (Fisch. & C.A.Mey.) Kuntze (Huoxiang), Magnolia officinalis Rehder & E. H.Wilson (Houpo), Atractylodes lancea (Thunb.) DC (Cangzhu), Amomum tsao-ko Crevost & Lema ric (Caoguo), Pinellia ternata (Thunb.) Makino (Bannixia), Poria cocos (Schw.) Wolf (Fuling), Paonia lactiflora Pall. (Chishao), Rheum palmatum L. (Dahuang), Scutellaria baicalensis Georgi (Huangqi), Descurainia sophia (L.) Webb ex Prantl (Tinglizi) and gypsym (Shigao). Clinical data suggested that combining HSBD with Western medicine (lopinavir-ritonavir) may shorten the disease course in COVID-19 patients who recovered within 16 days (Shi et al., 2021). HSBD combined with conventional treatment reduced the likelihood of worsening symptoms in mild COVID-19 patients after seven days of early treatment (Zhao et al., 2021). The use of HSBD and standard care combination therapy shortened the recovery time from fever and accelerated the resolution of symptoms such as cough, fatigue and chest discomfort in severe COVID-19 patients (Liu et al., 2021). HSBD has been officially approved by the National Medical Products Administration, and appointed as HSBD granules for clinical treatment of resolving dampness, removing toxin, diffusing lung and reducing heat. However, the precise mechanism of HSBD in treating sepsis-induced ALI has not been elucidated thus far.

In our research, we predicted the underlying pharmacological mechanism of HSBD against sepsis, which could supply a theoretical foundation for mechanistic studies. Then, we established a sepsis-induced ALI rat model via intraperitoneal lipopolysaccharide (LPS) to test the therapeutic effect of HSBD. Therefore, our research provides a specific investigation revealing the efficacy and mechanism of HSBD against sepsis-induced ALI.

Materials and methods

Materials and reagents

LPS (cat. L8880, from Escherichia coli 055: B5), pentobarbital sodium (cat. 8118025) and 4% paraformaldehyde solution (cat. P1110) were obtained from Solarbio Life Sciences (Beijing, China). Anti-NF-κB (#8242), anti-p-NF-κB (#3033), anti-Act (#2967), anti-p-Act (#4060) and anti-β-actin (#4970) antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-Pi3K (BS-5587R), anti-p-Pi3K (ab182651) and anti-TLR4 (sc-293072) antibodies were obtained from Bios Antibodies (Beijing, China), Abcam (Cambridge, MA, USA) and Santa Cruz Biotechnology (Dallas, TX, USA), respectively. Dexamethasone tablets (0.75 mg/Tablet) were obtained from Guangdong South Land Pharmaceutical Co., Ltd., China.

Network pharmacology analysis of HSBD against COVID-19 and sepsis

The chemical compounds of HSBD were obtained from the TCMSP database (https://tcmspw.com/tcmsp.php). The molecular targets of compounds from HSBD were mainly collected from the BATMAN-TANM database (http://bionet.ncpsb.org/batman-tcm-), and the target genes with high confidence (prediction score > 20) were considered potential targets of HSBD. The disease targets of sepsis were obtained from the GeneCards and OMIM (https://omim.org/) databases. Taking “corona-virus disease” as a keyword, the disease targets of COVID-19 were identified from the GeneCards database (https://www.genecards.org). Drug targets of HSBD and disease targets of sepsis or COVID-19 were merged and then uploaded into the STRING database (http://string-db.org) to construct the core protein–protein interaction (PPI) network. The PPI network was created by Cytoscape software (version 3.7.2, Boston, MA, USA). To determine the potential biological function of HSBD, the Metascape database (http://metascape.org/gp/index) was used to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses.

Network robustness assessment of HSBD against COVID-19 and sepsis

The interventional effect of HSBD against sepsis or COVID-19 was evaluated as previously reported (Xi et al., 2022). In brief, network robustness assessment reflecting drug perturbational potency on the disease network was estimated by the changes in the network topological characteristics after the removal of the drug targets. Meanwhile, to obtain reliable drug perturbation of the real disease network, a null distribution of drug interventions on a random disease network was used to correct the topological characteristics. Topological characteristics, including average shortest path length (ASPL), average degree (AD) and closeness centrality (CC), were used as the main metrics to evaluate the robustness of the network, and the above metrics were calculated by using the igraph package of R language (Ju et al., 2016). ASPL was negatively associated with network robustness, while the other three metrics were positively related to network robustness. The high robustness of the network indicated a smaller perturbation rate of the drug and a more stable network after drug intervention.

Experimental animals

Male Sprague–Dawley rats weighing 180–220 g were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (SCXK (Jing) 2016–0006, Beijing, China). All experiments were designed and conducted based on the local ethical guidelines for animal care and usage (ethics permit No. 2020B011). The rats were allowed to adapt to the environment for three days before the experiment.

HSBD preparation and chromatographic detection

HSBD comprises 14 different herbs: Mahuang, Kuxingren, Gancao, Huoxiang, Houpo, Cangzhu, Caoguo, Bannixia, Fuling, Chishao, Dahuang, Huangqi, Tinglizi and Shigao (weight ratio 6:9:3:10:10:15:10:9:15:10:5:10:10:15). HSBD granules were prepared by Guangdong Fong Biopharmaceutical Co., Ltd., China. The primary process of the production was as follows: Shigao was soaked in water (1:5, w/v) and boiled for 30 min. The other 11 herbs except Huoxiang and Dahuang were added and boiled in water (1:2, w/v) for 50 min. Next, Huoxiang and Dahuang together with previous herbal materials were boiled in water (1:8, w/v) for another 10 min, and the filtrates...
were acquired. The filtration was then condensed at 80 °C. The residues were boiled a second time in water (1:8, w/v) for 1 h to obtain the other filtrates. The filtration was also condensed at 80 °C. Two batches of the filtrates were mixed and concentrated to an extractum with the relative density of (1.07–1.09) at 80 °C. Finally, moderate dextrin and stevioside were combined with the extractum, dried in a vacuum freeze equipment, and thus HSBD granules were collected.

In order to ensure the stability of quality for the experimental sample, the content determinations of the four compounds including ephedrine hydrochloride and pseudoephedrine hydrochloride, amygdalin and paeoniflorin in HSBD were mainly quantitated by high performance liquid chromatography (HPLC) method (Wet et al., 2021). The HPLC conditions of the four compounds were all established on the basis of Chinese Pharmacopoeia 2020 edition. The HPLC analysis was performed by Waters 2695 HPLC System equipped with thermostat autosampler, diode array detector (DAD) and Empower 3 workstation (Waters Technologies, USA). The specific procedures of the corresponding HPLC determinations were listed in the attachment. The standard substances of the four compounds were all obtained from National Institutes for Food and Drug Control, Beijing, China (purity > 99.8% for all).

**Pulmonary histopathological assessment**

The middle lobe of the right lung was immediately removed and fixed in 4% paraformaldehyde solution. The lung tissues were embedded in paraffin, sliced at 5 μm, and then stained with hematoxylin and eosin (HE). The slides were subjected to histological examination under the light microscope (Olympus, Tokyo, Japan). The histopathological score was calculated based on the following aspects: i) alveolar congestion; ii) alveolar hemorrhage; iii) interstitial edema; iv) neutrophil infiltration; and v) thickening of the alveolar wall. Each item was scored 0 for no injury, 1 for slight injury, 2 for moderate injury, 3 for severe injury (Fahmi et al., 2016). The middle lobe of the right lung was isolated and the lobe of the left lung was removed. The corresponding MIP-3α (MIP), interferon (IFN)-γ, interleukin (IL)–1α, -1β, -2, -4, -5, -6, -7, -8 (GRO/KC), -10, -12 (p70), -13, -17A, -18, granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF), granulocyte colony stimulating factor (G-CSF), monocyte chemoattractant protein (MCP)–1, macrophage inflammatory protein (MIP)–1α, MIP-3α, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF) and regulated upon activation, normal T cell expressed and presumably secreted (RANTES).

**Statistical analysis**

SPSS 20.0 software was used for statistical analysis. Data were presented as mean ± standard deviation (SD). Student’s t-test and one-way analyses of variance (ANOVA) were used to compare the differences. Values of p < 0.05 were considered statistically significant.
Prediction of the potential therapeutic mechanism of HSBD against COVID-19 and sepsis

A total of 186 chemical ingredients and 237 putative targets of HSBD were identified. A total of 328 targets of COVID-19 and 816 targets of sepsis were collected. After Venn analysis, 24 and 103 targets between HSBD drug targets and corresponding disease targets were obtained, which may be potential therapeutic targets for HSBD in treating COVID-19 and sepsis, respectively. Among them, 16 overlapping therapy targets were shared by both COVID-19 and sepsis, indicating that there may be a common therapeutic mechanism existing of HSBD against COVID-19 and sepsis (Fig. 2A).

Two PPI networks were constructed for HSBD against COVID-19 and sepsis separately using the STRING database. According to the comparative rule of drug perturbation to real networks and random networks, the normalized topological features reflecting the changes of network robustness after drug intervention were evaluated. The total score of normalized topological features, including AD, ASPL and CC, was calculated; COVID-19 was 5.994, and sepsis was 7.627, which implied that HSBD might have a certain intervention effect on both COVID-19 and sepsis (Fig. 2B).

To further explain the potential pharmacological effect of HSBD in the treatment of sepsis, a PPI network was constructed. Fig. 2C shows that two inflammatory cytokines, TNF-α and IL-1β, were centrally located at the core position of the PPI network. These results demonstrated that these two molecular targets might be highly related to the treatment of HSBD. Meanwhile, GO enrichment analysis demonstrated that the BP category was mainly associated with inflammatory and...
immune responses, especially cytokine production, leukocyte activation and migration, the toll-like receptor signaling pathway, and coagulation-related function (Fig. 2D). KEGG enrichment analysis showed that the signaling pathways involved in HSBD against sepsis were mainly correlated with the TNF, Toll-like receptor, NF-κB, Fc epsilon RI, B-cell receptor, and NOD-like receptor signaling pathways (Fig. 2E). GO and KEGG enrichment analyses focused both on the same Toll-like receptor signaling pathway, which may play an important role in the therapeutic mechanism of HSBD against sepsis.

**HSBD alleviated acute lung injury induced by intraperitoneal LPS**

The structures of the control group were normal and intact. Intraperitoneal administration of LPS in the ALI group induced distinct histological changes, such as pulmonary congestion, interstitial edema, alveolar wall thickening, and many inflammatory cells infiltrating the alveolars (Fig. 3A). However, HSBD treatment remarkably alleviated the LPS-induced lung injuries, and the high dosage was the most effective. These histological changes led to significant increases in the lung injury score and lung W/D weight ratio in the ALI group. Moreover, the lung injury scores and lung W/D weight ratios of the HSBD- treated medium- and high-dose groups were decreased compared with those of the ALI group (Fig. 3B and C). The high-dose HSBD group had the strong protective effect among the three HSBD treated groups.

**HSBD reduced the levels of inflammatory cytokines in blood serum and lung tissue**

To determine whether the inflammatory cytokines (TNF-α and IL-1β) identified from the network pharmacology results were involved in the treatment of HSBD, we detected the levels of 23 inflammatory cytokines in the blood serum and lung tissue. Compared with the control group, the levels of 6 inflammatory cytokines, IL-1β, IL-10, GM-CSF, IFN-γ, TNF-α and VEGF, were obviously increased in the blood serum of ALI
group, and levels of 11 inflammatory cytokines such as IL-1β, IL-5, IL-6, IL-8, IL-10, IL-12, IL-18, GM-CSF, IFN-γ, TNF-α and VEGF were significantly increased in the lung tissue of the ALI group. Compared with the ALI group, the levels of 4 inflammatory cytokines, IL-1β, GM-CSF, IFN-γ and TNF-α, were decreased in the blood serum of HSBD-treated group, and the levels of 7 inflammatory cytokines, IL-1β, IL-5, IL-6, IL-18, GM-
CSF, IFN-γ and TNF-α, were decreased in the lung tissue of the HSBD-treated group (Fig. 4).

HSBD inhibited ALI by regulating the TLR4/NF-κB and PI3k/Akt signaling pathways

due to the network pharmacology results predicting the central involvement of the Toll-like receptor signaling pathway, the expression of the TLR4/NF-κB and PI3k/Akt pathways in lung tissue was examined by western blot (Fig. 5A). compared with the control group, the expression levels of TLR4 and NF-κB in the ALI group were markedly increased, and the expression levels of PI3k and Akt were significantly decreased. Simultaneously, compared with the ALI group, TLR4 and NF-κB expression in the HSBD-treated groups were obviously decreased, while PI3k and Akt protein expression was significantly elevated (Fig. 5B and C). These results suggested that HSBD may prevent the development of ALI by regulating the TLR4/NF-κB and PI3k/Akt signaling pathways.

Discussion

Our research investigated the protective effect of HSBD against sepsis-induced ALI using network pharmacology, on the basis of the clinical application of HSBD in the treatment of COVID-19 patients. A total of 16 overlapping HSBD therapy targets between COVID-19 and sepsis were acquired, which suggested that there may be a common therapeutic mechanism for HSBD treatment of COVID-19 and sepsis. The network robustness assessment results confirmed that HSBD exerted an interventional effect on the treatment of COVID-19 and sepsis. To further clarify the potential pharmacological effect of HSBD against sepsis, a PPI network was constructed. The PPI network demonstrated that two inflammatory cytokines, TNF-α and IL-1β, were the most critical molecular targets of HSBD. GO and KEGG enrichment analyses showed the same result that the Toll-like receptor signaling pathway may play a vital role in the therapeutic mechanism of HSBD against sepsis. The network pharmacology results provided the research foundation and offered a better guide for the following experimental study.

Sepsis can be caused by a range of pathogens, such as bacteria, fungi, viruses, and parasites.

LPS is a major component of the outer membrane of Gram-negative bacteria, and microbe-derived LPS has been confirmed to be the most common microbial mediator participating in the development of sepsis (Opal SM, 2010; Cabrera-Perez et al., 2016). LPS stimulation usually promotes an intense inflammatory response (Chen et al., 2010). Humans are very sensitive to LPS, and very small doses can trigger strong immune responses, leading to the symptoms of sepsis (Haudek et al., 2010).
To generate a sepsis-induced ALI model, a high-dose intraperitoneal LPS was utilized in our research, and the lung was defined as a key target organ to explore the actual therapeutic effect of HSBD against sepsis.

The animal experiments suggested that the levels of inflammatory cytokines such as IL-1β, IL-10, GM-CSF, IFN-γ, TNF-α and VEGF in the blood serum and IL-1β, IL-5, IL-6, IL-10, IL-12, IL-18, GM-CSF, IFN-γ, TNF-α and VEGF in the lung tissue were significantly increased in the ALI group. HSBD decreased the levels of IL-1β, GM-CSF, IFN-γ and TNF-α in the serum and IL-1β, IL-5, IL-6, IL-18, GM-CSF, IFN-γ and TNF-α in the lung tissue. The data for IL-1β and TNF-α in both blood serum and lung tissue were consistent with the network pharmacology results. Histopathological analysis further confirmed that HSBD protected the lung tissue from sepsis-induced injury. Simultaneously, HSBD suppressed pulmonary edema induced by sepsis according to the result of lung W/D weight ratio.

The pathogenesis underlying cytokine storms are still unclear. Studies have indicated that the occurrence of cytokine storms is mainly attributed to hyperactive immune system activation leading to a regulatory imbalance between proinflammatory and anti-inflammatory cytokines production (Shimabukuro-Vornhagen et al., 2018; Hay, 2018). Multiple viruses, including different subtypes of influenza virus, have been observed to cause cytokine storms and even viral sepsis (Li et al., 2020). In addition to viral sepsis, bacterial sepsis has also been associated with severe cytokine storms (Reyes et al., 2020). Furthermore, cytokine profiles vary from disease to disease (Hay, 2018). Overproduction of cytokines generally promotes downstream biological processes and ultimately results in fatal respiratory distress and multiple organ failure (Karki et al., 2021).

Cytokine storms and the resulting cytokine release syndrome are considered pathophysiological biomarkers and therapeutic targets in COVID-19 infections that are related to severe and fatal cases (Pum et al., 2021). The ATP competitive kinase inhibitor baricitinib reduces the viral entry into target cells and controls the cytokine storm, thus preventing damage to the lungs and possibly other organs in severe COVID-19 patients (Zhang et al., 2020). The sodium glucose cotransporter 2 (SGLT2) inhibitor canagliflozin improved acute lung injuries induced by LPS-treated mouse sepsis by inhibiting cytokine storms (Niu et al., 2020). To generate a sepsis-induced ALI model, a high-dose intraperitoneal LPS was utilized in our research, and the lung was defined as a key target organ to explore the actual therapeutic effect of HSBD against sepsis.

![Fig. 4. HSBD inhibited the levels of inflammatory cytokines in the serum (A, n = 7/group) and lung tissue (B, n = 6/group) of sepsis rats induced by intraperitoneal LPS. Data are expressed as the mean ± SD. *p < 0.05, **p < 0.01 vs. Control group; *p < 0.05, and **p < 0.01 vs. ALI group.

The animal experiments suggested that the levels of inflammatory cytokines such as IL-1β, IL-10, GM-CSF, IFN-γ, TNF-α and VEGF in the blood serum and IL-1β, IL-5, IL-6, IL-10, IL-12, IL-18, GM-CSF, IFN-γ, TNF-α and VEGF in the lung tissue were significantly increased in the ALI group. HSBD decreased the levels of IL-1β, GM-CSF, IFN-γ and TNF-α in the serum and IL-1β, IL-5, IL-6, IL-18, GM-CSF, IFN-γ and TNF-α in the lung tissue. The data for IL-1β and TNF-α in both blood serum and lung tissue were consistent with the network pharmacology results. Histopathological analysis further confirmed that HSBD protected the lung tissue from sepsis-induced injury. Simultaneously, HSBD suppressed pulmonary edema induced by sepsis according to the result of lung W/D weight ratio.

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et al., 2021). These pharmaceutical studies provide a scientific reference for the treatment of a series of diseases related to cytokine storms. Our research evaluated the modulatory effect of HSBD on multiple inflammatory cytokines, and found that HSBD had a dramatic and broad inhibitory effect on those inflammatory factors in the blood serum and lung tissue.

To further figure out the potential protective mechanism of HSBD in the treatment of sepsis induced ALI, two signaling pathways related to the inflammatory response were studied. Western blot analysis illustrated that HSBD downregulated the TLR4/NF-κB pathway and upregulated the PI3K/Akt pathway, respectively. The western blot results for the Toll-like receptor signaling pathway were similar to the predicted

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**Fig. 5.** HSBD regulated the expression of the TLR4/NF-κB and PI3K/Akt signaling pathways in the lung tissue of septic rats. (A) Typical band images of TLR4/NF-κB and PI3K/Akt. (B-C) Relative expression of the TLR4/NF-κB and PI3K/Akt pathways. n = 3/group. Data are expressed as the mean ± SD. *p < 0.05 vs. Control group; **p < 0.05, and ***p < 0.01 vs. ALI group.
network pharmacology results. The human toll-like receptor (TLR) family has 10 identified members. TLRs are type I transmembrane proteins involved in initiating the innate immune response and inflammation in mammals that plays significant roles in the generation of cytokine storms (Kumar V, 2020). Bacterial LPS can bind and activate TLR4 in the airway epithelium, resulting in the production of adhesion molecules, chemokines, colony-stimulating factors, and other cytokines necessary for proinflammatory responses (Jiang et al., 2015). The increased expression of TLR4 genes in lung tissue after LPS stimulation is closely correlated with the onset of ALI. LPS-induced upregulation of TLRs leads to the activation of downstream NF-κB, which may amplify innate immunity against pathogens in lung tissue (Oshikawa and Sugiyama, 2003). NF-κB activation is an intracellular signaling cascade beginning with recruitment of the proximal TLR ligands MyD88 and/or TRIF to the TLR membrane complex, contributing to the proinflammatory response (Takeuchi and Akira, 2010).

The PI3K family is divided into 3 types, Type I, Type II and Type III (Liu and Cheng, 2009), which participate in essential cellular functions such as cell survival, proliferation, differentiation and metabolism (Vanhaesebroeck et al., 2005). The PI3K/Akt signaling pathway may be an endogenous negative feedback or compensatory mechanism related to septic and inflammatory reactions in response to harmful stimuli (Fukao and Koyasu, 2003; Williams et al., 2004). This pathway is closely connected with pulmonary fibrosis after LPS-induced sepsis in both murine and human cells, and the fibrotic process can be reversed by treatment with the PI3K inhibitor LY294002 (Hu et al., 2020; Wan et al., 2018). Recent studies have shown that there is a cross-talk between TLRs and PI3K regulation of the proinflammatory response. After the recognition and binding of ligands, TLRs initiate a proinflammatory signaling cascade and finally activate several transcription factor families. Additionally, TLR signals cause the activation of PI3K/Akt through the combination of TLR ligands, affecting many aspects of the cellular response (Troutman et al., 2012a). The precise mechanism underlying how PI3K pathway regulates the host response remains unknown. Furthermore, the pharmacological effect of PI3K remains controversial. Some studies suggest PI3K contributes to NF-κB activation and thus promotes the inflammatory response, while others have indicated that the PI3K may inhibit the inflammatory response (Hazeki et al., 2007). Conversely, genetic evidence suggests that PI3K pathway serves as a crucial negative regulator of the proinflammatory response (Troutman et al., 2012b). Our research demonstrated that the expression of TLR4/NF-κB signaling was increased and the expression of PI3K/Akt signaling was decreased in the lung tissue of rats with sepsis-induced ALI. HSBD may inhibit the cytokine storm of sepsis by downregulating TLR4/NF-κB and upregulating PI3K/Akt, thus protecting the lung tissue from LPS-induced injuries.

Conclusion

In summary, we used network pharmacology to explore the potential therapeutic mechanism of HSBD in the treatment of COVID-19 and sepsis. Animal experiments on LPS-induced ALI rats demonstrated that HSBD suppressed cytokine storms and attenuated pulmonary injury by regulating the expression of TLR4/NF-κB and PI3K/Akt. HSBD mainly exhibited anti-inflammatory activity and lung-protective action to protect against sepsis-induced ALI. HSBD may inhibit the cytokine storm of sepsis by downregulating TLR4/NF-κB and upregulating PI3K/Akt, thus protecting the lung tissue from LPS-induced injuries.

Data availability statement

The datasets analyzed during the current study may be available upon reasonable request.
Karki, R., Sharma, B.R., Tuladhar, S., Williams, E.P., Zalduondo, L., Samir, P., Zheng, M., Jiang, Q., Yi, M., Guo, Q., Wang, C., Wang, H., Meng, S., Liu, C., Fu, Y., Ji, H., Chen, T., Hu, X., Xu, Q., Wan, H., Hu, Y., Xing, S., Yang, H., Gao, Y., He, Z., 2020. PI3K-Akt-Hazeki, K., Nigorikawa, K., Hazeki, O., 2007. Role of phosphoinositide 3-kinase in innate immun. Biol. Pharm. Bull. 30, 1617–1623. doi:10.1248/bpb.30.1617.

Hu, X., Xu, Q., Wan, H., Hu, Y., Xing, S., Yang, G., Hao, Y., He, Z., 2020. PI3K-Akt-mTOR/PPARβ pathway mediated lung fibroblast alcoholic glycolysis and collagen synthesis in lipopolysaccharide-induced pulmonary fibrosis. Lab. Invest. 100, 801–811. doi:10.1038/s41397-020-0404-9.

Jiang, Q., Yi, M., Guo, Q., Wang, C., Wang, H., Meng, S., Liu, C., Fu, Y., Ji, H., Chen, T., 2015. Protective effects of polyolatin on lipopolysaccharide-induced acute lung injury through TLR4-MYD88-NF-kappaB pathway. Int. Immunopharmacol. 29, 370–376. doi:10.1016/j.intimp.2015.02.027.

Ju, W., Li, J., Yu, W., Zhang, R., 2016. iGraph: an incremental data processing system for dynamic graph. Front. Comput. Sci. 10, 462–476. doi:10.1007/s11760-016-5485-7.

Karki, R., Sharma, B.B., Tuladhar, S., Williams, E.P., Zalduondo, L., Samir, P., Zheng, M., Sundaram, B., Banotth, B., Malireddi, R.K.S., Schreiner, P., Neale, G., Vogel, P., Weebly, R., Jonsson, C.B., Kanagam, T.D., 2021. Synergism of TNF-α and IFN-γ Triggers Inflammatory Cell Death, Tissue Damage, and Mortality in SARS-CoV-2 Infection and Cytokine Storm Syndrome. Cell 184, 149–168 e17. doi:10.1016/j.cell.2021.07.005.

Kim, K.A., 2018. Cytokine release syndrome and neurotoxicity after CD19 chimeric antigen receptor-modified (CAR-) T cell therapy. Br. J. Haematol. 183, 364–374. https://doi.org/10.1111/bjh.15644.

Reyes, M., Filbin, M.R., Bhattacharyya, R.P., Blistman, K., Eisenhaure, T., Hung, D.T., Levy, B.D., Baron, R.M., Blainey, P.C., Goldberg, M.B., Hochon, N., 2020. An immune-cell signature of bacterial sepsis. Nat. Med. 26, 333–340. doi:10.1038/s41591-020-0752-4.

Sadowitz, B., Roy, S., Gatto, L.A., Habashi, N., Nieman, G., 2011. Lung injury induced by sepsis: Lessons learned from large animal models and future directions for treatment. Expert Rev. Anti Infect. Ther. 9, 1169–1178. https://doi.org/10.1586/eri.11.141.

Shen, W., Gan, J., Xu, S., Jiang, G., Wu, H., 2009. Penicillolide hydrochloride attenuates LPS-induced acute lung injury involvement of NF-kappaB pathway. Pharmacol. Res. 60, 296–302. doi:10.1016/j.phrs.2009.04.007.

Shi, N., Guo, L., Liu, B., Bian, Y., Chen, R., 2021. Efficacy and safety of Chinese herbal medicine versus Lopinavir-Ritonavir in adult patients with coronavirus disease 2019: A non-randomized controlled trial. Phytomedicine, 153367. doi:10.1016/j.phymed.2021.696976.

Takatsuki, O., Akira, S., 2010. Pattern recognition receptors and inflammation. Cell 140, 805–826. doi:10.1016/j.cell.2010.01.022.

Thompson, B.T., Chambers, R.C., Liu, K.D., 2017. Acute respiratory distress syndrome. N. Engl. J. Med. 377, 562–572. doi:10.1056/NEJMra1608077.

Troutman, T.D., Bazan, J.F., Pasare, C., 2012a. Role for B-cell adapter for PI3K (BCAP) as a signaling adapter linking Toll-like receptors (TLRs) to serine/threonine kinases PI3K/Akt. Proc. Natl. Acad. Sci. U. S. A 109, 273–278. https://doi.org/10.1073/pnas.1011879109.

Troutman, T.D., Bazan, J.F., Pasare, C., 2012b. Toll-like receptors, signaling adapters and regulation of the pro-inflammatory response by PI3K. Cell Cycle 11, 3559–3567. doi:10.4161/cc.20434.12056.

Vanhaesebroeck, B., Ali, K., Bilancio, A., Geering, B., Foukas, L.C., 2005. Signalling by PI3K isoforms: insights from gene-targeted mice. Trends Biochem. Sci. 30, 194–204. doi:10.1016/j.tibs.2005.02.008.

Walker, B.R., 2007. Glucocorticoids and cardiovascular disease. Eur. J. Endocrinol. 157, 545–559. doi:10.1530/EJ.1.04016.

Wang, D.L., Li, C., Ha, T., Ozment-Skelton, T., Kalbfleisch, J.H., Preiszner, J., 2021. Lung injury induced by COVID-19. Int. Immunopharmacol. 86, 106749 https://doi.org/10.1016/j.intimp.2021.106749.

Wang, M.D., Chen, F., 2010. The anti-inflammatory effect of Hua Shi Bai Du granule (Q-14) and standard care in the treatment of patients with coronavirus disease 2019 (COVID-19): A single-center, open-label, randomized controlled trial. Phytomedicine, 153367. https://doi.org/10.1016/j.phymed.2020.153367.

Zhu, H., Shi, J., Leng, L., Li, D., Guo, L., Huang, L., 2021. Combination of Hua Shi Bai Du granule and IFN-α in treatment of COVID-19 patients. J. Integr. Med. 19, 473–480. https://doi.org/10.1016/s1877-7856(21)60046-8.

Zhang, X., Zhang, Y., Qiao, W., Zhang, J., Qi, Z., 2020. Baricitinib, a drug with potential anti-inflammatory effect, alleviates sepsis and improves the survival of sepsis mice. Acta Pharmacol. Sin. 41, 1821–1824. doi:10.1038/s41401-020-0284-5.

Wang, T., Devarakonda, P., Oza, P., Khan, A., 2021. Paclitaxel alleviated sepsis-induced acute lung injury by activating MUC1 and suppressing TLR4-NF-κB pathway. Drug Des. Devel. Ther. 15, 3391–3404. doi:10.2147/DDDT.S222296.

Wei, L.L., Wu, S.F., Li, H.J., Li, Z.W., Hu, Y., Yao, C.L., Zhang, J.Q., Li, J.Y., Wu, W.Y., Guo, D.A., 2021. Chemical profiling of Hua Shi Bai Du prescription, an effective anti-COVID-19 TCM formula, by UPLC-Q-TOF/MS. Chin. J. Nat. Med. 19, 473–480. https://doi.org/10.1016/s1877-7856(21)60046-8.