Baicalein suppresses inflammation and attenuates acute lung injury by inhibiting glycolysis via HIF-1α signaling

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Abstract. Baicalein, a flavonoid monomer compound isolated from the dried root of the traditional Chinese herb *Scutellaria baicalensis*, has several pharmacological activities, such as anti-inflammatory, anti-angiogenic, antitumor, antimicrobial and antiviral properties. Acute lung injury (ALI) is characterized by injury of the alveolar epithelium and capillary endothelium, which results in decreased lung volume, decreased lung compliance, ventilation/perfusion mismatch, intrapulmonary edema, alveolar edema and even acute hypoxemic respiratory failure. The present study aimed to investigate the effects of baicalein on lung injury and inflammation. Bioinformatics analysis using network pharmacology predicted that the hypoxia inducible factor-1α (HIF-1α) and glycolysis signaling pathways were involved in the mechanism underlying the therapeutic effects of baicalein. Further *in vitro* and *in vivo* experiments, such as immunohistochemistry, immunofluorescence and PCR, verified that baicalein could inhibit HIF-1α signaling, thus suppressing glycolysis, and improving inflammatory responses and ALI. Taken together, the results of the present study suggested that the anti-inflammatory effects of baicalein on treating ALI were associated with its ability to suppress glycolysis via the HIF-1α signaling pathway.

Introduction

Acute lung injury (ALI) is characterized by injury of the alveolar epithelium and capillary endothelium, eventually resulting in reduced lung volume and compliance, ventilation/perfusion mismatch, intrapulmonary and alveolar edema, and even acute hypoxemic respiratory failure (1,2). The most common treatment approaches for ALI include treating the primary disease and respiratory support (3). Antibiotics and corticosteroids used in the clinical treatment of ALI are not recommended for long-term use, due to their significant adverse reactions and the risk of drug dependency (4). Therefore, investigating the mechanisms underlying the development of ALI, and identifying novel drugs for the prevention and treatment of ALI, are of significant importance.

The pathogenesis of ALI is relatively complex. ALI is caused either by direct lung injury or by acute systemic inflammation, and is closely associated with the release of inflammatory signals (5). The inflammatory process is one of the core pathophysiological processes of ALI. During this process, inflammatory cells, such as macrophages (5), T lymphocytes (6) and neutrophils (7), are activated and trigger the release of various inflammatory mediators, including cytokines, chemokines, reactive oxygen species and proteolytic enzymes. In turn, the aforementioned inflammatory mediators can directly damage alveolar epithelial and capillary endothelial cells, thus leading to increased permeability of the alveolar-capillary barrier, pulmonary edema and severe impairment of lung function (5,8). Among these inflammatory cells, macrophages serve a key role in the inflammatory response, acting as both initiators and regulators. Recent studies have emphasized the significance of alveolar macrophages in the development of ALI and their potential as therapeutic targets (9-11).

Previous studies have also indicated that the glycolytic pathway could be involved in inflammatory responses and ALI (12,13). Glycolysis is a metabolic pathway that mediates the conversion of glucose into pyruvate, and it is upregulated under hypoxic conditions or in response to inflammation. As an intracellular enzyme, lactate dehydrogenase (LDH) is involved in enhanced aerobic glycolysis via catalyzing the reversible transformation of pyruvate to lactate, reflecting the metabolic changes and cellular damage associated with the inflammatory process in ALI (14). Under inflammatory conditions, there is a switch from cell metabolism towards increased glycolysis, known as aerobic glycolysis. This metabolic

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reprogramming supports the energy and biosynthetic demands of inflammatory cells, eventually facilitating the production and release of inflammatory mediators (15). In this process, inflammatory cells produce large amounts of lactate through aerobic glycolysis, which not only alters the intracellular and extracellular pH, but also intensifies the inflammatory response and tissue damage. The presence of cytokines, such as tumor necrosis factor-α (TNF-α) and IL-6, during inflammation can lead to cell damage and alterations in LDH activity, thereby affecting lactate production and its subsequent effects on the inflammatory process (16). Therefore, investigating glycolysis for the management and prognosis of patients with ALI is of clinical significance. Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor that becomes stabilized and active at low oxygen levels (17). It has been reported that HIF-1α not only promotes glycolysis via inducing the expression of genes encoding glycolytic enzymes and glucose transporters, but also serves a key role in the inflammatory response (18,19).

Baicalein, a flavonoid monomer compound isolated from the dried root of the traditional Chinese herb *Scutellaria baicalensis*, has a distinct structure characterized by a keto-enol tautomeric system (20). Baicalein has several roles in various processes, including scavenging of oxygen free radicals (21), anti-inflammatory action (22), inhibition of angiogenesis (23), antitumor activities (24), and antimicrobial and antiviral properties (25). However, whether baicalein can alleviate ALI via regulating glycolysis in macrophages remains to be elucidated. Therefore, the present study aimed to evaluate the potential therapeutic effects of baicalein via establishing models of ALI and macrophage inflammatory response. Additionally, bioinformatics network analysis was performed to identify the mechanism underlying the effects of baicalein on ALI.

Materials and methods

Chemicals. Baicalein (CAS no. 491-67-8) was purchased from Chengdu Must Bio-Technology Co., Ltd. Lipopolysaccharide (LPS) was obtained from MilliporeSigma and dexamethasone (DEX; CAS no. 50-02-2) from Shanghai Yuan Ye Bio-Technology Co., Ltd. The antibodies against hexokinase 2 (HK2; cat. no. 66974-1-Ig), phosphofructokinase-1 (PFK1; cat. no. 55028-1-AP), pyruvate kinase M2 (PKM2; cat. no. 10078-2-AP) and HIF1α (cat. no. 20960-1-AP) were purchased from Proteintech Group, Inc.

Animal studies. A total of 30 male C57BL/6 mice (weight, 18-22 g; age, 6-8 weeks) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. In the present study, animal welfare considerations included all possible efforts to minimize suffering and distress, as well as the use of anesthetics and controlled housing conditions. For example, mice were housed in a temperature-controlled (23.0±1.0˚C) and humidity-controlled (40-60%) cage under a 12-h light/dark cycle and with free access to food and water. The bedding material was composed of fine and softwood chips. To maintain a hygienic environment, the bedding material was changed at 2-day intervals. Animal health and behavior were monitored every day, and body weights were assessed weekly over the course of the study. Mice were randomly divided into the following five groups (n=6 mice/group): Normal, LPS, 10 mg/kg baicalein, 20 mg/kg baicalein and DEX groups. All groups received one-time tracheal instillation of LPS, with the exception of the normal group. A total of 7 days before modeling, the groups designated to receive medication were administered baicalein and DEX (2 mg/kg) by gavage. The ALI mouse model was established by a one-time tracheal instillation of LPS (5 mg/kg), which was a non-invasive procedure. Firstly, mice were anesthetized via intraperitoneal injection of pentobarbital sodium (50 mg/kg; MilliporeSigma). Subsequently, the mice were suspended on the experimental operating table, and their tongues were pulled to the side to expose the tracheal opening. A catheter was then inserted into the trachea along the opening. The needle core was immediately removed, and the corresponding LPS and saline solution was injected into the catheter using a 1-ml syringe. The normal group of mice was only injected with the corresponding volume of saline solution. Finally, the mice were placed under room temperature to recover from anesthesia before they were returned to their cages. At 24 h after modeling, the mice were euthanized by cervical dislocation, and bronchoalveolar lavage fluid (BALF), serum and lung tissue samples were collected. In addition, 0.3 ml blood was collected from the retro-orbital vein just before sacrifice and serum was obtained by centrifuging the blood at 1,500 x g for 15 min at 4˚C. The death of the mice was verified by lack of respiration, heartbeat and corneal reflex. The duration of the experiment was 15 days, including 7 days of adaptation, 7 days of dosing and 1 day of modeling. Animal health and behavior were monitored every day and no spontaneous death occurred during the present study. The present study was approved by the Ethics Committee of the Zhumadian Hospital of Traditional Chinese Medicine (approval no. 2024104001; Zhumadian, China). Mice that reached the humane endpoints (including but not limited to >15% weight loss, inability to eat or drink, and signs of extreme distress or pain) or completed the experiments were euthanized. In the present study, no mice reached the predefined humane endpoints before the end of the experiments.

Cell culture. MH-S mouse alveolar macrophages (cat. no. GNM43; The Cell Bank of Type Culture Collection of The Chinese Academy of Sciences) were cultured in RPMI 1640 complete medium (Beijing Solarbio Science & Technology Co., Ltd.) supplemented with 10% fetal bovine serum (Lonsera; Shanghai Shuangru Biotechnology Co., Ltd.). The RPMI 1640 medium used in the experiment contains 1% penicillin and streptomycin. The cells were divided into the following five groups: Control group; model group (100 ng/ml LPS); and baicalein groups (40, 20 and 10 μM). With the exception of the control group, the other groups were stimulated with LPS. Cells were stimulated with LPS (100 ng/ml) and baicalein (40, 20 and 10 μM) in an incubator at 37˚C and 5% CO_2. After 6 h of treatment, cells were harvested for testing.

Wet/dry (W/D) lung weight ratio. The left lungs obtained from the mice were washed with saline and dried, and the wet weight of the lungs was then measured. Subsequently, the left lungs were dried overnight in an oven at 65˚C and the dry weight was measured. The W/D ratio was calculated based on the wet and dry weights.

BALF collection and protein assay. The left lungs were lavaged three times with 0.6 ml PBS to collect BALF, and the protein concentration in the BALF was then determined using a BCA kit. In addition, BALF was centrifuged at 1,000 x g for 10 min at 4°C, and the cell pellet and supernatant of BALF were collected separately. Subsequently, the cell pellet was resuspended in PBS and total cells were counted using a hemocytometer.

Evaluation of pathological changes in lung tissues. The upper lobes of the left lungs were fixed in 4% paraformaldehyde for 48 h at room temperature, embedded in paraffin, sectioned (4 μm) and stained with 1% hematoxylin for 10 min at room temperature and 1% eosin for 1 min at room temperature. Subsequently, pathological changes in the lung tissues were observed under a light microscope. The lung tissues stained with hematoxylin and eosin (H&E) were analyzed based on the Szapiel score (26).

Measurement of lactic acid. The levels of lactic acid in lung tissues and cell supernatants were measured using a lactate colorimetric assay kit (cat. no. E-BC-K044-M; Elabscience®; Elabscience Bionovation Inc.), according to the manufacturer's instructions. The absorbance was then measured at 530 nm and lactic acid levels in the intervention groups were normalized to those of the control group, as previously described (27).

Immunohistochemistry (IHC). The aforementioned paraffin-embedded tissue sections from the upper lobes of the left lungs were deparaffinized with xylene and rehydrated in a graded alcohol series (100, 90 and 70%). The sections were then heated in 10 mM sodium citrate buffer (pH 6.0), for 15 min in a 95°C water bath for antigen retrieval. The sections were then washed with PBS three times (3 min each). Endogenous peroxidase activity was blocked by incubation with 3% H_2O_2 for 15 min at room temperature, and nonspecific immunoreactions were blocked using 5% inactivated goat serum (Beijing Solarbio Science & Technology Co., Ltd.) in PBS for 30 min at room temperature. The sections were then incubated with HK2 (1:200), PFK1 (1:200), PKM2 (1:400) and HIF-1α (1:200) antibodies overnight at 4°C. After washing with PBS, the sections were incubated with goat anti-rabbit IgG-HRP secondary antibody (1:500; cat. no. sc-2030; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. After counterstaining with hematoxylin for 5 min at room temperature, images of the sections were captured using the light microscope (Carl Zeiss AG).

Immunofluorescence staining. The paraffin-embedded lung tissue was cut in 4-μm sections. After dewaxing and rehydrating, the slides were subjected to antigen retrieval, by immersing in 10 mM sodium citrate (pH 6.0) and microwaving at 1,000 W for 30 min. The sections were then blocked with 5% inactivated goat serum at room temperature for 30 min. Subsequently, the sections were incubated with an antibody against HIF-1α (1:400) at 4°C overnight. Subsequently, Alexa Fluor® 488-conjugated Goat Anti-Rabbit IgG H&L (1:1,000; cat. no. 4412; Cell Signaling Technology, Inc.) was added and incubated at 37°C for 1 h. The sections were incubated with DAPI (cat. no. 4083; Cell Signaling Technology, Inc.) in the dark at room temperature for 5 min for nuclear counterstaining. The collected images were captured under a laser confocal microscope (LSM700; Carl Zeiss AG).

Enzyme-linked immunosorbent assay (ELISA). The concentrations of TNF-α and IL-6 in mouse BALF, serum and cell supernatants were measured using the TNF-α ELISA kit (cat. no. E-EL-M3063; Elabscience®; Elabscience Bionovation Inc.) and the IL-6 ELISA kit (cat. no. E-EL-M0044; Elabscience®; Elabscience Bionovation Inc.), according to the manufacturer's instructions and previous studies (28).

Cell viability assays. Cell viability was detected using the Cell Counting Kit-8 (CCK-8) assay kit (Dojindo Molecular Technologies, Inc.). Briefly, cells ($3x10^3$ cells/well) were seeded into 96-well plates and incubated at 37°C with 5% CO_2. After incubation with baicalein at varying concentrations (40, 20, 10, 5 and 2.5 μM) for 6 h, 10 μl CCK-8 solution was added to each well and incubated for an additional 4 h at 37°C. Absorbance was subsequently measured at a wavelength of 450 nm.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from cells using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and was reverse-transcribed into cDNA using a HiScript® II 1st Strand cDNA Synthesis Kit (cat. no. R211-01; Vazyme Biotech Co., Ltd.), according to the manufacturer's instructions. The mRNA expression levels of HK2, PFK1, PKM2 and HIF-1α were detected using AceQ® qPCR SYBR® Green Master Mix (cat. no. Q111-02; Vazyme Biotech Co., Ltd.). mRNA expression levels were normalized to ACTB and calculated using the $2^{-\Delta\Delta Cq}$ method (29). The primer sequences used are listed in Table I. The following thermocycling conditions were used for qPCR: 95°C for 30 sec, followed by 40 cycles at 95°C for 10 sec and 60°C for 30 sec, 95°C for 15 sec, and final extension at 60°C for 60 sec and 95°C for 15 sec.

Acquisition of ALI-related targets. ALI-associated genes were identified using the GeneCards (https://www.genecards.org) database with 'acute lung injury' used as the key word. The targets of baicalein were obtained through screening in the SwissTargetPrediction database (http://old.swisstargetprediction.ch).

Protein-protein interaction (PPI) network construction and core target screening. The overlapping targets between baicalein and ALI were imported into the STRING v11.5 database (https://string-db.org/) and a PPI network was constructed using Cytoscape software (version 3.7.1; https://cytoscape.org/). The top 100 genes were identified as core targets using the Cytohubba (https://apps.cytoscape.org/apps/cytohubba) (30), a plugin in Cytoscape software.

Gene Ontology (GO) term and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The overlapping targets were analyzed for GO term and KEGG pathway enrichment using the DAVID database (https://david.ncifcrf.gov). GO terms and KEGG pathways

Table I. Primers used in the present study.

Gene	Forward, 5'-3'	Reverse, 5'-3'
ACTB	GTGACGTTGACATCCGTAAAGA	GCCGGACTCATCGTACTCC
HIF-1α	TCTCGGCGAAGCAAAGAGTC	AGCCATCTAGGGCTTTCAGATAA
HK2	TGATCGCCTGCTTATTCACGG	AACCGCCTAGAAATCTCCAGA
PFK1	GGAGGCGAGAACATCAAGCC	CGGCCTTCCCTCGTAGTGA
PKM2	GCCGCCTGGACATTGACTC	CCATGAGAGAAATTCAGCCGAG
IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG
TNF-α	CTGAACTTCGGGGTGATCGG	GGCTTGTCACTCGAATTTTGAGA

HIF-1α, hypoxia inducible factor-1α; HK2, hexokinase 2; PFK1, phosphofructokinase-1; PKM2, pyruvate kinase M2.

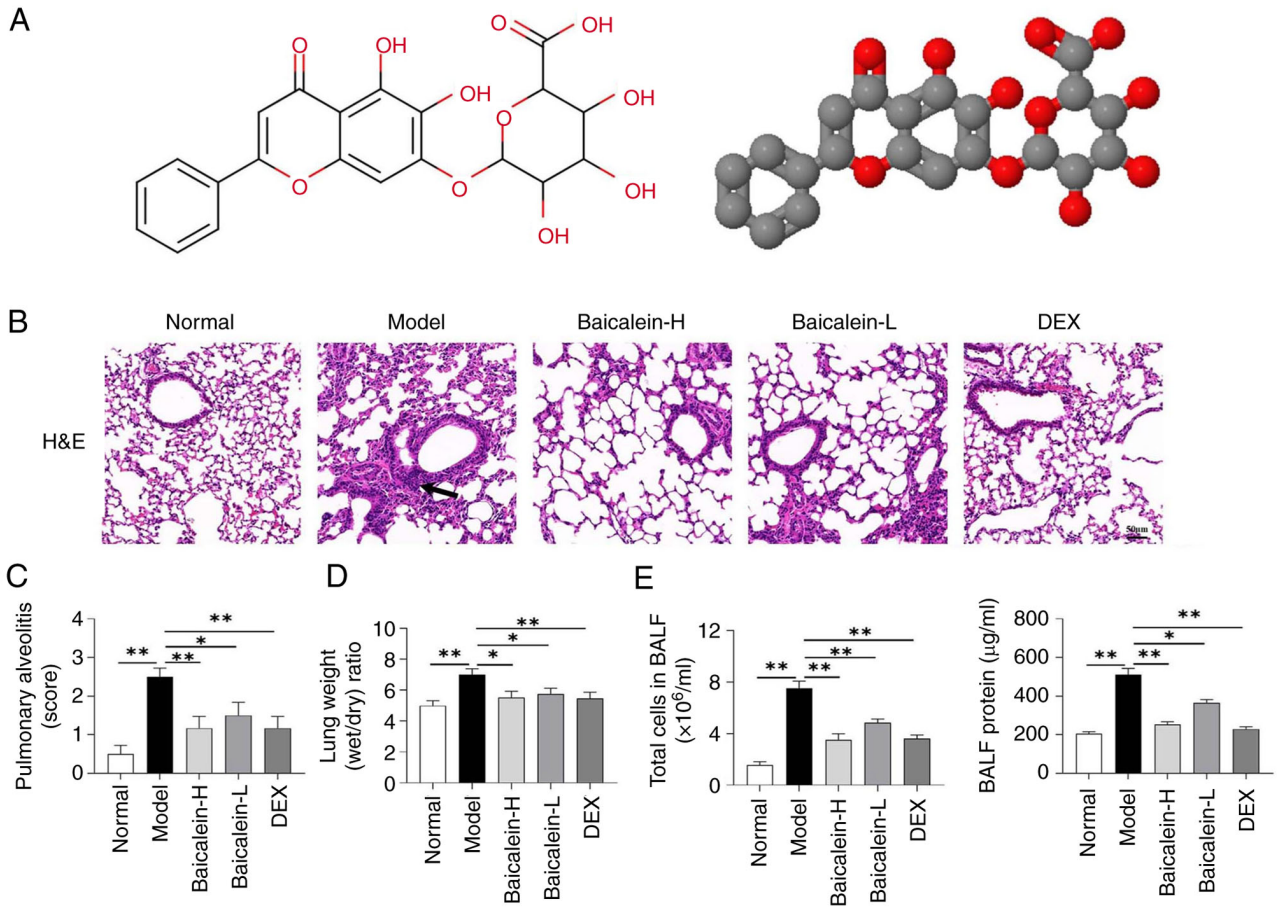

Figure 1. Baicalein ameliorates lung pathological damage in mice with lipopolysaccharide-induced acute lung injury. (A) 2D and 3D structure of baicalein. (B) H&E staining images of lung tissues (magnification, x200). The arrows represent inflammatory cell infiltration and thickening of the alveolar wall. (C) Inflammatory (alveolitis) score of the H&E staining images. (D) Lung wet/dry weight ratio of mice. (E) Number of total cells and total protein in the BALF of mice. Data are presented as the mean ± SEM, n=6. *P<0.05, **P<0.01. BALF, bronchoalveolar lavage fluid; DEX, dexamethasone; H, high; H&E, hematoxylin and eosin; L, low.

with P<0.05 were considered significantly enriched. Notably, no specific gene count thresholds were applied in the analysis. Component and target molecular docking were performed using AutoDock Tools 1.5.6 software (The Scripps Research Institute, La Jolla, CA, USA).

Statistical analysis. The data from cell experiments are from three independent experiments consisting of three replicates per experiment. All experimental data were analyzed using SPSS 23.0 software (IBM Corp.). Data are presented as the mean ± SEM. Non-parametric data were analyzed using the Kruskal-Wallis test and Dunn's multiple comparison test. The differences among multiple groups were analyzed by one-way ANOVA, followed by Dunnett's T3 for pairwise comparisons. P<0.05 was considered to indicate a statistically significant difference.

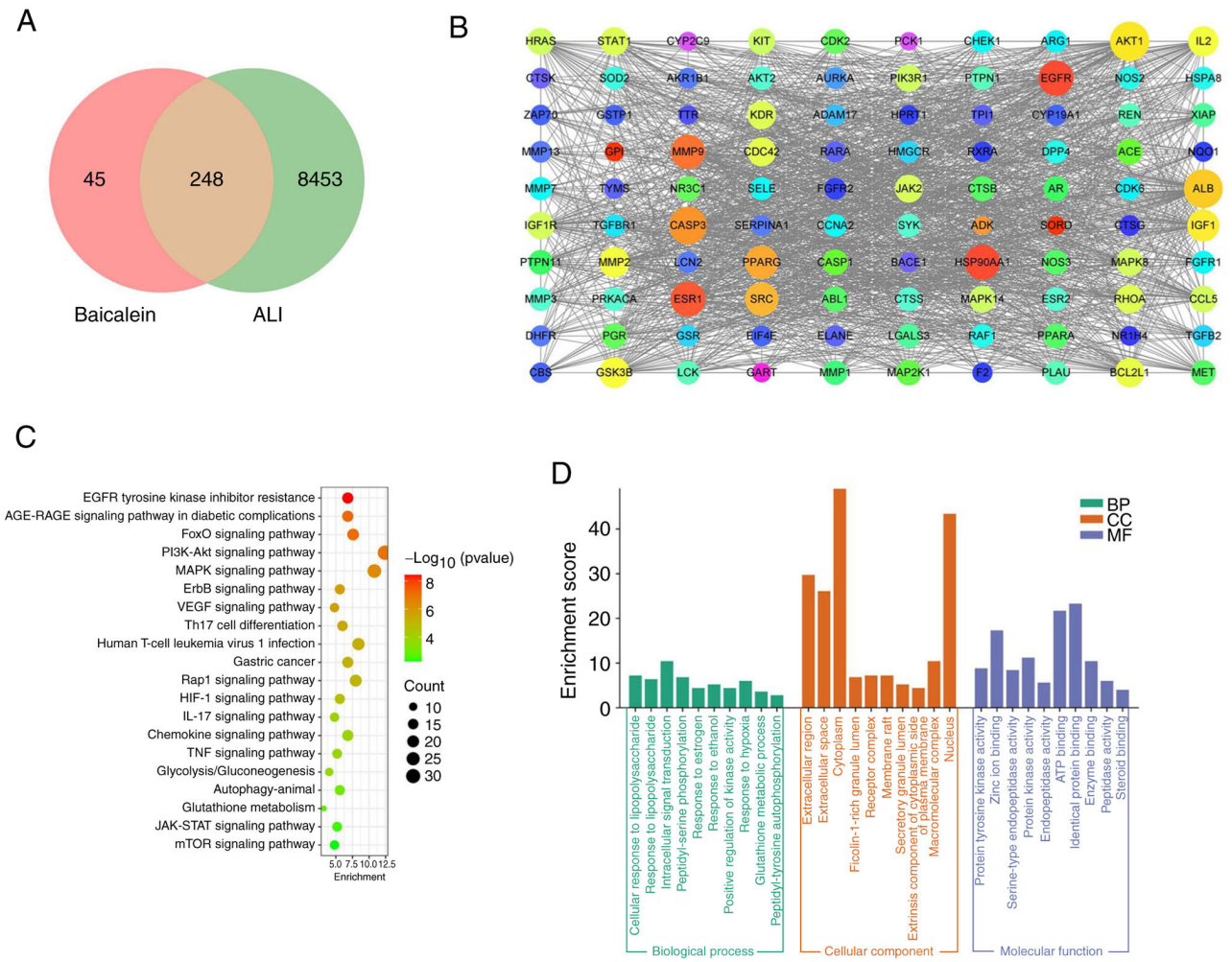

Figure 2. Network analysis of the baicalein-related targets and ALI-related genes. (A) Venn diagram showing 248 overlapping targets between baicalein-related targets and ALI-related genes. (B) Protein-protein interaction network of the top 100 overlapping targets. (C) KEGG pathway enrichment analysis of the overlapping targets. (D) GO analysis of the overlapping targets. GO terms and KEGG pathways with P<0.05 were considered significantly enriched. ALI, acute lung injury; BP, biological process; CC, cellular component; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; MF, molecular function.

Results

Baicalein improves LPS-induced ALI in mice. To evaluate the therapeutic effects of baicalein (Fig. 1A) on ALI, lung tissue pathology, W/D weight ratio, the number of cells and the total protein concentration in the BALF of mice were assessed. As a well-accepted drug in treating lung injury with strong clinical evidence, DEX was used as a positive control. H&E staining showed that baicalein could markedly inhibit LPS-induced inflammatory cell infiltration, alveolar wall edema and thickening (Fig. 1B and C). Furthermore, baicalein reduced the lung W/D weight ratio (Fig. 1D), the total number of cells and the total protein concentration in the BALF of mice (Fig. 1E) compared with those in the model group. These results suggested that baicalein could improve LPS-induced lung injury, alveolar-capillary barrier dysfunction and pulmonary edema.

Potential target screening and analysis of baicalein in ALI. Bioinformatics analysis using the SwissTargetPrediction and GeneCards databases predicted 293 targets of baicalein and 8,701 ALI-related genes, respectively. The intersection of baicalein-related targets and ALI-related genes revealed a total of 248 overlapping targets (Fig. 2A). The PPI of the top 100 overlapping targets was analyzed using PPI network analysis (Fig. 2B). KEGG enrichment analysis with P<0.05 showed that the aforementioned overlapping targets were enriched in 'MAPK signaling pathway', 'HIF-1 signaling pathway' and 'Glycolysis/Gluconeogenesis' (Fig. 2C). The GO functional enrichment analysis results with P<0.05 indicated that the overlapping targets were associated with 'cellular response to lipopolysaccharide' and 'response to lipopolysaccharide' (Fig. 2D).

Baicalein inhibits the inflammatory response in LPS-induced macrophages and mice with LPS-induced ALI. The inflammatory response is a key pathological process in ALI; therefore, the level of inflammation in mice with ALI was first evaluated. The results showed that baicalein significantly reduced the levels of IL-6 and TNF-α in the serum and BALF of mice compared with those in the model group mice (Fig. 3A and B). Additionally, cell viability was measured to assess the cytotoxicity of baicalein on MH-S cells. The result showed that the cell viability was not significantly affected by baicalein treatment (Fig. 3C). Furthermore, baicalein significantly inhibited

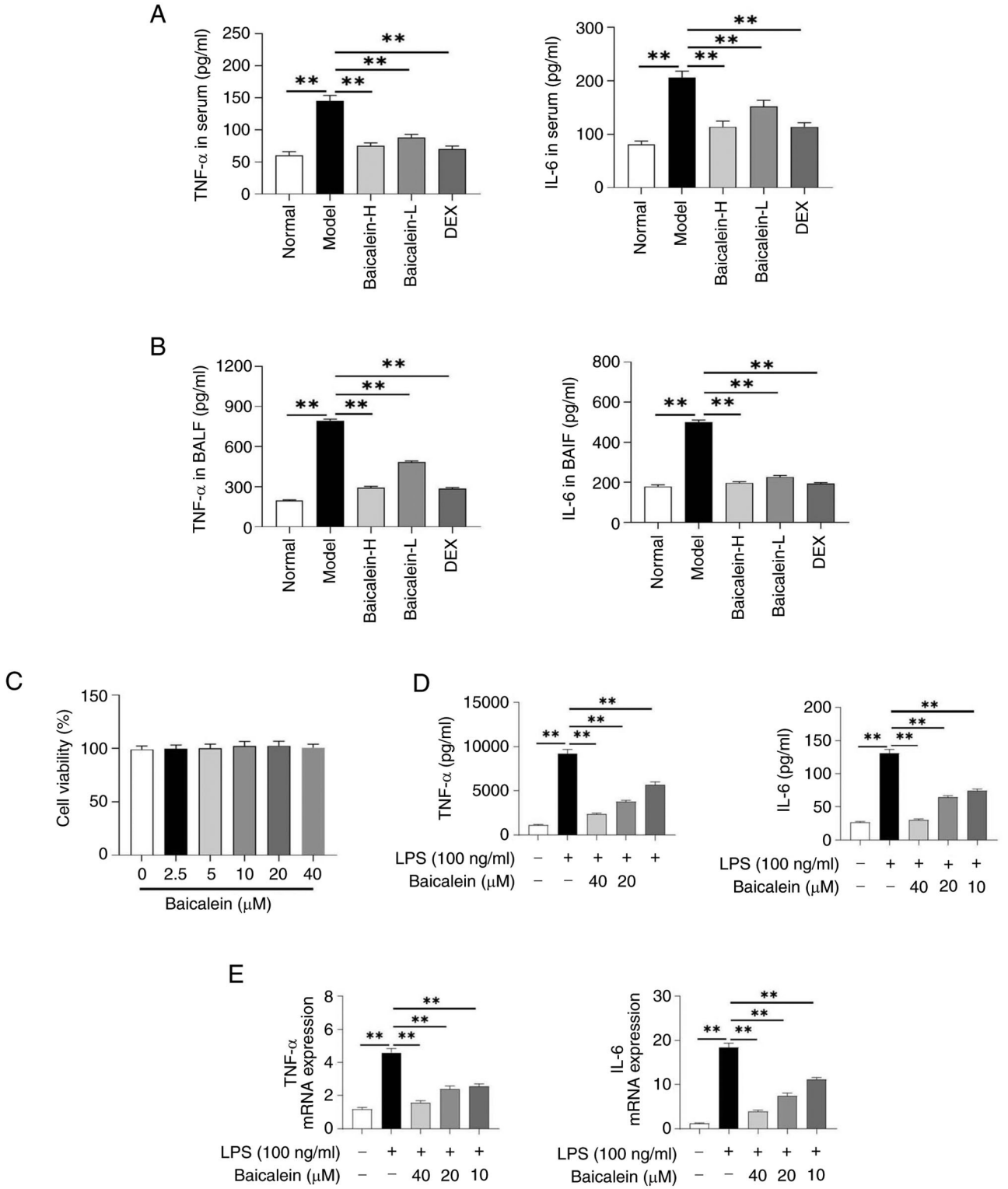

Figure 3. Baicalein inhibits the inflammatory response in LPS-induced macrophages and in mice with LPS-induced acute lung injury. Levels of IL-6 and TNF-α in the (A) serum and (B) BALF of mice. (C) Effects of baicalein at various concentrations on MH-S cell viability. Levels of IL-6 and TNF-α were assessed using (D) enzyme-linked immunosorbent assay and (E) quantitative PCR in MH-S cells. Data are presented as the mean ± SEM. **P<0.01. BALF, bronchoalveolar lavage fluid; DEX, dexamethasone; H, high; L, low; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α.

the levels of IL-6 and TNF-α in LPS-induced MH-S cells (Fig. 3D and E).

Baicalein inhibits glycolysis in LPS-induced macrophages and in the lung tissues of mice with LPS-induced ALI. Glycolysis serves a significant role in the inflammatory response; therefore, the present study aimed to explore whether baicalein could attenuate the inflammatory response via inhibiting glycolysis. The results showed that baicalein could inhibit the expression of key glycolysis-related enzymes (HK2, PFK1 and PKM2) in the lungs of mice with LPS-induced ALI and in LPS-induced macrophages (Fig. 4A-D and F). In

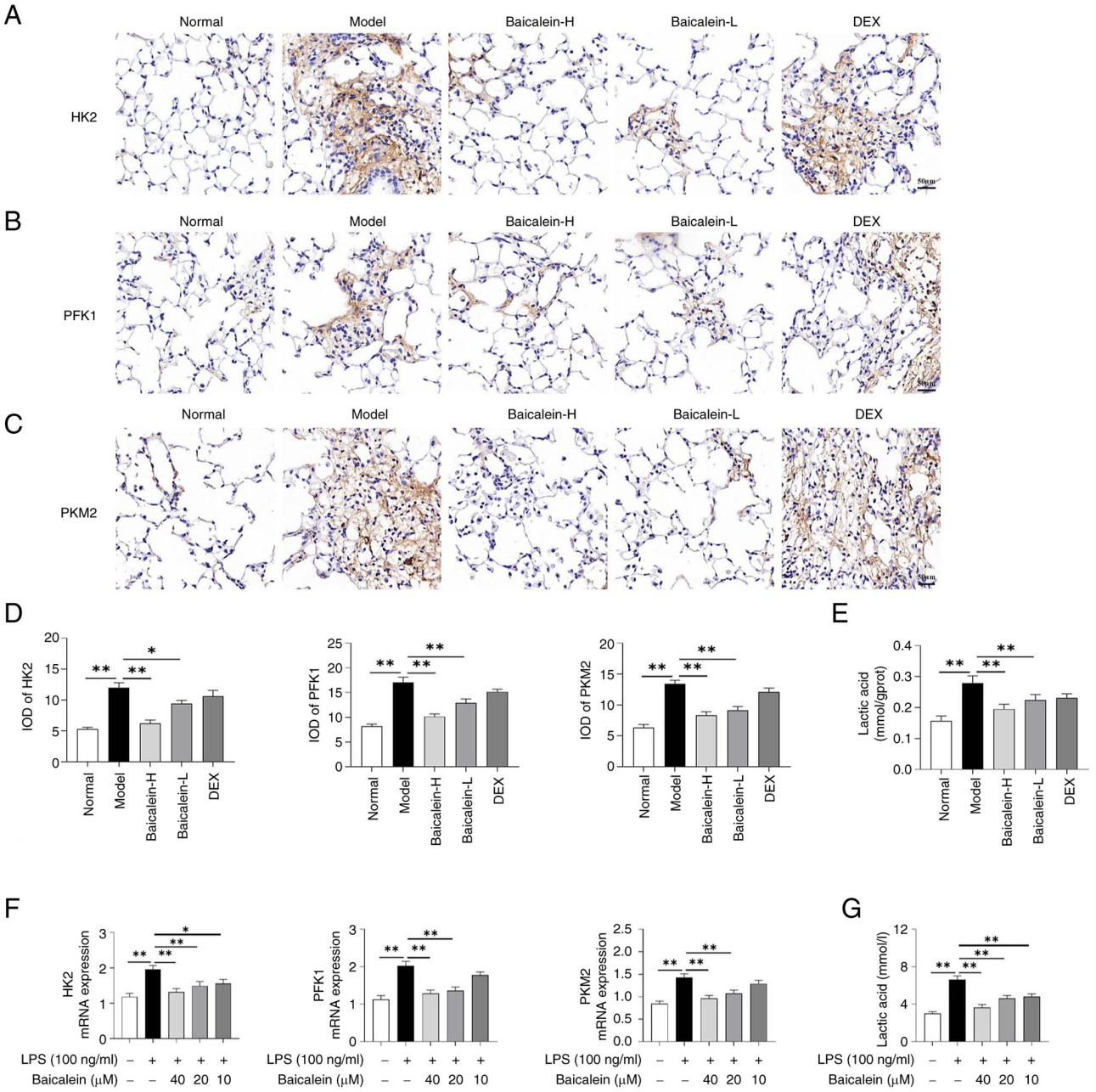

Figure 4. Baicalein inhibits glycolysis in LPS-induced macrophages and in the lung tissues of mice with LPS-induced acute lung injury. Immunohistochemical staining images of (A) HK2, (B) PFK1 and (C) PKM2 (magnification, x200) and (D) IOD values in the lungs. (E) Lactate content in the lung tissues. (F) mRNA expression levels of HK2, PFK1 and PKM2 in macrophages. (G) Lactate content in macrophages. Data are presented as the mean ± SEM. *P<0.05, **P<0.01. DEX, dexamethasone; H, high; HK2, hexokinase 2; IOD, integrated optical density; L, low; LPS, lipopolysaccharide; PFK1, phosphofructokinase-1; PKM2, pyruvate kinase M2.

addition, baicalein reduced lactate content in the lung tissues of LPS-induced ALI mice and in LPS-induced macrophages (Fig. 4E and G).

Baicalein suppresses HIF-1α signaling in LPS-induced macrophages and in the lung tissues of mice with LPS-induced ALI. HIF-1α is considered a significant activator of glycolysis and inflammatory responses. In the present study, the expression levels of HIF-1α were detected in the lung tissues of mice with ALI and in LPS-induced macrophages. Notably, baicalein could significantly inhibit the levels of HIF-1α in the lungs of LPS-induced ALI mice (Fig. 5A-C) and in LPS-induced macrophages (Fig. 5D). Furthermore, molecular docking experiments predicted that baicalein could interact with HIF-1α through the ASP-222 and ALA-312 residues (Fig. 5E).

Discussion

ALI is a respiratory disease associated with a high mortality rate, which has a notable impact on public health and is accompanied by acute inflammatory responses (31). Currently, the main interventions for patients with ALI include protective mechanical ventilation, anti-inflammatory drugs and corticosteroids (32). The primary pathological features of ALI commonly include release of inflammatory mediators, pulmonary edema and the destruction of alveolar structure (33).

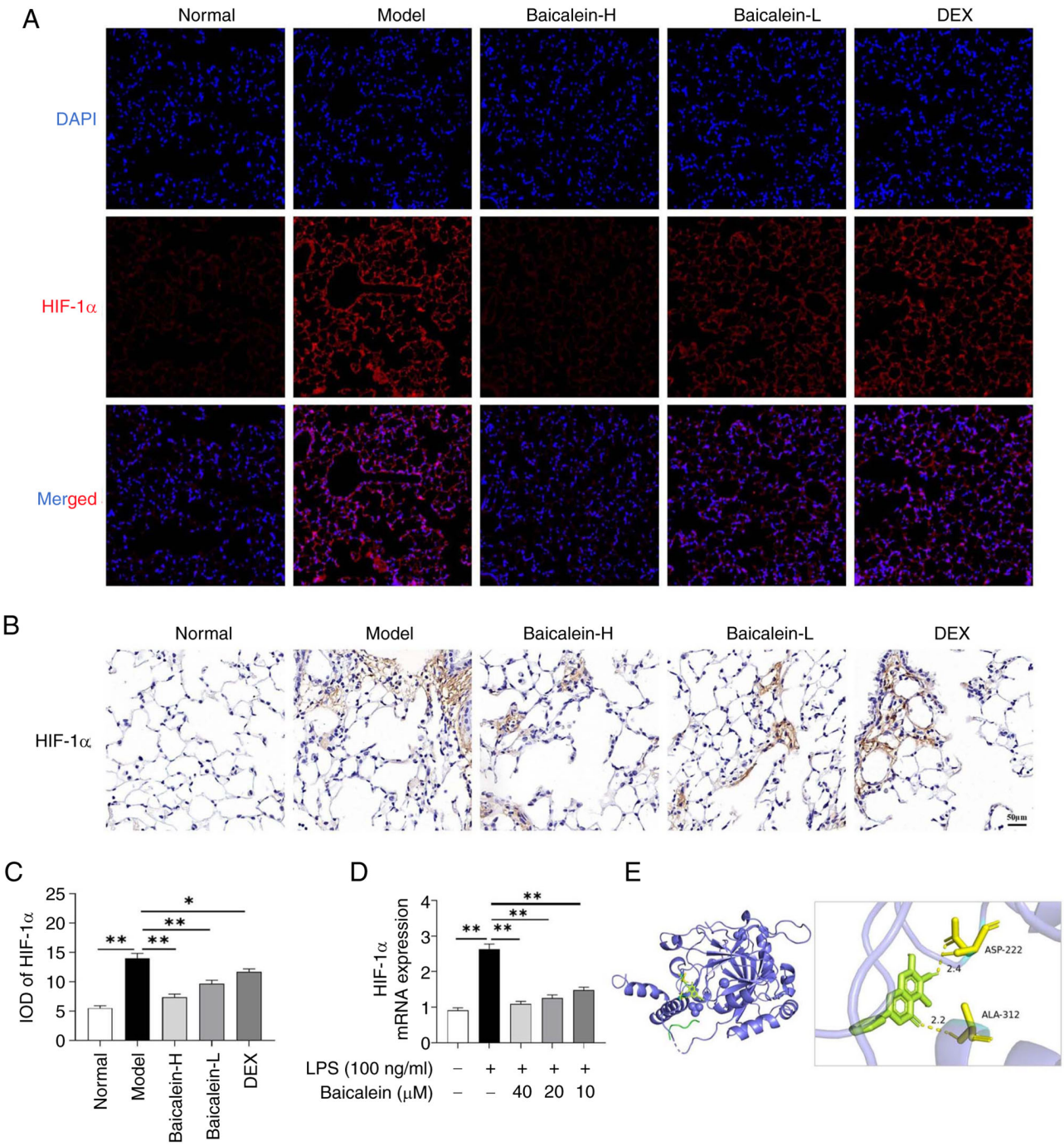

Figure 5. Baicalein suppresses HIF-1α signaling in LPS-induced macrophages and in mice with LPS-induced ALI. (A) Immunofluorescence staining images (magnification, x200) of HIF-1α in lung tissues. (B) Immunohistochemical staining images (magnification, x200) and (C) IOD values of HIF-1α in the lungs. (D) mRNA expression levels of HIF-1α in LPS-induced macrophages. (E) Molecular docking of baicalein and HIF-1α. Data are presented as the mean ± SEM. *P<0.05, **P<0.01. DEX, dexamethasone; H, high; HIF-1α, hypoxia inducible factor-1α; IOD, integrated optical density; L, low; LPS, lipopolysaccharide.

Therefore, intervening in inflammatory responses could be beneficial for patients with ALI. Baicalein possesses various pharmacological effects, such as scavenging oxygen free radicals (21), antipyretic and analgesic effects, and anti-inflammatory (22), anti-angiogenic (23) and antitumor properties (24). In addition, it can alleviate intestinal disorders through exerting regulatory effects on intestinal microorganisms and short-chain fatty acid production (34). Therefore, the present study aimed to evaluate the therapeutic effects of baicalein on ALI. The mechanism underlying the effects of baicalein intervention on ALI was predicted using network analysis. Finally, *in vitro* and *in vivo* experiments suggested that baicalein could improve ALI by inhibiting the inflammatory response through suppressing glycolysis via HIF-1α signaling.

The mouse model of LPS-induced ALI has been widely used in basic research on ALI-related diseases due to its maturity, reliability and high reproducibility (35,36). After entering the body, LPS binds to cell surface receptors to stimulate the recruitment and infiltration of inflammatory cells, and to

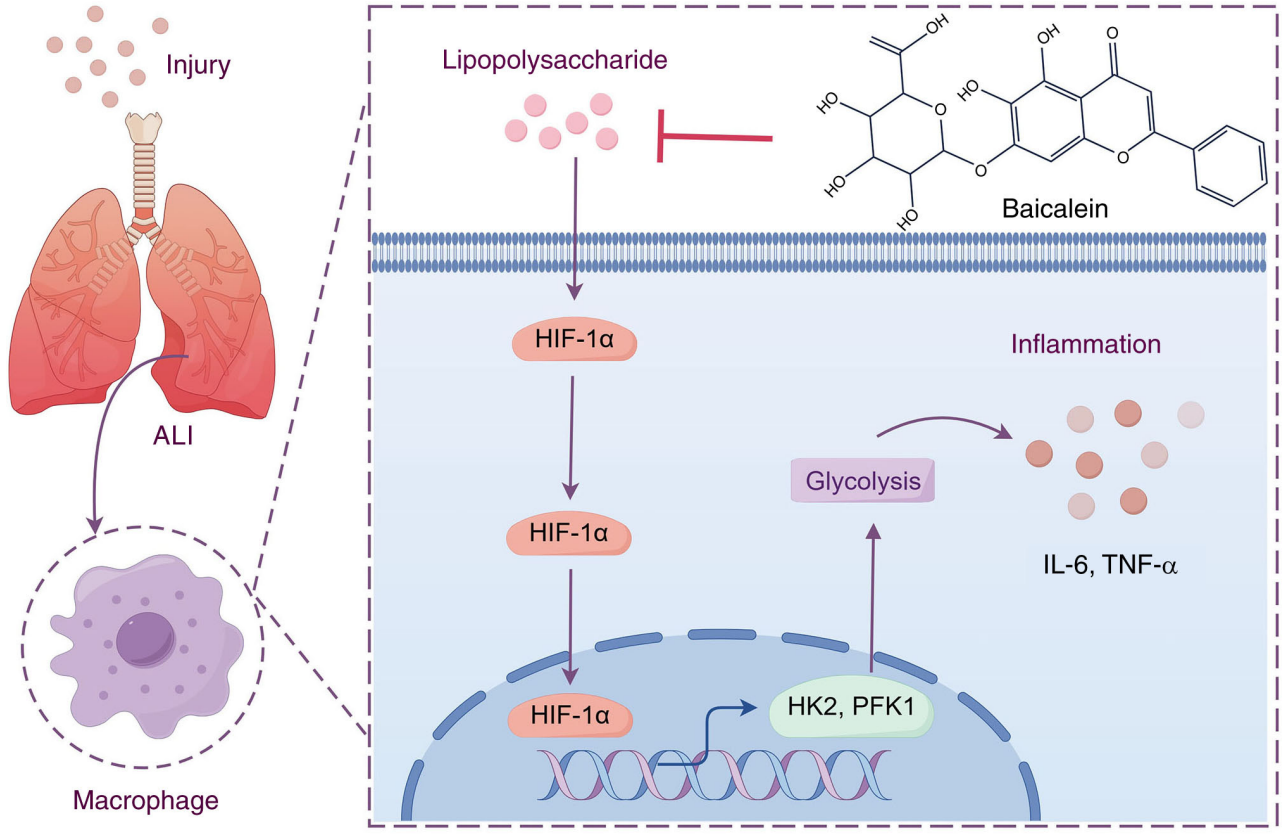

Figure 6. Diagram of the molecular mechanism underlying the therapeutic effects of baicalein on ALI. This picture was drawn by Figdraw (https://www.figdraw.com/; ID: PIPRId8c78). ALI, acute lung injury; HIF-1α, hypoxia inducible factor-1α; HK2, hexokinase 2; PFK1, phosphofructokinase-1; TNF-α, tumor necrosis factor-α.

increase the synthesis and release of inflammatory mediators, eventually leading to the destruction of lung tissue structure and functional impairment (37,38). Therefore, inhibition or alleviation of the inflammatory response may be an effective intervention measure for ALI. Herein, the results showed that baicalein could improve LPS-induced ALI in mice.

Network analysis is commonly used to predict the targets of active ingredients in treating corresponding diseases (39,40). In the present study, network analysis was used to predict the targets of baicalein and genes associated with ALI. The results showed that the overlapping baicalein-related targets and ALI-related genes were mainly associated with the 'Glycolysis/Gluconeogenesis' and 'HIF-1 signaling pathway'. Emerging evidence has suggested that crosstalk exists between inflammatory processes and metabolic dysregulation (41). During inflammation, cells experience metabolic shifts, including a heightened rate of glucose uptake and an increased production of lactic acid (42). This metabolic change, also known as glycolysis, induces the recruitment of inflammatory cells to the site of the inflammation (43). Glycolysis is a metabolic pathway, which provides the energy necessary for cellular survival, proliferation and differentiation (44,45). HK2 is considered the key metabolic enzyme that catalyzes the first step of glycolysis via phosphorylating glucose to produce glucose-6-phosphate (46). PFK1 is the rate-limiting enzyme in the glycolytic pathway, which converts fructose-6-phosphate to fructose-1,6-bisphosphate (47). The third key enzyme in glycolysis is pyruvate kinase, which catalyzes the production of pyruvate, thus promoting energy supply and amino acid synthesis metabolism (48). In addition to providing energy to cells, the glycolytic pathway has been reported to be associated with the occurrence of inflammation (49). Under inflammatory stimuli, such as infection, endotoxins and hypoxia, resting macrophages can polarize into pro-inflammatory macrophages, glycolysis metabolism becomes active and large amounts of pro-inflammatory factors, such as TNF-α and IL-1β, can be released (15). A previous study suggested that the aforementioned inflammatory factors could aggravate cell membrane damage, thus leading to LDH leakage (50), which in turn could further catalyze the conversion of pyruvate to lactate and aggravate lung injury. LDH is a marker of cell necrosis, as it is released into the extracellular space upon cell membrane disruption. The role of cytokines in inducing cell necrosis and the subsequent release of LDH have been addressed to connect the inflammatory processes with cellular damage (51). As a metabolic by-product, lactate accumulation is influenced by cytokine activity and is associated with metabolic disorders (52). Therefore, lactate accumulation could serve as a biomarker reflecting the interplay between inflammation and metabolic dysregulation. In the current study, baicalein reduced the levels of glycolysis-related enzymes and lactate content in the lung tissues of ALI mice and LPS-induced macrophages.

Glycolysis is regulated by several glycolytic key enzymes and HIF-1α (53,54). HIF-1α upregulation can directly induce the excessive expression of glycolysis-related genes, such as

HK and LDH, thus accelerating the glycolytic process (55). Enhanced expression of HIF-1α may also increase the expression of IL-1β and activate immune cells in the local environment (55). In the present study, baicalein down-regulated HIF-1α in the lung tissue of mice with ALI and in LPS-induced macrophages.

In conclusion, the results of the present study indicated that baicalein could improve ALI by suppressing inflammatory responses through inhibiting glycolysis via HIF-1α signaling (Fig. 6). These results supported the anti-inflammatory effects of baicalein on ALI and suggested that targeting glycolysis-related catalytic enzymes to inhibit glycolysis could be a promising therapeutic strategy for ALI.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

ZL participated in the study design and manuscript writing. XZ performed experiments, analyzed data and participated in manuscript writing. NL and ZW participated in statistical analysis and manuscript writing. ZL and XZ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Zhumadian Hospital of Traditional Chinese Medicine (approval no. 2024104001; Zhumadian, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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