Article

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Glycyrrhizic Acid Ameliorates Sepsis-Induced Acute Lung Injury Through Suppression of Endoplasmic Reticulum Stress

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Background: Sepsis-mediated acute lung injury (ALI) has a high mortality rate, and glycyrrhizic acid (GA) possesses diverse pharmacologic activities. Herein, we investigated the role of GA in attenuating sepsis-triggered ALI.
Methods: Septic ALI was induced by cecal ligation and puncture (CLP). Mice were assigned into 5 groups with varied treatments. Their time of death was recorded every 6 hours after surgery. The wet/dry (W/D) weight ratio of the lung was measured. Observation of lung tissues was conducted by hematoxylin and eosin (HE) staining. Protein concentration in bronchoalveolar lavage fluid (BALF), levels of inflammatory cytokines, and production of reactive oxygen species (ROS) were detected by bicinchoninic acid (BCA), enzyme-linked immunosorbent assay, and dihydroethidium (DHE) staining, respectively. Endoplasmic reticulum (ER) stress-related genes, heme oxygenase-1 (HO-1), Janus kinase 2 (JAK2), and signal transducer and activator of transcription 3 (STAT3) levels were quantified by western blot.
Results: GA remarkably elevated survival rate and mitigated lung injury ($p < 0.05$). CLP markedly increased the W/D weight ratio and BALF protein concentration, while GA did the opposite ($p < 0.05$). CLP promoted interleukin-1β (IL-1β), tumor necrosis factor α (TNF-α), interleukin 6 (IL-6), protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), phosphorylation of eIF2α (p-eIF2α), activating transcription factor 4 (ATF4), C/EBP homologous protein (CHOP), the phosphorylation level of JAK/STAT3, along with DHE intensity, while GA showed opposite effects ($p < 0.01$). Additionally, GA markedly enhanced the HO-1 level ($p < 0.05$).
Conclusion: GA holds promise for future improvements in treating sepsis-induced ALI.

Keywords: glycyrrhizic acid; endoplasmic reticulum stress; sepsis; acute lung injury

Introduction

Sepsis refers to a dysregulated host response to an ongoing or suspected infection [1]. As one of the oldest but unpredictable conditions in medicine, sepsis poses a considerable burden across all economic regions, contributing to 5.3 million deaths globally every year [2]. Sepsis is usually accompanied by respiratory dysfunction resulting from acute lung injury (ALI), characterized by acute hypoxemic respiratory failure with bilateral pulmonary infiltrates [3]. As a result of the progress made by modern medicine, ALI's lethality has fallen in the past decade, remaining at 30–40% [4]. Therefore, reducing the global burden of sepsis and taking adequate preventive measures against ALI are global health challenges.

The endoplasmic reticulum (ER) is a complex central intracellular organelle in the secretory pathway, commonly known as the protein folding factory [5]. It is a critical structure in protein folding, Ca^{2+} storage, and lipid and carbohydrate metabolism. In the ER lumen, unfolded or misfolded proteins can be accumulated under multiple cellular stresses [6], the increase of which is termed ER stress, and this is detrimental to cells [7]. Mounting evidence has proved that ER stress is associated with diverse disorders, such as inflammatory diseases [8–10]. Noteworthily, ER stress has been observed in sepsis-induced ALI [11]. Nevertheless, there is a knowledge gap in the interplay between ER stress and sepsis-mediated ALI. Hence, our study explored whether ER stress could be a new target for alleviating sepsis-induced ALI.

Chinese materia medica has latent potential in treating multiple diseases through its antioxidant, anti-inflammatory, antiviral, and immunoregulatory effects [12, 13]. *Glycyrrhizae Radix et Rhizoma*, belonging to the Leguminosae family, is one of the most popular medicinal plants [14], in which glycyrrhizic acid (GA) is the primary bioac-

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tive component, possessing various pharmacological and biological effects [15]. Recent research has revealed the protective effects of GA on the lung; for instance, GA can attenuate ALI and inflammation during sepsis in experimental rats [16]. Glycyrrhizin also shows hepatoprotective activity in total parenteral nutrition-induced ALI by inhibiting ER stress and pro-inflammatory cytokines [17]. Additionally, GA ameliorates sepsis-associated acute kidney injury by repressing the NF-κB signaling pathway [18]. Signal transducer and activator of transcription 3 (STAT3) activation can inhibit ER stress in chronic stimuli [19]. In acute injury responses, such as sepsis-induced ALI, JAK/STAT3 is activated, and JAK/STAT3 pathway inactivation can alleviate sepsis [20]. Interestingly, GA can block STAT3 activation [21]. Thus, our study explored whether GA can alleviate sepsis-associated ALI by suppressing ER stress via regulating the JAK/STAT3 pathway.

In the current study, experiments were performed using the cecal ligation and puncture (CLP) sepsis model to expound GA's role and mechanism in sepsis-associated ALI. The findings suggest that GA can alleviate sepsis-mediated ALI by repressing ER stress.

Materials and Methods

Animals

Male C57 mice (n = 61, 20–25 g, 6–8 weeks old, Hangzhou Medical College, Hangzhou, China) were housed in Specific Pathogen Free (SPF) plastic cages (60–65% humidity, 22–24 °C, circadian rhythm) and allowed food and water *ad libitum*. All experiments complied with the guidelines of the China Council on Animal Care and Use on the premise of obtaining ratification from the Zhejiang Baiyue Biotechnology Co., Ltd. Experimental Animal Ethics Committee (ZJBYLA-IACUC-20230407).

Experimental Design

In brief, 12 h before the experiment, mice were fasted, with access to water. Subsequently, they were assigned into 5 groups: (1) the sham operation group (n = 5); (2) the CLP group (n = 14); (3) the GA (PHR1516, Sigma-Aldrich, St. Louis, MO, USA) (35 mg/kg) intervention plus CLP group (GA-35 group, n = 15); (4) the GA (70 mg/kg) intervention plus CLP group (GA-70 group, n = 10); (5) the GA (100 mg/kg) intervention plus CLP group (GA-100 group, n = 17). In the CLP, GA-35, GA-70, and GA-100 groups, mice underwent anesthetization by intraperitoneal injection of 2% sodium pentobarbital (80 mg/kg, Y0002194, Sigma-Aldrich, St. Louis, MO, USA) [22]. The mice were laid in the supine position on the laboratory bench, and their abdominal area was sterilized after shaving. The cecum was exposed by creating a 1-cm incision in the middle of the abdomen and ligated 1 cm away from the tail with a sterile sewing silk no. 4. Infection was induced with a drop of the intestinal content squeezed out post insertion of a 21-gauge needle into cecum. Finally, the cecum was repositioned, and the wound was closed by simple running sutures to the abdominal musculature and metallic clips to the skin. The same operation was conducted in the sham group but without CLP [22,23]. Animals in the GA-35, GA-70, and GA-100 groups were intraperitoneally administered with 35, 70, or 100 mg/kg GA immediately after CLP, while intraperitoneal injection with isovolumetric 10% DMSO was exploited in sham and CLP groups [24]. The mice were sacrificed with a high concentration of CO_2 36 hours after surgery. The lung tissues in all groups were harvested for subsequent assessments. Injection of bronchoalveolar lavage fluid (BALF) into a tracheal cannula with sterile phosphate-buffered saline (PBS, 1.5 mL) (C0221A, Beyotime, Shanghai, China) thrice and centrifugation (1500 rpm, 5 min, 4 °C) were performed for BAL.

Survival Analysis

For survival analysis, the time of death was recorded after surgery by observing mice's survival status every 6 h after CLP surgery. The survival rate was dissected by a log-rank test.

Lung Wet/Dry (W/D) Weight Ratio

The lung W/D weight ratio is a pulmonary edema index that reflects the severity of endothelial permeability and lung injury. The weight of lung tissues was measured immediately after surgery and after 48-h heating in a 60 °C oven. Finally, the W/D weight ratio of the lung tissues was calculated.

Hematoxylin and Eosin (HE) Staining

The HE Staining Kit (MHS1, Sigma-Aldrich, St. Louis, MO, USA) was used to pathologically examine lung tissues. Lung tissues were immersed in 10% formalin (A5472, Sigma-Aldrich, St. Louis, MO, USA) for at least 48 h, followed by dehydration in gradient ethanol, embedment in paraffin, and slicing to generate 5-µm-thick sections for histological analyses. The sections were dewaxed in xylene, rehydrated (gradient ethanol), and stained (hematoxylin [5 min] and eosin [2 min]). A neutral resin was mounted, and the histopathological changes were observed under an optical microscope (100×, ECLIPSE 80i, Nikon, Tokyo, Japan). Building upon a previous study with minor modifications, the lung injury score was assessed by observing the inflammation, edema, hemorrhage, and alveolar septal thickening of lung tissue with a total score of 5 points.

Protein Concentration in BALF

After centrifugation of BALF (1000 rpm, 10 min, 4 °C) and resuspension of cells in 0.5 mL of PBS, the cell number in BALF was assessed by a hemocytometer. A cell suspension (10 µL) was collected for differential leukocyte cell count and stained on a glass slide with Giemsa stain.

The protein concentration in BALF was measured using the bicinchoninic acid (BCA) protein assay kit (P0012S, Beyotime, Shanghai, China).

Enzyme-Linked Immunosorbent Assay (ELISA)

BALF was collected for analyzing inflammatory cytokines. Mouse interleukin-1β (IL-1β) (PI301, Beyotime, Shanghai, China), Mouse tumor necrosis factor α (TNF-α) (PT512, Beyotime, Shanghai, China), and Mouse interleukin 6 (IL-6) (PI326, Beyotime, Shanghai, China) ELISA Kits were procured to quantify protein levels. In brief, samples (100 µL/well) in 96-well plates were probed with biotinylated antibodies (100 µL/well) (room temperature [RT], 60 min) and streptavidin-horseradish peroxidase (HRP)-conjugated secondary antibodies (RT, 20 min). Finally, plates were developed with tetramethylbenzidine (TMB) (P0209, Beyotime, Shanghai, China), and the color development was stopped with TMB stop reagent (P0215, Beyotime, Shanghai, China), followed by the detection of optical density (OD) (450 nm) utilizing a microplate reader (1681135, Bio-Rad, Hercules, CA, USA). A standard curve was drawn with the concentration of the standard substance as the horizontal coordinate and OD value as the vertical coordinate. Specimen OD values were used to identify the cytokine level of samples to be tested.

DHE Determination of Reactive Oxygen Species (ROS) Production

Lung sections were prepared as described above and incubated with dihydroethidium (DHE) (2 µmol/L) (D7008, Sigma-Aldrich, St. Louis, MO, USA) (darkness, 30 min, RT). After washing three times with Krebs-Henseleit buffer (KHB), sections were placed on coverslips. A Nikon A1R confocal microscope (Nikon, Tokyo, Japan) was used to obtain fluorescent images, and results were analyzed with ImageJ software (1.52s version, National Institutes of Health, Bethesda, MD, USA). The relative DHE intensity was obtained using the formula: average fluorescence intensity = total fluorescence intensity of the region/area of the region, which was normalized to the data in the sham group.

Western Blot

After washing with PBS, cells were resuspended in protease or phosphatase inhibitor supplemented RIPA Buffer (89901, ThermoFisher, Waltham, MA, USA) to extract total protein. The protein concentration was measured using a BCA protein assay kit (P0012S, Beyotime, Shanghai, China). Proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels (P1203, Solarbio, Beijing, China) and transferred to 0.2-µm polyvinylidene fluoride membranes (FFP24, Beyotime, Shanghai, China). Membranes were blocked with 5% skim milk (P0216, Beyotime, Shanghai, China) (RT, 1 h) and incubated with primary antibodies (RT, 1.5 h or 4 ℃, overnight) procured from Abcam (Cambridge, UK), including protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) (1/1000, ab229912, 150 kDa), activating transcription factor 4 (ATF4) (1/1000, ab216839, 38 kDa), C/EBP homologous protein (CHOP) (5 µg/mL, ab11419, 31 kDa), heme oxygenase-1 (HO-1) (1/2000, ab52947, 33 kDa), phosphorylated Janus kinase 2 (p-JAK2) (1/1000, ab32101, 120 kDa), Janus kinase 2 (JAK2) (1/5000, ab108596, 130 kDa), p-STAT3 (1/2000, ab76315, 88 kDa), STAT3 (1/1000, ab109085, 88 kDa) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (1/500, ab8245, 36 kDa), except phosphorylation of eIF2α (p-eIF2α) (1/1000, 9721S, Cell Signaling Technology, Danvers, MA, USA, 38 kDa). After washing with TBST, membranes were incubated with secondary antibodies (Abcam, London, UK), Goat Anti-Rabbit IgG H&L (HRP) (1/2000, ab205718) and Rabbit Anti-Mouse IgG H&L (HRP) (1/2000, ab6728) (RT, 1 h), followed by processing with an enhanced chemiluminescence kit (WB7106, Thermo Scientific, Waltham, MA, USA). Protein band intensities were detected with an imaging system (FLUORCHEMFC3, ProteinSimple, Minneapolis, MN, USA), and digitization was achieved by ImageJ software. The relative protein expression level was calculated: x' (normalized data) = (x (original data) - mean data (x)) / std data (x, internal control), which was normalized to the data in the sham group.

Statistical Analyses

All data were depicted by means ± standard deviation (SD). Multi-group comparisons and statistical analyses were separately achieved by one-way analysis of variance (ANOVA) and GraphPad Prism 8.0 (GraphPad Software Inc., San Diego, CA, USA), with the post-hoc analyses of chi-square test (only for survival rate test) and Tukey test. A statistically significant difference was defined by $p < 0.05$.

Results

GA Remarkably Improved Survival in Septic Mice

Fig. 1A depicts the chemical structure of GA. During the 36-h observation window on mice survival status, we noticed that the survival rate of mice was markedly reduced by CLP (Fig. 1B, $p = 0.004$) but was elevated after GA intervention (Fig. 1B, $p = 0.023$).

GA Mitigated Lung Injury in Sepsis Mice

In agreement with Fig. 2A, the W/D weight ratio was remarkably increased by CLP but decreased by GA intervention (Fig. 2A, $p < 0.05$). Sham-operated mice showed a clear reticular structure, whereas CLP-induced mice displayed strikingly damaged alveolar structures (Fig. 2B, $p < 0.001$). Lung tissues were less damaged in the GA groups than in the CLP group (Fig. 2B). The protein con-

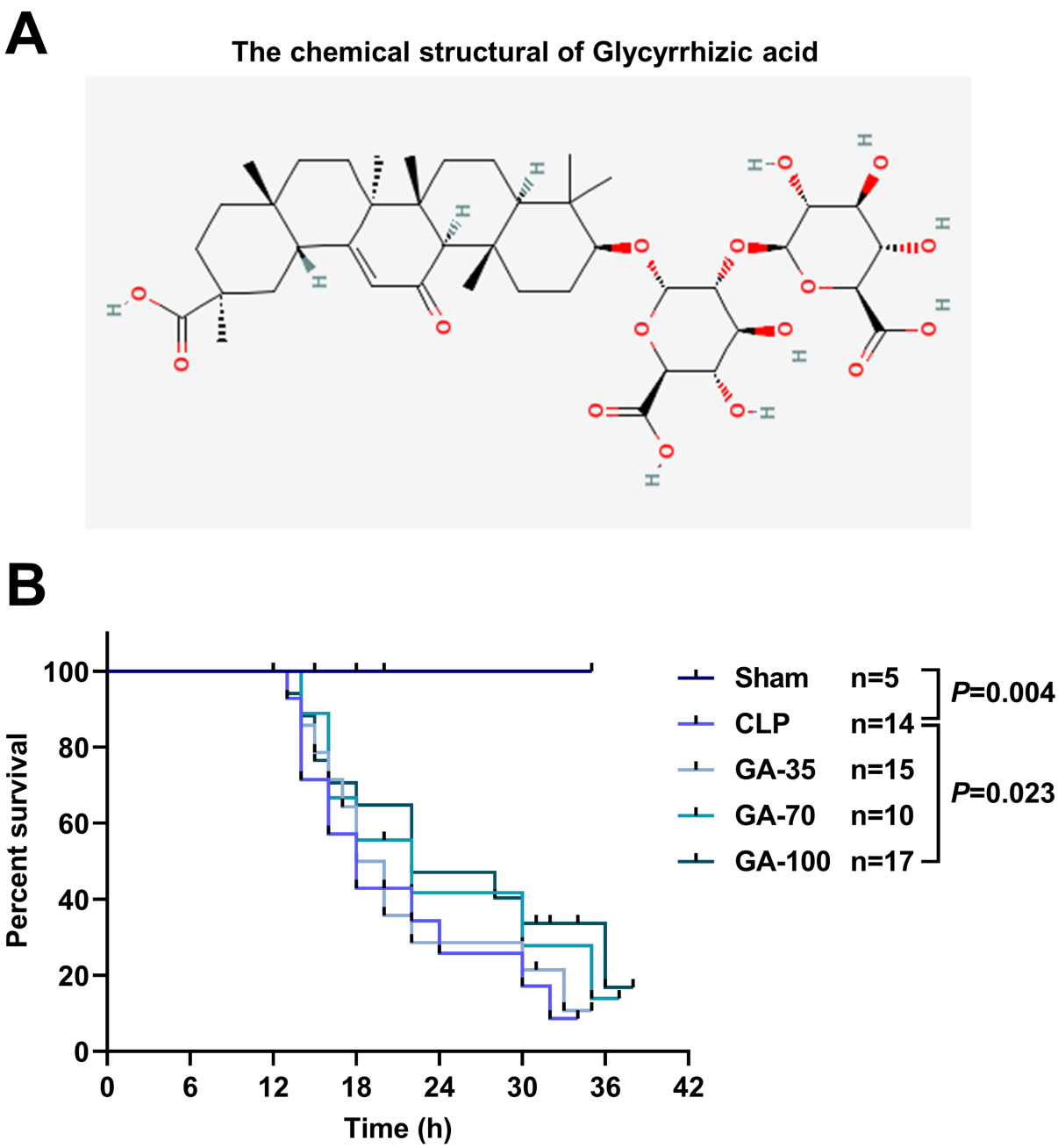

Fig. 1. Glycyrrhizic acid (GA) improved the survival rate of septic mice. (A) The chemical structure of GA. (B) The survival curves in the sham, cecal ligation and puncture (CLP), GA-35, GA-70, and GA-100 groups. The survival rate was analyzed using the log-rank test. $n_{Sham} = 5$, $n_{CLP} = 14$, $n_{GA\text{-}35} = 15$, $n_{GA\text{-}70} = 10$, $n_{GA\text{-}100} = 17$.

centration change in BALF was in agreement with the W/D weight ratio, suggesting that sepsis can contribute to severe lung damage (Fig. 2C, $p < 0.001$). The associated inflammatory cytokines in BALF, IL-1β, TNF-α, and IL-6 were measured using ELISA (Fig. 2D–F). The cytokine levels were much higher in the CLP group than in the sham group, while GA dose-dependently reduced the levels of pro-inflammatory factors (Fig. 2D–F, $p < 0.001$). DHE staining results indicated that GA repressed CLP-induced DHE intensity (Fig. 2G, $p < 0.001$).

GA Reduced ER Stress-Related Protein Levels in Lung Tissues of Sepsis Mice

Levels of ER stress-associated genes, PERK, p-eIF2α, ATF4, and CHOP, were examined by western blot. Considering Fig. 3, PERK, p-eIF2α, ATF4, and CHOP protein expression were higher in the CLP group than in the sham group (Fig. 3, $p < 0.001$). Nevertheless, after GA intervention, ER stress-associated protein expression was markedly decreased (Fig. 3, $p < 0.01$). Notably, the higher dose of GA exerted a greater effect on these proteins (Fig. 3, $p < 0.01$).

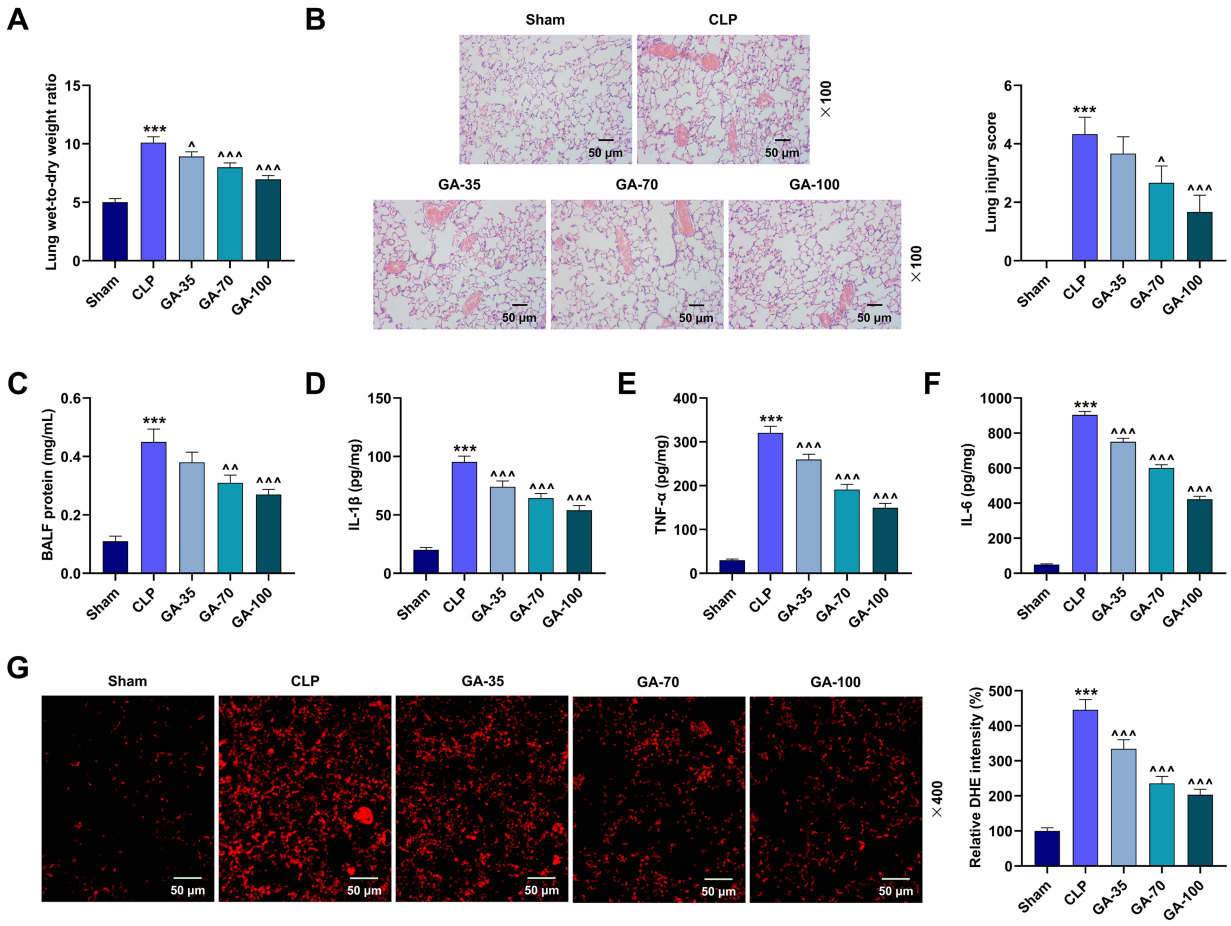

Fig. 2. GA mitigated lung injury in sepsis mice. (A) The lung wet/dry (W/D) weight ratio. (B) Representative images of HE-stained lung tissues (magnification 100×) and the lung injury scores of the sham, CLP, GA-35, GA-70, and GA-100 groups. (C) The bronchoalveolar lavage fluid (BALF) protein concentration (bicinchoninic acid (BCA) protein assay kit). (D) Interleukin-1β (IL-1β) level (Mouse IL-1β enzyme-linked immunosorbent assay (ELISA) Kit). (E) Tumor necrosis factor α (TNF-α) level (Mouse TNF-α ELISA Kit). (F) Interleukin 6 (IL-6) level (Mouse IL-6 ELISA Kit). (G) Representative fluorescent images of dihydroethidium (DHE) detection of reactive oxygen species (ROS) production in CLP mice (magnification 400×). Lung sections were incubated with freshly prepared DHE (2 μmol/L, darkness, 30 min, room temperature (RT)). Nikon A1R confocal microscope was used to obtain fluorescent images. Bar = 50 μm. Data were presented as means ± standard deviation (SD). ***$p < 0.001$ vs. Sham; $^{\wedge\wedge\wedge}p < 0.001$, $^{\wedge\wedge}p < 0.01$, $^{\wedge}p < 0.05$ vs. CLP. n = 3.

GA Enhanced HO-1 Expression While Inactivating JAK2/STAT3 Signaling Pathways

According to western blot data, HO-1, p-JAK2/JAK2, and p-STAT3/STAT3 expression were elevated in the CLP group (Fig. 4, $p < 0.05$). GA strikingly promoted the HO-1 expression level while inactivating the JAK2/STAT3 signaling pathway (Fig. 4, $p < 0.05$).

Discussion

The morbidity and mortality rates of sepsis continue to increase in the context of incompletely defined pathogenesis. Furthermore, sepsis contributes to multiple organ injuries, including ALI [25]. The lack of adequate clinical methods to prevent the onset of ALI is associated with the poor prognosis of sepsis. GA, the primary bioactive component of *Glycyrrhizae Radix et Rhizoma*, has shown latent solid potential in treating various diseases. Therefore, we examined the impact of GA on sepsis-mediated ALI and its potential mechanism of action, and we provide the first line of evidence that GA can ameliorate sepsis-mediated ALI by repressing ER stress.

Recent research has demonstrated that GA can modulate ALI. For instance, Zhao *et al.* [24] demonstrated that GA can attenuate sepsis-associated ALI by inactivating NF-κB, JNK, and p38 MAPK signaling pathways. In addition, GA can ameliorate lipopolysaccharide (LPS)-associated ALI and reduce the secretion of inflammatory factors via the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway [26]. To confirm the effect of GA on sepsis-induced ALI, the CLP-caused sepsis model was used to

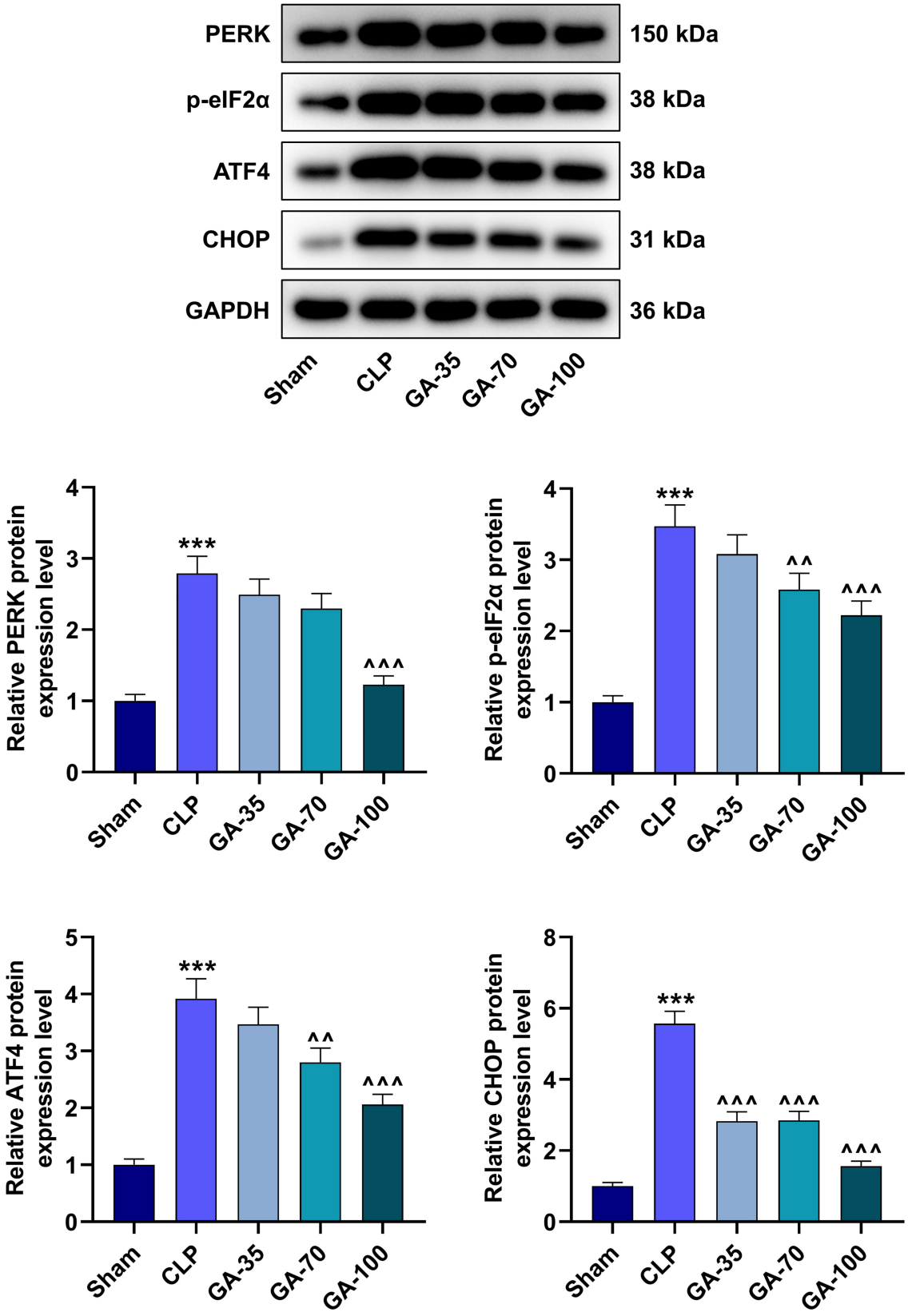

Fig. 3. GA reduced PERK, p-eIF2α, ATF4, and CHOP expression in CLP-treated lung tissues of mice. The levels of protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK), phosphorylation of eIF2α (p-eIF2α), activating transcription factor 4 (ATF4), and C/EBP homologous protein (CHOP) (western blot). Data were presented by means ± SD. ***$p < 0.001$ vs. Sham; $^{\wedge\wedge\wedge}p < 0.001$, $^{\wedge\wedge}p < 0.01$ vs. CLP. n = 3. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

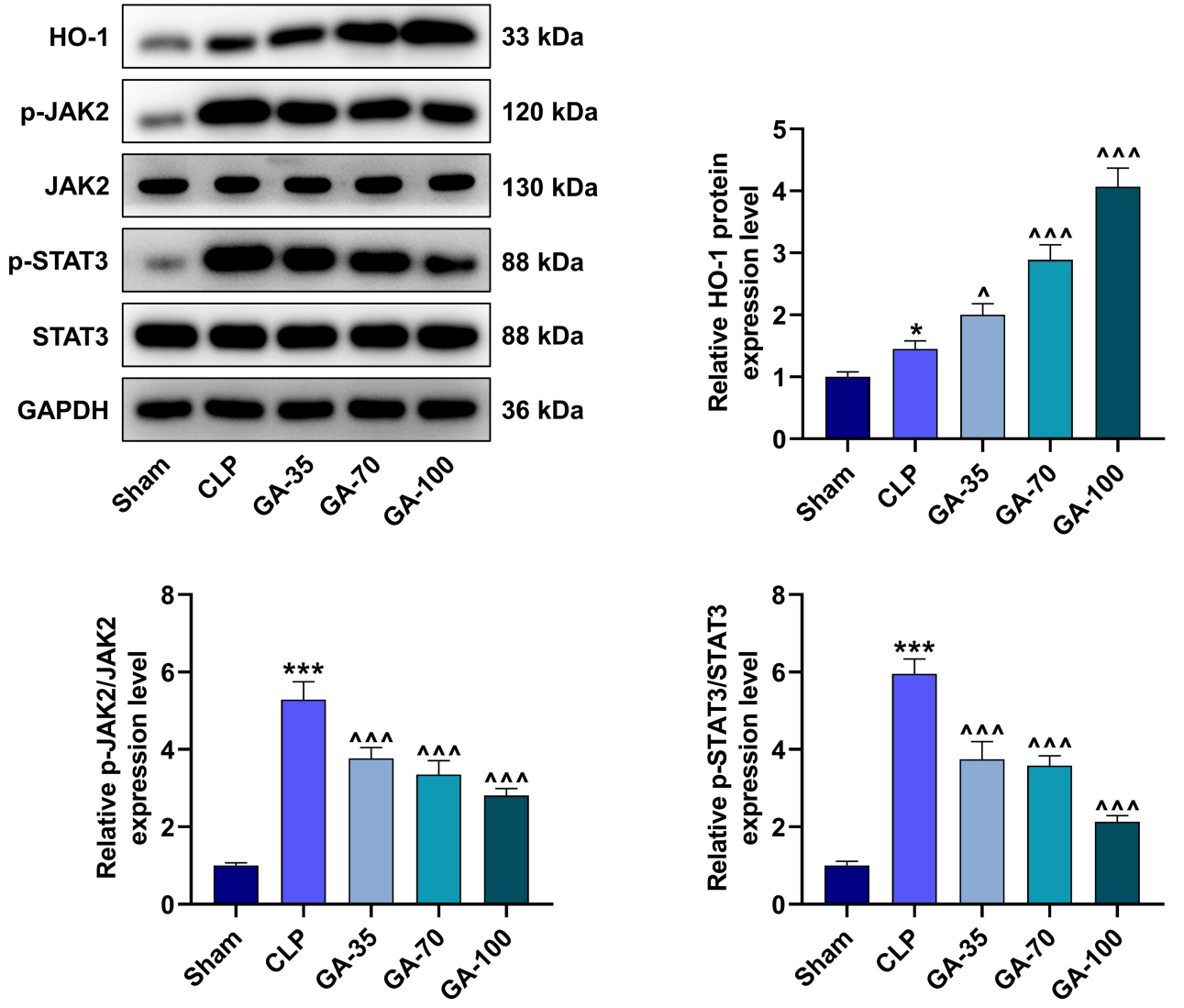

Fig. 4. GA enhanced HO-1 expression while inactivating JAK2/STAT3 signaling. Heme oxygenase-1 (HO-1), phosphorylated Janus kinase 2 (p-JAK2), Janus kinase 2 (JAK2), phosphorylated signal transducer and activator of transcription 3 (p-STAT3), and signal transducer and activator of transcription 3 (STAT3) expression in lung tissues of mice (western blot). Data were presented by means ± SD. ***$p < 0.001$, *$p < 0.05$ vs. Sham; ^^^$p < 0.001$, ^$p < 0.05$ vs. CLP. n = 3.

carry out related research, and the results revealed that GA can markedly improve the survival rate of mice by alleviating sepsis-induced lung injury. Inflammatory factor secretion has been studied in the context of reducing the damage caused by ALI. For instance, GA can attenuate ALI by inhibiting the production of inflammatory factors TNF-α, IL-1β, and high mobility group box 1 (HMGB1) [16]. In addition, Wang *et al.* [27] revealed that GA ameliorates ALI in mice via regulating ROS/PI3K/AKT pathway to dampen the activation of NLR family pyrin domain containing 3 (NLRP3) inflammasomes. Herein, we probed other pathways in which GA can mitigate the inflammatory response caused by sepsis and different mechanisms of GA in sepsis-mediated ALI.

ALI causes uncontrolled lung inflammation that is related to high morbidity and mortality, and inflammation, a kind of cellular stress contributing to misfolded proteins, can lead to ER stress [28]. ER stress is associated with various diseases. For instance, the farnesoid X receptor mitigates hepatocyte apoptosis and liver injury by regulating ER stress [29], and ER stress enhancement is a vital factor for aggravating myocardial ischemia/reperfusion (I/R) injury by Wilms' tumor 1-associating protein [30]. Moreover, ER stress is instrumental in the etiology of lung injury. It has been reported that Cereblon (CRBN) knockdown participates in inhibiting the progression of ALI by suppressing ER stress [31], and cold-inducible RNA-binding protein can cause sepsis-mediated ALI by inducing ER stress [32]. Herein, we measured ER stress-related proteins and found

that sepsis increased PERK, p-eIF2α, ATF4, and CHOP levels in the lung, which was reversed by GA intervention. Thus, we inferred that GA alleviated sepsis-induced ALI by suppressing ER stress.

HO-1 has been reported to be efficient in inhibiting ER stress [33]. HO, a cytoprotective enzyme, is conducive to maintaining a healthy vascular endothelium. HO-1, an isoform of HO, possesses antioxidative and cytoprotective effects [34]. The induction of HO-1 is considered a positive response in multiple tissues and cellular injury models, such as sepsis and ischemia-reperfusion [35]. Furthermore, HO-1 can inhibit sepsis-mediated ER stress and apoptosis of intrapulmonary cells by suppressing the PERK/eIF2-α/ATF4/CHOP pathway [36]. Of note, GA can regulate the HO-1 pathway [37]. In our study, the HO-1 level was upregulated after the intervention of GA, indicating that GA may suppress ER stress by increasing the generation of HO-1.

STAT3, an acute-phase response gene in the lung, is critical in early inflammatory responses [38]. IL-6 can elevate STAT3 phosphorylation, which, in turn, may enhance the levels of IL-6 and downstream acute-phase genes, including TNF-α and IL-1β, thus intensifying the inflammatory response [39]. As a transcription factor, STAT3 is also critical in cross-talk with other signaling pathways [38]. JAK/STAT signaling pathways participate in multiple pathological processes [20]. The JAK2/STAT3 signaling pathway can be sensitized by TNF-α, IL-1β, and IL-6 [40], thus potentiating inflammatory responses and providing a foundation for sepsis [41]. Herein, our results suggested that IL-1β, TNF-α, and IL-6 levels in BALF and JAK2/STAT3 pathway-related protein levels were significantly increased after CLP, while the GA intervention downregulated their expression levels. Hence, we conclude that GA inactivated JAK2/STAT3 signaling pathway.

However, this study only investigated how GA influences sepsis-induced ALI in mice. Rescue experiments will be performed in the future to overexpress or silence HO-1 and further pinpoint the mechanism of GA in sepsis-triggered ALI mice.

Conclusion

In summary, our data suggests that GA can mitigate sepsis-triggered ALI by suppressing ER stress, which may be related to the activation of HO-1 induced by GA. This provides a novel treatment route for sepsis-induced ALI. We also report GA's inhibitory impact on STAT3, but its potential molecular mechanism still needs further research.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Substantial contributions to conception and design: LF. Data acquisition, data analysis and interpretation: XPW, WG. Drafting the article or critically revising it for important intellectual content: LF, XPW and WG. Final approval of the version to be published: LF, XPW and WG. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of the work are appropriately investigated and resolved: LF, XPW and WG.

Ethics Approval and Consent to Participate

All experiments were carried out in line with the Chinese Animal Research Guidelines and were approved by the Committee of Zhejiang Baiyue Biotech Co., Ltd. (ZJBYLA-IACUC-20230407).

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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