The mechanism underlying hypaconitine-mediated alleviation of pancreatitis–associated lung injury through up-regulating aquaporin-1/TNF-α

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ABSTRACT

Background/Aims: *Acute pancreatitis–associated lung injury (APALI) is one of the most common and most dangerous form of extra-pancreatic organ damage in severe acute pancreatitis (SAP). The treatment options for SAP were limited thus far; as a result, approximately 60%–80% of patients with SAP would die within a week. Hypaconitine (HC), one of the most important active ingredients in a Mongolian traditional medicine Radix Aconiti Kusnezoffii has an excellent anti-inflammatory effect.*

Materials and Methods: *To ascertain whether HC has a protective effect against APALI, we investigated the therapeutic effects and the underlying mechanisms in vivo and in vitro and attempted to elucidate the mechanism in detail. In this study, APALI rats and human pulmonary microvascular endothelial cells were treated with therapeutic doses of HC after establishing a model with sodium taurocholate and lipopolysaccharide, respectively.*

Results: *Serum amylase and lipase activity, lung wet/dry weight ratio, lung myeloperoxidase activity, and pancreatic and lung histopathological changes showed that HC alleviated APALI in a dose-dependent way, which can be abolished by an aquaporin-1 (AQP-1) knockdown. The results of the reverse transcriptase polymerase chain reaction, Western blot, and immunohistochemical staining confirmed the expression of AQP-1, a kind of transmembrane protein that mainly distributed in the membranes of pulmonary cells and contributed to maintain water balance in the body by interacting with tumor necrosis factor-alpha (TNF-α), is negatively associated with APALI. On the contrary, HC treatment up-regulated AQP-1 expression and down-regulated the TNF-α expression as a consequence in APALI.*

Conclusion: *These results suggest that HC has a good anti-inflammatory therapeutic effect on APALI with a possible underlying mechanism that affects the AQP-1/TNF-α pathway.*

Keywords: *Acute pancreatitis, lung injury, Radix Aconiti, aquaporin-1, tumor necrosis factor-α*

INTRODUCTION

Severe acute pancreatitis (SAP) is inflammation of pancreas that is characterized by rapid onset and deterioration of symptoms, multiple complications, and high mortality. At present, the mortality rate is 20%-40% given that no specific and effective treatment is available (1, 2). Among the SAP-associated multiple organ dysfunction syndrome, acute pancreatitis–associated lung injury (APALI) is one of the most common and most dangerous form of extra-pancreatic organ damage in SAP (3, 4). APALI, as a syndrome of pulmonary edema and atelectasis caused by diffuse injury of alveolar capillary membrane, which is associated with the development of acute respiratory distress syndrome (ARDS) and manifests as respiratory distress and refractory hypoxemia (3, 4), plays a key role in shock, sepsis, and death in patients undergoing multiple transfusions. Studies have shown that 60%-80% of SAP patients who die within a week have a lung injury, indicating that APALI is the leading cause of early SAP death (5, 6).

The specific pathogenesis of APALI has not yet been fully elucidated. It is widely believed that SAP-induced activation of the systemic inflammatory response system and release of a large number of cytokines, inflammatory mediators, proteases, and reactive oxygen species, which in turn affect human pulmonary microvascular endothelial cells' (HPMVECs) permeability and water trans-pulmonary epithelial cell transport make the greatest contribution (7, 8). In particular, tumor necrosis factor-alpha (TNF-α), whose serum and tissue expression levels are closely related to the degree of lung injury, is identified as a most important inflammatory mediator in the formation of APALI. TNF-α is known to induce endothelial cell activation, leukocyte migration, neutrophil degranulation and capillary leakage, alveolar cell perfusion, and oxygen exchange (9). Thus, inhibition of TNF-α may be a potential therapeutic target for lung injury.

Radix Aconiti Kusnezoffii is the dried leaf of *Aconitum kusnezoffii Reichb*. It has long been used as a traditional

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medicinal material in Mongolia for cardiovascular diseases (CVDs) such as heart failure, hypertension, and arrhythmias. (10, 11) and known for its anti-inflammatory and analgesic effects for rheumatism and joint pain (12). Hypaconitine (HC), one of the most important active ingredients in *Radix Aconiti Kusnezoffii* is considered to be effective against CVDs despite its toxicity, which can be reduced by specific processing such as boiling or addition of other ingredients to improve pharmacokinetic parameters (13-16). More in-depth studies on its mechanism of action have shown that HC protects endothelial cells from oxidative damage via the histone deacetylase 3-high mobility group box 1 pathway and reduces necrosis and apoptosis of myocardium by oxygen and glucose deprivation via the PI3K/Akt signaling pathway (17, 18). Recent studies have shown that HC has an excellent anti-inflammatory effect, such as inhibition of cyclooxygenase-2, TNF-α, interleukin-1, and prostaglandin E2 in arthritic rats (19); inhibition of mouse cochlear edema and rat paw swelling; and increased peritoneal capillary permeability in mice (20). Total aconitine containing HC extracted from natural medicinal plants has obvious protective effects on rat lung tissue against acute lung injury (ALI) (21). In addition, studies have shown that HC dose-dependently inhibits TNF-α-mediated cytotoxicity in the dose range of 50-200 μg/mL.

To determine whether HC has a protective effect against APALI, we investigated the therapeutic effects of APALI *in vivo* and *in vitro* and attempted to elucidate the detailed mechanisms in this study. Our findings may provide a pharmacological basis for the use of HC in the treatment of APALI (22).

MATERIALS AND METHODS

Animal

Adult male Sprague Dawley rats (provided by the Experimental Animal Center of Inner Mongolia Medical University) and AQP-1 knockout rats (Sun Yat-Sen University Animal Care and Use Committee, SuZhou, China) weighing between 180 g and 230 g were used to establish an SAP model. After rising in a no-special pathogen environment with a constant temperature of 23°C±2°C and a relative humidity of 55%±2% under a 12 h/12 h light-dark cycle for 1 week, rats were restricted from eating 12 h before surgery, without restricting water intake. All experimental procedures were approved by the Medical Ethics Committee of Inner Mongolia Medical University on July 20, 2018 (No.YKD201801128).

MAIN POINTS

- *Mongolian traditional medicine effective component Hypaconitine (HC) has a good anti-inflammatory therapeutic effect on Acute pancreatitis associated lung injury. (APALI) in vivo and in vitro.*
- *HC exerts its anti-inflammatory effect on APALI through the AQP-1 /TNF-α pathway.*
- *What needs further research is the safe dose of HC because of its toxicity.*

Animal Models and Grouping

A total of 60 rats were divided into 6 groups: sham operation group (control group), model group, AQP-1$^{-/-}$ group, dexamethasone (DXM) group, HC group, and AQP-1$^{-/-}$ +HC group. All rats were treated in sterile conditions. After being anesthetized by subcutaneous injection of 10% chloral hydrate solution (0.3 mL/100 g), rats' skin was prepared and disinfected. The SAP model was established by a retrograde injection of 5% sodium taurocholate (1 mL/kg, Sigma Chemical Company, St Louis, MO, USA) into the main pancreatic duct through a microinjection pump at a speed of 0.1 mL/min for 30 s as previous research showed (23, 24). The rats in the control group had the same surgical procedure, but without the cannulation procedure and the retrograde injection of 5% sodium taurocholate. After constructing the model, 2 mg/kg DXM was injected from the femoral vein for the DXM group. The rats in the HC group and the AQP-1$^{-/-}$ +HC group were intragastrically administered with HC at a dose of 2.5 mg/kg. Normal saline was administered in the same way with the rats in the other 4 groups. Rats were thoroughly anesthetized with ether at 24 h. Orbital blood and pancreatic and lung tissues were collected and stored at −80°C for further analysis.

Serum Activity of Amylase, Lipase, and TNF-α

Serum amylase and lipase activity was determined by means of iodine-amylum colorimetry using kits provided by the manufacturer (JianCheng Biotechnology Research Institute, Nanjing, China) and expressed in U/L.

TNF-α Activity in Lung Tissue

Lung tissue was homogenized and centrifuged, and the supernatants (expressed in pg/mL) were collected to detect TNF-α by means of enzyme-linked immunosorbent assay using kits provided by the manufacturer (JianCheng Biotechnology Research Institute, Nanjing, China).

Lung Wet/Dry Weight Ratio

The superior lobe of left lung each rat was taken immediately after sacrifice, washed with phosphate buffered

saline (PBS), and then weighed (wet weight). The tissues were dried for 24 h at 60°C in an electric oven and were then reweighed (dry weight). Tissue water content was determined by calculating the wet/dry (W/D) weight ratio according to the following formula: water content = (wet weight − dry weight)/dry weight × 100%.

Pathology of Lung Tissue

Fresh rats' pancreas and superior lobe of right lung were fixed in 4% paraformaldehyde and embedded in paraffin, and cut into 5-μm-thick sections for hematoxylin-eosin staining. Two pathologists who were blinded to the group status randomly selected multiple microscopic fields from at least 3 rats in each group to observe if vacuolar tissue, interstitial edema, interstitial inflammation, and acinar cell necrosis were present in pancreatic tissue and to observe whether the alveoli had congestion, hemorrhage, neutrophil infiltration, and the thickness of the alveolar wall/transparent membrane formation.

Lung Tissue Myeloperoxidase Activity

The supernatants of the middle and inferior lobe of right lung were gathered after homogenization and centrifugation. Neutrophil sequestration within the lung was evaluated by quantitating tissue myeloperoxidase (MPO) activity using kits provided by the manufacturer (JianCheng Biotechnology Research Institute, Nanjing, China).

Cell Culture, Grouping, and Cell Viability Assay

HPMVECs (American Type Culture Collection, Manassas, Virginia, USA) were cultured for 24 h. After cells grow adherently and reach a density of approximately 90% or more), discarded the supernatant of cells in each group after rinsing with Roswell Park Memorial Institute (RPMI) 1640 medium except for the normal HPMVEC group. Lipopolysaccharide (LPS; Sigma-Aldrich Co., St Louis, MO, USA) was used to stimulate the cells for 0.5 h to establish an APALI model in HPMVECs (25). Then 20% sample solution was added separately, whereas only maintenance solution was added in the HPMVEC group. Cells in each group were incubated in 5% CO_2 and 95% air at 37°C till they reached the logarithmic growth phase to conduct the experiment. All cells were divided into 7 groups: normal HPMVEC group (control group), LPS-treated HPMVEC group (ie LPS-HPMVEC group, model group), and LPS-HPMVECs treated with 5 different doses of HC (0.01, 0.1, 1.0, 10.0, and 100.0 μg/mL). In the logarithmic growth phase, cells in each groups were collected, digested with 0.25% trypsin-ethylenediaminetetraacetic acid (Keygentec, Nanjing, China), and washed with PBS. Next, single cells were suspended in Dulbecco's modified eagle medium high-glucose growth culture (Gibco, Gaithersburg, MD, USA) containing 10% fetal bovine serum, which were then seeded in 96-well plates (1×10 4 cells/well) at 37°C and incubated overnight in an incubator with CO_2 5% and 95% air. Thereafter, different concentrations of HC (0.01, 0.1, 1.0, 10.0, and 100.0 μg/mL) were added after the supernatant was discarded. Blank well plates (only growth medium added) were prepared as controls. Each concentration group consisted of 3 replicate wells, which were incubated for 8, 24, and 48 h. cell counting kit-8 (CCK-8) solution (10 μL, Dojindo, Japan) was added to each well, and the cells were incubated at 37°C for 4 h. The optical density value of each well at 450 nm was measured using a microplate reader.

Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from the inferior lobe of left lung and cells. Reverse transcription was carried out based on the AQP-1 primer (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and detection was carried out using reverse transcription polymerase chain reaction (RT-PCR) reagent and universal primers and probes for a total of 40 cycles. After the reaction was completed, the standard template was diluted as a standard curve, and the PCR reagents was recorded and analyzed by agarose gel electrophoresis and gel recording system to calculate the expression levels of TNF-α and AQP-1 mRNA to β-actin as an internal control.

Western Blot

Total protein was extracted from the right lung and cells. The protein concentration was determined by the bicinchoninic acid method. The protein was subjected to sodium dodecyl sulfonate-polyacrylamide gel electrophoresis, then transferred semi-dry to a nitrocellulose membrane and blocked with 2% skim milk powder, and gently shaken at 37°C for 1 h. After washing 3 times with tris-buffered saline (TBS), the first antibody (AQP-1 antibody 1:1000, TNF-α antibody 1:1000, Santa Cruz Biotechnology Inc., Dallas, TX, USA) was incubated overnight at 4°C. The samples were washed with TBS and incubated with horseradish oxidase–labeled secondary antibody for 1 h at 37°C. The color bands were quantitatively analyzed using Gel-Pro gel analysis software after developing by the diaminobenzidine method.

Statistical Analysis

All data are shown as mean±standard deviation. All analyses were evaluated using the Statistical Package for the Social Sciences (SPSS) 18.0 software (IBM Corp.; Armonk, NY, USA). Analysis of variance was used to analyze

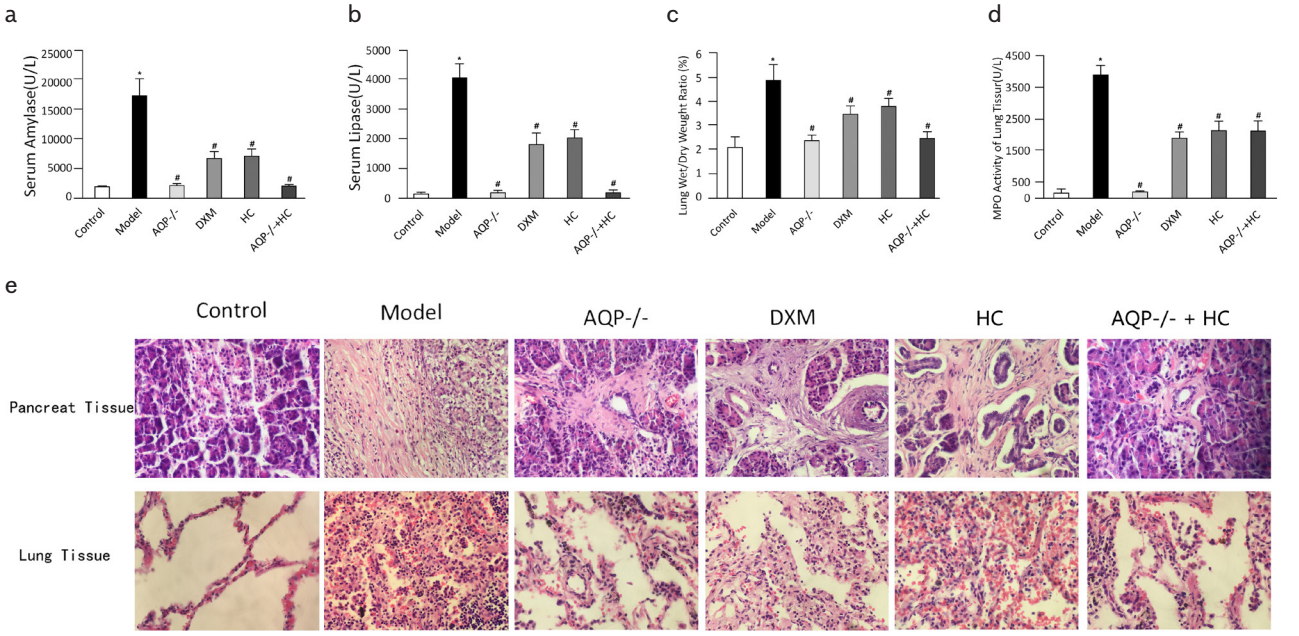

Figure 1. a-e. HC alleviated APALI. (a, b) Iodine-amylum colorimetry method was used to detect serum amylase and lipase of rats in each group. (c) Whole left lung tissue wet weight and dry weight of rats in each group were measured, and the lung W/D ratio was calculated. (d) MPO activity of lung tissue of rats in each group was measured. The * symbol represents $p<0.05$ compared with control group, and # represents $p<0.05$ compared with the model group. (e) Pathological examination of pancreatic tissue and lung tissue of rats in each group (×400).
APALI: acute pancreatitis–associated lung injury; MPO: myeloperoxidase; W/D: wet/dry weight.

the mean of each group. $P<0.05$ was considered to be a significant difference.

RESULTS

Hypaconitine Treatment Relieves APALI

As shown in Figure 1a-c, serum amylase, lipase, and W/D of the model group were significantly higher than those of the control group ($p<0.05$). Increased lung MPO activity indicated that neutrophils were sequestered within the lungs. Treatment with HC decreased the lung MPO activity ($p<0.05$; Figure 1d). It was observed that the APALI induced by sodium taurocholate was associated with elevated lung tissue MPO activity. No morphological changes were observed in the pancreas and lung tissues of the rats in the control group. In the model group, pancreatic cell necrosis, cytoplasmic vacuolation, edema, inflammatory cell infiltration, and hemorrhage were observed in the pancreas tissue; hyperemia and hemorrhage, inflammatory cell infiltration, and a little reddish edema fluid in some alveolar spaces were observed simultaneously in pulmonary interstitial (Figure 1e). Compared with the model group, HC treatment significantly reduced serum amylase, serum lipase, lung MPO activity, and lung W/D ratio ($p<0.05$; Figure 1a-d) and led to an alleviation of pathological changes of pancreas and lung tissue (Figure 1e). There was no significant difference in lung tissue between the HC group and the DXM group (Figure 1e), but the lung injury of rats in the AQP-1$^{-/-}$ group and the AQP-1$^{-/-}$+HC group did not improve (Figure 1a-e). Thus, HC alleviated APALI, which may be related to AQP-1 expression.

Hypaconitine Treatment Promotes the Proliferation of LPS-HPMVECs

CCK-8 assay showed that HC promoted HPMVEC proliferation in a time- and dose-dependent manner in the range of 1.0-100.0 μg/mL (Figure 2).

Hypaconitine Alleviates APALI by Up-regulating AQP-1 Expression

AQP-1 expression was significantly decreased in the model group compared with the control group, and it was barely expressed in the AQP-1$^{-/-}$ group ($p<0.05$, Figure 3a). According to the results of the experiment *in vitro*, AQP-1 expression levels were significantly reduced in LPS-HPMVEC group compared with the HPMVEC group ($p<0.05$, Figure 3b). These results suggested that AQP-1

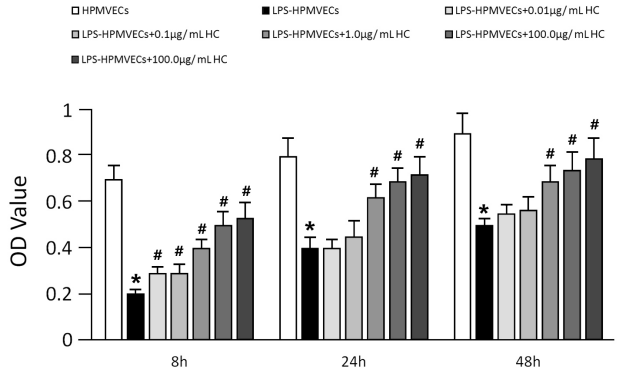

Figure 2. HC promotes proliferation of HPMVECs. After administration of different doses of HC (0.01, 0.1, 1.0, 10.0, 100.0 μg/mL) at 8, 24, and 48 h, LPS-HPMVECs exhibited HC dose- and time-dependent proliferation in the HC dose range of 1.0-100.0 μg/mL. The * symbol represents $p<0.05$ compared with the control group, and # represents $p<0.05$ compared with the model group.
HC: hypaconitine; HPMVEC: human pulmonary microvascular endothelial cell; LPS: lipopolysaccharide.

expression is reduced in APALI. According to the results of the experiment *in vivo*, HC treatment reversed APALI-induced decrease of AQP-1 expression levels in lung tissue of rats. However, this effect was canceled by AQP-$1^{-/-}$ ($p<0.05$, Figure 3a). The effect of HC on the up-regulation of decreased AQP-1 expression levels induced by APALI was also observed in cell experiments. ($p<0.05$, Figure 3b). Furthermore, RT-PCR and immunohistochemistry of AQP-1 expression yielded similar results to the Western blot (Figure 3c-e). These results indicated that HC can alleviate APALI by restoring reduced AQP-1 expression levels.

Hypaconitine Reduces APALI by AQP-1-Dependent Reduction of TNF-α Expression

In animal experiments, the protein and mRNA expression level of TNF-α in lung tissue and serum activity was significantly increased in the model group compared with the control group, but no increase was observed in the AQP-$1^{-/-}$ group. HC treatment up-regulated this decreased expression, but this up-regulating effect can be abolished by AQP-$1^{-/-}$ ($p<0.05$, Figure 4a-d). In cell experiments, LPS-HPMVECs showed significantly elevated TNF-α expression levels compared with untreated HPMVECs, whereas treatment with 1.0-100.0 μg/mL HC significantly reduced the increase in expression ($p<0.05$, Figure 4e, f). Furthermore, immunohistochemistry of TNF-α expression yielded similar results to RT-PCR and Western blot (Figure 4g). These data indicate that HC may reduce the expression of TNF-α in lung tissue in an AQP-1-dependent manner.

DISCUSSION

Consistent with the histological analysis of the pancreas and lung tissue, HC successfully reduced elevated serum amylase and lipase activity in APALI rats. At the same time, we found that HC reduced the lung W/D ratio, suggesting that HC can inhibit the infiltration of serum into lung tissue and reduce the development of pulmonary edema. In addition, treatment with HC inhibited SAP-induced neutrophils from being isolated in the lung, an indication of increased lung MPO activity. Neutrophils play an important role in the development of most ALI cases and are considered to be central to the pathogenesis of ALI/ARDS (22-26). These findings confirm that the protective effect of HC on APALI rats may be related to the isolation of inflammatory cells and the attenuation of lung tissue migration. In addition, cell experiments demonstrated that the therapeutic effect of HC on APALI is time- and dose-dependent over a range of doses.

AQPs are a family of transmembrane proteins closely related to the rapid transport of water (27). By localization on lipid rafts and affecting permeability to water, AQPs play an important role in maintaining water balance in the body (27). ALI-induced pulmonary edema is related to not only increased pulmonary capillary permeability due to alveolar wall damage but also reduced alveolar fluid scavenging, which can be restored by AQPs by providing the principal route for fluid transport between pulmonary alveoli and blood capillary (28). AQP-1 and AQP-5, which are the most studied members of AQPs because they play a particularly important role in the permeability of water under high osmotic gradients, were found to be elevated in SAP (29). It was showed AQP-1 may play a crucial role in ventilator-induced lung injury, resulting in the appearance of inflammatory mediators such as TNF-α in the lung and subsequently in edema. APALI has a similar mechanism, in which we supposed AQP-1 is also likely to play some role (30). Currently, 13 subtypes have been identified in mammals, namely AQP0-12, in which main distributers in lung tissue are AQP-1, AQP-3, AQP-4, and AQP-5 (27). Recently, it was reported that AQP-1 and AQP-5 in HPMVECs in which the AQP-1 content is relatively high are involved in the occurrence of APALI, which may be related to their removal of excess fluid in lung tissue during injury (31). AQP-1 is mainly distributed in the membranes of pulmonary capillary endothelial cells, alveolar type I epithelial cells, and airway epithelial cells. Studies have shown that those who lack the *AQP-1*

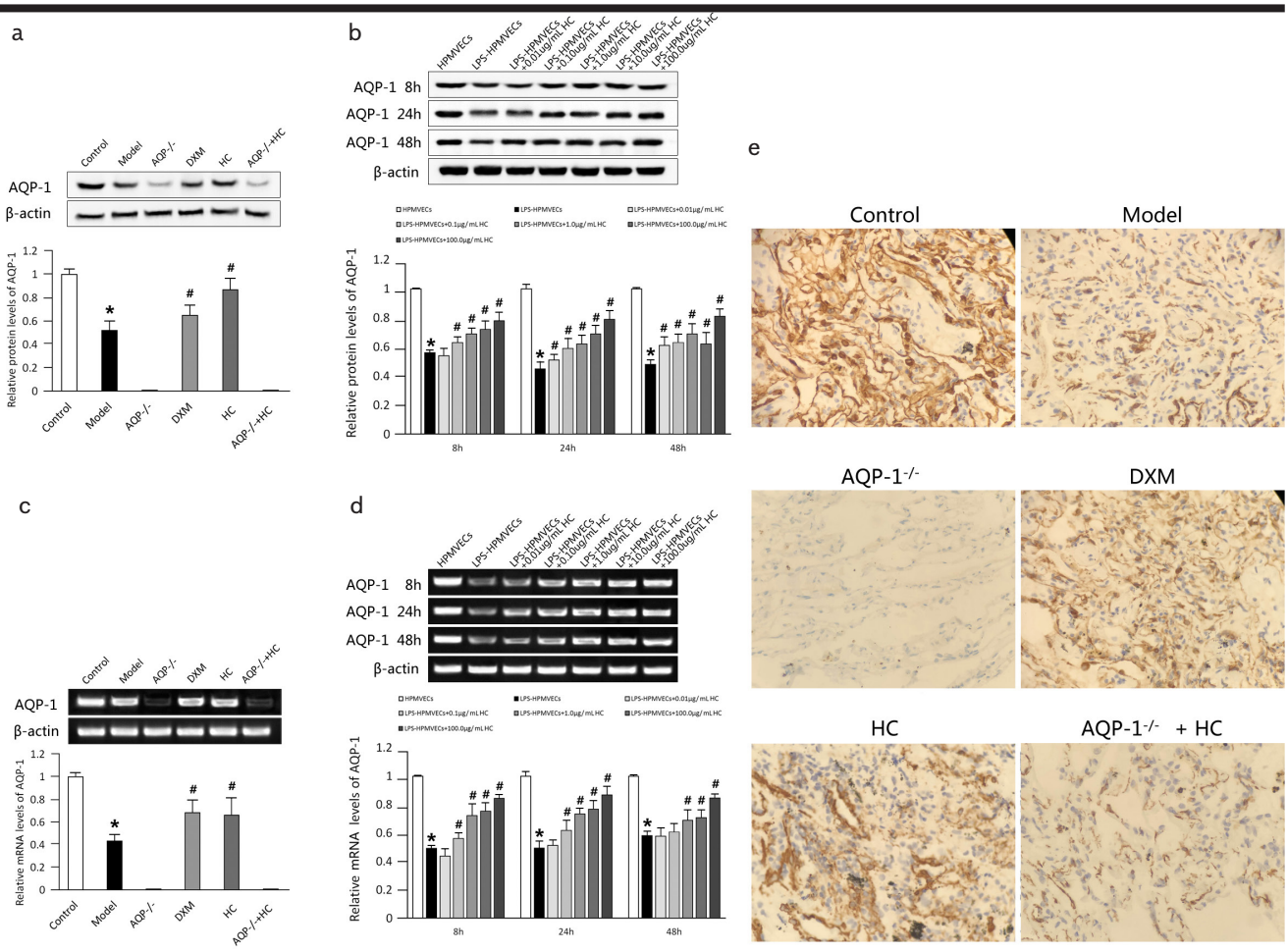

Figure 3. a-e. AQP-1 protein and mRNA expression levels in lung tissue animal and cell model of APALI. (a, b) Western blot was used to detect the expression of AQP-1 protein in lung tissue and cells of each group. (c, d) RT-PCR was used to detect the expression of AQP-1 mRNA in lung tissue and cells of each group. The * symbol represents $p<0.05$ compared with the control group, and # represents $p<0.05$ compared with the model group. (e) Immunohistochemical staining for proteins expression of AQP-1 in lung tissue of rats. Positive cells were stained dark brown (×400).

APALI: acute pancreatitis–associated lung injury; AQP-1; aquaporin-1; RT-PCR: reverse transcription polymerase chain reaction.

gene or the *AQP-5* gene are 10 times less able to detect water transport osmotic concentrations and bypass the blood-gas barrier than normal cell genotypes. In addition, knockout of the 2 genes resulted in a 30-fold reduction in water permeability (32). Our study also showed that at both of the protein and gene levels, the expression levels of AQP-1 were lower in APALI, confirming that AQP-1 is associated with APALI.

Inflammatory factors play an important role in the pathogenesis of APALI. In particular, as an important inflammatory mediator in the formation of APALI, TNF-α, which occurs in the early stages of SAP, exacerbates damage to lung cells and its level in serum and lung tissue is closely related to the degree of lung injury (23). It can be used as an early indicator to determine the extent and prognosis of SAP (23). We also found a significant increase in TNF-α expression in APALI, whereas its inhibition may reduce the extent of APALI. Most of the biological functions of TNF-α are mediated through TNF receptor-1 (TNFR-1), which is localized in the caveolae. Caveolae is a 50–100 nm flask-like plasma membrane invagination, a type of lipid raft that is present on the plasma membrane of most tissue cells (33). Caveolin-1 (Cav-1) is a marker protein of caveolae's structure and function localized inside lipid rafts and plays an important role in signal transduction, cholesterol transport, and endocytosis (34). TNF-α is the major mediator of the regulation of Cav-1 expression, and

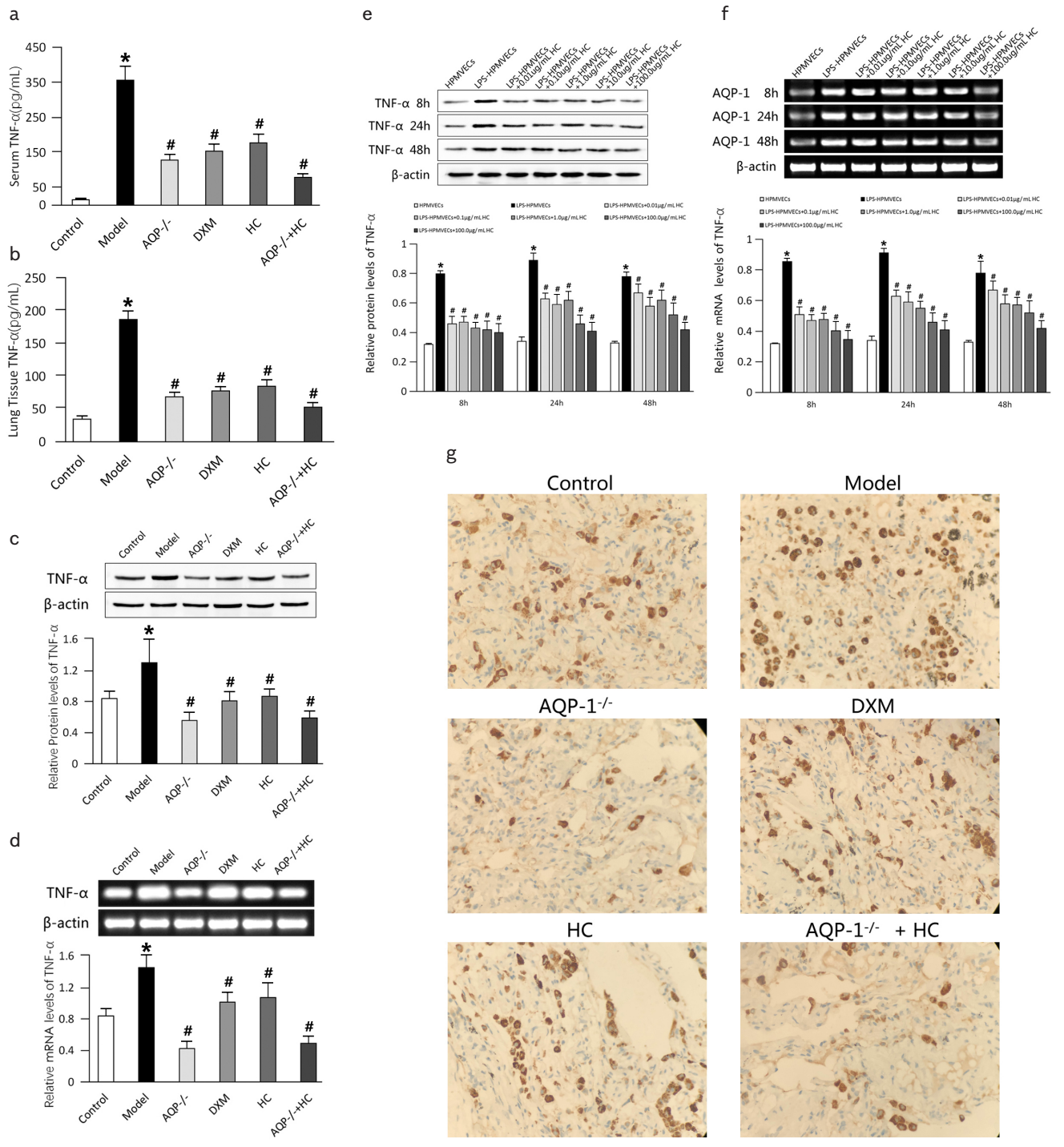

Figure 4. a-g. TNF-α protein and mRNA expression levels in serum and lung tissue of animal and cell models of APALI. (a, b) ELISA method was used to detect TNF-α activities in serum and lung tissue of each group of rats. (c, e) Western blot method was used to detect the expression of TNF-α protein in lung tissue and cells of each group. (d, f) RT-PCR was used to detect the expression of TNF-α mRNA in lung tissue and cells of each group. The * symbol represents $p<0.05$ compared with the control group, and # represents $p<0.05$ compared with the model group. (g) Immunohistochemical staining for protein expression of TNF-α in lung tissue of rats. Positive cells were stained dark brown (×400).

APALI: acute pancreatitis–associated lung injury; ELISA: enzyme-linked immunosorbent assay; RT-PCR: reverse transcription polymerase chain reaction; TNF-α: tumor necrosis factor-alpha.

its receptor TNFR-1 has a caveolin-binding motif, which is located in caveolae (35). Therefore, TNF-α can affect the expression and function of caveolae/Cav-1 through TNFR-1 and further regulate the activity of various signaling pathways to produce different effects. Studies have shown that TNF-α released by alveolar macrophages can down-regulate the expression of Cav-1 by TNFR-1, thereby activating various inflammatory signaling pathways leading to lung damage (36). Our study suggests that HC can alleviate the extent of APALI, which is likely to be associated with AQP-1 and is directly related to decreased expression of TNF-α by APALI.

Although HC is stable in plasma under 3 conditions (room temperature for 8 h, -40°C for 30 days, freeze-thaw for 3 times) and showed a good alleviation of APALI (16), we faced the problem that HC has a narrow margin of safety between a therapeutic and toxic dose. The diester alkaloids in *Radix Aconiti Kusnezoffii*, including HC, are both physiologically active components and the main toxic substance causing cardiotoxicity, neurotoxicity, genotoxicity, and reproductive toxicity, most frequent weakness, perioral and limb numbness, arrhythmia, hypotension, and gastrointestinal disorders (37, 39). After steaming or boiling, the content of HC in unprocessed *Radix Aconiti Kusnezoffii* is reduced by hydrolysis, which in turn reduces its therapeutic effect (38). Although the excellent therapeutic effect of HC on APALI was confirmed in our study, the dose of HC (2.5 mg/kg) used was close to its reported median lethal dose (LD50=5.8 mg/kg) (39). Although no rats were killed by the toxicity of HC in this study, the safety of this dose needs further proof by future expanding studies. Some toxicokinetics (TK) models have been established to assess the dynamic characteristics of toxic substances in the body (39). In this study, we did not conduct an in-depth TK study, but it should be necessary before the clinical application of HC and will be a part of our future research.

Although the clinical trial of infliximab for acute pancreatitis—an approved monoclonal antibody to TNF-α has shown significant effectiveness on Crohn's disease, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, and ulcerative colitis—is still recruiting in the United Kingdom (NCT03684278), the role of infliximab and its analogous antibody adalimumab in acute pancreatitis animal models were investigated in several studies (40-43). As expected, infliximab treatment suppressed neutrophil infiltration and MPO activity of the pancreatic tissue, mitigating the necrotic pathological changes in acute pancreatitis animal model (40). In another model, adalimumab was demonstrated to have a protective effect against methotrexate-induced pancreatic injury (43). These results give us confidence that HC has potential application in the clinical treatment of acute pancreatitis.

In summary, we used HC, a compound extracted from the Mongolian traditional medicine *Radix Aconiti Kusnezoffii* to treat APALI and explored the potential mechanism of its therapeutic effect. It was found that HC had a good anti-inflammatory therapeutic effect on APALI with a possible underlying mechanism that affected the expression of AQP-1 and down-regulated the expression of TNF-α as a consequence. Clearly taking into account the narrow therapeutic window, further study is required to find a safe and effective therapeutic dose and also analyze the premise of wide use of HC as a potential treatment of APALI.

Ethics Committee Approval: The study was approved by the Ethics Committee of Inner Mongolia Medical University with the approval number: 20190401, which was adopted on March 20, 2019.

Informed Consent: N/A.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – S.L.; Design – S.L., A.Z.; Supervision – S.L., A.Z.; Resource – L.B.; Materials – L.B.; Data Collection and/or Processing – J.G.; Analysis and/or Interpretation – J.G., L.B.; Literature Search – J.G.; Writing – J.G.; Critical Reviews – S.L., A.Z.

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Conflict of Interest: The authors have no conflict of interest to declare.

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