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Research Article

Effects of Daphnetin on Experimental Acute Pancreatitis-Associated Acute Lung Injury in Mice

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Background and Aim. Daphnetin, an active monomer ingredient extracted from *D. marginata*, is proved to have anti-inflammatory and antioxidant effect. The aim of this study is to explore the effect and possible mechanism of daphnetin on acute lung injury (ALI) associated with acute pancreatitis (AP) in mice. *Methods*. A total of 36 mice were randomly assigned into three groups: control group, AP group, and daphnetin group. The mouse model of AP was induced by caerulein and lipopolysaccharide. Animals were sacrificed at 6 and 12 h after daphnetin treatment, respectively. The pathological changes of lung and pancreas were determined by hematoxylin-eosin staining and the pathological scores. Levels of IL-1β, IL-6, and TNF-α in serum and lung and the activity of myeloperoxidase (MPO) in lung tissue homogenate were detected by ELISA. The protein level of toll-like receptor 4 (TLR4), phospho-nuclear factor-kappa B p65 (p-NF-κB p65), nuclear factor-kappa B p65 (NF-κB p65), and hypoxia-inducible factor 1 alpha (HIF-1α) in the lung was detected by Western blot. *Results*. Results showed extensive neutrophil infiltration, hemorrhage, and edema in the pancreas tissues or lung tissues in mice with AP. The daphnetin treatment improved pathological changes in the lung tissues of AP mice. The MPO activity and the levels of inflammatory cytokines including IL-1β, TNF-α, and IL-6 of lung tissues and serum in the AP group were significantly higher than those in the control group ($P < 0.05$), and daphnetin intervention significantly reversed the changes ($P < 0.05$). Compared with the control mice, the protein levels of TLR4, p-NF-κB p65, and HIF-1α were significantly higher in the lung tissue of the AP mice ($P < 0.05$), while daphnetin treatment decreased these protein expression levels. No significant difference was observed in the NF-κB p65 level among control, AP, and daphnetin groups ($P > 0.05$). *Conclusions*. Daphnetin exerted a protective effect on the acute lung injury induced by SAP in mice. The mechanism may be related to the regulation of TLR4/NF-κB/HIF-1α pathway to reduce the release of inflammatory factors.

1. Introduction

Acute pancreatitis (AP) is a relatively common abdominal organ disorder, and severe acute pancreatitis (SAP) is associated with a high mortality rate of 20%–30% [1]. Acute lung injury (ALI) is a major complication of SAP, with approximately 20% of patients developing acute respiratory distress syndrome (ARDS) [2, 3]. ALI and ARDS are two types of severe pancreatitis-associated acute lung injury (PALI), which is one of the predominant causes of death during the early stages of SAP [4, 5]. Vascular endothelial cells and pulmonary alveolar epithelial cells are compromised by oxidative stress, which is the main pathophysiologic characteristic [6, 7]. However, the mechanisms of PALI are complicated and incompletely understood. Thus, greatly understanding its regulatory network is essential for the treatment of ALI induced by SAP.

SAP starts as an inflammation of local pancreatic acini [8]. Nuclear factor (NF)-κB activation is considered to be a key link in the pathogenesis of SAP [9]. TLR4 is a transmembrane protein that is mainly expressed in immune cells such as macrophages and activates NF-kB through biological signals; studies have reported that it is essential for the activation of NF-kB [10–12]. Hypoxia-inducible factor 1α (HIF-1α) is

activated by lipopolysaccharide (LPS) in a TLR4-dependent manner and promotes the production of inflammatory cytokines, including TNF-α, IL-1, and IL-6 [13, 14]. Besides, a large quantity of proinflammatory cytokines can be released as a result of NF-κB activation in the process of pancreatitis, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and IL-6 [11, 15]. Inflammatory factors such as TNF-α, IL-1, and IL-6 are closely related to SAP-related acute lung injury. In the early stage of SAP inflammatory response, the damaged pancreatic tissue produces and releases a large amount of TNF-α, IL-1, and IL-6, promoting the systemic inflammatory response, leading to systemic inflammatory response syndrome (SIRS) and even multiple organ dysfunction syndrome (MODS), and lung is the most common organ involved in SAP [16, 17].

Daphnetin, an active monomer ingredient extracted from *D. marginata*, has been widely used to treat rheumatoid arthritis and systemic lupus erythematosus [18]. Daphnetin can decrease the TNF-α and IL-1 level of serum and is proved to have anti-inflammatory and antioxidant effect [18]. Recently, it has been shown that daphnetin can inhibit the secretion of inflammatory factors in pancreatic inflammatory response and reduce oxidative stress and inflammatory response, thereby alleviating pancreatic injury in SAP rats [19]. However, the relevant role of daphnetin in acute lung injury in mice with SAP needs to be further explored. The present study was planned to explore the effect of daphnetin on SAP-related acute lung injury and further investigate its potential mechanism.

2. Materials and Methods

2.1. Animals. Male C57/BL mice, 6–8-week-old, weighing 18–20 g, were purchased from SPF Biotechnology Co., Ltd. (Beijing, China), and housed in rooms that controlled temperature (21–24°C) and maintained light/dark cycle (12 : 12) for 1 week to acclimate to the surroundings, with free access to tap water and standard laboratory chow. The animal experiments were approved by the Animal Care and Welfare Committee of Southwest Medical University (ethical protocol code: SWMU20210418) and conducted according to the guidelines of the Local Animal Use and Care Committees of Luzhou as well as the National Animal Welfare Law of China.

2.2. Reagents. Caerulein (Cae) was obtained from MCE (China), and LPS was purchased from Sigma-Aldrich (USA). Daphnetin was supplied by Shanghai Tauto Biotech Co. Ltd. (Shanghai, China). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-1β, and IL-6 were purchased from ELK Biotechnology (Wuhan, China). Myeloperoxidase (MPO) kits were from Jiancheng Company (Nanjing, China). Antibodies against TLR-4 (#14358), NF-κB p56 (#8242), p-NF-κB p65 (#3033), and HIF-1α (#36169) were obtained from Cell Signaling Technology (CST, USA). Antibodies for HMGB1 (ab18256) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, ab8245) were obtained from Abcam (USA).

2.3. Animal Treatment. Mice were divided randomly into experimental groups as follows: control group ($n = 12$), AP group ($n = 12$), and daphnetin group ($n = 12$, treated with daphnetin). Each group was then randomly subdivided into two subgroups as 6 and 12 h according to the time course in the experiment, with 6 rats in each subgroup. Before the induction of AP, mice were fasted for 12 h, but drinking water was available ad libitum. The mice model of AP was established by Cae and LPS as follows [17, 20]: Cae (50 μg/kg) was intraperitoneally injected 13 times, followed by intraperitoneal injection of LPS (15 mg/kg). The control group was administered an equivalent volume of saline. In the daphnetin group, daphnetin was administered at 4 mg/kg 30 min after the last injection of Cae. The mice were randomly sacrificed by a lethal dose of pentobarbital at respective time points following the induction of pancreatitis. The blood, pancreas, and lung of each mouse were obtained for subsequent analysis.

2.4. Histological Assessment. Tissue samples of lung and pancreas were fixed in 4% paraformaldehyde overnight. Then, the samples were embedded in paraffin and sliced into 4 μm sections. These sections were stained with hematoxylin-eosin (H-E) for morphological observation and pathological scoring. For this study, specimens were given a score according to the criteria described previously [2, 21].

2.5. Measurement of the Levels of Inflammatory Factors. ELISA was performed to detect the concentrations of TNF-α, IL-6, and IL-1β of the Lung tissue homogenate and serum according to the instructions of the ELISA kits. The values were normalized with protein contents.

2.6. Determination of the MPO Activity. Myeloperoxidase (MPO) activity was determined in the lung tissues according to the manufacturer's instruction. The MPO activity values were normalized with protein contents. Values are expressed as MPO units per g tissue.

2.7. Western Blot Analysis. The protein expressions of HMGB1, TLR4, p-NF-κB p65, NF-κB p65, HIF-1α, and GAPDH were examined by Western blot analysis. Total protein from lung tissues was extracted by RIPA buffer, and the concentrations were determined by a BCA protein assay kit (ASPEN, Wuhan, China). Proteins (40 μg) were loaded and separated in 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE). The proteins were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). The membrane was blocked with 5% fat-free milk for 1 h at room temperature and further incubated at 4°C overnight with primary antibodies for NF-κB (1 : 3000 dilution), p-NF-κB p65 (1 : 500 dilution), HMGB1 (1 : 1000 dilution), TLR4 (1 : 300 dilution), HIF-1α (1 : 1,000 dilution), and GAPDH (1 : 10000 dilution). The next day, the membranes were incubated with HRP-conjugated secondary antibody (ASPEN, Wuhan, China) diluted to 1 : 10000 at room temperature for 30 minutes. Eventually, the proteins

were visualized with a chemiluminescence kit (Millipore, USA).

2.8. Statistical Analysis. The results were depicted as mean ± standard deviation. $P < 0.05$ was considered to indicate a statistically significant variation. All statistical tests were performed using SPSS software program.

3. Results

3.1. Acute Lung Injury Was Induced in AP Mice. As shown in Figure 1(a), the typical pathological sections of pancreatic tissue in AP mice at 6 h after LPS injection showed acinar edema, interstitial hemorrhage, widening of interlobular space structure, and increased neutrophil exudation. Twelve hours after modeling, pancreatic tissues of AP mice showed obvious inflammatory cell infiltration, bleeding, destruction of pancreatic lobule structure, and acinar cell necrosis. Figure 1(c) showed pulmonary alveolar and interstitial edema, hemorrhage, and inflammatory cell infiltration in the lung tissues of AP group. The pathological changes of lung tissues in AP mice worsened at 12 h after LPS injection, which showed the lung tissues of AP group were characterized by aggravated pulmonary alveolar and interstitial edema, increased bleeding areas, alveolar septa thickening, and increased leukocyte infiltration. Microscopic evaluation indicated the pathological structure of pancreas and lung was normal in the control group. Pathological scores of the pancreas and lung of pancreatitis mice were assessed further, and statistical analysis showed that the pancreatic and lung tissue damage tended to worsen progressively over time (Figures 1(b) and 1(d)).

3.2. Daphnetin Prophylaxis Mitigates the Severity of AP-Induced ALI. The effects of daphnetin on the mouse model of AP were examined. Compared with untreated mice with AP, treatment with daphnetin ($4\ mg.kg^{-1}$) clearly decreased the severity of ALI induced by AP, as shown by reduced congestion, edema, and alveolar interstitial thickening (Figure 1(c)). The pathological scores of lung in AP mice treated with daphnetin were also lower than those in the AP group ($P < 0.05$), indicating that the impaired lungs were relieved by daphnetin intervention (Figure 1(d)).

Myeloperoxidase is an enzyme derived from white blood cells and is present mainly in neutrophils and macrophages. It is also a marker of neutrophil activation. Then, the MPO activity in the lung homogenate was investigated. The results showed that MPO activity increased at 6 and 12 h after modeling in AP mice ($P < 0.05$). Compared with mice in AP group, the MPO activity was reduced in the daphnetin-treated mice ($P < 0.05$) (Figure 2).

3.3. Daphnetin Reduces the Levels of IL-1β, IL-6, and TNF-α. These inflammatory cytokines have been demonstrated in association with the development of systemic complications of AP in early phase. In the present study, the levels of IL-1β, IL-6, and TNF-α in lung homogenate and serum were increased significantly in the AP group than those in the control group. But the levels of IL-1β, IL-6, and TNF-α decreased at 12 h after modeling. Daphnetin treatment could reduce the levels of IL-1β, IL-6, and TNF-α in AP mice ($P < 0.05$), and the inhibiting effect of IL-6 and TNF-α continues till 12 h after modeling, indicating the anti-inflammatory role of daphnetin in ALI induced by AP (Figures 3 and 4).

3.4. Daphnetin Decreases the TLR4, p-NF-κB p65, NF-κB p65, and HIF-1α Expression in Lung. The effects of daphnetin on the expression of TLR4, p-NF-κB p65, and HIF-1α in AP-induced lung injury were further investigated. As shown in Figure 4, compared with the control group, the expression of TLR4, p-NF-κB p65, and HIF-1α was increased in lung tissues of AP mice, and the increase of TLR4 and HIF-1α expression was more significant at 12 h than 6 h after modeling ($P < 0.05$), while the TLR4, p-NF-κB p65, and HIF-1α protein expression in lungs could be suppressed by daphnetin treatment. In contrast, there was no significant difference in NF-κB P65 protein expression between control and AP mice (Figure 5).

4. Discussion

ALI is the most common and earliest distant organ complication of SAP. Unfortunately, approximately 45% of SAP patients develop respiratory failure, which is unacceptably high in morbidity and mortality and imposes a heavy economic burden on the general population [22–24]. The pathogenesis of PALI is rather complicated [25]. Studies have shown that inflammatory cytokines play an important role in the occurrence and development of PALI. During acute lung injury, the permeability of the alveolar-microvascular barrier increases; therefore neutrophils and macrophages, as the two most critical immune cells in the progression of PALI, will transfer to the lung tissue by means of inflammatory mediators. Neutrophils and macrophages then release a series of inflammatory mediators, aggravating pulmonary microvascular disorder and inflammatory response, forming a vicious circle and causing atelectasis and pulmonary edema [26–28]. The current clinical treatment strategies for acute lung injury mainly include lung protective ventilation in ventilator strategy [29], prone position to improve oxygenation [30], fluid conservative therapy in fluid management [31], nutritional support [32], and other conservative strategies. The efficacy of other pharmacological interventions such as the use of systemic corticosteroids, β-2 agonists, statins, exogenous surfactants, and inhaled vasodilators is uncertain [33]. Therefore, it is of great significance to deeply explore the pathophysiological mechanism of acute lung injury and new treatment methods.

Daphnetin is the monarch herb of Zushima-Pian, which is used for the treatment of collagen-induced arthritis and autoimmune diseases. Recent research studies have confirmed that daphnetin has analgesic, anti-inflammatory, antihypoxic, and antiparasitic effects [19, 34, 35]. In an animal randomized controlled trial in SAP treatment in rats

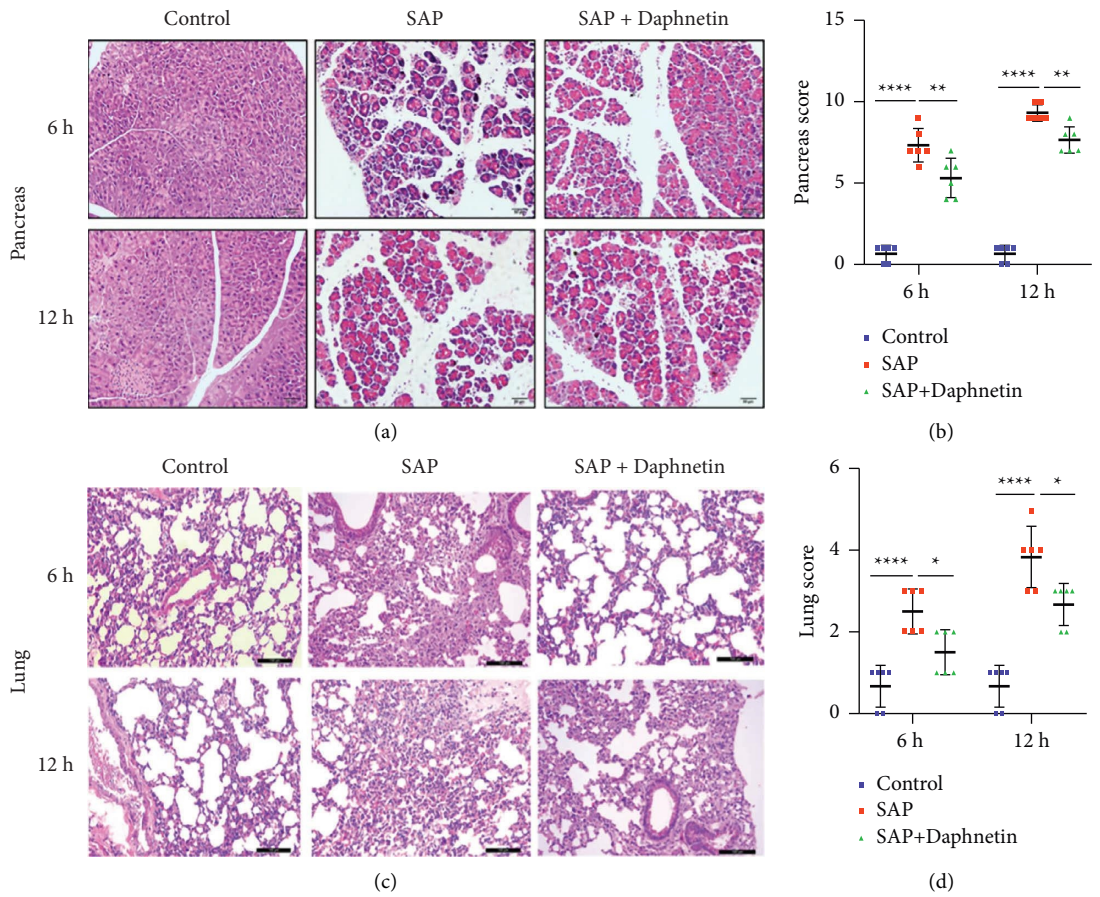

Figure 1: Representative sections were stained with hematoxylin-eosin (H-E). The original magnification is ×200. (a) Pancreas sections of mice in control, SAP, and daphnetin groups. (b) The pathological scores of pancreas. (c) Lung tissue sections of mice in control, SAP, and daphnetin groups. (d) The pathological scores of lung. $^{*}P < 0.05$, versus control group; $^{**}P < 0.05$, versus control group; $^{****}P < 0.05$, versus SAP group.

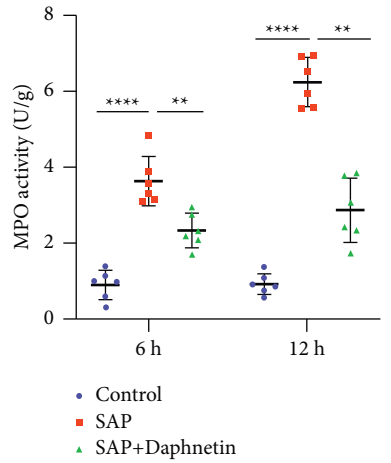

Figure 2: The MPO activity of lung tissue. $^{**}P < 0.05$, versus control group at the same time point; $^{****}P < 0.05$, versus SAP group at the same time point.

receiving daphnetin intervention, daphnetin was shown to inhibit pancreatic inflammatory response, reduce the production of inflammatory factors, and have a protective effect on the pancreas [36]. This study was designed to investigate the possible protective effect of daphnetin in SAP-associated lung injury and explore its underlying potential mechanisms. A mice model of PALI was established by using lipopolysaccharide and caerulein, and the experimental

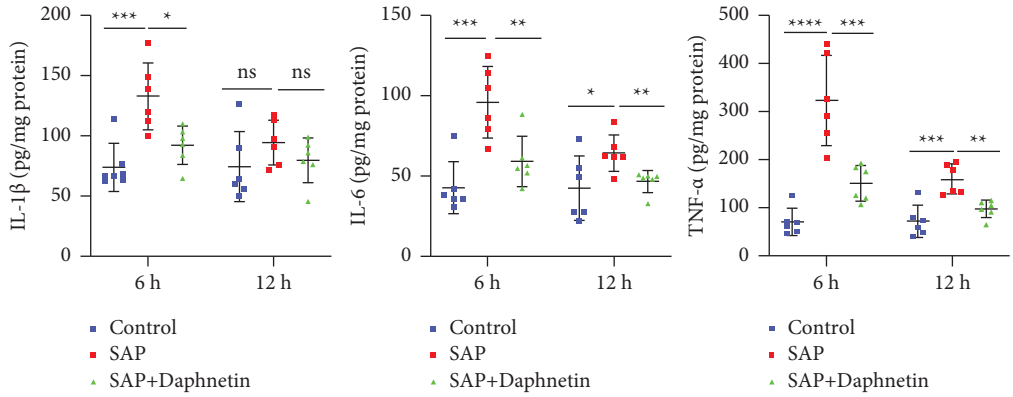

Figure 3: Effects of daphnetin on proinflammatory cytokine production in lungs. The levels of TNF-α, IL-1β, and IL-6 were quantified by ELISA. Results are expressed as mean ± standard error of the mean. ns, results not statistically significant.

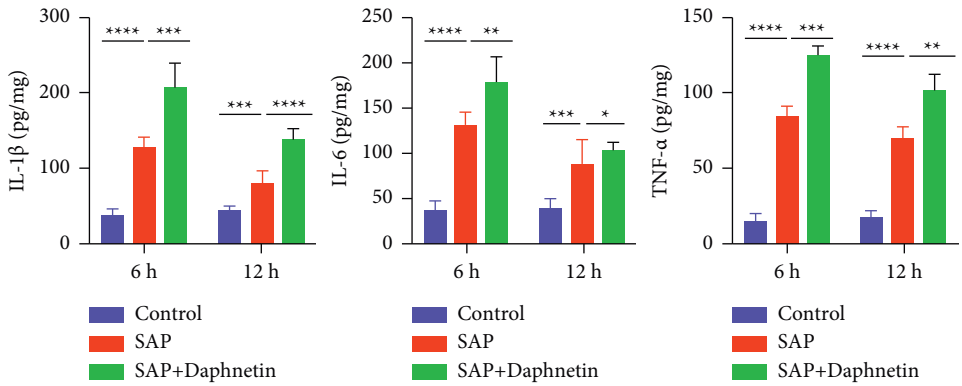

Figure 4: Effects of daphnetin on proinflammatory cytokine production in the serum. The levels of TNF-α, IL-1β, and IL-6 were quantified by ELISA. Results are expressed as mean ± standard error of the mean. $^{**}P < 0.05$, versus control group at the same time point; $^{***}P < 0.05$, versus SAP group at the same time point; $^{****}P < 0.05$, versus SAP group at the same time point.

results showed that neutrophil infiltration, hemorrhage, edema, and alveolar septum thickening in the lung tissue of AP mice, while daphnetin treatment could improve the pathological lung injury induced by AP. Since MPO is a marker of tissue neutrophil infiltration, the activity of MPO in lung tissues was also examined. The results showed that MPO activity was strongly activated in the lung tissue of the mice in the AP group. In addition, it is also investigated that intervention of daphnetin could decrease the MPO activity in AP mice, suggesting that daphnetin can effectively reduce the activity of MPO in the lung tissue, reduce the infiltration of neutrophils, attenuate the inflammatory response of lung tissue in AP mice, and have a protective effect on AP-related acute lung injury in mice.

In the early stage of SAP, the main feature is systemic inflammatory response syndrome as well as related organ failure [37, 38]. The proinflammatory cytokines including IL-1β, TNF-α, and IL-6 are considered to be determining mediators for initiating, amplifying, and perpetuating lung injury, which play a vital role in the occurrence and development of early ALI [39–41]. TNF-α and IL-6 are potent proinflammatory factors that are consistent with the severity of ALI, and IL-1β can induce monocytes and macrophages to promote the process of inflammation that plays a role in the formation of SAP [1, 2, 42]. The development of inflammation is often accompanied by the infiltration of inflammatory cells and the release of inflammatory cytokines [43]. Therefore, the levels of inflammatory mediators including IL-1β, IL-6, and TNF-α in lung tissue and serum were examined. The results showed that the IL-1β, IL-6, and TNF-α levels in the lung homogenate and serum were increased in AP mice, suggesting that the inflammatory response was induced in the early stage of AP. As we expected, the treatment of daphnetin reduced the levels of these inflammatory cytokines. These results showed that daphnetin attenuated the inflammatory response in the lung injury induced by AP, and these anti-inflammatory effects may explain the protective role of daphnetin in SAP-related acute lung injury.

TLRs play a pivotal role in the innate immune system, which may also play a central role in the initiation of signal transduction in the inflammatory reaction during SAP [11, 44]. In an animal experiment of SAP-related acute lung injury in rats, Wang et al. found that in the early stage of acute lung injury, effectively inhibiting the TLR4/NF-κB pathway could reduce the expression of inflammatory mediators in lung tissue, thereby reducing lung injury [44]. Extracellular activation of TLR4 could lead to ALI and lethal

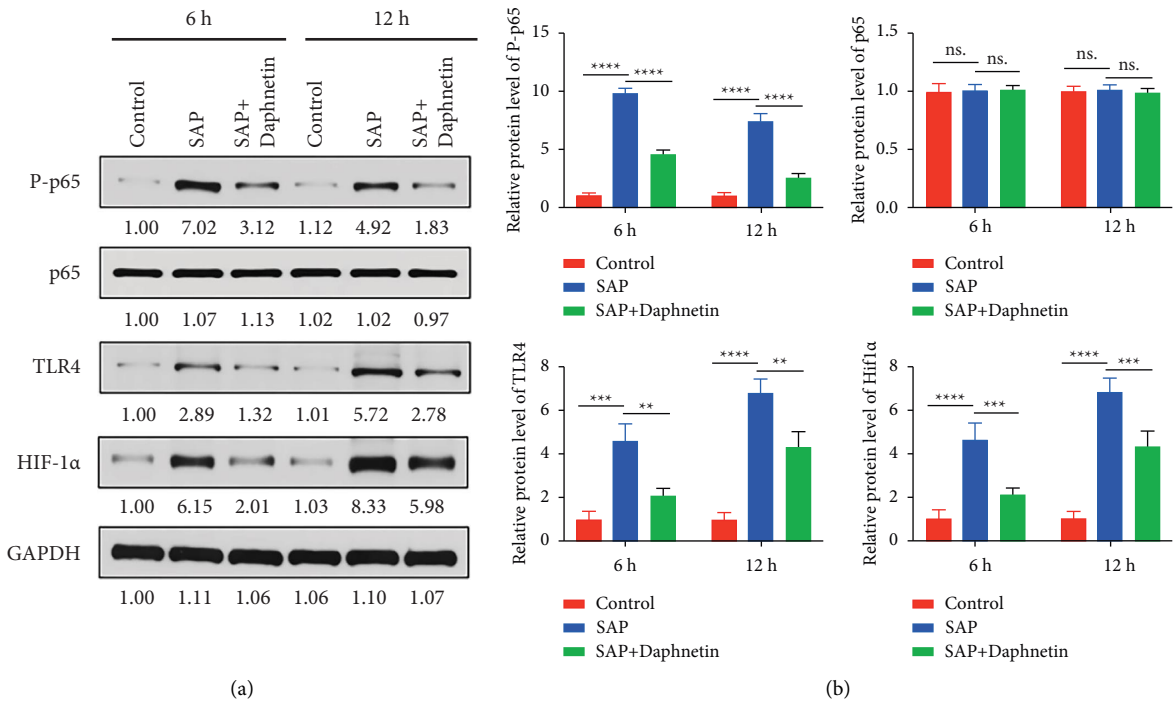

Figure 5: Effects of daphnetin on the protein expressions of TLR4, p-NF-κB p65, NF-κB p65, and HIF-1α in lungs. Daphnetin decreases the TLR4, p-NF-κB p65, NF-κB p65, and HIF-1α expression in lung. $^{**}P < 0.05$, versus control group at the same time point; $^{***}P < 0.05$, versus SAP group at the same time point; $^{****}P < 0.05$, versus SAP group at the same time point; ns, results not statistically significant.

inflammatory processes by inducing NF-κB translocation to the nucleus [42, 45]. NF-κB is a key transcription factor mediating the release of multiple proinflammatory cytokines, playing a vital role in controlling the inflammatory cascade [46]. NF-κB also mediates apoptosis, cell growth, and immune responses and controls the production of inflammatory factors such as TNF-α, IL-1β, and IL-6 [47, 48]. Previous studies have shown that selective inhibition of NF-κB activation efficiently suppresses expression levels of cytokines and reduces the degree of lung injury in laboratory murine acute pancreatitis [49, 50]. In the present study, the effect of daphnetin on TLR4 and NF-κB expression in ALI induced by AP was detected. AP can lead to the phosphorylation of NF-κB p65 and subsequent upregulation of NF-κB p65 [46]. The total NF-κB p65 protein expression did not differ significantly among the three groups. The result showed that the protein expressions of p-NF-κB p65 and TLR4 in lung tissue of mice in AP group were significantly increased compared to those in control mice. Importantly, the levels of p-NF-κB p65 and TLR4 in the lung were suppressed by daphnetin treatment.

HIF-1α can adapt to hypoxic and inflamed microenvironments during infection and is a heterodimeric protein. HIF-1α is involved in inflammation and pulmonary vascular barrier dysfunction and plays an important role in ALI, innate immunity, and host defense [13, 51]. An animal experiment has showed that LPS raises the level of HIF-1α in a TLR4-dependent fashion. Moreover, HIF-1α contributes to the production of inflammatory cytokines, including TNF-α, IL-1, and IL-6, which are vital biological mediators of sepsis [13]. Previous study has found that HIF-1α plays an important role in the pathogenesis of acute pancreatitis [52]. Qi et al. have confirmed that blocking the expression of HIF-1α can avoid the destruction of the alveolar-capillary membrane barrier and achieve the effect of alleviating SAP-related acute lung injury [53]. Western blot demonstrated that the HIF-1α expression of lung tissue was increased in AP mice, while daphnetin significantly downregulated its protein expression in this experiment. NF-κB pathway can activate HIF-1α and promote HIF-1α expression under hypoxic or inflammatory conditions [54]. The inhibition of the NF-κB/HIF-1α pathway can reduce the production of inflammatory factors such as TNF-α and IL-1β and have a protective effect on LPS-induced ALI in mice [55]. Jiang et al. believed that LPS increased the level of HIF-1α in a TLR4-dependent manner and found that the induction of HIF-1α during acute lung injury in mice requires the participation of the TLR4/NF-κB pathway [56]. In this study, the data implied that the expression of TLR4/NF-κB/HIF-1α in lung tissue was consistent with the expression of inflammatory mediators and MPO. These results suggest that daphnetin may downregulate the expression of HIF-1α through the TLR4/NF-κB pathway to inhibit the synthesis and release of inflammatory mediators during the progression of PALI, thereby reducing the pathological damage and inflammatory response of lung caused by AP.

In conclusion, the current study showed that daphnetin was identified to have a therapeutic effect on PALI. It could alleviate the pathological injury and inflammatory reactions of lung caused by PALI. The mechanism may be related to the reduction of inflammatory factor release by regulation of TLR4/NF-κB/HIF-1α. However, further studies are needed

to elucidate the exact mechanism by which daphnetin mediates SAP-related acute lung injury and provide a theoretical basis for the future clinical application of daphnetin.

Data Availability

The data generated in the present study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

TC and OC designed and completed the study, interpreted the data, and wrote the first draft. MZ did the statistical analysis and provided manuscript design. XC designed the study and did the critical revision of the manuscript. All authors have approved the publication of this article. Tao Chen and Ou Chen these authors contributed equally to this work.

References

[1] R. Zhu, Y. Zhao, X. Li et al., "Effects of penehyclidine hydrochloride on severe acute pancreatitis-associated acute lung injury in rats," *Biomedicine and Pharmacotherapy*, vol. 97, pp. 1689–1693, 2018.

[2] Y. Meng, S. Sha, J. Yang, and H. Ren, "Effects of tec tyrosine kinase inhibition on the inflammatory response of severe acute pancreatitis-associated acute lung injury in mice," *Digestive Diseases and Sciences*, vol. 64, no. 8, pp. 2167–2176, 2019.

[3] T. Dombernowsky, M. O. Kristensen, S. Rysgaard, L. L. Gluud, and S. Novovic, "Risk factors for and impact of respiratory failure on mortality in the early phase of acute pancreatitis," *Pancreatology*, vol. 16, no. 5, pp. 756–760, 2016.

[4] L. Interdonato, R. D'amico, M. Cordaro et al., "Aerosol-administered adelmidrol attenuates lung inflammation in a murine model of acute lung injury," *Biomolecules*, vol. 12, no. 9, p. 1308, 2022.

[5] V. Fanelli and V. M. Ranieri, "Mechanisms and clinical consequences of acute lung injury," *Annals of the American Thoracic Society*, vol. 12, no. 1, pp. S3–S8, 2015.

[6] A. S. Elder, G. T. Saccone, and D. L. Dixon, "Lung injury in acute pancreatitis: mechanisms underlying augmented secondary injury," *Pancreatology*, vol. 12, no. 1, pp. 49–56, 2012.

[7] M. A. Matthay, L. B. Ware, and G. A. Zimmerman, "The acute respiratory distress syndrome," *Journal of Clinical Investigation*, vol. 122, no. 8, pp. 2731–2740, 2012.

[8] L. Wang, T. Xu, R. Wang, X. Wang, and D. Wu, "Hypertriglyceridemia acute pancreatitis: animal experiment research," *Digestive Diseases and Sciences*, vol. 67, no. 3, pp. 761–772, 2022.

[9] G. Li, X. Wu, L. Yang et al., "TLR4-mediated NF-κB signaling pathway mediates HMGB1-induced pancreatic injury in mice with severe acute pancreatitis," *International Journal of Molecular Medicine*, vol. 37, no. 1, pp. 99–107, 2016.

[10] V. Scalise, C. Sanguinetti, T. Neri et al., "PCSK9 induces tissue factor expression by activation of TLR4/NFkB signaling," *International Journal of Molecular Sciences*, vol. 22, no. 23, Article ID 12640, 2021.

[11] X. Shen and W. Q. Li, "High-mobility group box 1 protein and its role in severe acute pancreatitis," *World Journal of Gastroenterology*, vol. 21, no. 5, pp. 1424–1435, 2015.

[12] M. T. Kuipers, T. van der Poll, M. J. Schultz, and C. W. Wieland, "Bench-to-bedside review: damage-associated molecular patterns in the onset of ventilator-induced lung injury," *Critical Care*, vol. 15, no. 6, p. 235, 2011.

[13] C. Peyssonnaux, P. Cejudo-Martin, A. Doedens, A. S. Zinkernagel, R. S. Johnson, and V. Nizet, "Cutting edge: essential role of hypoxia inducible factor-1α in development of lipopolysaccharide-induced sepsis," *The Journal of Immunology*, vol. 178, no. 12, pp. 7516–7519, 2007.

[14] B. Crifo and C. T. Taylor, "Crosstalk between toll-like receptors and hypoxia-dependent pathways in health and disease," *Journal of Investigative Medicine*, vol. 64, no. 2, pp. 369–375, 2016.

[15] D. M. Booth, R. Mukherjee, R. Sutton, and D. N. Criddle, "Calcium and reactive oxygen species in acute pancreatitis: friend or foe?" *Antioxidants and Redox Signaling*, vol. 15, no. 10, pp. 2683–2698, 2011.

[16] L. Owusu, C. Xu, H. Chen et al., "Gamma-enolase predicts lung damage in severe acute pancreatitis-induced acute lung injury," *Journal of Molecular Histology*, vol. 49, no. 4, pp. 347–356, 2018.

[17] Q. Fu, Z. Zhai, Y. Wang et al., "NLRP3 deficiency alleviates severe acute pancreatitis and pancreatitis-associated lung injury in a mouse model," *BioMed Research International*, vol. 2018, Article ID 1294951, 10 pages, 2018.

[18] M. Javed, A. Saleem, A. Xaveria, and M. F. Akhtar, "Daphnetin: a bioactive natural coumarin with diverse therapeutic potentials," *Frontiers in Pharmacology*, vol. 13, Article ID 993562, 2022.

[19] Z. Y. Liu, J. Liu, K. L. Zhao et al., "Protective effects of daphnetin on sodium taurocholate-induced severe acute pancreatitis in rats," *Molecular Medicine Reports*, vol. 9, no. 5, pp. 1709–1714, 2014.

[20] X. Yan, T. Lin, Q. Zhu, Y. Zhang, Z. Song, and X. Pan, "Naringenin protects against acute pancreatitis-associated intestinal injury by inhibiting NLRP3 inflammasome activation via AhR signaling," *Frontiers in Pharmacology*, vol. 14, Article ID 1090261, 2023.

[21] A. S. Elder, G. T. Saccone, A. D. Bersten, and D. L. Dixon, "Evaluation of lung injury and respiratory mechanics in a rat model of acute pancreatitis complicated with endotoxin," *Pancreatology*, vol. 12, no. 3, pp. 240–247, 2012.

[22] P. A. Banks, T. L. Bollen, C. Dervenis et al., "Classification of acute pancreatitis—2012: revision of the Atlanta classification and definitions by international consensus," *Gut*, vol. 62, no. 1, pp. 102–111, 2013.

[23] C. E. Forsmark, S. Swaroop Vege, and C. M. Wilcox, "Acute pancreatitis," *New England Journal of Medicine*, vol. 375, no. 20, pp. 1972–1981, 2016.

[24] Y. Zhu, X. Pan, H. Zeng et al., "A study on the etiology, severity, and mortality of 3260 patients with acute pancreatitis according to the revised Atlanta classification in jiangxi, China over an 8-year period," *Pancreas*, vol. 46, no. 4, pp. 504–509, 2017.

[25] Z. S. Lin, C. F. Ku, Y. F. Guan et al., "Dihydro-resveratrol ameliorates lung injury in rats with cerulein-induced acute pancreatitis," *Phytotherapy Research*, vol. 30, no. 4, pp. 663–670, 2016.

[26] S. Chooklin, A. Pereyaslov, and I. Bihalskyy, "Pathogenic role of myeloperoxidase in acute pancreatitis," *Hepatobiliary and*

Pancreatic Diseases International, vol. 8, no. 6, pp. 627–631, 2009.

[27] M. Wei, Y. J. Gong, L. Tu, J. Li, Y. H. Liang, and Y. H. Zhang, "Expression of phosphatidylinositol-3 kinase and effects of inhibitor Wortmannin on expression of tumor necrosis factor-α in severe acute pancreatitis associated with acute lung injury," *World Journal of Emergency Medicine*, vol. 6, no. 4, pp. 299–304, 2015.

[28] D. Liu, L. Wen, Z. Wang et al., "The mechanism of lung and intestinal injury in acute pancreatitis: a review," *Frontiers of Medicine*, vol. 9, Article ID 904078, 2022.

[29] E. C. Goligher, E. L. V. Costa, C. J. Yarnell et al., "Effect of lowering vt on mortality in acute respiratory distress syndrome varies with respiratory system elastance," *American Journal of Respiratory and Critical Care Medicine*, vol. 203, no. 11, pp. 1378–1385, 2021.

[30] C. Lai, X. Monnet, and J. L. Teboul, "Hemodynamic implications of prone positioning in patients with ARDS," *Critical Care*, vol. 27, no. 1, p. 98, 2023.

[31] H. Wiedemann, A. Wheeler, G. Bernard et al., "Comparison of two fluid-management strategies in acute lung injury," *Journal of Vascular Surgery*, vol. 44, no. 4, pp. 909–975, 2006.

[32] T. W. Rice, A. P. Wheeler, B. T. Thompson, B. P. deBoisblanc, J. Steingrub, and P. Rock, "Enteral omega-3 fatty acid, γ-linolenic acid, and antioxidant supplementation in acute lung injury," *JAMA*, vol. 306, no. 14, pp. 1574–1581, 2011.

[33] N. T. Mowery, W. H. Terzian, and A. C. Nelson, "Acute lung injury," *Current Problems in Surgery*, vol. 57, no. 5, Article ID 100777, 2020.

[34] L. Tu, S. Li, Y. Fu et al., "The therapeutic effects of daphnetin in collagen-induced arthritis involve its regulation of Th17 cells," *International Immunopharmacology*, vol. 13, no. 4, pp. 417–423, 2012.

[35] A. Feily and H. Reza Fallahi, "Potential utility of daphnetin as a novel treatment for pemphigus vulgaris," *Giornale Italiano di Dermatologia e Venereologia*, vol. 145, no. 4, pp. 557-558, 2010.

[36] Z. Liu, J. Liu, K. Zhao et al., "Role of daphnetin in rat severe acute pancreatitis through the regulation of TLR4/NF-[Formula: see text]B signaling pathway activation," *American Journal of Chinese Medicine*, vol. 44, no. 01, pp. 149–163, 2016.

[37] M. T. Zhou, C. S. Chen, B. C. Chen, Q. Y. Zhang, and R. Andersson, "Acute lung injury and ARDS in acute pancreatitis: mechanisms and potential intervention," *World Journal of Gastroenterology*, vol. 16, no. 17, pp. 2094–2099, 2010.

[38] S. S. Vege, M. J. DiMagno, C. E. Forsmark, M. Martel, and A. N. Barkun, "Initial medical treatment of acute pancreatitis: American gastroenterological association institute technical review," *Gastroenterology*, vol. 154, no. 4, pp. 1103–1139, 2018.

[39] Y. Chen, L. Wang, Q. Kang et al., "Heat shock protein A12B protects vascular endothelial cells against sepsis-induced acute lung injury in mice," *Cellular Physiology and Biochemistry*, vol. 42, no. 1, pp. 156–168, 2017.

[40] Q. H. Yu, J. F. Guo, Y. Chen, X. R. Guo, Y. Q. Du, and Z. S. Li, "Captopril pretreatment protects the lung against severe acute pancreatitis induced injury via inhibiting angiotensin II production and suppressing Rho/ROCK pathway," *The Kaohsiung Journal of Medical Sciences*, vol. 32, no. 9, pp. 439–445, 2016.

[41] Q. Hu, R. Tao, X. Hu, H. Wu, and J. Xu, "Effects of piperlonguminine on lung injury in severe acute pancreatitis via the TLR4/NF-κB pathway," *European Journal of Histochemistry*, vol. 67, no. 2, p. 3639, 2023.

[42] Z. G. Luan, X. J. Zhang, X. H. Yin et al., "Downregulation of HMGB1 protects against the development of acute lung injury after severe acute pancreatitis," *Immunobiology*, vol. 218, no. 10, pp. 1261–1270, 2013.

[43] S. Burstein, "Cannabidiol (CBD) and its analogs: a review of their effects on inflammation," *Bioorganic and Medicinal Chemistry*, vol. 23, no. 7, pp. 1377–1385, 2015.

[44] B. Wang, X. W. Wu, M. X. Guo et al., "Effects of ω-3 fatty acids on toll-like receptor 4 and nuclear factor-κB p56 in lungs of rats with severe acute pancreatitis," *World Journal of Gastroenterology*, vol. 22, no. 44, pp. 9784–9793, 2016.

[45] P. Asavarut, H. Zhao, J. Gu, and D. Ma, "The role of HMGB1 in inflammation-mediated organ injury," *Acta Anaesthesiologica Taiwanica*, vol. 51, no. 1, pp. 28–33, 2013.

[46] H. Z. Jin, X. J. Yang, K. L. Zhao et al., "Apocynin alleviates lung injury by suppressing NLRP3 inflammasome activation and NF-κB signaling in acute pancreatitis," *International Immunopharmacology*, vol. 75, Article ID 105821, 2019.

[47] T. Lawrence, "The nuclear factor NF- B pathway in inflammation," *Cold Spring Harbor Perspectives in Biology*, vol. 1, no. 6, 2009.

[48] Y. Liang, Y. Zhou, and P. Shen, "NF-kappaB and its regulation on the immune system," *Cell Molecular Immunology*, vol. 1, no. 5, pp. 343–350, 2004.

[49] X. Zhao, B. Jin, B. Yang et al., "Gadolinium chloride ameliorates acute lung injury associated with severe acute pancreatitis in rats by regulating CYLD/NF-κB signaling," *Biochemical and Biophysical Research Communications*, vol. 492, no. 2, pp. 255–261, 2017.

[50] Z. G. Luan, J. Zhang, X. H. Yin, X. C. Ma, and R. X. Guo, "Ethyl pyruvate significantly inhibits tumour necrosis factor-α, interleukin-1β and high mobility group box 1 releasing and attenuates sodium taurocholate-induced severe acute pancreatitis associated with acute lung injury," *Clinical and Experimental Immunology*, vol. 172, no. 3, pp. 417–426, 2013.

[51] K. M. Shepardson, A. Jhingran, A. Caffrey et al., "Myeloid derived hypoxia inducible factor 1-alpha is required for protection against pulmonary Aspergillus fumigatus infection," *PLoS Pathogens*, vol. 10, no. 9, 2014.

[52] G. Gomez, E. W. Englander, G. Wang, and G. H. Greeley Jr, "Increased expression of hypoxia-inducible factor-1α, p48, and the notch signaling cascade during acute pancreatitis in mice," *Pancreas*, vol. 28, no. 1, pp. 58–64, 2004.

[53] B. Qi, H. L. Chen, D. Shang, Y. Dong, G. X. Zhang, and L. Yu, "Effects of hypoxia-inducible factor-1α and matrix metalloproteinase-9 on alveolar-capillary barrier disruption and lung edema in rat models of severe acute pancreatitis-associated lung injury," *Experimental and Therapeutic Medicine*, vol. 8, no. 3, pp. 899–906, 2014.

[54] A. B. Zepeda, A. Pessoa Jr, R. L. Castillo, C. A. Figueroa, V. M. Pulgar, and J. G. Farías, "Cellular and molecular mechanisms in the hypoxic tissue: role of HIF-1 and ROS," *Cell Biochemistry and Function*, vol. 31, no. 6, pp. 451–459, 2013.

[55] H. L. Sun, M. L. Peng, S. S. Lee et al., "Endotoxin-induced acute lung injury in mice is protected by 5,7-dihydroxy-8-methoxyflavone via inhibition of oxidative stress and HIF-1α: endotoxin induced acute lung injury in mice," *Environmental Toxicology*, vol. 31, no. 12, pp. 1700–1709, 2016.

[56] H. Jiang, R. Hu, L. Sun, D. Chai, Z. Cao, and Q. Li, "Critical role of toll-like receptor 4 in hypoxia-inducible factor 1α activation during trauma/hemorrhagic shock–induced acute lung injury after lymph infusion in mice," *Shock*, vol. 42, no. 3, pp. 271–278, 2014.