Emodin Ameliorates Acute Pancreatitis-associated Lung Injury Through Inhibiting the Alveolar Macrophages Pyroptosis

Xiajia Wu

Sichuan University West China Hospital

Jiaqi Yao

Sichuan University West China Hospital

Qian Hu

Sichuan University West China Hospital

Hongxin Kang

Sichuan University West China Hospital

Yifan Miao

Sichuan University West China Hospital

Lu Zhu

Sichuan University West China Hospital

Cong Li

Sichuan University West China Hospital

Xianlin Zhao

Sichuan University West China Hospital

Juan Li

Sichuan University West China Hospital

Meihua Wan

Sichuan University West China Hospital

Wenfu Tang (✉ tangwf@scu.edu.cn)

Sichuan University West China Hospital <https://orcid.org/0000-0001-9294-6634>

Research

Keywords: emodin, NLRP3 inflammasome, alveolar macrophage, pyroptosis, acute pancreatitis, acute lung injury

Posted Date: September 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-835790/v1>

Abstract

Background: Emodin, one of the most dominant components of the traditional Chinese medicine *rhubarb*, has been utilized to treat acute pancreatitis (AP). Acute lung injury (ALI) is one of the most common complications of acute pancreatitis, leading to a serious mortality rate. However, the specific mechanism of emodin in the treatment of AP-associated lung injury remains unclear. Therefore, we investigated the protective roles of emodin in AP-ALI and its underlying mechanisms in two clinically relevant experimental AP models.

Methods: NaT-AP model in rats was constructed using 3.5% sodium taurocholate, and CER+LPS-AP model in mice was constructed using caerulein combined with Lipopolysaccharide. Animals were divided randomly into sham, AP, Ac-YVAD-CMK (caspase-1 specific inhibitor, AYC), and emodin groups. AP-associated lung injury was assessed through H&E staining, inflammatory cytokine levels, and myeloperoxidase activity. Alveolar macrophages (AMs) pyroptosis was evaluated by flow cytometry. In bronchoalveolar lavage fluid, the levels of lactate dehydrogenase and inflammatory cytokines were measured by enzyme-linked immunosorbent assay. Pyroptosis-related protein expressions were detected by Western Blot.

Results: Emodin, similar to the positive control AYC, significantly alleviated pancreatic and lung damage in rats and mice. Additionally, emodin decreased the pyroptotic rates of AMs, inflammatory cytokines, and the lactate dehydrogenase level. More importantly, the protein expressions of NLRP3, ASC, Caspase1 p10, GSDMD, and GSDMD-NT in AMs were significantly downregulated after emodin intervention.

Conclusion: Emodin has a therapeutic effect on AP-associated lung injury, which is at least partially due to the inhibition of NLRP3/Caspase1/GSDMD-mediated AMs pyroptosis signaling ways.

1 Introduction

Acute pancreatitis (AP) is one type of inflammatory disease of the pancreas, followed by early local symptoms (e.g., pancreatic edema, abdominal pain, and distension *et al*) and systemic inflammatory response [1]. Progression of AP into severe acute pancreatitis (SAP) usually leads to a life-threatening condition with multiple organ dysfunction, particularly acute lung injury (ALI), which is the cause of most deaths during the early phase of AP [2]. Accumulating evidence has indicated that the overactivated immune system combined with excessive inflammatory response plays a leading role in this progression [3, 4]. Therefore, how to effectively restrict the local inflammatory response and attenuate AP-related lung injury (SAP-ALI) is key to improving the prognosis of AP and reducing the early mortality rate.

Emodin is an anthraquinone compound, mainly derived from *rhubarb*, *Polygonum cuspidatum*, and other natural plants [5], which has been confirmed to possess anti-inflammatory bioactivity [6, 7]. In recent years, numerous researches have reported the beneficial effects of emodin against AP progression *in vivo* and *in vitro* [8, 9]. For example, emodin could ameliorate LPS-induced ALI by inhibiting the release of pulmonary inflammatory cytokines in rats and significantly reduce CIRR-activated inflammatory signaling

pathways in rat alveolar macrophages (AMs) [10, 11]. However, the effect and working mechanisms of emodin on SAP-ALI remain elusive.

Recently, The NOD-like receptor protein 3 (NLRP3) inflammasome, an intracytoplasmic multiprotein complex, containing NLRP3, apoptosis-associated speck-like protein (ASC) and procaspase-1, has been involved in mediating the inflammatory damage process of ALI [12, 13]. Activation of the NLRP3 inflammasome triggers caspase1 maturation and eventually induces caspase1-dependent pyroptosis, a new form of programmed cell death, characterized by pore-formation in the cell membrane, cell rupture, and the leakage of cytoplasmic contents [14]. Moreover, a caspase-1 inhibitor Ac-YVAD-CMK (AYC) has been reported to alleviate LPS-induced lung injury by inhibiting caspase1-dependent AMs pyroptosis [15]. AMs account for the major leukocyte population in the airways, through secreting a variety of inflammatory cytokines, which seriously affects the development of ALI under infection or non-infectious factors [16, 17]. A growing body of evidence suggests that inflammation and cell death interplay with each other and create a cycle of auto-amplification that leads to further expansion of the inflammatory response. Extensive studies have demonstrated that AMs pyroptosis plays critical roles in acute lung inflammatory injury under various pathogenic conditions (e.g., endotoxin, mechanical ventilation, and *Streptococcus aureus*) [18–20]. Notably, a recent study has demonstrated exosome-mediated activation of NLRP3 inflammasome and consequent AMs pyroptosis could be considered as a potential target for AP treatment [21]. However, the effect of emodin on NLRP3 inflammasome-dependent AMs pyroptosis during SAP-ALI remains unclear.

To examine this issue, we established two AP models of pancreatic ductal infusion of sodium taurocholate (NaT-AP) in rats and intraperitoneal injections with caerulein plus LPS (CER + LPS-AP) in mice and furthermore investigated the specific mechanism of emodin on AP-related ALI.

2 Materials And Methods

2.1 *Animals*

Thirty-two male Sprague-Dawley rats (200-250g) and thirty-two C57BL/6J mice (22–25 g) were purchased from the Jiangnan Laboratory Animal Center of the Sichuan University (Chengdu, China). All animals were housed in a controlled environment (temperature: $22 \pm 2^{\circ}\text{C}$, humidity: $65\% \pm 10\%$) and received standard chow and water. Rats and mice received adapted feeding for 7 days and fasted for 24 h before AP induction. All the animal experiments were conducted in West China Science and Technology Park of Sichuan University and approved by the Experimental Animal Ethics Committee of the Sichuan University of West China Hospital (protocol number: 2020013A).

2.2 *Establishment of experimental SAP-ALI models*

Animals were randomly divided into four groups ($n = 8$ for each group): (1) sham group, (2) AP group, (3) AP + AYC group, and (4) AP + emodin group. NaT-AP was induced in rats as described in our previous work [22]. In brief, rats were anesthetized by intraperitoneally (i.p.) injecting 2% sodium pentobarbital

(40mg/kg.BW), then 3.5% sodium taurocholate (1 mL/kg) was retrogradely injected into the biliopancreatic duct of rats using a micro-perfusion pump at a rate of 0.1 mL/min. CER + LPS-AP mice were i.p. injected with caerulein 6 times hourly (50 µg/kg) followed by LPS (10 mg/kg) challenge immediately after the last caerulein injection [23]. Controls were injected with an equal amount of saline. AYC (5 mg/kg), as a positive control, was i.p. injected 1 h before modeling and emodin (10 mg/kg) was intragastrically administered twice at 6 h and 12 h post modeling.

Animals were anesthetized with i.p. injection of 2% sodium pentobarbital (40mg/kg.BW) the next day (24 h after AP induction), blood was collected by cardiac puncture for the measurement of serum amylase and lipase activity. Lung tissue samples were collected to analyze the myeloperoxidase (MPO) activity and inflammatory cytokines. Pancreas and lung tissue samples were fixed for histological analysis. Bronchoalveolar lavage fluid (BALF) was extracted for AMs separation, and the proinflammatory cytokines and lactic dehydrogenase (LDH) released in the supernatant of BALF were evaluated by ELISA, then the collected AMs were subjected to western blot and flow cytometry.

2.3 Detection of inflammatory factors in lung tissues

After BALF collection, lung homogenates were prepared by the following methods. Briefly, lung tissues were added with 1 ml RIPA lysis buffer, then homogenized with a high-flux tissue homogenizer (MX-S, SCILOGEX, USA). After homogenization, the mixer was incubated at 4°C for half an hour and then centrifugated at 13000 × *g* for 15 min. Finally, the collected supernatants were used to measure inflammatory cytokine levels (including IL-1β, IL-18, and TNF-α) by corresponding ELISA kits (Neobioscience, China) following the manufacturer's protocol.

2.4 Lung MPO activity

To assess the degree of infiltrated neutrophils, myeloperoxidase (MPO) activity in lung samples was measured by the MPO activity assay kit (Nanjing Jiancheng Bioengineering Institute, China), following the manufacturer's instructions.

2.5 Collection of bronchoalveolar lavage fluid(BALF)

Intratracheal injection of 10 ml (1ml for mice) phosphate-buffered saline (PBS), which also means bronchoalveolar lavage, was performed 4 times on the animals following the anesthesia. Then the collected BALF from the syringe was mixed and centrifugated at 250 × *g* for 5 min. IL-1β and IL-18 in the supernatant were measured using the corresponding ELISA kit (Neobioscience, China), and the level of lactate dehydrogenase (LDH) was measured by an LDH Assay Kit (Beyotime Biotechnology, Haimen city, China). Besides, the pelleted cells were resuspended in conditioned RPMI 1640 medium (fetal bovine serum: 10%; penicillin and streptomycin: 1%). Then they were incubated in 60-mm sterilized dishes (37°C; 5% CO₂) for 2h and then washed twice with a warm medium to remove nonadherent cells. Finally, the whole cells were harvested and subjected to western blotting.

2.6 Western blot

Collected AMs were resuspended on ice with RIPA lysis buffer containing proteinase inhibitors, phosphatase inhibitors, and PMSF for 30 min, then the supernatants were obtained after centrifugation for 15 min (12,000 × g, 4°C). Quantification of protein concentrations was determined by a bicinchoninic assay protein assay kit (Beyotime Biotechnology, Haimen city, China). Next, each lysate samples (25 µg/lane) were resolved on 12% SDS-PAGE gels and transferred onto nitrocellulose (NC) or polyvinylidene difluoride (PVDF) membranes. After being blocked in the blocking solution (EpiZyme Biotech, Shanghai, China) for 1 h, membranes were incubated with primary antibodies against NLRP3 (1:1000; abcam), Caspase 1 p10 (1:1000; abcam), ASC (1:200; affinity), GSDMD (1:1000; abcam) GSDMD-NT (1:1000; abcam), and GAPDH (1:2000; affinity) all night at 4°C. The next day, the membranes were washed with TBST 3 times and incubated with horseradish peroxidase (HRP)-labeled secondary antibody at room temperature for 1 h. Finally, blots were visualized through the Bio-Rad imaging system. The relative expression of pyroptosis-related proteins to GAPDH was analyzed and processed by ImageJ software.

2.7 Determination of AMs pyroptosis

The collected AMs from BALF were analyzed by flow cytometry to evaluate the ratio of pyroptotic cell death. Firstly, AMs were stained using FLICA® 660 Caspase-1 Assay reagent (Immunochemistry Technologies, USA) at 37°C for 30min in the dark. Pelleted cells were washed two times and then stained with propidium iodide (PI) in the dark at room temperature for 5 min. Flow cytometry (Cytoflex, Beckman) analyzed the rates of double-positive staining (caspase-1 and PI) cells, which were also regarded to be pyroptotic cells.

2.8 Histological examination

The histopathological injury of pancreatic and lung tissues was evaluated after H&E staining. Two independent pathologists scored pancreatic injury by acinar cells edema, infiltrated inflammatory cells, focal hemorrhage, and necrosis (from 0 to 4) as described [24]. Assessment of pulmonary injury was scored by interstitial/intra-alveolar edema, alveolar wall thickening, inflammation, and hemorrhage (from 0 to 4) as described [25].

2.9 Statistical analysis

All Data were presented as mean ± standard deviation (SD) and analyzed using GraphPad Prism 8 Software. Differences between multiple groups were evaluated by ANOVA and post hoc Tukey's test. $P < 0.05$ was considered significantly different.

3 Results

3.1. Emodin attenuated AP-associated lung injury in NaT-AP rats

In this study, two animal models (NaT-AP and CER + LPS-AP) were established. We first tested the effects of emodin on AP-associated lung damage in rats induced by 3.5% sodium taurocholate. As shown in

Fig. 1A, H&E staining results showed obvious acinar cells edema, infiltrated inflammatory cells, focal hemorrhage and necrosis in the pancreas of NaT-AP rats. Conversely, emodin treatment distinctly lowered the pathological injury of the pancreas, with reduced pathological scores. Meanwhile, elevated serum amylase and lipase levels are a common diagnostic criteria in the diagnosis of pancreatitis [26], the serum amylase and lipase levels of NaT-AP rats were markedly higher compared with those in the control group, while they were downregulated by emodin administration, the effect of AYC on serum amylase and lipase is similar to emodin (Fig. 1B).

Then, the effects of emodin on pulmonary damage and inflammatory response were evaluated. NaT-AP rats showed significant pathological changes in the lung tissues, characterized by interstitial/intra-alveolar edema, hemorrhage, alveolar wall thickening, and inflammation, accompanied by increased histopathological scores. After emodin and AYC administration, the lung injury was effectively ameliorated (Fig. 1C). Moreover, the inflammatory mediators like IL-1 β , IL-18, and TNF- α within the lungs significantly decreased after emodin and AYC intervention (Fig. 1D). In addition, the MPO activity of lung tissue in the AP group was enhanced, indicating extensive immersion of neutrophils, while the intervention of emodin and AYC had an inhibitory effect on neutrophil immersion in the lung tissue of AP rats (Fig. 1E).

3.2. Emodin inhibited AMs pyroptosis and secreted inflammatory factors in SAP-ALI rats

After collecting the BALF, the rates of pyroptotic AMs were assayed by flow cytometry. NaT-AP group displayed a significantly higher frequency of caspase-1 + PI AMs, a hallmark of cell pyroptosis, compared with those from control animals. Of note, emodin and AYC significantly prevented AMs pyroptosis in NaT-AP rats (Fig. 2A). Meanwhile, we quantified proinflammatory cytokines and analyzed the LDH level in BALF. As a result, serum IL-1 β and IL-18 levels were much higher in AP rats compared with those in the controls, while these levels decreased after emodin and AYC treatment (Fig. 2B). Additionally, the increased levels of LDH released in BALF supernatant revealed a higher rate of cell damage or death. Moreover, emodin and AYC treatment markedly reversed the AP-induced increased LDH release in rats (Fig. 2C).

3.3. Emodin inhibited AMs pyroptosis by targeting NLRP3-Caspase1-GSDMD pathway in SAP-ALI rats

Further, we examined the NLRP3 inflammasome and pyroptosis-related proteins by western blotting. Our results indicated that the protein expression levels of NLRP3, ASC, Caspase1 p10, GSDMD, GSDMD-NT in AMs were markedly elevated in NaT-AP rats, and both effects were prevented by caspase 1 blockade AYC. Interestingly, emodin showed the same inhibitory effect on NLRP3-Caspase1-GSDMD pathway. The expressions of NLRP3, ASC, Caspase1 p10, GSDMD, GSDMD-NT were significantly decreased after emodin administration. (Fig. 3A-E).

3.4. Emodin attenuated AP-associated lung injury in CER + LPS-AP mice

After exploring the therapeutic roles and mechanism of emodin in NaT-AP rats, we investigated its effect on another AP model. H&E staining results showed obvious acinar cells edema, infiltrated inflammatory cells, focal hemorrhage and necrosis in the pancreas of CER + LPS-AP mice. Conversely, emodin treatment effectively improved pulmonary injury and lowered pathological scores. Meanwhile, the levels of serum amylase and lipase activity were markedly increased in the CER + LPS-AP group than those in the controls, while they were downregulated by emodin and AYC administration (Fig. 4B).

In the next step, pulmonary inflammatory damage was also evaluated in CER + LPS-AP mice. Significant pathological changes together with increased histopathological scores were observed in the lung tissues, the specific changes were consistent with those of NaT-AP rats. After emodin and AYC administration, the lung injury was effectively ameliorated (Fig. 3C). Moreover, the inflammatory cytokines like IL-1 β , IL-18, and TNF- α within the lung exhibited a significantly downregulated trend through emodin and AYC intervention (Fig. 3D). In addition, the MPO activity of lung tissue in the AP group was enhanced, while the intervention of emodin and AYC had an inhibitory effect on neutrophil immersion in CER + LPS-AP mice (Fig. 4E).

3.5. Emodin inhibited AMs pyroptosis and secreted inflammatory factors in SAP-ALI mice

Similarly, AMs from CER + LPS-AP mice displayed a higher ratio of pyroptosis compared with the controls. Of note, emodin and AYC significantly prevented the AMs pyroptosis in CER + LPS-AP mice (Fig. 5A). Meanwhile, serum IL-1 β and IL-18 levels were higher in CER + LPS-AP mice compared with those in the sham group, while these levels decreased after emodin and AYC treatment (Fig. 5B). In addition, emodin and AYC treatment obviously reversed the AP-induced increase in LDH release (Fig. 5C). Taken together, these findings suggested that acute pancreatitis induced AMs pyroptosis in the lung environment, meanwhile, emodin and AYC reversed this form of cell death.

3.6. Emodin inhibited AMs pyroptosis by targeting NLRP3-Caspase1-GSDMD pathway in SAP-ALI mice

Finally, the pyroptosis-related protein expressions were detected by western blot. The results showed that NLRP3, ASC, Caspase1 p10, GSDMD, and GSDMD-NT expressions in AMs were markedly elevated in CER + LPS-AP mice. Moreover, emodin has the same effects as AYC, both significantly inhibiting the NLRP3, ASC, Caspase1 p10, GSDMD, GSDMD-NT protein expression in AMs (Fig. 6A-E).

Discussion

Acute lung injury (ALI) is one kind of primary or secondary disease caused by different kinds of intrapulmonary insults and extrapulmonary factors (including but not limited to sepsis, shock, pneumonia, pancreatitis etc) [27]. Despite many methods have been widely used for the treatment of AP-associated ALI, the mortality and morbidity remain high, with no approved effective therapies and specific drugs. Previous researches have revealed that emodin could alleviate the AP-induced lung inflammatory response syndrome and acute lung injury via Nrf2/HO-1 signaling [11, 28]. However, systematic elucidation of the underlying therapeutic mechanism is still further detected. In this study, we established two clinically relevant SAP-ALI models, including primary lung injury caused by caerulein combined with LPS (CER + LPS) and secondary lung injury induced by 3.5% sodium taurocholate (NaT). Based on the results, we demonstrated that emodin treatment ameliorated AP-associated lung injury and inflammatory response by inhibition of NLRP3/Caspase1/GSDMD-mediated AMs pyroptosis.

Consistent with previous findings [28], we found that emodin attenuated pancreatitis-associated lung injury and mitigated excessive inflammatory response, as assessed by H&E staining, inflammatory factors and MPO activity. IL-1 β and IL-18, as important promoters of inflammation, have the ability to recruit immune cells like neutrophils and accelerate the release of other inflammatory mediators like TNF- α and IL-6, which trigger cascades of inflammation and amplify the inflammatory response during the course of AP [29, 30]. MPO is seen as a reliable marker for neutrophil activation. In this study, we found that emodin significantly downregulated the levels of IL-1 β , IL-18, TNF- α , and MPO activity within the lung in two types of SAP-ALI models, an effect consistent with the protective role of emodin in oxidative stress and inflammasome signals [9]. Therefore, the interaction between pulmonary and systemic inflammation enhances the cascade of inflammation within the lungs and therefore exaggerates the progression of SAP-ALI.

Pyroptosis, a caspase1-dependent highly inflammatory programmed cell death, a molecular pattern leading to activation of procaspase-1 and secretion of IL-1 β and IL-18, which is inherently associated with the inflammasome activation [31]. Cell death ways and activation of alveolar macrophages are considered major factors responsible for the progression of uncontrolled pulmonary inflammation during ALI [32, 33]. Accumulating evidence has suggested that inflammasome-dependent AMs pyroptosis is closely related to ALI induced by a variety of challenges (e.g., lipopolysaccharide, cardiopulmonary bypass, and ischemia-reperfusion *et al*) [19, 34, 35]. It has been reported that AMs are involved in the progression of AP from local pancreatic injury to pulmonary dysfunction, through the release of various substances like inflammatory cytokines, nitric oxide (NO), and arachidonic acid metabolites [36]. Therefore, in this trial, AMs were accordingly selected as the target cells in the treatment strategy for AP-related lung injury. Moreover, Cheng *et al*/reported that a large amount of NO produced by AMs in the lung tissues could be a cause of pulmonary inflammatory damage secondary to sodium taurocholate-induced AP rats [37]. However, the specific regulatory role of AMs during SAP-ALI remains not fully clarified. To identify this type of pyroptotic cells, active caspase-1 and PI positivity, downstream inflammatory factors and the LDH level were all performed. In this study, the percentages of pyroptotic AMs were increased, which indicated AMs pyroptosis was related to the ALI that was induced in NaT-AP rats and CER + LPS-AP mice. Emodin was previously reported to effectively inhibit GSDMD-mediated pyroptosis induced by

myocardial ischemia/reperfusion in cardiomyocytes [38]. Our findings disclosed that the emodin could effectively inhibit AMs pyroptosis in two kinds of AP models, and its inhibitory effect was similar to that of AYC.

Activation of NLRP3 inflammasome in macrophages played critical roles in the pathogenesis during SAP-ALI [21]. NLRP3 deficiency or inhibitor attenuated excessive local and systemic inflammation in experimental SAP-ALI model [39]. In line with other findings, our results showed the NLRP3 inflammasome in AMs was significantly activated both in NaT-AP rats and CER + LPS-AP mice. Emerging evidence showed that emodin had an inhibitory effect on the activation of NLRP3 inflammasome in myocardial injury combined with cardiovascular dysfunction [38, 40, 41]. Importantly, the administration of emodin and AYC markedly inhibited AP-associated activation of NLRP3 inflammasome in AMs, which was supported by downregulated expression of NLRP3, ASC, and Caspase1 p10. The assembly of this multimeric protein complex triggers the automatic cleavage of pro-caspase-1, which converts into caspase-1. Additionally, N-terminal GSDMD fragment (GSDMD-NT) was generated in the process after gasdermin D (GSDMD) was cleaved by active caspase-1, thus furthermore promoting membrane pore formation and consequent inflammatory cascades. Initiated by activation of the inflammasome, pyroptosis did occur in AMs and exaggerated ALI during the progression of acute pancreatitis. Consistent with other findings, we similarly observed that the cleavage of GSDMD was promoted under acute pancreatitis. Moreover, emodin and AYC downregulated the expressions of GSDMD and GSDMD-NT in AMs. Taken together, all evidence suggested the therapeutic effect of emodin on NaT-AP rats and CER + LPS mice was tightly related to the inhibition of AMs pyroptosis by targeting the NLRP3-Caspase1-GSDMD pathway.

Conclusions

In summary, the discovery of pyroptosis has greatly broadened our understanding of the pathogenesis of ALI, and targeting this manner of cell death provides new avenues for the treatment and management of SAP-ALI. This study used two experimental AP models to explore the functions of AMs pyroptosis during SAP-ALI and pointed out a significant correlation between emodin and AMs pyroptosis signaling pathways. In conclusion, we revealed emodin exhibited its therapeutic effect at least partially via regulating NLRP3-Caspase1-GSDMD signaling pathway. Accordingly, the present study may provide certain novel signaling ways for explaining the pathogenesis of SAP-ALI and develop a novel therapeutic approach for this disease.

Abbreviations

AP, acute pancreatitis; SAP, severe acute pancreatitis; ALI, acute lung injury; SAP-ALI, acute pancreatitis-associated lung injury; NaT, sodium taurocholate; CER, caerulein; LPS, Lipopolysaccharide; AYC, Ac-YVAD-CMK; MPO, myeloperoxidase; BALF, bronchoalveolar lavage fluid; LDH, lactate dehydrogenase; TNF- α , tumor necrosis factor α ; IL-1 β , interleukin 1 β ; IL-18, interleukin 18; AMs, alveolar macrophages; NLRP3, NOD-like receptor protein 3; ASC, apoptosis-associated speck-like protein; GSDMD, gasdermin D; GSDMD-

NT, N-terminal GSDMD fragment; NO, nitric oxide; SDS-PAGE, SDS-polyacrylamide electrophoresis gel; ELISA, enzyme-linked immunosorbent assay.

Declarations

Ethics approval and consent to participate

All the animal experiments were conducted in West China Science and Technology Park of Sichuan University and approved by the Experimental Animal Ethics Committee of the Sichuan University of West China Hospital (protocol number 2020013A).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

Funding

This work was supported by National Natural Science Foundation of China (grant number: 81873203, 81774160, 81974552).

Author Contributions

Xiajia Wu drafted the main part of the manuscript. Jiaqi Yao and Xiajia Wu conducted the experiments with help from Qian Hu, Yifan Miao, Lv Zhu and Cong Li. Xiajia Wu, Jiaqi Yao, Hongxin Kang, Xianlin Zhao, and Juan Li designed the experiments and analyzed the data. Qian Hu and Meihua Wan edited the manuscript. Meihua Wan and Wenfu Tang provided funding support and experimental supervision.

Acknowledgments

Thanks to Li Fu, Jiangrong Deng, Xiangyi Ren, and Yan Wang from the Research Core Facility of West China Hospital for their great assistance in our experiments.

References

1. Xiao AY, Tan ML, Wu LM, Asrani VM, Windsor JA, Yadav D, et al. Global incidence and mortality of pancreatic diseases: a systematic review, meta-analysis, and meta-regression of population-based cohort studies. *Lancet Gastroenterol Hepatol*. 2016;1(1):45–55.

2. Akbarshahi H, Rosendahl AH, Westergren-Thorsson G, Andersson R. Acute lung injury in acute pancreatitis—awaiting the big leap. *Respiratory medicine*. 2012;106(9):1199–210.
3. Kumar V. Pulmonary Innate Immune Response Determines the Outcome of Inflammation During Pneumonia and Sepsis-Associated Acute Lung Injury. *Frontiers in immunology*. 2020;11:1722.
4. Zhang Y, Li X, Grailer JJ, Wang N, Wang M, Yao J, et al. Melatonin alleviates acute lung injury through inhibiting the NLRP3 inflammasome. *J Pineal Res*. 2016;60(4):405–14.
5. Dong X, Fu J, Yin X, Cao S, Li X, Lin L, et al. Emodin: A Review of its Pharmacology, Toxicity and Pharmacokinetics. *Phytother Res*. 2016;30(8):1207–18.
6. Xue J, Chen F, Wang J, Wu S, Zheng M, Zhu H, et al. Emodin protects against concanavalin A-induced hepatitis in mice through inhibiting activation of the p38 MAPK-NF- κ B signaling pathway. *Cell Physiol Biochem*. 2015;35(4):1557–70.
7. Li H, Yang T, Zhou H, Du J, Zhu B, Sun Z. Emodin Combined with Nanosilver Inhibited Sepsis by Anti-inflammatory Protection. *Front Pharmacol*. 2016;7:536.
8. Xu J, Huang B, Wang Y, Tong C, Xie P, Fan R, et al. Emodin ameliorates acute lung injury induced by severe acute pancreatitis through the up-regulated expressions of AQP1 and AQP5 in lung. *Clin Exp Pharmacol Physiol*. 2016;43(11):1071–9.
9. Xia S, Ni Y, Zhou Q, Liu H, Xiang H, Sui H, et al. Emodin Attenuates Severe Acute Pancreatitis via Antioxidant and Anti-inflammatory Activity. *Inflammation*. 2019;42(6):2129–38.
10. Li X, Shan C, Wu Z, Yu H, Yang A, Tan B. Emodin alleviated pulmonary inflammation in rats with LPS-induced acute lung injury through inhibiting the mTOR/HIF-1 α /VEGF signaling pathway. *Inflamm Res*. 2020;69(4):365–73.
11. Xu Q, Wang M, Guo H, Liu H, Zhang G, Xu C, et al. Emodin Alleviates Severe Acute Pancreatitis-Associated Acute Lung Injury by Inhibiting the Cold-Inducible RNA-Binding Protein (CIRP)-Mediated Activation of the NLRP3/IL-1/CXCL1 Signaling. *Front Pharmacol*. 2021;12:655372.
12. Wu J, Yan Z, Schwartz DE, Yu J, Malik AB, Hu G. Activation of NLRP3 inflammasome in alveolar macrophages contributes to mechanical stretch-induced lung inflammation and injury. *J Immunol*. 2013;190(7):3590–9.
13. Grailer JJ, Canning BA, Kalbitz M, Haggadone MD, Dhond RM, Andjelkovic AV, et al. Critical role for the NLRP3 inflammasome during acute lung injury. *J Immunol*. 2014;192(12):5974–83.
14. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. *Nature*. 2015;526(7575):660–5.
15. Wu D, Pan P, Liu B, Su X, Zhang L, Tan H, et al. Inhibition of Alveolar Macrophage Pyroptosis Reduces Lipopolysaccharide-induced Acute Lung Injury in Mice. *Chin Med J*. 2015;128(19):2638–45.
16. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdts S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity*. 2014;41(1):14–20.
17. Gordon S, Plüddemann A, Martinez Estrada F. Macrophage heterogeneity in tissues: phenotypic diversity and functions. *Immunol Rev*. 2014;262(1):36–55.

18. Yang J, Zhao Y, Zhang P, Li Y, Yang Y, Yang Y, et al. Hemorrhagic shock primes for lung vascular endothelial cell pyroptosis: role in pulmonary inflammation following LPS. *Cell Death Dis.* 2016;7(9):e2363.
19. Wu D-D, Pan P-H, Liu B, Su X-L, Zhang L-M, Tan H-Y, et al. Inhibition of Alveolar Macrophage Pyroptosis Reduces Lipopolysaccharide-induced Acute Lung Injury in Mice. *Chin Med J.* 2015;128(19):2638–45.
20. Yao L, Sun T. Glycyrrhizin administration ameliorates *Streptococcus aureus*-induced acute lung injury. *Int Immunopharmacol.* 2019;70:504–11.
21. Wu X-B, Sun H-Y, Luo Z-L, Cheng L, Duan X-M, Ren J-D. Plasma-derived exosomes contribute to pancreatitis-associated lung injury by triggering NLRP3-dependent pyroptosis in alveolar macrophages. *Biochim Biophys Acta Mol Basis Dis.* 2020;1866(5):165685.
22. Zhang Y-M, Ren H-Y, Zhao X-L, Li J, Li J-Y, Wu F-S, et al. Pharmacokinetics and pharmacodynamics of Da-Cheng-Qi decoction in the liver of rats with severe acute pancreatitis. *World journal of gastroenterology.* 2017;23(8):1367–74.
23. Ding S-P, Li J-C, Jin C. A mouse model of severe acute pancreatitis induced with caerulein and lipopolysaccharide. *World journal of gastroenterology.* 2003;9(3):584–9.
24. Kusske AM, Rongione AJ, Ashley SW, McFadden DW, Reber HA. Interleukin-10 prevents death in lethal necrotizing pancreatitis in mice. *Surgery.* 1996;120(2).
25. Mikawa K, Maekawa N, Nishina K, Takao Y, Yaku H, Obara H. Effect of lidocaine pretreatment on endotoxin-induced lung injury in rabbits. *Anesthesiology.* 1994;81(3):689–99.
26. Rompianesi G, Hann A, Komolafe O, Pereira SP, Davidson BR, Gurusamy KS. Serum amylase and lipase and urinary trypsinogen and amylase for diagnosis of acute pancreatitis. *Cochrane Database Syst Rev.* 2017;4:CD012010.
27. Cao Y, Lyu YI, Tang J, Li Y. MicroRNAs. Novel regulatory molecules in acute lung injury/acute respiratory distress syndrome. *Biomed Rep.* 2016;4(5):523–7.
28. Gao Z, Sui J, Fan R, Qu W, Dong X, Sun D. Emodin Protects Against Acute Pancreatitis-Associated Lung Injury by Inhibiting NLRP3 Inflammasome Activation via Nrf2/HO-1 Signaling. *Drug design, development and therapy.* 2020;14:1971-82.
29. Bunbupha S, Prachaney P, Kukongviriyapan U, Kukongviriyapan V, Welbat JU, Pakdeechote P. Asiatic acid alleviates cardiovascular remodelling in rats with L-NAME-induced hypertension. *Clin Exp Pharmacol Physiol.* 2015;42(11):1189–97.
30. Ma H-J, Huang X-L, Liu Y, Fan Y-M. Sulfur dioxide attenuates LPS-induced acute lung injury via enhancing polymorphonuclear neutrophil apoptosis. *Acta Pharmacol Sin.* 2012;33(8):983–90.
31. Zeng C, Wang R, Tan H. Role of Pyroptosis in Cardiovascular Diseases and its Therapeutic Implications. *Int J Biol Sci.* 2019;15(7):1345–57.
32. Niesler U, Palmer A, Fröba JS, Braumüller ST, Zhou S, Gebhard F, et al. Role of alveolar macrophages in the regulation of local and systemic inflammation after lung contusion. *J Trauma Acute Care Surg.* 2014;76(2):386–93.

33. Aggarwal NR, King LS, D'Alessio FR. Diverse macrophage populations mediate acute lung inflammation and resolution. *Am J Physiol Lung Cell Mol Physiol*. 2014;306(8):L709-L25.
34. Hou L, Yang Z, Wang Z, Zhang X, Zhao Y, Yang H, et al. NLRP3/ASC-mediated alveolar macrophage pyroptosis enhances HMGB1 secretion in acute lung injury induced by cardiopulmonary bypass. *Lab Invest*. 2018;98(8):1052–64.
35. Fei L, Jingyuan X, Fangte L, Huijun D, Liu Y, Ren J, et al. Preconditioning with rHMGB1 ameliorates lung ischemia-reperfusion injury by inhibiting alveolar macrophage pyroptosis via the Keap1/Nrf2/HO-1 signaling pathway. *J Transl Med*. 2020;18(1):301.
36. Gea-Sorlí S, Closa D. Role of macrophages in the progression of acute pancreatitis. *World J Gastrointest Pharmacol Ther*. 2010;1(5):107–11.
37. Cheng S, Yan W-M, Yang B, Shi J-d, Song M-m, Zhao Y. A crucial role of nitric oxide in acute lung injury secondary to the acute necrotizing pancreatitis. *Hum Exp Toxicol*. 2010;29(4):329–37.
38. Ye B, Chen X, Dai S, Han J, Liang X, Lin S, et al. Emodin alleviates myocardial ischemia/reperfusion injury by inhibiting gasdermin D-mediated pyroptosis in cardiomyocytes. *Drug Des Devel Ther*. 2019;13:975–90.
39. Fu Q, Zhai Z, Wang Y, Xu L, Jia P, Xia P, et al. NLRP3 Deficiency Alleviates Severe Acute Pancreatitis and Pancreatitis-Associated Lung Injury in a Mouse Model. *Biomed Res Int*. 2018;2018:1294951.
40. Dai S, Ye B, Chen L, Hong G, Zhao G, Lu Z. Emodin alleviates LPS-induced myocardial injury through inhibition of NLRP3 inflammasome activation. *Phytother Res*. 2021.
41. Zhang Y, Song Z, Huang S, Zhu L, Liu T, Shu H, et al. Aloe emodin relieves Ang II-induced endothelial junction dysfunction via promoting ubiquitination mediated NLRP3 inflammasome inactivation. *J Leukoc Biol*. 2020;108(6):1735–46.

Figures

Figure 1

Emodin attenuated AP-associated lung injury in Nat-AP rats. (A) H&E staining images ($\times 200$) and pathological scores in pancreas from sham rats, NaT-AP rats, NaT-AP+AYC rats, and NaT-AP+emodin rats. Arrows (blue, yellow, red, black) indicated pancreatic edema, inflammation, hemorrhage, and necrosis respectively. (B) Serum amylase and lipase levels of each group. (C) H&E staining images ($\times 200$) and pathological scores in lung tissues of each group. Arrows (blue, yellow, red, black) indicated lung edema, hemorrhage, alveolar septal thickening, and inflammation respectively. (D) The levels of IL-1 β , IL-18, and TNF- α within lung tissues of each group. (E) The expression of MPO activity within lung tissues of each group. * $P < 0.05$ vs the controls. # $P < 0.05$ vs NaT-AP rats.

Figure 2

Emodin inhibited AMs pyroptosis and secreted inflammatory factors in SAP-ALI rats. (A) The rate of pyroptotic AMs determined by flow cytometry from sham rats, NaT-AP rats, NaT-AP+AYC rats, and NaT-AP+emodin rats. Double-positive staining cells (caspase-1 and PI) were regarded to be pyroptotic cells. (B) the levels of IL-1 β and IL-18 of each group in BALF. (C) The LDH level of each group in BALF. *P < 0.05 vs the controls. #P < 0.05 vs NaT-AP rats.

Figure 3

Emodin inhibited AMs pyroptosis by targeting NLRP3-Caspase1-GSDMD pathway in SAP-ALI rats. (A)-(E) Western blots and quantitative analysis of pyroptosis-related proteins (NLRP3, ASC, Caspase1 p10, GSDMD, and GSDMD-NT) relative to GAPDH from sham rats, NaT-AP rats, NaT-AP+AYC rats, and NaT-AP+emodin rats. * $P < 0.05$ vs the controls. # $P < 0.05$ vs NaT-AP rats.

Figure 4

Emodin attenuated AP-associated lung injury in CER+LPS-AP mice. (A) H&E staining images ($\times 200$) and pathological scores in pancreas from sham mice, CER+LPS-AP mice, CER+LPS-AP+AYC mice, and CER+LPS-AP+emodin mice. Arrows (blue, yellow, red, black) indicated pancreatic edema, inflammation, hemorrhage, and necrosis respectively. (B) Serum amylase and lipase levels of each group. (C) H&E staining images ($\times 400$) and pathological scores in lung tissues of each group. Arrows (blue, yellow, red, black) indicated lung edema, hemorrhage, alveolar septal thickening, and inflammation respectively. (D) The levels of IL-1 β , IL-18, and TNF- α within the lung tissues of each group. (E) The expression of MPO activity of each group. * $P < 0.05$ vs the controls. # $P < 0.05$ vs CER+LPS-AP mice.

Figure 5

Emodin inhibited AMs pyroptosis and secreted inflammatory factors in SAP-ALI mice. (A) The rate of pyroptotic AMs determined by flow cytometry from sham mice, CER+LPS-AP mice, CER+LPS-AP+AYC mice, and CER+LPS-AP+emodin mice. Double-positive staining cells (caspase-1 and PI) were regarded to be pyroptotic cells. (B) the levels of IL-1 β and IL-18 of each group in BALF. (C) The level of LDH of each group in BALF. *P < 0.05 vs the controls. #P < 0.05 vs CER+LPS-AP mice.

Figure 6

Emodin inhibited AMs pyroptosis by targeting NLRP3-Caspase1-GSDMD pathway in SAP-ALI mice. (A)-(E) Western blots and quantitative analysis of pyroptosis-related proteins (NLRP3, ASC, Caspase1 p10, GSDMD, and GSDMD-NT) relative to GAPDH from sham mice, CER+LPS-AP mice CER+LPS-AP+AYC mice, and CER+LPS-AP+emodin mice. * $P < 0.05$ vs the controls. # $P < 0.05$ vs CER+LPS-AP mice.