International Immunopharmacology 72 (2019) 204–210

Contents lists available at ScienceDirect

International Immunopharmacology

journal homepage: www.elsevier.com/locate/intimp

# Baicalein attenuates pancreatic inflammatory injury through regulating MAPK, STAT 3 and NF-κB activation

Wei-ling Pu[a,b], Ru-yu Bai[a], Kun Zhou[a,b,c], Yan-fei Peng[d], Meng-ying Zhang[a,d], Micheal O. Hottiger[e], Wen-hua Li[f], Xiu-mei Gao[a,b,c,*], Li-kang Sun[b,d,**]

[a] Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China
[b] Tianjin Key Laboratory of Chinese Medicine Pharmacology, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China
[c] Key Laboratory of Pharmacology of Traditional Chinese Medical Formulae, Ministry of Education, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China
[d] School of Integrative Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China
[e] Department of Molecular Mechanisms of Disease, University of Zurich, Winterthurerstrasse 190, 8057 Zurich, Switzerland
[f] College of Medicine, Xizang Minzu University (Tibetan National University), Xianyang 712082, Shaanxi, China

ARTICLE INFO

*Keywords:*
Baicalein
Acute pancreatitis
NF-κB
STAT 3
MAPK

ABSTRACT

Acute pancreatitis (AP) is a common acute abdominal disease with local or systemic inflammatory response, caused by abnormal activation of digestive enzymes. Baicalein has been shown to exert anti-inflammatory effects and to attenuate the pathological changes of AP. The aim of the research was to investigate the effects of baicalein on caerulein induced pancreatitis, and to elucidate the putative underlying mechanism. In this study, the therapeutic potential of baicalein and its mechanism were investigated in a caerulein-induced AP *in vivo* and *in vitro* model. The results indicate that baicalein treatment alleviates the caerulein-induced pathological damage in the pancreas. Baicalein decreased the expression level of pro-inflammatory cytokines and chemokines of the pancreas in caerulein treated mice and of isolated pancreatic acinar cells. Moreover, baicalein inhibited the expression of NF-κB p65 and the phosphorylation of p38 MAPK, ERK (extracellular signal-regulated kinase) as well as STAT 3, which indicates that baicalein exerts its anti-inflammatory effects *via* dampening the NF-κB, MAPK and STAT 3 signaling pathways. Together, this study provides experimental evidence for the clinical application of *Scutellaria baicalensis* Georgi or baicalein and indicates that baicalein may be a promising candidate for treatment of AP patients in the future.

## 1. Introduction

Acute pancreatitis (AP), a common clinical acute abdominal emergency, is a reversible inflammatory process of the exocrine pancreas mainly caused by over-activation of pancreatic digestive enzymes [1]. AP may lead to local destruction of acini or systemic inflammatory syndromes. The annual incidence of AP is about 4.9 to 40 cases per year per 100, 000 humans, with a mortality rate of 1%. Furthermore, about 30% of AP patients may develop a more severe form [2]. The current clinical treatments of AP include conservative approaches (aggressive fluids replacement, nutrition and antibiotics) and invasive methods according to the severity of AP. However, there is still a need of a specific therapy for clinical treatment of AP [3]. Traditional Chinese medicinal herbs have been used for the treatment of AP in China for many years [4]. Clinical observations have provided strong evidence that TCM adjuvant treatment of AP has positive effects on multiple organ failure, mortality and systemic infection [5].

*Scutellaria baicalensis* Georgi, listed in the Chinese Pharmacopoeia, is a commonly-used traditional Chinese herb for treatment of pancreatitis in China and other East Asian countries. Baicalein (5,6,7-trihydroxy-2-phenyl-4H-1-benzopyran-4-one) (Fig. 1), a major active compound isolated from the root of *S. baicalensis* Georgi, has been reported to possess many pharmacological effects including anti-inflammatory, anti-bacterial, anti-hypertension, and anti-tumor activities [6,7]. Inflammation plays a critical role in the process of AP. Inflammatory mediators such as TNFα or IL-6 and various pathways (including NF-κB and STAT 3) play a key role in the initial pancreatic injury and the subsequent immune response [8]. Baicalein exerts anti-inflammatory effects in various diseases (including diabetes, cardiovascular diseases, inflammatory bowel diseases, mastitis and pancreatitis) *via* multiple

* Correspondence to: X.-m. Gao, Institute of Traditional Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China.
** Correspondence to: L.-k. Sun, School of Integrative Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 301617, China.
*E-mail addresses:* gaoxiumei@tjutcm.edu.cn (X.-m. Gao), sunlikang_tjutcm@163.com, likang.sun@bluewin.ch (L.-k. Sun).

https://doi.org/10.1016/j.intimp.2019.04.018
Received 9 December 2018; Received in revised form 4 April 2019; Accepted 8 April 2019
Available online 15 April 2019
1567-5769/ 

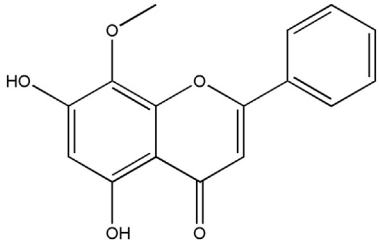



**Fig. 1.** Chemical structure of baicalein.

pathways [6]. Baicalein could alleviate experimental pancreatitis by inhibiting the expression of pancreatic enzyme and cytokines in sodium taurocholate induced severe acute pancreatitis (SAP) mice [9]. Although many studies have confirmed that baicalein can improve pathological changes of AP, the mode of action of baicalein still not fully understood. The aim of the research was thus to investigate the effects of baicalein on caerulein (cholecystokinin analogue) induced AP and to elucidate the mechanism providing protective activity.

## 2. Materials and methods

### 2.1. Reagents

Baicalein (purity > 98%, endotoxin-free) was purchased from Top High Bio Technology Co., Ltd. (Nanjing, China). Lipopolysaccharides (LPS), collagenase IA, and trypsin inhibitor were purchased from Sigma-Aldrich (St Louis, MO). Caerulein was purchased from Yeasen Biotech Co., Ltd. (Shanghai, China). Ficoll and dimethylsulfoxide (DMSO) were purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Primary antibodies against phosphorylated IκBα (p-IκBα), IκBα, NF-κB p65, p38, p-p38, JNK, p-JNK, ERK, p-ERK, STAT 3, p-STAT 3, and β-actin were purchased from CST Inc. (Danvers, MA).

### 2.2. Cell culture and preparation of conditioned medium (CM)

RAW264.7 murine macrophages cells, obtained from the Cell Culture Center of the Chinese Academy of Medical Sciences (Beijing, China), were cultured in Dulbecco's Modified Eagle Medium supplemented with 10% FBS as the manufacturer's suggested. To prepare the macrophage conditioned medium (CM), RAW264.7 cells were plated in 24-well plates ($2.5 \times 10^5$ cells/mL) for 24 h and then treated with or without LPS (0.5 μg/mL) for 18 h. The supernatant was centrifuged and collected as the CM. 40 inflammatory mediators (cytokines, chemokines, and more) in CM were analyzed by Mouse Cytokine Antibody Array (R&D Systems, Minneapolis, USA).

### 2.3. Animals and treatment

C57BL/6 mice (about 20 ± 2 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China), and cultured with free access to food and water for 2 weeks. All procedures of animal experiments were approved by animal ethics committee of Tianjin University of Traditional Chinese Medicine (No. TCM-LAEC2018022) and performed according to the National Institutes of Health Guidelines on Laboratory Research and Guide for the Care and Use of Laboratory Animals. The pancreatitis model was established by intraperitoneally injecting caerulein (50 μg/kg) every hour for 5 consecutive hours a day for three days. Baicalein was administered intravenously (20 mg/kg per day) 1 h after the last caerulein injection for baicalein group. The mice were randomly divided to three experimental groups (n = 6). Control: intraperitoneally injecting equal volume of normal saline instead of caerulein; Model (Cae group): intraperitoneally injecting caerulein for AP induction; Baicalein group (Bai group): intraperitoneally injected with 20 mg/kg baicalein 1 h after caerulein administration. After 24 h all mice were anesthetized, sacrificed and their serum was collected for further analysis. The pancreas of every animal was also collected and separated into two parts, one for pathological analysis and another for biochemical analysis.

### 2.4. Histological examination and immunohistochemical staining

For histological examination, pancreas samples were fixed in 4% paraformaldehyde for H&E staining. After dehydration in graded alcohol, the tissues were cut into slices of 3 to 4 μm and stained with H&E solution. The tissues were examined under an optical microscope. The histopathological changes were graded as previously described: edema (0–3), inflammatory infiltration (0–3), necrosis (0–7) and hemorrhage (0–7) [10]. The pathological changes were evaluated and scored by two pathologists in blinded manner.

For immunohistochemical staining, pancreas samples were cut as above, deparaffinized and rehydrated according to standard histological protocols. These sections were then rinsed in 3% hydrogen peroxide to inactivate endogenous peroxidase, followed by antigen retrieval with sodium citrate buffer at 95 °C for 20 min. After blocking with 3% BSA serum, the sections were incubated with either anti-F4/80 antibody (macrophages) or anti-Ly6G antibody (neutrophils) at a dilution of 1:200 each. After subsequent incubation with biotinylated secondary antibody, horseradish peroxidase conjugated streptavidin and 2, 4-diaminobenzidine substrates, the sections were counterstained with hematoxylin. The F4/80 or Ly6G positive cells were observed by optical microscope. Positive cells in five high power fields were counted in each section (magnification 400×). The quantitative analysis was determined by the proportion of positive cells of total cells in each section.

### 2.5. Enzymatic activity of α-amylase

The enzymatic activities of amylase in blood samples were determined by α-amylase assay kit from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the instructions of the manufacturer. Briefly, amylase activity was normalized to the activity required to hydrolyze 10 mg starch in 30 min at 37 °C. Each assay was performed in duplicates.

### 2.6. Isolation and culture of mouse pancreatic acinar cells

Pancreases from C57 mice were quickly washing in ice-cold Hanks' buffered saline solution (HBSS) and diced into small pieces (approx. 1–2 mm). For digestion, the pancreatic pieces were shaken by hand in collagenase IA solution (HBSS 1× containing 10 mM HEPES, 0.2 mg/mL of collagenase IA, and 0.25 mg/mL of trypsin inhibitor). The digestion period was variable until the tissue suspension appeared homogeneous to the eye. The enzymatic reaction was stopped by adding ice-cold buffered washing solution (HBSS 1× containing 5% FBS and 10 mM HEPES). The cell pellet was then resuspended in 10 mL F12K medium (containing 20% FBS and 0.25 mg/mL of trypsin inhibitor) and the cell suspension was filtrated through a 70 μm filter to retain the non-digested fragments. The isolated acini were seeded in 25 $cm^2$ culture flasks to eliminate contaminant cells (*i.e.* fibroblasts) and cellular remnants that had adhered. After 4 h acini were carefully transferred and layered on 40% Ficoll for further purification by gradient centrifugation. Cell viability was tested with the trypan blue method immediately after preparation of acini. Preparations were accepted for study only if > 95% of the cells excluded the dye.

### 2.7. Cell viability assay

Cell viability of pancreatic acinar cells treated with baicalein was measured by MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl

tetrazolium bromide) assay. After treatment with baicalein (5–80 μM), cells were incubated with MTT reagent (at a final concentration of 0.5 mg/mL) at 37 °C for 4 h. After rinsing of the wells, DMSO was used to dissolve the dark blue crystals. Plates were read out on a Microplate Reader (Molecular Devices, Sunnyvale, CA) at 570 nm wavelength and a reference wavelength of 630 nm.

### 2.8. Determine of cytokines and chemokines in pancreas

The concentrations of six cytokines and chemokines (TNFα, IL-1β, IL-6, IP-10, MCP-1, MIP-1α) in acinar cells or pancreatic tissues lysates were measured by Milliplex mouse cytokines/chemokines magnetic bead panel kit (Millipore, USA) according to the manufacturer's instructions. In brief, samples were diluted 1:2 with analyte dilution buffer and assayed in duplicate.

### 2.9. Statistical analysis

All data are presented as mean ± SD values. Data were analyzed by one-way analysis of variance and *t*-test was used for comparison between any two groups. *p* values of $< 0.05$ were considered as statistically significantly different.

## 3. Results

### 3.1. Baicalein alleviated the severity of pancreatic injury

To explore the effect of baicalein on caerulein treated mice, the pathological changes of pancreas and level of amylase were investigated. There were no obvious changes in the pancreas of control group (Fig. 2A). The pancreatic lobular structure was clear and there were no obvious interstitial edema and necrosis. In the model group (Cae), there were common acini edema and disruption of lobular structure, and sporadic necrotic areas. The score of the model group was significantly higher than that of the controls ($p < 0.01$). In the baicalein co-treated group, the lobular structure was clear and a few acini edema. Necrosis was rarely observed. The pathological score decreased markedly compared to Cae group ($p < 0.01$). The activity of amylase was increased about 2.5 times in caerulein group compared with that of control group (Fig. 2B), suggesting that administration of baicalein significantly suppressed the caerulein induced pancreatitis in mice.

### 3.2. Baicalein inhibited the infiltration of macrophages and neutrophils

As the infiltration of macrophages is a major manifestation in AP onset, paraffin sections of pancreatic tissue were stained using a monoclonal anti-F4/80-antibody. As Few F4/80 positive cells (macrophages) were observed in the pancreas (Fig. 3A). In the caerulein treated group (Cae), macrophages were observed at the edge of the tissue and interlobular ducts, with occasional infiltration of the parenchyma. The number of macrophages in the pancreas was decreased in baicalein treated group (Cae + Bai). Neutrophils are another essential factor in the progress of AP and the infiltration of neutrophils (Ly6G) in pancreas. Caerulein increased the amount of neutrophils in pancreas and the effect was inhibited by baicalein, which was similar with the filtration of macrophages (Fig. 3B), strengthening the protective effects of baicalein on caerulein-induced pancreatic injury and inflammatory response.

### 3.3. Baicalein inhibited the expression of pancreatic cytokines in vivo and in vitro

Cytokines play a key role in the progress of AP. The research investigated six vital cytokines or chemokines using the caerulein-induced mice model and CM-induced acinar cells. The concentration of cytokines and chemokines were all increased in caerulein group, indicating the inflammatory response of pancreas (Fig. 4A). MTT analyses provided evidence that the treatment with increasing concentrations (5–80 μM) of baicalein resulted not in a measurable difference in the viability of isolated pancreatic cells compared to control samples (Fig. 4B). Furthermore, baicalein treatment decreased the expression level of TNFα, IL-1β and MIP-1α within the pancreas (Fig. 4A), but had no effects on IL-6, MCP-1 and IP-10. Similar effects were found in CM induced pancreatic acinar cells *in vitro*. CM comparably induced the over-expression of cytokines (TNFα, IL-1β, IL-6) in acinar cells (Fig. 4C), which could be reduced by baicalein. All these results indicate that baicalein attenuates the inflammatory response of pancreatic injury.

To better illustrate the cytokines of CM, we monitored the relative

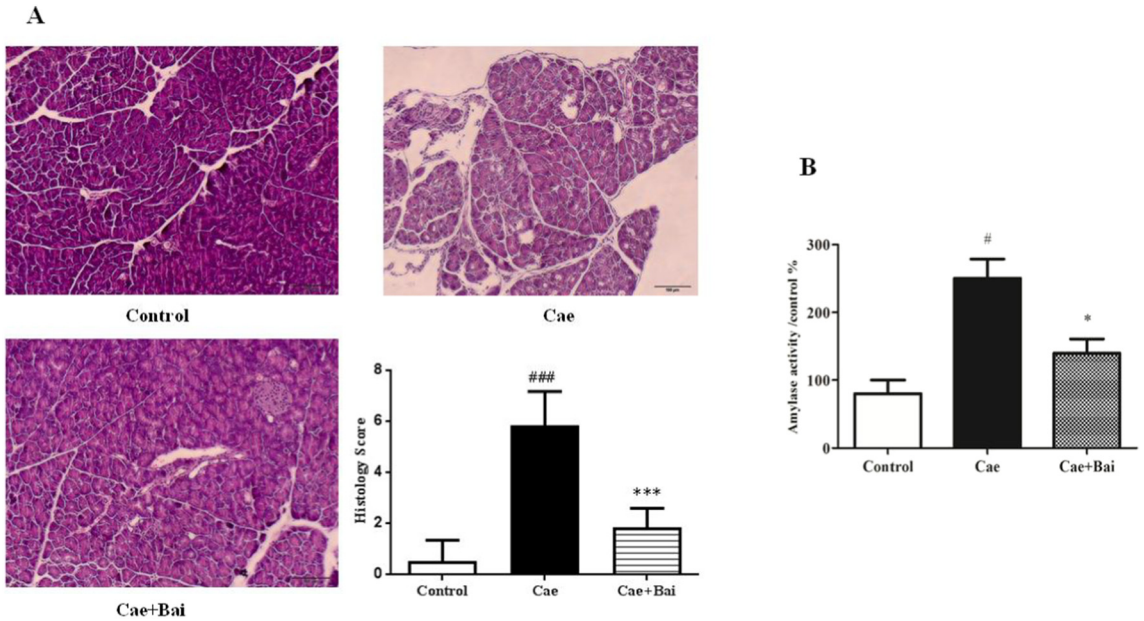
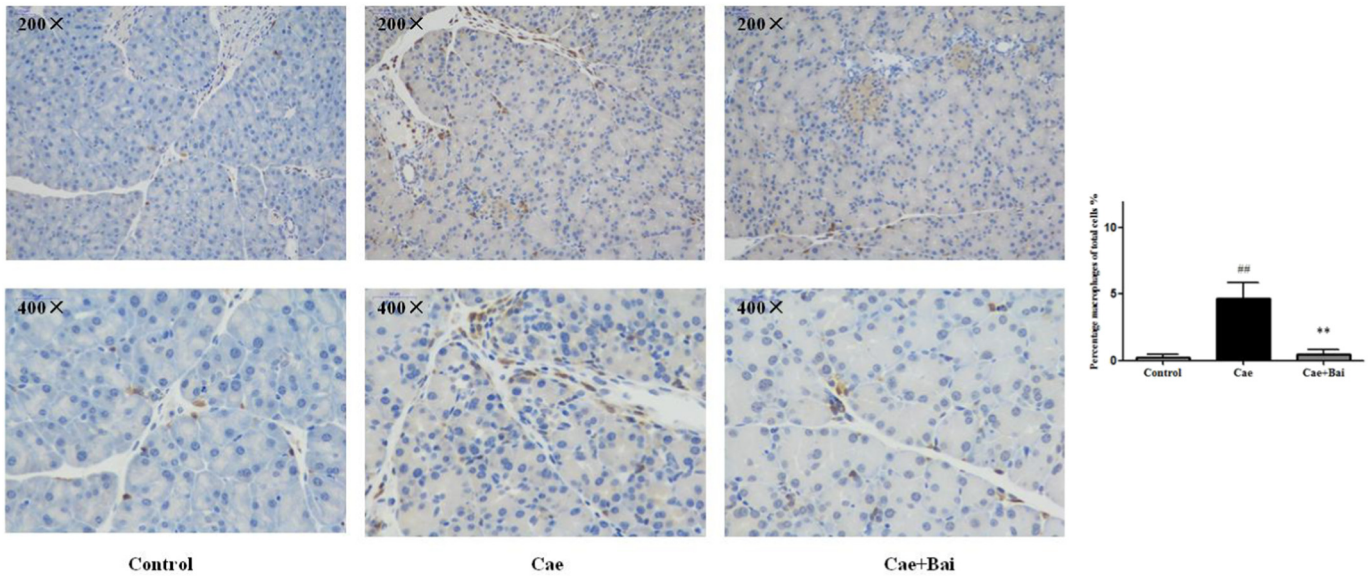
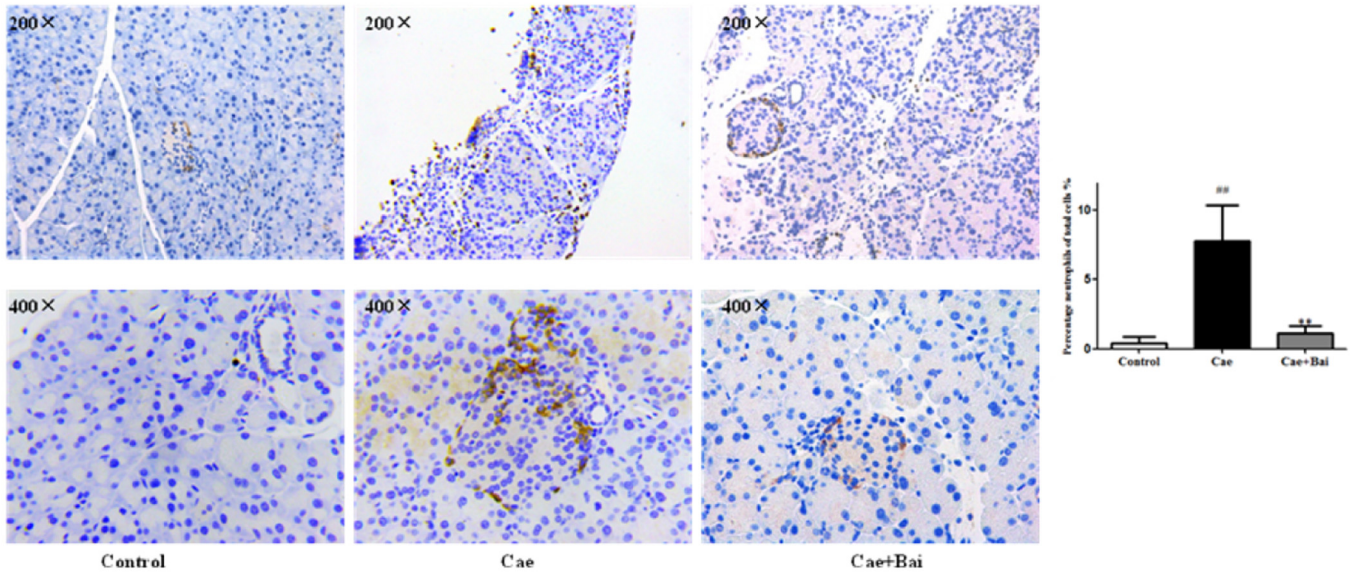



**Fig. 2.** Effects of baicalein on pancreatic injury induced by caerulein. (A) Pathological changes and the histology score of the pancreas; (B) activity of serum α-amylase. $^{\#}p < 0.05$ *vs.* control, $^{\#\#\#}p < 0.001$ *vs.* control; $^{*}p < 0.05$ *vs.* Cae, $^{**}p < 0.01$ *vs.* Cae.




(A)




(B)

**Fig. 3.** Effects of baicalein on infiltration of immune cells by immunohistochemical staining. (A) The infiltration of macrophages (F4/80 positive) and the quantitative analysis; (B) The infiltration of macrophages (Ly6G positive) and the quantitative analysis. $^{\#\#}p < 0.05$ *vs.* control; $^{**}p < 0.01$ *vs.* Cae.

levels of 40 inflammatory mediators in CM. LPS induced changes in secretion of 11 inflammatory mediators of macrophage, and baicalein could inhibit the secretion of IL-6, IL-10, IL-27 and TNFα (Fig. 5).

### *3.4. Baicalein inhibited the activation of MAPK, NF-κB and STAT 3 signaling pathways*

To further explore the mechanism of baicalein during the pancreatic inflammatory response, we examined the protein expression of three important signal pathways that often correlate with inflammation. Caerulein increased the degradation of IκBα and phosphorylation of NF-κB subunit p65 in the NF-κB signal pathway, the levels of p38, ERK and JNK phosphorylation in the MAPK signal pathway, and JAK and STAT 3 phosphorylation in the STAT 3 signal pathway compared to control samples (Fig. 6A), indicating that the tested three signaling pathways were activated. In contrast, baicalein co-treatment inhibited the degradation of IκBα and up-regulation of phosphorylation of p65, p38, ERK, JAK, STAT 3 induced by caerulein. The similar results were observed *in vitro* (Fig. 6B). Briefly, CM induced the activation of NF-κB, MAPK, and STAT 3 signaling pathway of pancreatic acinar cells which was inhibited by baicalein.

## 4. Discussion

AP is a common acute disease caused by abnormal activation of pancreatic enzymes and the subsequent local or systemic inflammatory

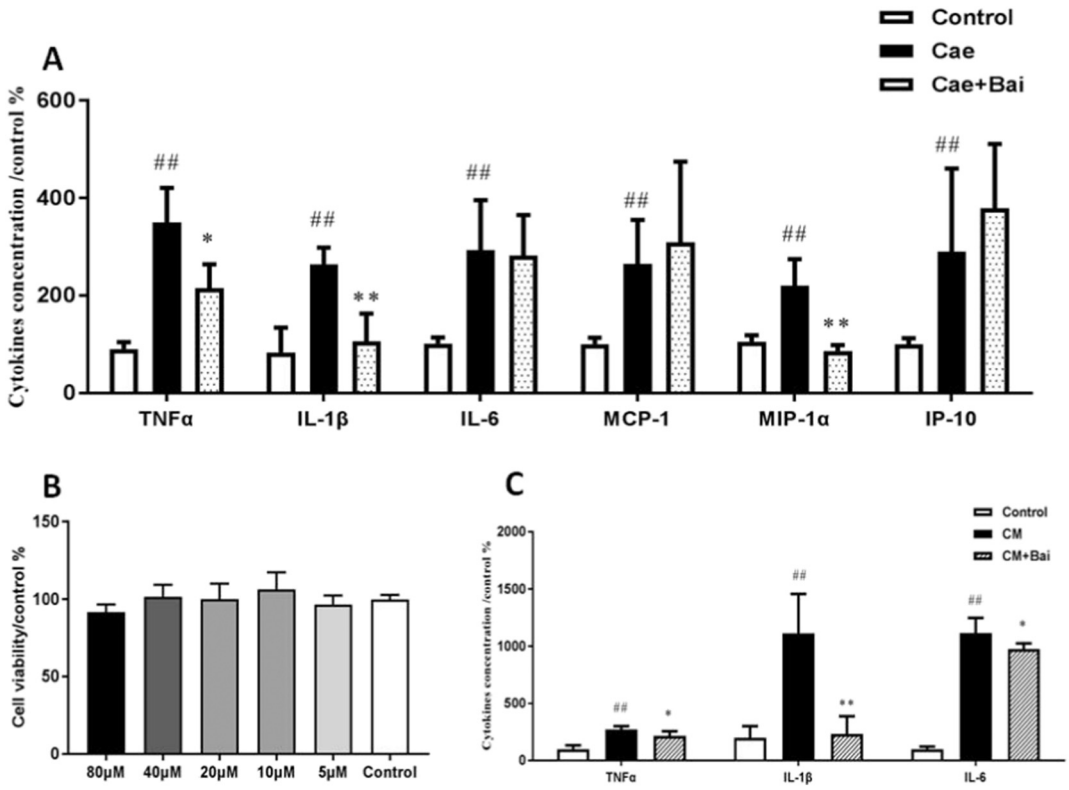
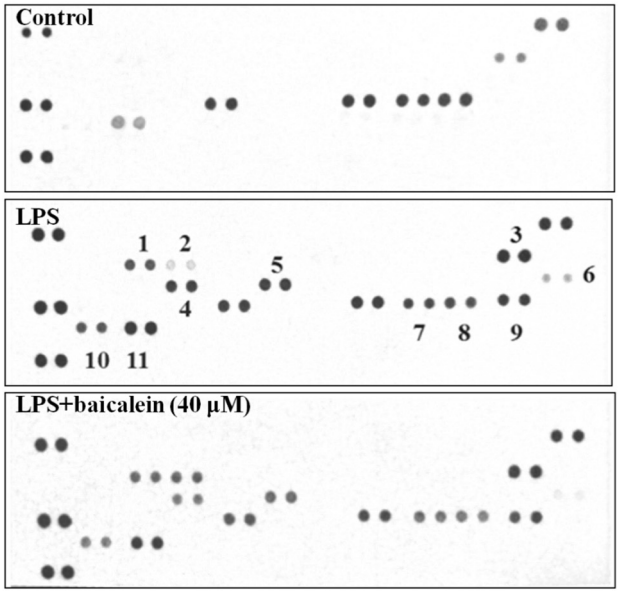



**Fig. 4.** Baicalein inhibited the cytokines expression of pancreas. (A) Expression levels of pro-inflammatory cytokines and chemokines in pancreas; (B) cell viability of isolated pancreatic acinar cells treated with different concentrations of baicalein; (C) expression levels of pro-inflammatory cytokines in isolated pancreatic acinar cells. $^{\#\#}p < 0.01$ *vs.* control; $^{*}p < 0.05$ *vs.* Cae, $^{**}p < 0.01$ *vs.* Cae.




| Number | Target |
|---|---|
| 1 | G-CSF |
| 2 | GM-CSF |
| 3 | IL-1ra |
| 4 | **IL-6** |
| 5 | **IL-10** |
| 6 | **IL-27** |
| 7 | MIP-1β |
| 8 | MIP-2 |
| 9 | RANTES |
| 10 | TIMP-1 |
| 11 | **TNF-α** |

**Fig. 5.** Cytokines array of the culture medium of LPS treated RAW 264.7 cells with or without baicalein.

response [11]. The severity of AP is largely attributed to the immune cells and a variety of inflammatory mediators. Macrophages play a vital role in the progression of pancreatitis by enhancing local damage or inducing systemic complications. In the early stage of pancreatic injury, several enzymes and inflammatory mediators are released from the damaged acinar cells; these activate immune cells, especially the monocyte-macrophage system, which in turn produces a series of cytokines and chemokines to induce the inflammatory cascade [12–14]. Clinical and experimental research has demonstrated that the severity of AP correlates to the number of macrophages mobilized to the bloodstream and the activation state of macrophages [15–17]. Furthermore, depletion of macrophages was reported to attenuate pancreatic inflammation and associated damage in C57BL/6 mice [18]. The damaged pancreatic cells and the activated macrophages secrete a variety of inflammatory mediators. Accumulating research has demonstrated that the release of endogenous inflammatory mediators (mainly TNFα, IL-1β, IL-8, and IL-6) from the damaged pancreas is an early event of AP, and pro-inflammatory cytokines and oxidative stress

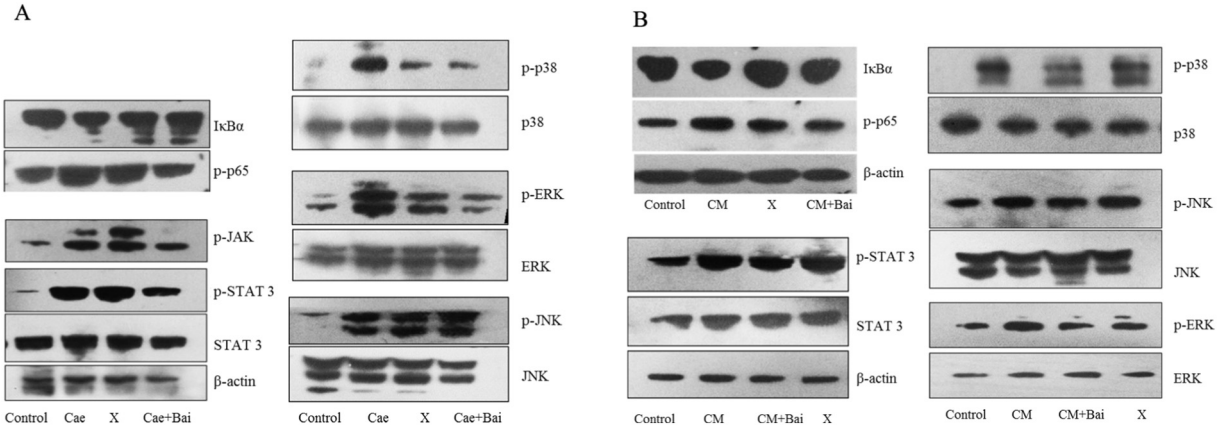



**Fig. 6.** Protein levels of IκB, phosphorylated p65, p38, ERK, JNK, STAT 3 and JAK in pancreatic tissues (A) and isolated pancreatic acinar cells (B) as determined by Western blot analysis. (Note: X was another compound irrelevant to the paper.)

contribute to pathogenesis of pancreatitis and the subsequent inflammatory response [19]. In a previous study, four proinflammatory cytokines (IL-6, IL-8, IL-18, and TNFα) were monitored in AP patients with organ failures. It was found that all of the cytokines were particularly elevated and that IL-6 and IL-8 could be markers for organ failures in AP [20]. The results obtained in this study are in accordance with previous reports indicating that baicalein could attenuate the caerulein-induced pancreatic damage, inhibit the infiltration of macrophage and the secretion of inflammatory mediators *in vivo* and *in vitro* [9]. Furthermore, baicalein could decrease the level of MIP-1α *in vivo* in addition to decreased levels of TNFα and IL-1β secretion, which confirmed that baicalein could inhibit the infiltration of macrophages as MIP-1α is a relevant chemokine for this process. The contradictory results regarding the effects of baicalein on IL-6 expression *in vivo* and *in vitro* need further investigations.

During AP, the abnormal activation of pancreatic enzymes leads to auto digestion, causing the activation of inflammatory signaling pathways and transcription factors, including NF-κB, STAT 3, and activator protein 1 (AP-1). NF-κB, a common early transcription factor which regulates immune responses, is activated rapidly during AP and induces the expression of cytokines (such as TNF, IL-6, IL-1β) and chemokines (including MCP-1 and MIP-1α). Accumulating evidence suggests that NF-κB activation is an early and central event in the progression of AP [21]. The activation of AP-1 is mainly related to mitogen-activated protein kinases (MAPKs), a family of signal transduction proteins including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinases (JNK), and p38. Pro-inflammatory cytokines and other stimuli during progress of AP induce the MAPK signaling cascades and regulate immune response [22]. Clinical research demonstrated that the phosphorylation levels of p38 MAPK are significantly increased in peripheral blood mononuclear cells (PBMCs) from patients with acute pancreatitis and are related to the severity of AP [23]. Meanwhile, the p38 MAPK signaling pathway is involved in the NF-κB activation by phosphorylating MK2 (a downstream protein kinase of p38) in the progression of AP [24]. The Janus kinase/signal transducers and activators of transcription 3 (JAK/STAT 3) is a critical inflammatory signaling pathway that mediates inflammatory responses in AP progress. JAK2/STAT 3 can be activated by many inflammatory factors including IL-6 and trypsinogen activation peptide (TAP), which leads to aggravated AP [25,26]. In agreement with these findings, our studies provide evidence that the NF-κB, MAPK and STAT 3 pathways are activated in the pancreatic tissues of caerulein induced AP mice and in isolated pancreatic acinar cells treated with conditional medium of classically activated macrophages.

Baicalein is one of the active components of *Scutellaria baicalensis* Georgi, a common herb used in treatment of pancreatitis in Chinese medicine practice. Baicalein has been reported to be effective against various inflammatory diseases including pancreatitis, diabetes, cardiovascular diseases, and inflammatory bowel diseases with almost no toxicity [6]. Xi-ping Zhang, Jun Li and colleagues investigated the effects of baicalein in a severe acute pancreatitis (SAP) rat model (induced by 5% sodium taurocholate) and in isolated pancreatic acinar cells *in vitro*. Their results demonstrated that baicalein could attenuate pancreatic injury and improved survival by inhibiting the expression of pro-inflammatory cytokines (IL-6 and TNFα) [9,27,28]. Furthermore, our previous research indicated that baicalein can also inhibit the expression of various inflammatory mediators in LPS induced macrophages (unpublished) and in inflammatory acinar-to-ductal metaplasia of pancreatic acinar cells through improving the inflammatory microenvironment [29]. In this study, baicalein also attenuated caerulein-induced pancreatic injury and macrophage infiltration through inhibiting the phosphorylation of NF-κB p65, JAK2, STAT 3, p38 and ERK1/2 *in vivo* and *in vitro*, suggesting that the underlying mechanism of the baicalein anti-inflammatory activities includes the inhibition of the production of key inflammatory mediators through NF-κB, JAK2/STAT 3 and MAPK signaling pathways.

In conclusion, these data indicate that baicalein significantly inhibits the inflammatory response of the pancreas and attenuates the caerulein-induced pancreatitis in mice by dampening the various inflammatory signaling pathways. However, the specific molecular mechanisms of baicalein on pancreatitis need to be further elucidated. This study provides experimental evidence on the clinical application of *Scutellaria baicalensis* Georgi or baicalein and indicates that baicalein may be a candidate for treatment of AP patients in the future.

## Conflicts of interest

The authors declare no conflicts of interest.

## Acknowledgment

The authors appreciate Mrs. Hong SHI for her excellent technical support and thank Prof. Matthias BUREIK for the kind linguistic editing. This work was supported by Science & Technology Development Fund of Tianjin Education Commission for Higher Education (Grant No. 2018KJ005); National Natural Science Foundation of China (81630106, 81673826); Natural Science Foundation Tibetan Autonomous Region of China (XZ2017ZRG-67(Z)); Sino Swiss Science and Technology Cooperation Programme (EG 05-032015); Tianjin University of Traditional Chinese Medicine Foundation (to SLK).

## References

[1] S.J. Pandol, et al., Acute pancreatitis: bench to the bedside, Gastroenterology 132

(3) (2007) 1127–1151, https://doi.org/10.1053/j.gastro.2007.01.055.
[2] A.Y. Hammad, M. Ditillo, L. Castanon, Pancreatitis, Surg. Clin. 98 (5) (2018) 895–913 https://doi.org/*Pancreatitis.* Surgical Clinics.
[3] J.-L. Frossard, M.L. Steer, C.M. Pastor, Acute pancreatitis, Lancet (Lond. Engl.) 371 (9607) (2008) 143–152, https://doi.org/10.1016/S0140-6736(08)60107-5.
[4] H. Xiang, et al., Chinese herbal medicines attenuate acute pancreatitis: pharmacological activities and mechanisms, Front. Pharmacol. 8 (2017) 216, https://doi.org/10.3389/fphar.2017.00216.
[5] Q. Wang, et al., Chinese herbal medicines for acute pancreatitis, Cochrane Database Syst. Rev. 1 (2005), https://doi.org/10.1002/14651858.CD003631.pub2.
[6] B. Dinda, et al., Therapeutic potentials of baicalin and its aglycone, baicalein against inflammatory disorders, Eur. J. Med. Chem. 131 (2017) 68–80, https://doi.org/10.1016/j.ejmech.2017.03.004.
[7] M. Li-Weber, New therapeutic aspects of flavones: the anticancer properties of Scutellaria and its main active constituents Wogonin, Baicalein and Baicalin, Cancer Treat. Rev. 35 (1) (2009) 57–68, https://doi.org/10.1016/j.ctrv.2008.09.005.
[8] P. Shamamian, et al., Pathophysiology of acute pancreatitis, in: L.A.L. Baert, G.M. Knauth, H.K. Sartor (Eds.), Medical Radiology (Diagnostic Imaging), Springer, Berlin, Heidelberg, 2009, pp. 3–13, , https://doi.org/10.1007/978-3-540-68251-6_1.
[9] J. Li, et al., Baicalein protect pancreatic injury in rats with severe acute pancreatitis by inhibiting pro-inflammatory cytokines expression, Biochem. Biophys. Res. Commun. 466 (4) (2015) 664–669, https://doi.org/10.1016/j.bbrc.2015.09.094.
[10] H. Spormann, A. Sokolowski, G. Letko, Experimental acute pancreatitis–a quantification of dynamics at enzymic and histomorphologic levels, Pathol. Res. Pract. 185 (3) (1989) 358–362, https://doi.org/10.1016/S0344-0338(89)80013-5.
[11] F. Basile, et al., Pancreatitis, in: A. Crucitti (Ed.), Surgical Management of Elderly Patients, Springer International Publishing, Cham, 2018, pp. 327–334, , https://doi.org/10.1007/978-3-319-60861-7_22.
[12] X. Wang, et al., Gut origin Sepsis, macrophage function, and oxygen extraction associated with acute pancreatitis in the rat, World J. Surg. 20 (3) (1996) 299–308, https://doi.org/10.1007/s002689900048.
[13] J. Yang, et al., The physiologic consequences of macrophage pacification during severe acute pancreatitis, Shock 10 (3) (1998) 169–175, https://doi.org/10.1097/00024382-199809000-00004.
[14] L.K. Sun, et al., Prostaglandin E2 modulates TNF-alpha-induced MCP-1 synthesis in pancreatic acinar cells in a PKA-dependent manner, 293 (6) (2007) G1196, https://doi.org/10.1152/ajpgi.00330.2007.
[15] S. Merilainen, et al., Portal vein cytokines in the early phase of acute experimental oedematous and necrotizing porcine pancreatitis, Scand. J. Gastroenterol. 47 (11) (2012) 1375–1385, https://doi.org/10.3109/00365521.2012.722675.
[16] C. McKay, C.W. Imrie, J.N. Baxter, Mononuclear phagocyte activation and acute pancreatitis, Scand. J. Gastroenterol. Suppl. 219 (1996) 32–36, https://doi.org/10.3109/00365529609104997.
[17] A. Dabrowski, et al., Monocyte subsets and natural killer cells in acute pancreatitis, Pancreatology 8 (2) (2008) 126–134, https://doi.org/10.1159/000123605.
[18] A.L. Shifrin, et al., Macrophage ablation attenuates adenoviral vector-induced pancreatitis, Surgery 137 (5) (2005) 545–551, https://doi.org/10.1016/j.surg.2005.01.004.
[19] S. Chooklin, O. Usach, Proinflammatory cytokines and splanchnic blood flow in acute pancreatitis, Cytokine 63 (3) (2013) 254, https://doi.org/10.1016/j.cyto.2013.06.050.
[20] M.L. Malmstrøm, et al., Cytokines and organ failure in acute pancreatitis: inflammatory response in acute pancreatitis, Pancreas 41 (2) (2012) 271–277, https://doi.org/10.1097/MPA.0b013e3182240552.
[21] A. Jakampudi, et al., NF-κB in Acute Pancreatitis: Mechanisms and Therapeutic Potential, 16 (2016), https://doi.org/10.1016/j.pan.2016.05.001.
[22] M.M. Murr, et al., Regulation of Kupffer cell TNF gene expression during experimental acute pancreatitis: the role of p38-MAPK, ERK1/2, SAPK/JNK, and NF-kappaB, J. Gastrointest. Surg. 7 (1) (2003) 20–25, https://doi.org/10.1016/S1091-255X(02)00053-7.
[23] L. Gui-fen, G. Ji-qiang, Character of p38 MAPK signal pathway in peripheral blood mononuclear cells of patients with acute pancreatitis, J. Jiangsu Univ. (Med. Ed.) 22 (1) (2012) 51–54.
[24] C. Li-jia, et al., Effect of Ang 1-7 on the p38 MAPK/NF-κB signaling pathway in caerulein- stimulated pancreatic acinar AR42J cell, J. Clin. Exp. Med. 13 (24) (2014) 2085–2089.
[25] K.D. Ju, et al., Potential role of NADPH oxidase-mediated activation of Jak2/STAT 3 and mitogen-activated protein kinases and expression of TGF-β1 in the pathophysiology of acute pancreatitis, Inflamm. Res. 60 (8) (2011) 791–800, https://doi.org/10.1007/s00011-011-0335-4.
[26] L. Jin, The Negative Regulation of SOCS-3 in JAK/STAT Signal Transduction Pathway Induced by TAP in Rat Pancreatic Acinar Cells, Zunyi Medical University, Zunyi, 2014.
[27] X.P. Zhang, et al., Effects of emodin and baicalein on rats with severe acute pancreatitis, World J. Gastroenterol. 11 (14) (2005) 2095–2100, https://doi.org/10.1111/j.1365-2036.2005.02408.x.
[28] J. Li, et al., Emodin and baicalein inhibit sodium taurocholate-induced vacuole formation in pancreatic acinar cells, World J. Gastroenterol. 24 (1) (2018) 35–45, https://doi.org/10.3748/wjg.v24.i1.35.
[29] W.L. Pu, et al., Baicalein inhibits acinar-to-ductal metaplasia of pancreatic acinal cell AR42J via improving the inflammatory microenvironment, J. Cell. Physiol. 233 (8) (2018) 5747–5755, https://doi.org/10.1002/jcp.26293.