

## Modified Da-chai-hu Decoction regulates the expression of occludin and NF- $\kappa$ B to alleviate organ injury in severe acute pancreatitis rats

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**[ABSTRACT]** Modified Da-chai-hu Decoction (MDD), a traditional Chinese medicinal formulation, which was empirically generated from Da-chai-hu decoction, has been utilized to treat severe acute pancreatitis (SAP) for decades. The aim of the present study was to explore its potential organprotective mechanism in SAP. In the present study, rat SAP model was induced by retrograde injection of 3.5% sodium taurocholate into the biliopancreatic duct, MDD (23.35 g/kg body weight, twelve times the clinical dose) were orally given at 2 h before and 10 h after injection. At 12 h after model induction, blood was taken from vena cava for analysis of amylase, diamine oxidase (DAO), pulmonary surfactant protein-A (SP-A), and C-reactive protein (CRP). Histopathological change of pancreas, ileum and lung was assayed by H&E staining, myeloperoxidase (MPO) activity were determined using colorimetric assay, and the expressions of occludin and nuclear factor- $\kappa$ B (NF- $\kappa$ B) were detected by real-time RT-PCR and western blot, respectively. In addition, the tissue concentrations of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and monocyte chemoattractant protein-1 (MCP-1) were measured by enzyme-linked immunosorbent assay (ELISA). The results showed that in SAP rats, MDD significantly alleviated histopathological damage, depressed the MPO activity and the concentrations of TNF- $\alpha$ , IL-1 $\beta$ , and MCP-1 of pancreas, ileum and lung, and reduced the serum levels of amylase [(3283.4  $\pm$  585.5) U·L<sup>-1</sup> vs (5626.4  $\pm$  795.1) U·L<sup>-1</sup>], DAO [(1100.1  $\pm$  334.3) U·L<sup>-1</sup> vs (1666.4  $\pm$  525.3) U·L<sup>-1</sup>] and CRP [(7.6  $\pm$  1.2)  $\mu$ g·mL<sup>-1</sup> vs (17.8  $\pm$  3.8)  $\mu$ g·mL<sup>-1</sup>]. However, the serum SP-A concentration [(106.1  $\pm$  16.6) pg·mL<sup>-1</sup> vs (90.1  $\pm$  14.9) pg·mL<sup>-1</sup>] was elevated when treated SAP rats with MDD. Furthermore, MDD increased the occludin expression and reduced the NF- $\kappa$ B expression in pancreas, ileum and lung of SAP rats. Our findings suggested that MDD administration was an effective therapeutic approach for SAP treatment. It could up-regulate occludin expression to protect intercellular tight junction and down-regulate NF- $\kappa$ B expression to inhibit inflammatory reaction of pancreas, ileum and lung.

**[KEY WORDS]** Severe acute pancreatitis; Modified Da-chai-hu Decoction; Occludin; Nuclear factor- $\kappa$ B; Ileum; Lung

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### Introduction

Severe acute pancreatitis (SAP) is an acute intra-abdominal

disease which can lead to systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS). It is widely accepted that uncontrolled systemic inflammation is the most important event in the pathogenesis of SAP, which promotes the development of MODS<sup>[1-2]</sup>. At the early stage of SAP, pathogenic factors destroys the intercellular junction of acinus and causes cell injury, which provides an opportunity for the leakage of pancreatic enzyme and activates monocytes/macrophages, resulting in releases of pro-inflammatory cytokines, chemokines, and adhesion factors. Large quantities of inflammatory mediators further cause

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distant organs damage, in which intestinal and lung are the most common involved organs<sup>[3-4]</sup>.

Tight junctions, the primary apical structures in epithelium and endothelium, play an important roles in barrier function by forming cell-to-cell contacts and sealing paracellular pathway. Occludin is a transmembrane protein which provides most of the barrier function of the tight junction<sup>[5]</sup>. Previous studies have demonstrated that occludin showed a linear staining pattern delineating the apical membranes of normal ductal epithelium and of acinar cells under confocal laser scanning microscopy. Disruption of tight junctions resulting in leakage of amylase and lipase is an early event in acute caerulein-pancreatitis, which could increase production of proinflammatory cytokines<sup>[6]</sup>; cytokines further disrupt pancreatic epithelial barrier *via* expressional downregulation of key structural components of tight junctions<sup>[7]</sup>. It is more likely to aggravate the severity of SAP and resulting MODS. Others have reported that occludin was expressed in intestinal epithelia and pulmonary epithelia, impairment of intestinal barrier function and alveolar barrier function are both associated with the downregulation and localization shift of occludin<sup>[8-9]</sup>.

The molecular mechanisms involved in the pathogenesis of SAP include several signaling pathways and transcription factors, in which nuclear factor- $\kappa$ B (NF- $\kappa$ B) is the most striking one<sup>[10]</sup>. NF- $\kappa$ B is a collective name of transcriptional proteins which belongs to the Rel family and consists of RelA (also known as p65), RelB and c-Rel. These proteins are synthesized in their mature forms and contain transcription-modulating domains. Activated NF- $\kappa$ B induces the transcription of many genes including genes coding tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ) and monocyte chemoattractant protein-1 (MCP-1), which accelerate systemic inflammatory response and aggravate organ damage<sup>[11]</sup>. Therefore, targeted suppression of NF- $\kappa$ B has been considered as effective therapeutic strategy in acute inflammatory diseases<sup>[12-13]</sup>.

Modified Da-chai-hu Decoction (MDD) was empirically generated from Da-chai-hu decoction, which were recorded in the classical literature of *Treatise on Febrile Diseases*. MDD, also known as compound Da-chai-hu Decoction or Qing-yi Decoction, has been used as an effective treatment of SAP in clinical practice for nearly a half century<sup>[14]</sup>, but the underlying mechanism is still not fully elucidated. Several experimental studies have demonstrated that it could reduce the pancreatic expression of secreted phospholipase A2 and protect intestinal mucosa barrier<sup>[15-16]</sup>. In present study, the protective effects of MDD on multi-organ and the underlying mechanisms were investigated in SAP rats.

## Materials and Methods

### Animals

Specific Pathogen Free male Wistar rats weighing 190–210 g were provided by the Beijing Vital River Laboratory Animal Technology Co., Ltd., Beijing, China [SCXK(jing)

2016-0006]. The animals were acclimatized to laboratory environment for a week prior to start study and were kept in standard environmental conditions (23–25 °C, 12 h/12 h light/dark cycle) with a standard chow and free access to water. All experimental procedures were identical to those recommended in the Guidelines for Laboratory Animal Care and Use from the Chinese Ministry of Science and Technology and were approved by the Animal Ethical and Welfare Committee (AEWC) of Tianjin Medical University.

### Preparation of MDD

MDD mainly includes six kinds of traditional Chinese medicine: Radix Bupleuri, Radix Scutellariae, rhubarb, Fructus Aurantii Immaturus, Pinellia Ternate and Paeonia lactiflora. The mixed proportion of respective medicine in MDD is 4 : 3 : 4 : 3 : 2 : 3. These raw medicines were prepared by boiling water extraction, decompression, concentration, distillation filtration and then drying granulation. All the mentioned medicine names can be checked in *Pharmacopoeia of People's Republic of China* (2015 edition, Chinese medical science and Technology Press), and were obtained from Anguo Longlian Chinese Herbal Medicine Co., Ltd. (Hebei Province, China).

### Experimental grouping and processing

After a 7-day acclimation, thirty-four rats were randomly divided into 3 groups, namely sham ( $n = 10$ ), SAP ( $n = 12$ ), and SAP + MDD group ( $n = 12$ ). Rat SAP model was induced by retrograde injection of 3.5% sodium taurocholate into the biliopancreatic duct, and the sham operation group was injected only with saline in the same way. Rats in the SAP + MDD group were orally given with MDD (1.868 g·mL<sup>-1</sup>, 12.5 mL/kg body weight) at 2 hours before and 10 hours after the induction of SAP. All rats were anaesthetized at 12 hours after model induction, blood was taken from vena cava for analysis of amylase, diamine oxidase (DAO), pulmonary surfactant protein-A (SP-A), and C-reactive protein (CRP). The tissues of lung, ileum and pancreas were harvested individually for the observation of histopathological changes and the determination of myeloperoxidase (MPO) activity. In addition, the expression of NF- $\kappa$ B and occludin were determined by western blot and real-time RT-PCR.

### Induction of SAP

Rats were fasted but had free access to water starting at 12 h before the surgery. SAP model was induced consistent with previous literature<sup>[17]</sup>. Skin was prepared and disinfected after anesthesia, a midline incision was made to open the abdominal cavity. The pancreas was exposed, and the common bile duct was occluded with a small bulldog clamp in the hepatoduodenal ligament. The biliopancreatic duct was cannulated through mammary papilla from the anterior wall of the duodenum and 3.5% solution of sodium taurocholate (Sigma, St.Louis, MO, USA) was injected with an even speed of 0.15 mL·min<sup>-1</sup> (1.0 mL/kg body weight) using syringe pump (LP240, Lifepum, Beijing, China). Then, the atraumatic bulldog clamp was removed in 5 minutes after injection. The

abdominal incision were finally closed in two layers. All procedures were performed with sterile technique.

#### *Histologic examination and grading*

Tissue samples of lung, ileum, and pancreas were embedded in paraffin and cut in 3  $\mu\text{m}$ -thick sections. After de-paraffinization, sections were stained with hematoxylin and eosin (H&E) stain and examined. The specimens were scored by two investigators in a blinded manner according to the criteria described previously<sup>[17-19]</sup>.

#### *Determination of the MPO activity*

The samples of lung, ileum and pancreas were homogenized in an extraction buffer and MPO activity were determined in accordance with the manufacturer's instruction (Jiancheng Bioengineering Institute of Nanjing, Jiangsu, China). The protein concentrations of the corresponding tissues were measured and the MPO activity values were normalized with protein contents.

#### *Analysis of serum amylase, DAO, SP-A and CRP*

Serum amylase activity and CRP level were measured with automatic biochemical analyzer. DAO activity and SP-A concentration were determined respectively using ELISA kit (Sangon Biotech, Shanghai, China and R&D, Systems Minneapolis, MN, USA).

#### *Measurement of the levels of Inflammatory factors*

The levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1) and monocyte chemoattractant protein-1 (MCP-1) in homogenates of lung, ileum and pancreas were measured by enzyme-linked immunosorbent assay (ELISA) kits (R&D, Systems Minneapolis, MN, USA) according to the manufacturer. The values were normalized with protein contents.

#### *Western blot analysis*

Total protein were extracted by using radio-Immunoprecipitation assay (RIPA) and the concentrations were determined by the BCA protein assay kit. Equal amounts of protein (20  $\mu\text{g}$ ) were separated using 10 % SDS-polyacrylamide gel electrophoresis and following electroblotted onto a polyvinylidene fluoride (PVDF) membrane (Millipore, USA). The membrane was blocked with 5 % fat-free milk in Tris-buffered saline (TBS) and the blot was probed using a primary antibody against NF- $\kappa\text{B}$  (Cell Signaling Technology, USA), occludin and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Abcam Inc., USA, USA). Eventually, the proteins were visualized by utilization of an enhanced chemiluminescence kit (Millipore, USA) and normalized to the expression level of GAPDH.

#### *Real-time quantitative PCR*

Total RNA was isolated respectively from the homogenization of lung, ileum, and pancreas using SV Total RNA Isolation System (Promega, USA) subsequently 1.0  $\mu\text{g}$  of total RNA was reverse-transcribed into cDNA with Reverse Transcription System (Promega, USA). PCR amplification was performed on iCycler iQ<sup>TM</sup> Multicolor Real-Time PCR Detection System (Bio-Rad, USA) using real-time Master Mix (Tiangen Biotech, China). The relative levels of mRNA were normalized to GAPDH. The following primers were used for

PCR: NF- $\kappa\text{B}$ : Forward 5'-ATGCCAACGCCCTCTT-CGACT-3', Reverse 5'-ACACTGTCCCCGTTCTCATCC-3'; occludin: Forward 5'-AGTACATGGCTGCTGCTGATG-3', Reverse 5'-CCGACCATCGTCTTGATGTGT-3'; GAPDH: Forward 5'-TTCAACGGCACAGTCAAG-3', Reverse 5'-CC-ACGACATACTCAGCAC-3'.

#### *Statistical Analysis*

All quantitative values were depicted as means  $\pm$  standard deviation (SD) and were analyzed by one-way analysis of variance (ANOVA), with individual group means being compared with LSD multiple comparison test.  $P < 0.05$  was considered to be statistically significant. All the statistical calculations were performed in SPSS 17.0 software program.

## **Results**

### *MDD ameliorates histopathological injury of pancreas, ileum and lung*

None of the rats died until twelve hours after SAP induction in the sham group. However, There were 3 and 1 rats died in the SAP and SAP + MDD group, respectively. In the SAP group, extensive necrosis and intense hemorrhage in pancreatic tissues, mucous membrane exfoliated and exuviations of microvillus top in ileal tissues, and diffuse pulmonary capillary congestion and part of alveolar structural damage in lung tissue were found in SAP rats, but in SAP + MDD group, the histopathological injury were mitigated significantly compare to those of SAP group (Fig. 1A). The mean histopathologic scores of pancreas, ileum, and lung in SAP + MDD group were significantly decreased compared to those of SAP group (pancreas:  $5.4 \pm 1.2$  vs  $7.3 \pm 1.9$ , ileum:  $3.0 \pm 0.9$  vs  $4.7 \pm 0.8$  and lung:  $2.3 \pm 0.4$  vs  $3.3 \pm 0.8$ ) ( $P < 0.05$ , Fig. 1B).

### *MDD reduces MPO activity of pancreas, ileum and lung*

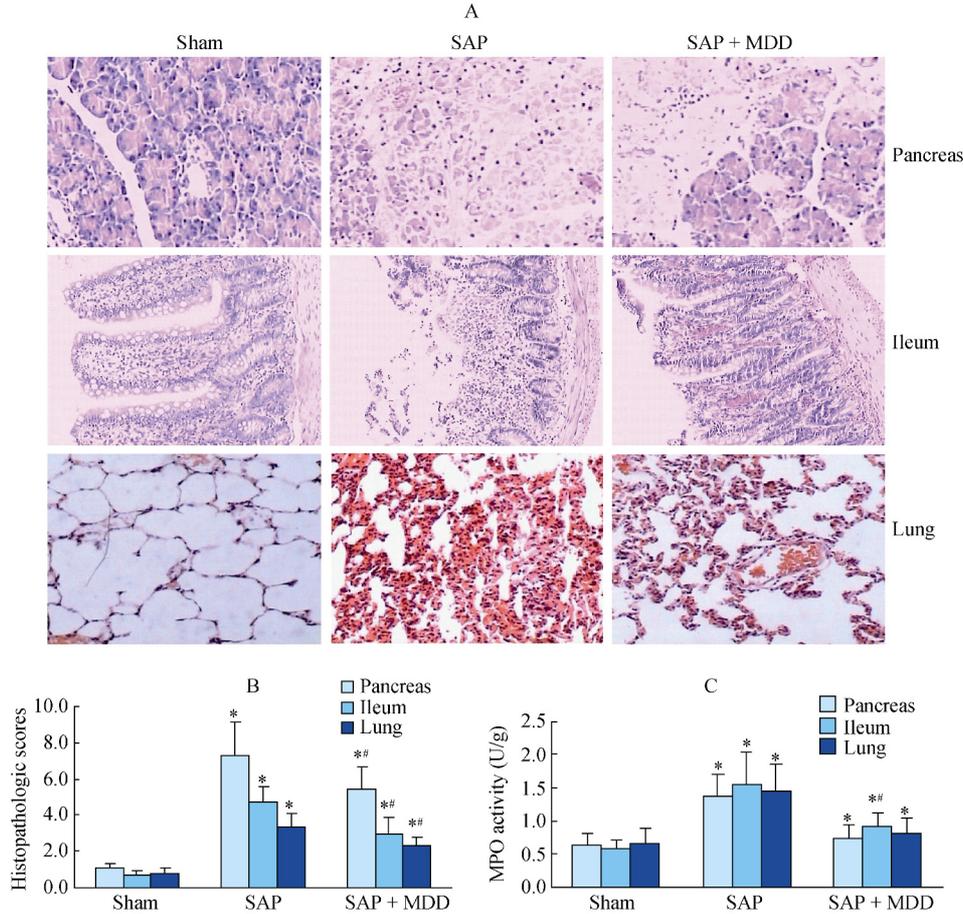
MPO is a major protein constituent of the primary granules of neutrophils, which represents the degree of neutrophils infiltration in tissue. Our present data showed that the MPO activity levels of pancreas, ileum, and lung were higher in SAP rats, administration of MDD could significantly decreased the MPO activity levels [pancreas:  $(1.4 \pm 0.3) \text{ U}\cdot\text{g}^{-1}$  vs  $(0.7 \pm 0.2) \text{ U}\cdot\text{g}^{-1}$ , ileum:  $(1.6 \pm 0.5) \text{ U}\cdot\text{g}^{-1}$  vs  $(0.9 \pm 0.2) \text{ U}\cdot\text{g}^{-1}$  and lung:  $(1.4 \pm 0.4) \text{ U}\cdot\text{g}^{-1}$  vs  $(0.8 \pm 0.2) \text{ U}\cdot\text{g}^{-1}$ ] ( $P < 0.05$ , Fig. 1C).

### *MDD reduces the activities of amylase and DAO, and raises SP-A concentration in serum*

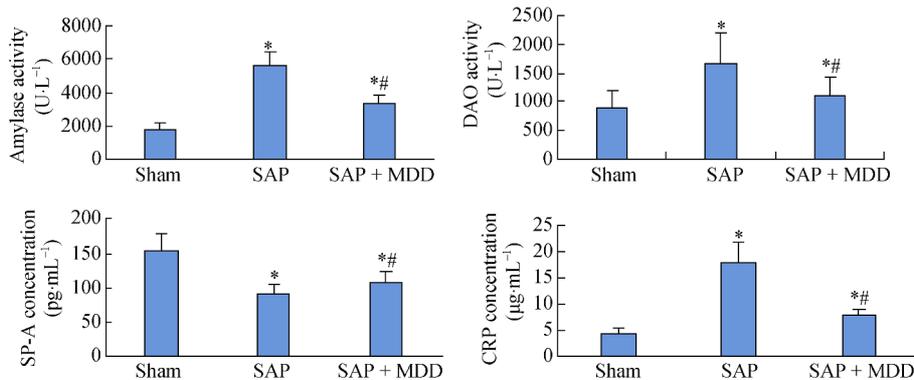
Amylase and DAO are marker enzymes of pancreatic acinar cells and intestinal epithelial cells, respectively. They are released into the blood when the pancreatic tissue and intestinal mucosal barrier are destroyed. SP-A is a glycoprotein secreted by alveolar type II epithelial cells, which can be used as an index for the integrity of alveolar structural. So we detected the activities of amylase and DAO, and the concentration of SP-A in serum. Twelve hours after SAP induction, the activities of amylase and DAO were increased, and the SP-A concentration were decreased in the SAP group. However, administration of MDD could significantly reduce the activities

of amylase [(3283.4 ± 585.5) U·L<sup>-1</sup> vs (5626.4 ± 795.1) U·L<sup>-1</sup>] and DAO [(1100.1 ± 334.3) U·L<sup>-1</sup> vs (1666.4 ± 525.3) U·L<sup>-1</sup>],

and raise the SP-A concentration [(106.1 ± 16.6) pg·mL<sup>-1</sup> vs (90.1 ± 14.9) pg·mL<sup>-1</sup>] in rats with SAP (Fig. 2).



**Fig. 1** Effects of MDD on the histopathological change and MPO activity of pancreas, ileum, and lung in SAP rats. **A:** Extensive necrosis and intense hemorrhage in pancreatic tissues, mucous membrane exfoliated and exuviations of microvillus top in ileal tissues, and diffuse pulmonary capillary congestion and part of alveolar structural damage in lung tissue were found in SAP group, but in SAP + MDD group, the histopathological injury were mitigated (H & E, × 400). **B:** The mean histopathologic scores of pancreas, ileum, and lung in SAP + MDD group were significantly decreased compared to those of SAP group. **C:** The MPO activities of pancreas, ileum and lung were significantly decreased in the SAP + MDD group than in the SAP group. \**P* < 0.05 vs the sham group, #*P* < 0.05 vs the SAP group. SAP: severe acute pancreatitis, MDD: modified Da-chai-hu Decoction



**Fig. 2** Effects of MDD on serum amylase, DAO, SP-A and CRP in SAP rats. Twelve hours after SAP induction, the serum activities of amylase and DAO, and CRP concentration were increased, the serum SP-A concentration was decreased, treated SAP rats with MDD decreased the activities of amylase and DAO, and CRP concentration, raised SP-A concentrations. \**P* < 0.05 vs the sham group, #*P* < 0.05 vs the SAP group. SAP: severe acute pancreatitis, MDD: modified Da-chai-hu Decoction, DAO: diamine oxidase, SP-A: pulmonary surfactant protein-A diamine oxidase, CRP: C-reaction protein

### MDD reduces serum CRP concentration

Increased serum CRP levels strongly predict inflammation aggravation. Twelve hours after SAP induction, we detected serum CRP concentration and found that the serum CRP level increased by three times in the SAP group. However, administration of MDD significantly reduced the serum CRP level [(7.6 ± 1.2) µg·mL<sup>-1</sup> vs (17.8 ± 3.8) µg·mL<sup>-1</sup>] (Fig. 2).

### MDD reduces the levels of TNF-α, IL-1β and MCP-1

Twelve hours after SAP induction, we evaluated the effect of MDD on the concentrations of proinflammatory cytokines and chemokines in tissue homogenate supernatants of pancreas, ileum and lung. As shown in table 1 and Fig. 3, the levels of TNF-α, IL-1β and MCP-1 in the SAP group were both higher, administration of MDD could significantly re-

duce the the levels of TNF-α, IL-1β and MCP-1.

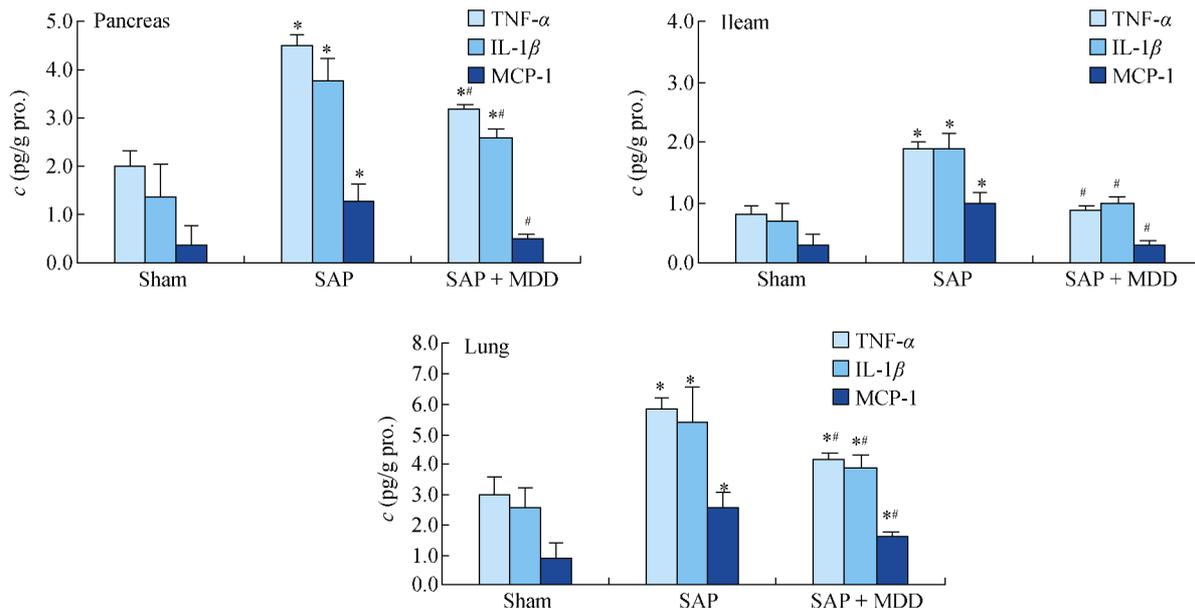
### MDD promotes occludin expression and inhibits NF-κB expression

To further explore the underlying mechanisms of MDD for the preservation of cells structural integrity and for the reduction of proinflammatory cytokines and chemokines, the expressions of occludin and NF-κB in pancreas, ileum and lung were detected using western blot and real-time quantitative PCR. As shown in Fig. 4 and Fig. 5, the mRNA and protein expressions of occludin expression were down-regulated, and the NF-κB were up-regulated in the SAP group; when treated SAP rats with MDD, the occludin expression were increased and the NF-κB expression were decreased.

**Table 1** MDD reduces the levels of TNF-α, IL-1β and MCP-1 of pancreas, ileum and lung (pg/g pro., mean ± SD)

Groups	Pancreas			Ileum			Lung		
	TNF-α	IL-1β	MCP-1	TNF-α	IL-1β	MCP-1	TNF-α	IL-1β	MCP-1
sham	2.0 ± 0.2	1.4 ± 0.2	0.4 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.3 ± 0.1	3.0 ± 0.6	2.6 ± 0.4	0.9 ± 0.2
SAP	4.5 ± 0.6*	3.8 ± 0.5*	1.3 ± 10.2*	1.9 ± 0.3*	1.9 ± 0.3*	1.0 ± 0.1*	5.8 ± 0.6*	5.4 ± 1.2*	2.6 ± 0.4*
SAP + MDD	3.2 ± 0.4*#	2.6 ± 0.4*#	0.5 ± 0.1#	0.9 ± 0.2#	1.0 ± 0.2#	0.3 ± 0.1#	4.2 ± 0.5*#	3.9 ± 0.5*#	1.6 ± 0.2*#

\*P < 0.05, vs the sham group; #P < 0.05, vs the SAP group



**Fig. 3** Effects of MDD on the levels of TNF-α, IL-1β and MCP-1 in pancreas, ileum and lung. Twelve hours after SAP induction, the levels of TNF-α, IL-1β and MCP-1 of pancreas, ileum and lung in the SAP group were higher. When compared with the SAP group, the levels of TNF-α, IL-1β and MCP-1 were lower in the SAP + MDD group. \*P < 0.05 vs the sham group, #P < 0.05 vs the SAP group. SAP: severe acute pancreatitis, MDD: modified Da-chai-hu Decoction, TNF-α: tumor necrosis factor-α, IL-1β: interleukin-1β, MCP-1: monocyte chemoattractant protein-1

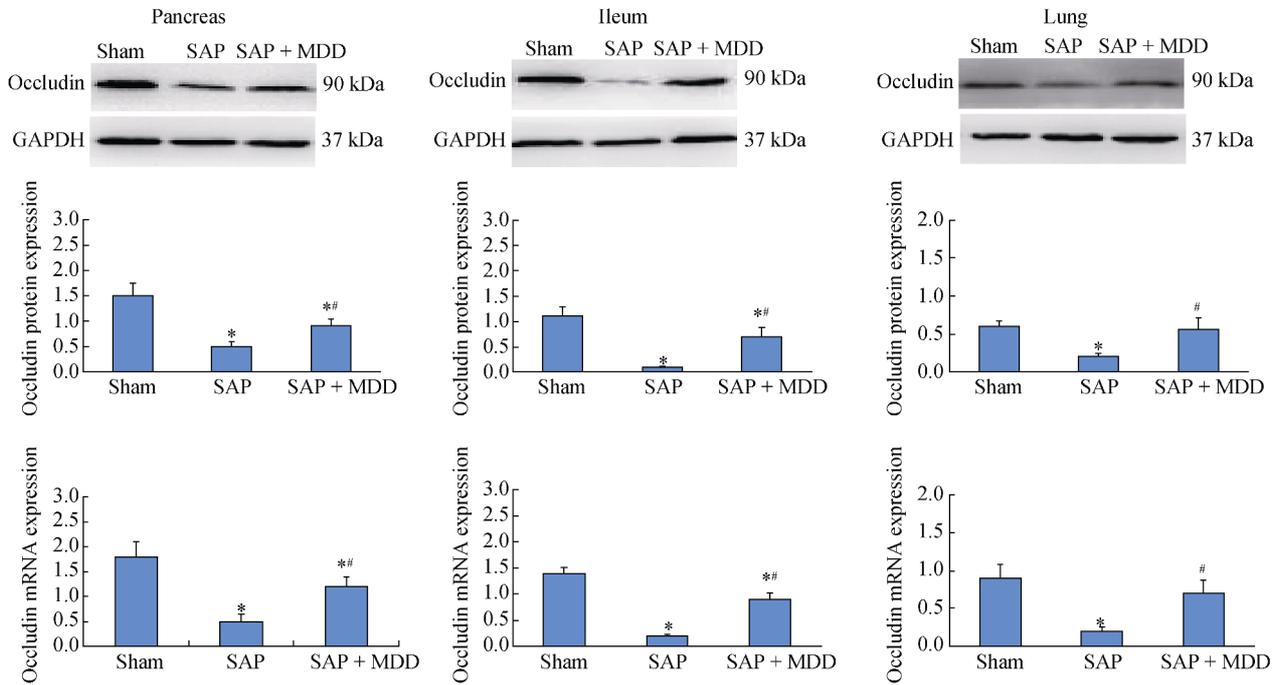
## Discussion

When SAP occurs, acinar cells were destroyed and leucocytes were excessively activated. A large number of enzymes and inflammatory mediators were released, leading to the cascade of proinflammatory cytokines, which further ag-

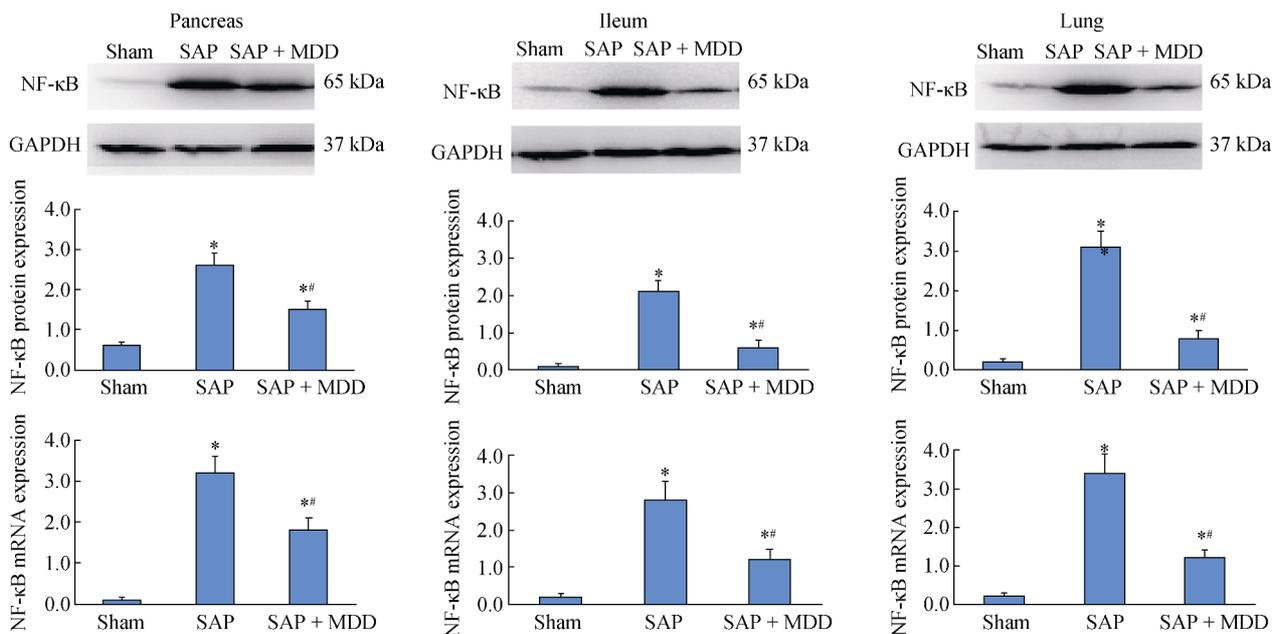
gravate the pancreatic injury and cause multiple organ failure<sup>[1, 6, 8]</sup>. Seeking new therapeutic strategies is the key to improving the cure rate of SAP. The clinical application and research of traditional Chinese medicine has provided a novel treatment for SAP. MDD, which was adopted from traditional Chinese medicinal formulation, has been used as an effective

treatment for SAP in clinical. In present study, we explored its potential organprotective mechanism in SAP rats induced by

retrograde injection of 3.5% sodium deoxycholate into the biliopancreatic duct.



**Fig. 4** Effects of MDD on the protein and mRNA expressions of occludin in pancreas, lung and ileum. Twelve hours after SAP induction in rats, the protein and mRNA expressions of occludin of pancreas, lung and ileum were decreased. Compared with the SAP group, the expressions were increased significantly in the SAP + MDD group. \* $P < 0.05$  vs the sham group, # $P < 0.05$  vs the SAP group. SAP: severe acute pancreatitis, MDD: modified Da-chai-hu Decoction



**Fig. 5** Effects of MDD on the protein and mRNA expressions of NF-κB in pancreas, lung and ileum. Twelve hours after SAP induction in rats, the protein and mRNA expressions of NF-κB of pancreas, lung and ileum were stronger. Compared with the SAP group, the expressions were decreased significantly in the SAP + MDD group. \* $P < 0.05$  vs the sham group, \*\* $P < 0.05$  vs the SAP group. SAP: severe acute pancreatitis, MDD: modified Da-chai-hu Decoction, NF-κB: nuclear factor-κB

*Rhubarb* is the the monarch herb of MDD, which is used singly or in combination for the treatment of digestive diseases in traditional medicinal practice [20-21]. Recent researches have confirmed that *rhubarb* and its active components, such as emodin, have anti-bacterial, anti-inflammatory, and anti-cancer effects [22-23]. In the present study, we studied the protective effects of MDD on pancreas, ileum and lung in experimental rat SAP model.

Intestinal barrier dysfunction and acute lung injury can occur at an early stage that may predispose to severity of disease and poor prognosis [24-25]. Our findings are in agreement with previous reports illustrating that there were histopathological injury in pancreas, ileum and lung tissue at 12 hours after retrograde injecting sodium deoxycholate into biliopancreatic duct. The serum amylase activity is usually elevated as a result of pancreatic acinar destruction. DAO, as a highly active intracellular enzyme located in the upper part of intestinal mucosa, is released into the blood as mucosal cells are necrotized [26]. SP-A has two important roles: stabilize the alveoli and prevent them from collapse by reducing alveolar surface tension, and as an important component of the innate immune system, which can prevent virus and bacteria infection [27]. Thus, serum amylase, DAO and SP-A assays have been used as indexes of the structural integrity of acinar, small intestinal mucosal and pulmonary alveolus, respectively. In the present study, the activities of serum amylase and DAO were markedly higher, and the SP-A concentration was decreased in SAP rats. It is suggested that the cytoarchitecture was destroyed. However, administration of MDD could significantly reduce the activities of amylase and DAO, and raise the SP-A concentration in serum. In addition, we also found the mRNA and protein expressions of occludin in pancreas, ileum and lung were both up-regulated when treated SAP rats with MDD. These results indicated that MDD preserved cells structural integrity and reduced the injury of *via* promoting the expression of tight junction protein.

Since MPO is a marker of tissue neutrophils infiltration, we mensurated MPO activities of the tissues, and found the MPO activities of pancreas, ileum and lung were increased in rats with SAP, MDD treatment could lower the MPO activities, as well as the histopathological scores. Combination of serum CRP levels, this study indicates that MDD has an anti-inflammatory effect. Inflammatory mediators are thought to play a pivotal role in the pathogenesis and progression of SAP. Previous studies have shown that the endogenous pro-inflammatory cytokines, such as TNF- $\alpha$  and IL-1 $\beta$ , are up-regulated in pancreatic tissue during the early phase of SAP in rats, which in turn activate a series of inflammatory mediators including cytokines, chemokines, adhesion factors, and lipid mediators, as well as gaseous mediators. These inflammatory mediators can cause distant organs second injury even MODS. MCP-1, as a kind of chemokines, with potent leukocyte-activating properties and have been shown to be

involved in the pathophysiological process of experimental acute pancreatitis, and blockage of MCP-1 activity by injecting mutated MCP-1 vector into thigh muscle attenuated the severity of SAP [28]. In addition, a close correlation has been found between the degree of MCP-1 elevation and the severity of remote organ failure in SAP patients [29]. Our findings were in consistent with previous reports showing that the levels of TNF- $\alpha$ , IL-1 $\beta$  and MCP-1 in tissue homogenate supernatants of lung, ileum and pancreas were both higher in SAP rats. Furthermore, treatment SAP rats with MDD significantly reduced the levels of TNF- $\alpha$ , IL-1 $\beta$  and MCP-1. These results suggested that MDD could alleviate systemic inflammatory response caused by SAP.

To further explore the underlying mechanism by which MDD inhibits the productions of TNF- $\alpha$ , IL-1 $\beta$  and MCP-1, we examined its effect on NF- $\kappa$ B expression. NF- $\kappa$ B is a transcription factor that is necessary for the transcription of numerous proinflammatory mediators, including TNF- $\alpha$ , IL-1 $\beta$  and MCP-1. Previous reports have shown that selective inhibition of NF- $\kappa$ B activation efficiently suppresses ICAM-1 expression and reduced the degree of pancreas and lung injury in experimental murine acute pancreatitis induced by cerulein [30-31]. We found that the NF- $\kappa$ B expressions of pancreas, ileum and lung tissues were both increased in SAP rat induced by sodium taurocholate and MDD significantly down-regulated the mRNA and protein expressions of NF- $\kappa$ B. These results suggested that MDD could suppress the synthesis and release of inflammatory mediators in the progression of SAP by inhibition the expression of NF- $\kappa$ B.

In conclusion, the current study confirmed that MDD was an effective therapeutic approach for SAP treatment. It could alleviate the pathological damage and inflammatory reactions of pancreas, ileum and lung in experimental SAP rat model, the mechanism should include the preservation of cell structural integrity by promoting the expression of tight junction protein and the reduction of inflammatory factors release by inhibiting NF $\kappa$ B pathway activation.

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