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Emodin diminishes immune stress in rats with severe acute pancreatitis.

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Abstract

Objectives: The aim was to observe the immune stress in rats with Severe Acute Pancreatitis (SAP) and diminish by emodin.
Methods: Rats were divided into four groups: sham operation group (SO), SAP model group (SAP), emodin treatment group (ET), and two times dose emodin treatment group (HT). Samples of blood, terminal ileum and lung were harvested on 6 h, 12 h, 18 h and 24 h for examination. Serum amylase, and high-mobility group box 1 (HMGB 1), cyclooxygenase-2 (COX-2), and peroxisome proliferator activated receptor gamma-γ (PPAR-γ) in terminal ileum and lung was determined.
Results: The values for emodin treated showed distinctly treatment effect, with lower levels of serum amylase, HMGB 1 and COX-2 on 12, 18 and 24 h and higher levels of PPAR-γ in the ET and HT groups compared with the SAP group on four time-points. The levels of detection indicators in the ET group were quite different from the HT group, with higher levels of serum amylase, HMGB 1 and COX-2 on 12, 18 and 24 h and lower expression of PPAR-γ on each time points.
Conclusions: Emodin can diminish immune stress in rats with SAP. The strategy of two times may be a better therapeutic effect.

Keywords: Severe acute pancreatitis, Rats, emodin, Immune stress.

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Introduction

Severe Acute Pancreatitis (SAP) is one of the most serious inflammatory disorders of the exocrine pancreas with Systemic Inflammatory Response Syndrome (SIRS), multiple organ dysfunction syndrome (MODS), secondary infection, and subsequent septic complications. [1] The marked change in the immune stress is thought to have a central role in the development of SAP, with excessive inflammatory immune response-mediated pathological damage and cell-mediated immunity [2,3]. systemic inflammation triggers by pancreatitis itself, fuels by the gut, results in exacerbating damage of systemic organs (especially for lung) in the early phase of SAP [4,5].

In spite of decades of intensive study, with the clinical treatment technology and development of the pathogenesis of SAP, we are still in lack of positive and effective therapy for the deleterious impact of multiple organ dysfunctions trigged by SIRS with significant morbidity and mortality. How to survive more patients with SIRS and MODS in the first phase, most gastroenterologists have a challenge for the management of SAP. In China, traditional Chinese herb is widely used in the treatment of SAP for many years [6]. Emodin (6-methyl-1,3,8-tanthragallol), the main active monomer of rhubarb, as a special kind of the selective gut decontamination, can significantly decrease the mortality and had significant therapeutic effects on SAP rats by promoting intestinal peristalsis and inhibiting inflammatory cytokine release [7].

In the present experiment, we aimed to observe the levels of high-mobility group box (HMGB) 1, cyclooxygenase-2 (COX-2), and peroxisome proliferator activated receptor gamma-γ (PPAR-γ) in terminal ileum and lung for rats with severe acute pancreatitis, and tried to explore possible mechanisms of emodin in the treatment for SAP.

Materials and Methods

Animal

Healthy adult male Wistar rats (200-250 g body weight) were purchased from the Experimental Animal Research Center of Hubei. All rats were allowed to acclimatize for at least a week, housed in cages with controlled temperature, humidity and 12-h light-dark cycles, fed standard laboratory feed and water. The Animal Studies Ethics Committee of Hubei University of Chinese Medicine approved all of the experiments. All experimental procedures were carried out in accordance with established International Guiding Principles for Animal Research.

Reagents

Emodin was obtained from Hubei Institute for Food and Drug Control. Sodium taurocholate was purchased from Sigma Chemical Company (St. Louis, MO, USA).

ELISA kit for HMGB1was purchased from Elabscience Biotechnology Co., Ltd (Wuhan, China). COX-2 Enzyme-Linked Immunosorbent Assay (ELISA) kit was provided by R&D Systems (Shanghai, China). Phenylmethanesulfonyl Fluoride (PMSF), Radio Immunoprecipitation Assay (RIPA) lysis buffer and BCA Protein Assay kit were provided by Beyotime Biotechnology (Shanghai, China). GAPDH antibody was obtained from Goodhere Biotechnology Co., Ltd (Hanzhou, China). PPAR-γ antibody was purchased from Proteintech group, Inc. (Wuhan, China). HRP-conjugated sheep-anti-rabbit secondary antibody was provided by Boster Biotechnology Co., Ltd (Wuhan, China). Electrochemiluminescence (ECL) substrate solution was obtained from Thermo Scientific™ (Shanghai, China).

Experimental model

The rats were fasted overnight with free access to water before experiments. All procedures were performed under aseptic conditions. Animals were anaesthetized by intraperitoneal injection of 3% pentobarbital sodium (40 mg/kg body weight) as routine. SAP models were induced by retrograde injection 5% sodium taurocholate into biliary-pancreatic duct according to the method by Aho et al. [8]. After entering the abdomen *via* median epigastric incision and closing hepatic duct by a small bulldog clamp, 5% sodium taurocholate was retrograde injected into the biliary-pancreatic duct at 0.1 ml/min speed to induce the SAP model according to the calculation of 1 ml/ 1000 g. Meanwhile, the sham operation rat was performed according to the above procedure, and stroke-physiological saline solution instead of sodium taurocholate. After that, emodin was injected into the rats of each correspondent group *via* the tail vein immediately. After surgery, the animals were fed ad libitum water.

Experimental design

Rats were randomly divided into four groups: sham operation group (SO), SAP model group (SAP), emodin (0.25 mg/100 g body weight) treatment group (ET), and two times dose emodin (0.50 mg/100 g) treatment group (HT).

Our previous study [9] had shown that we confirmed the successful induction of the pancreatitis model, and evaluated the trends of these measurements at the designated time-points. The time-course of experiments was 24 h. Zero time refers to the point of injection of sodium taurocholate. Rats of four group (n=48 per group) were killed at 6, 12, 18 and 24 h after the induction of SAP (n=12 each group at each time point), and samples were collected immediately.

Samples of terminal ileum and lung were infused by phosphate-buffered saline (PBS) immediately, frozen and maintained at -80°C until assayed. Blood samples were obtained from the abdominal aorta by direct puncture, and sent to testing straightway.

Ethics

The study was approved by the university and the hospital ethics committee.

Measurement of serum amylase

Serum Amylase (AMY) activity in serum was determined by clinical laboratory of Hubei Hospital of Chinese Medicine using AMY kits by automated clinical biochemistry analysis equipment (Hitachi Co., Tokyo, Japan).

HMGB 1 and COX-2 measurements

Samples of terminal ileum and lung were thawed and prepared to 10% tissue homogenate respectively. The suspension was centrifuged at 10000 rpm for 20 min, and carefully drawn the supernatant to marked EP tube for measurement. The concentrations of HMGB 1 and COX-2 were checked by ELISA kits according to the instructions of the manufacturer, and all were detected spectrophotometrically at 450 nm on a microplate reader.

PPAR-γ measurements

Immunohistochemistry was performed to examine the protein expression levels of PPAR-γ and to localize PPAR-γ expression in the terminal ileum and lung of rats. The frozen samples from each rat were homogenized in ice-cold lysis buffer containing a cocktail of protease inhibitors. After removing nuclei and cell debris, then protein was separated by 10% SDS polyacrylamide gel electrophoresis (50 μg/lane) and electrophoretically transferred to nitrocellulose membranes. After nonspecific binding blocking with 5% milk, membranes were stained overnight at 4°C with the specific primary antibody, rabbit-anti-rat, followed by HRP-conjugated sheep-anti-rabbit secondary antibody. The following day, membranes were washed five times and incubated for 2 h at 25°C with antirabbit immunoglobulin G horseradish peroxidase-conjugated secondary antibody (1:5,000). The specific bands were detected by use of an ECL plus western blotting detection system. The membranes were then stripped using stripping buffer and probed with antibodies specific for GAPDH to ensure equal loading of protein on the gel. PPAR-γ expression was quantified with the use of GelExpert version 3.5 software.

Statistical analysis

Continuous data are presented as the mean ± Standard Error of the Mean (SEM). Mann-Whitney U test and the χ^2 test with Yates correction, respectively. The one-way ANOVA test was performed by the software package SPSS 11 (Chicago, IL, USA). Comparisons of continuous and categorical variables between two groups used the nonparametric Correlations were evaluated with the Spearman rank test. A $P<0.05$ was considered statistically significant.

Results

Survival rate

On four time points, the rats in the SAP, ET and HT groups occurred mortality. On 12 h and 24 h, the survival rates of the rats in the SAP group were 50%, and significantly lower than in the other groups (P<0.05) (Table 1). On 18 h and 24 h, the survival rates of the rats in the HT group were 83.3% and 83.3%, and significantly higher than in the ET groups (P<0.05) (Table 1).

Table 1. *Survival rate of each group. ●(P<0.05) at 12 h, differences between the SO group vs. the SAP group was significant; ★(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the SAP group vs. the HT group were significant; ▲P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6h (n=12)	12 h (n=12)	18 h (n=12)	24 h (n=12)
SO	12	12●	12★	12★▲
SAP	11	9	6	6
ET	10	10	8★	8★▲
HT	11	10	10★[b]	10★▲

Table 2. *Comparison of the dynamic serum amylase levels (U/ml). ★(P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲(P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ○(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6 h	12 h	18 h	24 h
SO	2890.5 ± 765.9	1761.5 ± 273.6★	1735.5 ± 300.2	1688.0 ± 275.7
SAP	10012.0 ± 1788.5 ♀	8495.8 ± 1557.7▲☆	7598.4 ± 1325.5▲◎	5752.3 ± 859.5▲○
ET	8098.3 ± 1425.2b ♀	6780.3 ± 1365.8■☆	4948.7 ± 1125.7■◎	3787.3 ± 959.7■○
HT	8075.3 ± 1505.1b	5873.0 ± 1125.6●☆	3884.7 ± 989.3●◎	2887.9 ± 595.5●○

Serum amylase

Inflammation expression in terminal ileum

In the SAP, ET and HT groups, the levels of HMGB 1 and COX-2 expression in terminal ileum increased significantly on 12 h, but was markedly depleted on 24 h (Tables 3 and 4). In the SAP, ET and HT groups, the levels of HMGB 1 and COX-2 expression in terminal ileum increased significantly compared with those in the SAP group on four time-points (P<0.05, Tables 3 and 4). The values for emodin treated showed distinctly treatment effect, with lower levels of HMGB 1 and COX-2 (P<0.05, Tables 3 and 4) and higher levels of PPAR-γ (P<0.05, Table 5) in the ET and HT groups compared with those in the SAP group at four time-points. The levels of inflammation expression in the ET group were quite different from the HT group, with higher expression of HMGB 1 and COX-2 on 12 h, 18 h and 24 h (P<0.05, Tables 3 and 4) and lower expression of PPAR-γ on four time points (P<0.05, Table 5).

Table 3. *Comparison of the dynamic HMGB 1 levels in terminal ileum (pg/ml). ★(P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲ (P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; °(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6 h	12 h	18 h	24 h

SO	27.5 ± 7.2	33.5 ± 8.7★	26.5 ± 9.2★	28.0 ± 8.1
SAP	65.0 ± 28.2♀	73 ± 17.1▲☆	67.4 ± 25.8▲◎	57.3 ± 19.6▲○
ET	58.3 ± 20.2♀	68.7 ± 18.7■☆	49.4 ± 19.1■◎	37.4 ± 17.9■○
HT	57.6 ± 21.3	63.1 ± 15.5●☆	42.5 ± 19.3●◎	33.1 ± 15.3●○

Table 4. *Comparison of the dynamic COX-2 levels in terminal ileum (pg/ml). ★(P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲ (P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ○(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6 h	12 h	18 h	24 h
SO	397.5 ± 115.9	417.5 ± 113.6★	359.5 ± 100.4★	357.0 ± 117.1
SAP	514.2 ± 158.4♀	594.8 ± 277.2▲☆	516.4 ± 145.7▲◎	468.3 ± 152.3▲○
ET	481.7 ± 142.3♀	501.3 ± 136.8■☆	472.7 ± 126.3■◎	449.3 ± 119.7■○
HT	457.5 ± 150.5♀	473.0 ± 112.7●☆	457.7 ± 114.1●◎	435.9 ± 105.9●○

Table 5. *Comparison of the dynamic PPAR-γ levels in terminal ileum. ★(P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲ (P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎ (P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ○(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6 h	12 h	18 h	24 h
SO	0.276 ± 0.057	0.300 ± 0.066★	0.250 ± 0.058★	0.246 ± 0.063
SAP	0.527 ± 0.054♀	0.587 ± 0.071▲☆	0.511 ± 0.047▲◎	0.466 ± 0.059▲○
ET	0.548 ± 0.057♀	0.601 ± 0.064■☆	0.547 ± 0.057■◎	0.493 ± 0.097■○
HT	0.611 ± 0.089♀	0.622 ± 0.094●☆	0.586 ± 0.079●◎	0.549 ± 0.073●○

Inflammation expression in lung

In the SAP, ET and HT groups, the levels of HMGB 1 and COX-2 expression in lung increased significantly compared with those in the SAP group on four time-points (P<0.05, Tables 6 and 7). In the SAP, ET and HT groups, the levels of HMGB 1 and COX-2 expression in lung increased significantly on 12 h, but was markedly depleted on 24 h (Tables 6 and 7). The levels of inflammation expression in the HT group were quite different from the ET group, with lower expression of HMGB 1 and COX-2 on 12 h, 18 h and 24 h (P<0.05, Tables 6 and 7) and higher expression of PPAR-γ on each time points (P<0.05, Table 8). The values for emodin treated showed distinctly treatment effect, with lower levels of HMGB 1 and COX-2 on 12 h, 18 h and 24 h (P<0.05, Tables 6 and 7) and higher levels of PPAR-γ (P<0.05, Table 8) compared with those in the SAP group on four time-points.

Table 6. *Comparison of the dynamic HMGB 1 levels in lung (pg/ml). ★(P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲ (P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group and the SAP group vs. the ET group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ○(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6 h	12 h	18 h	24 h
SO	17.5 ± 9.2	23.2 ± 7.9★	17.3 ± 9.2★	18.0 ± 9.3
SAP	62.7 ± 22.8♀	70.0 ± 19.2▲☆	67.1 ± 20.1▲◎	59.0 ± 16.9▲○
ET	54.6 ± 20.2♀	68.7 ± 18.7■☆	49.4 ± 19.1■◎	44.0 ± 17.9■○
HT	53.8 ± 22.0	61.3 ± 16.5●☆	43.0 ± 17.3●◎	32.0 ± 16.5●○

Table 7. *Comparison of the dynamic COX-2 levels in lung (pg/ml). ★ (P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲ (P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group and the SAP group vs. the ET group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the*

ET group vs. the HT group were significant; ○(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.

Groups	6 h	12 h	18 h	24 h
SO	496.5 ± 120.9	515.7 ± 126.3★	488.0 ± 104.0★	499.0 ± 118.1
SAP	813.9 ± 188.9♀	981.2 ± 296.1▲☆	836.7 ± 167.3▲◎	783.4 ± 139.8▲○
ET	718.7 ± 159.6♀	890.3 ± 207.1■☆	792.4 ± 167.1■◎	603.0 ± 120.1■○
HT	707.5 ± 172.1	830.7 ± 191.4●☆	710.2 ± 147.5●◎	576.3 ± 111.8●○

Table 8. *Comparison of the dynamic PPAR-γ levels in lung. ★ (P<0.05) in the SO group, differences between 6 h vs. 12 h and 12 h vs. 18 h were significant; ▲(P<0.05) in the SAP group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ■(P<0.05) in the ET group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ●(P<0.05) in the HT group, differences between 6 h vs. 12 h, 12 h vs. 18 h, and 18 h vs. 24 h were significant; ♀(P<0.05) at 6 h, differences between the SO group vs. the SAP group and the SAP group vs. the ET group were significant; ☆(P<0.05) at 12 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ◎(P<0.05) at 18 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant; ○(P<0.05) at 24 h, differences between the SO group vs. the SAP group, the SAP group vs. the ET group, and the ET group vs. the HT group were significant.*

Groups	6 h	12 h	18 h	24 h
SO	0.296 ± 0.075	0.330 ± 0.073★	0.289 ± 0.047★	0.285 ± 0.076
SAP	0.572 ± 0.084♀	0.667 ± 0.074▲☆	0.501 ± 0.086▲◎	0.349 ± 0.089▲○
ET	0.578 ± 0.075 ♀	0.701 ± 0.068■☆	0.578 ± 0.068■◎	0.404 ± 0.097■○
HT	0.689 ± 0.096♀	0.757 ± 0.099●☆	0.602 ± 0.089●◎	0.582 ± 0.08●○

Discussion

SAP is one of the most serious pancreatic diseases, is usually accompanied by obvious systemic inflammatory reactions, and still has a substantial mortality, which depends on the severity and course of the disease [10]. The development of SAP is complicated and varied, triggered by local pathological pancreatic injuries, started the SIRS, aggravated by the gut, and resulted in exacerbating damage of systemic organs [11,12]. Excessive inflammatory response during SAP is the main cause of the intestinal mucosa injury, increased the permeability of intestinal mucosa, and aggravated the body invasion of intestinal bacteria continuously, which lead to bacterial translocation during inflammatory responses [13,14].

Researchers have found that SIRS contributes to early SAP causing systemic epithelial barrier dysfunction and increased vascular endothelial permeability [15]. The alteration of vascular permeability is represented systemically by the development of other distal organ dysfunction including lung, renal and hepatic failure [16]. Acute lung injury with SAP can be attributed to an increase in lung vascular permeability, which is characterized by an intense inflammatory process in the lung, with the development of interstitial edema and accumulation of activated neutrophils [17,18].

Extracellular HMGB1, one of the families of non-histone nuclear proteins, is already known as a novel pro-inflammatory cytokine in humans. In the last decade, many studies have claimed a positive relationship between extracellular HMGB1 expression and SAP severity [19]. Cyclooxygenase (COX), has two isoforms: COX-1 and COX-2, is a key enzyme in the synthesis of prostaglandins from arachidonic acid [20]. COX-2, induced by mitogens, proinflammatory cytokines, growth factors, and tumor promoters, is a crucial and central mediator in the development and severity of acute pancreatitis [21]. Peroxisome Proliferator-Activated Receptors (PPARs) are ligand activated transcription factors, which play a modulatory role in the inflammatory response of different organs, belonging to the nuclear receptor superfamily. Activation of PPAR-γ can suppress inflammatory gene targets and provide potent protection against organ injury [22].

As an effective Chinese medicine, emodin has significant therapeutic effects on SAP, correcting intestinal flora disturbances, facilitating bowel movement, maintaining morphologic changes in the intestine, protecting intestinal mucosal barrier, preventing translocation for bacteria and endotoxin, inhibiting inflammatory cytokine release and pancreatin activity, scavenging oxygen free radicals, and ameliorating the systemic inflammatory response and multiorgan failure [23-25].

The excessive release of inflammatory substances during the course of SAP is the main cause of the intestinal barrier injury. The inflammatory mediators can increase the permeability of intestinal mucosa, aggravate the body invasion of proinflammatory cytokines continuously, which lead to distant organ injury during inflammatory responses [26]. Our founding shows that rats in the SAP group have higher mortality, increase significantly serum amylase and the expression of HMGB 1, COX-2 and PPAR-γ in terminal ileum and lung, by compared those in the SO group. The gut maybe play a key role in the development of SAP's SIRS and MODS [27]. The treatment for SAP can focus on recovery of intestinal function, which would moderate the disease and restore the damage of distant organ damage. Our experiment suggests that emodin treatment can reduce mortality, ameliorate serum amylase and the expression of HMGB 1, COX-2 and PPAR-γ in terminal ileum and lung, compared the ET group with the SAP group. In this report, we also find that different doses emodin can achieve different therapeutic effect, which the HT group had lower mortality on 18 h and 24 h, decreased significantly levels of serum amylase on 12 h, 18 h and 24 h, had lower levels of HMGB 1 and COX-2 on 12 h, 18 h and 24 h and higher levels of PPAR-γ on four time-points, comparing with the SAP group.

Our results show that the therapeutic values for emodin showed distinctly treatment effect, by reducing mortality, ameliorating serum amylase and the inflammation expression in terminal ileum and lung, and two times dose emodin may be better than one time. Wang et al. [28] reported that emodin can exert protective effects on SAP rats and remarkably alleviate the severity of experimental SAP, by protecting the intestinal barrier, inhibiting over-inflammatory reaction and abating oxidative stress. Xia et al. [29] found that emodin can enhance alveolar epithelial barrier function, attenuate pulmonary edema and inflammation, and promote expression of claudin-4, claudin-5 and occludin in lung tissue samples from rats with acute pancreatitis. Wu et al. [30] reported that emodin could reduce pancreatic injury and restrain inflammatory reaction in SAP rats, *via* inhibiting endoplasmic reticulum stress transducers IRE1α and its downstream molecules.

In conclusion, the development and progression of SAP have high mortality, increase significantly serum amylase and the expression of HMGB 1, COX-2 and PPAR-γ in terminal ileum and lung. Emodin can diminish immune stress in rats with SAP. Therapeutic mechanism of emodin might account for the beneficial effects include inhibiting over-inflammatory reaction of HMGB 1, COX-2 and increasing the expression of PPAR-γ in terminal ileum and lung. The strategy of two times dose emodin proved to be better therapeutic effect than one time dose alone. Future large-scale, high quality, animal experiments and clinical trials are required to clarify the therapeutic effects of emodin for the throughout course of SAP.

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Conflict of Interest

None

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