Emodin alleviates intestinal mucosal injury in rats with severe acute pancreatitis via the caspase-1 inhibition

Jian-Wen Ning, Yan Zhang, Mo-Sang Yu, Meng-Li Gu, Jia Xu, Ali Usman and Feng Ji

Hangzhou, China

BACKGROUND: Emodin, a traditional Chinese medicine, has a therapeutic effect on severe acute pancreatitis (SAP), whereas the underlying mechanism is still unclear. Studies showed that the intestinal mucosa impairment, and subsequent release of endotoxin and proinflammatory cytokines such as IL-1β, which further leads to the dysfunction of multiple organs, is the potentially lethal mechanism of SAP. Caspase-1, an IL-1β-converting enzyme, plays an important role in this cytokine cascade process. Investigation of the effect of emodin on regulating the caspase-1 expression and the release proinflammatory cytokines will help to reveal mechanism of emodin in treating SAP.

METHODS: Eighty Sprague-Dawley rats were randomly divided into four groups (n=20 each group): SAP, sham-operated (SO), emodin-treated (EM) and caspase-1 inhibitor-treated (ICE-I) groups. SAP was induced by retrograde infusion of 3.5% sodium taurocholate into the pancreatic duct. Emodin and caspase-1 inhibitor were given 30 minutes before and 12 hours after SAP induction. Serum levels of IL-1β, IL-18 and endotoxin, histopathological alteration of pancreas tissues, intestinal mucosa, and the intestinal caspase-1 mRNA and protein expressions were assessed 24 hours after SAP induction.

RESULTS: Rats in the SAP group had higher serum levels of IL-1β and IL-18 (P<0.05), pancreatic and gut pathological scores (P<0.05), and caspase-1 mRNA and protein expressions (P<0.05) compared with the SO group. Compared with the SAP group, rats in the EM and ICE-I groups had lower IL-1β and IL-18 levels (P<0.05), lower pancreatic and gut pathological scores (P<0.05), and decreased expression of intestine caspase-1 mRNA (P<0.05). Ultrastructural analysis by transmission electron microscopy found that rats in the SAP group had vaguer epithelial junctions, more disappeared intercellular joints, and more damaged intracellular organelles compared with those in the SO group or the EM and ICE-I groups.

CONCLUSIONS: Emodin alleviated pancreatic and intestinal mucosa injury in experimental SAP. Its mechanism may partly be mediated by the inhibition of caspase-1 and its downstream inflammatory cytokines, including IL-1β and IL-18. Our animal data may be applicable in clinical practice.

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KEY WORDS: severe acute pancreatitis; intestinal mucosa; emodin; caspase-1 inhibitor

Introduction

Severe acute pancreatitis (SAP) is a potentially lethal disease characterized by sudden onset, rapid progression, multiple organ failure and high mortality rate.[1-3] The underlying pathogenesis remains incompletely elucidated. As main anatomic and functional barrier, gut mucosa impairment is considered one of the major pathologies in SAP,[4, 5] which will increase intestinal permeability, bacteria translocation as well as release of endotoxin, inflammatory mediators and cytokines (IL-1β, IL-18).[6]

Caspase-1 (IL-1β-converting enzyme/ICE) is a common intracellular cysteine protease that converts the precursors of IL-1β and IL-18 into active cytokines.[7, 8] Blockade of caspase-1 suppresses inflammatory mediator expressions, such as IL-1β and IL-18, which will alleviate inflammation cascade reaction and ameliorate the overall severity and mortality of patients with SAP.[9, 10] The

Author Affiliations: Division of Emergency (Ning JW and Xu J) and Division of Gastroenterology (Yu MS, Gu ML, Usman A and Ji F), First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China; Division of Gastroenterology, Yixing People's Hospital, Yixing 214200, China (Zhang Y)

Corresponding Author: Feng Ji, MD, Division of Gastroenterology, First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou 310003, China (Tel: +86-571-87236522; Fax: +86-571-87236863; Email: jifeng@zju.edu.cn)

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study by Wang et al[11] demonstrated that therapeutic effect of emodin (1, 3, 8-trihydroxy-6-methyl-anthraquinone) is partly mediated by IL-1β suppression, whereas the underlying mechanism is still unclear.

In this study, we hypothesized that emodin may have similar mechanism as caspase-1 inhibitor and investigated whether emodin showed analog inflammation cascade blockade with caspase-1 inhibitor in preventing intestinal mucosa injury in SAP.

Methods

Ethical approval

The study was approved by the Animal Care and Use Committee of Zhejiang University and conformed with the regulations of the Chinese guidelines for the care and use of laboratory animals.

Animals

Eighty adult male Sprague-Dawley rats weighing 250±50 g were supplied by Animal Center of Zhejiang Chinese Medical University. The rats were caged in a controlled-temperature of 25 ℃ and 12 hours light-dark cycles with free access to a standard rat chow.

Induction of pancreatitis and treatment

The rats were randomly divided into four groups (n=20 for each group): SAP, sham-operated (SO), emodin-treated (EM) and caspase-1 inhibitor-treated (ICE-I) groups. The rats were fasted for 12 hours before experiment with water access. Anesthesia was induced with 4% chloral hydrate intraperitoneal injection (1 mL/100 g, Solarbio Science & Technology Co, Ltd., Beijing, China). Rats in the SAP group had retrograde infusion of 3.5% sodium taurocholate (0.1 mL/100 g body weight, Sigma-Aldrich, St. Louis, MO, USA) into the pancreatic duct. Normal saline (0.2 mL) replaced sodium taurocholate in rats of the SO group. Rats in the EM and ICE-I groups were administered with emodin (0.5 mg/100 g) and ICE-I (0.1 mg/100 g) intra-gastric 30 minutes before and 12 hours after the induction of SAP. Meanwhile, rats in the SO and SAP groups were treated with normal saline solution of equivalent volume. The survived animals were sacrificed 24 hours after SAP induction with prolonged anesthesia and the obtained specimens from venous blood, pancreas body and gut tissues (5 cm from terminal ileac segments) were cut and stored in liquid nitrogen.

Histology

The specimens of pancreas and ileum were fixed with formalin solution, embedded in paraffin, stained with hematoxylin & eosin (HE), and analyzed under light microscope. The severity of acute pancreatitis and intestinal mucosal damage was assessed semi-quantitatively based on the scoring method defined by previous literatures.[12, 13] Two experienced pathologists, who were blinded with the treatment, assessed each tissue sample in 5 microscopic fields randomly and totalled those fields to calculate the final scores.

Transmission electron microscopy (TEM)

Terminal ileac segments specimens (5 mm×5 mm in size) were conventional fixed by 2.5% glutaraldehyde and 2% osmium tetroxide, dehydrated by ethanol, and subjected to isoamyl acetate transition. AHCP-2 type critical point drying apparatus was used for critical point drying. The specimens were gilded target alloy using an IB-5 ion sputter coater. The cell morphology of the intestinal mucosa, the junctions of the intestinal mucosa epithelial cells, the ultrastructural changes of the intracellular mitochondria, the Golgi apparatus and other organelles were observed under TEM.

Serum IL-1β and IL-18 levels

Blood samples were collected from the inferior vena cava and centrifuged at 800 g for 10 minutes at 4 ℃. Serum levels of IL-1β and IL-18 were measured by enzyme-linked immunosorbent assays (ELISA) according to the manufacturer's instructions (IL-1β: e-Bioscience Company, Vienna, Austria; IL-18: Abnova Company, Heidelberg, Germany).

Caspase-1 mRNA levels

Total RNA of ileum tissues was extracted with chloroform and TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Two μg of RNA was reverse transcribed into complementary deoxyribonucleic acid (cDNA) using PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China). cDNA was stored at -80 ℃ until use. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the housekeeping gene. Specific primers were as follows: caspase-1 forward 5'-CCA GAG CAC AAG ACT TCT GAC-3' and reverse 5'-TGG TGT TGA AGA GCA GAA AGC-3'; GAPDH forward 5'-ACA GCA ACA GGG TGG TGG AC-3' and reverse 5'-TTT GAG GGT GCA GCG AAC TT-3'. DNA products were amplified with SYBR® Premix Ex Taq™ (Tli RNaseH Plus) (Takara). The amplifications were done with an initial denaturation at 95 ℃ for 10 minutes, followed by 40 thermal cycles at 95 ℃ for 30 seconds and 60 ℃ for 34 seconds. The threshold cycle (Ct) value was