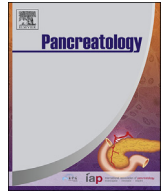




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Biliary tract external drainage protects against multiple organs injuries of severe acute pancreatitis rats via heme oxygenase-1 upregulation

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ABSTRACT

Objective: To investigate the effect of biliary tract external drainage (BTED) on severe acute pancreatitis (SAP) in rats and the relationship with heme oxygenase-1 (HO-1) pathway.

Methods: Thirty SD rats weighing 250–300 g were randomly assigned into five groups (n = 6): sham surgery (SS) group, SAP group, SAP + BTED group, SAP + zinc protoporphyrin IX (ZnPP) group, SAP + BTED + ZnPP group. The SAP model was induced via retrograde injection of 4% sodium taurocholate (1 mL/kg) into biliopancreatic duct through duodenal wall. BTED was performed by inserting a cannula into the bile duct of SAP rats. Tissue and blood samples were collected 24 h after surgery. Pathological changes in organs were scored. The level of amylase, alanine transaminase (ALT), aspartate aminotransferase (AST), diamine oxidase (DAO), lipopolysaccharide (LPS), myeloperoxidase (MPO) and ability to inhibit hydroxyl radical ($\cdot\text{OH}$) in serum were measured. The expression of hemeoxygenase-1 (HO-1), tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6) in tissues were analyzed by RT-PCR and western-blot.

Results: Organs damage in SAP rats was significantly alleviated by BTED ($p < 0.05$). Compared to the SAP group, the serum level of amylase, ALT, AST, DAO, MPO, and LPS were significantly lower in the SAP + BTED group, and the ability to inhibit $\cdot\text{OH}$ was significantly higher ($p < 0.05$). The BETD treatment led to a significant reduction of TNF- α , IL-6 level and a significant increase of HO-1 level in tissues than in SAP rats ($p < 0.05$). ZnPP significantly inhibited all above mentioned changes.

Conclusions: BTED protected multiple organs against SAP related injuries via HO-1 upregulation.

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1. Introduction

Acute pancreatitis (AP) is one of the most common seen acute abdominal diseases requiring emergency hospitalization all over the world [1]. Although Most of AP is mild, however, severe acute

pancreatitis (SAP) develops in about 15–20% of patients with AP, characterized by acute onset, rapid change, local complications, early progressing multiple organ failure, high morbidity and mortality [2–5].

In the pathogenesis of SAP, the levels of the pro-inflammatory factors TNF- α and IL-6 increase in the ileum, which contributes to intestinal tissue injury. On the one hand, Kupffer cells in the liver is activated by TNF- α and IL-6 from intestinal tissue via the portal vein and produce pro-inflammatory TNF- α and IL-6, thus inducing early liver injury and the further release of pro-inflammatory factors from hepatocytes. On the other hand, gut-derived cytokines reach the lungs by permeating the mesenteric lymph vessels, the thoracic duct, the subclavian artery and the right atrium, resulting in ARDS [6–8]. Liver-derived cytokines enter gut via bile to establish the inflammatory loop of the gut-liver axis and enter the lungs via systemic circulation to activate monocytes/macrophages in the

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lungs to increase inflammatory cytokine production, leading to ARDS and increased intestinal and hepatic inflammation [9–12]. These processes constitute “gut-liver-lung” cycle that ultimately induces a cytokine cascade, resulting in multiple organ dysfunctions, which may lead to MODS and even death [13].

Hemeoxygenase-1 (HO-1), which is also referred to as heat shock protein-32, is a rate-limiting enzyme in heme catabolism to engender carbon monoxide, biliverdin, and ferrous iron [14,15]. It protects cells by anti-inflammatory, anti-apoptotic and antioxidant activities under stress. HO-1 pathway has been drawing the attention from basic and clinical researchers for more than four decades [16,17]. Previous studies showed that HO-1 can be strongly induced under several critical conditions, such as hemorrhagic shock, sepsis, and graft pancreatitis [18–20], etc. Since HO-1 is considered as a protective factor in the scenario of stress, the targeted induction of HO-1 is emerging as an important therapeutic method for the protection against the development of inflammatory and oxidant activities [21,22]. Particularly, pretreatment with HO-1-inducing agent in animal models of hemorrhagic shock exerted protective effects on multiple organs, including the liver, the kidney and the lungs [23–25]. In clinical practice, the organ function in patients with shock due to SAP was significantly improved when received biliary tract external drainage (BTED) either by endoscopic naso-biliary drainage, cholecystectomy or gallbladder percutaneous catheter drainage. The incidence of infection and morbidity of MODS were significantly decreased as well. Animal studies have also indicated that BTED can alleviate the injure of vital organs and improve the survival of rats with shock [26,27]. Based on the above knowledge, we hypothesized that bilirubin might play a role of negative feedback in the formation of HO-1. We deduce that BTED may induce compensatory increase in HO-1 expression via interrupting the enterohepatic circulation of bilirubin. Since shock occurs more frequently among SAP patients, we hypothesized that BTED may exert a protective effect on SAP patients via the up-expression of HO-1 in vital organs. Therefore, this study was designed to explore the effect of BTED on HO-1 expression in these organs of rats subjected to SAP.

2. Materials and methods

2.1. Animal model

Thirty adult male Sprague-Dawley rats (250–300 g) were purchased from Slac Laboratory Animal Corporation, Shanghai. The animals were housed and fed in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources that were approved by the Shanghai Jiao Tong University School of Medicine Animal Care and Ethics Committee and were provided with free access to food and water. Before surgery, the rats were fasted overnight but were allowed access to water.

The rats were randomly divided into five groups ($n = 6$): sham surgery (SS) group, SAP group, SAP + BTED group, SAP + zinc protoporphyrin IX (ZnPP) group, SAP + BTED + ZnPP group. Rats in the SS group were subjected to pentobarbital anesthesia, laparotomy and closure of abdomen only. SAP model rats were anaesthetized and ventral laparotomy, then 4% sodium taurocholate (1 ml/kg body weight) was retrograde injected into the pancreatic duct using a micro-injection pump at an even rate of 3 ml/h, and the atraumatic vascular clamp was removed 5 min later. Finally, the abdominal incisions were closed with sutures. All procedures were performed using a sterile technique. BTED model rats were performed bile duct cannulation and ligation under pentobarbital anesthesia [11]. A laparotomy was performed, and a catheter (inner diameter 0.4 mm; outer diameter 0.8 mm; length 20 cm) was inserted into the bile duct. The distal end of the bile duct was

ligated, and the catheter was passed through the flank of the rat to avoid the permeation of bile into the gut and to facilitate external collection of the bile. Subsequently, the abdomen was closed, and the rats were housed in restraining cages with free access to food and water. Animals in the SAP + ZnPP group and SAP + BTED + ZnPP group were given ZnPP, a specific HO-1 blocker (30 mg/kg body weight), by intraperitoneal injection 24 h prior to surgery. All rats received resuscitation with a single injection (40 ml/kg of body weight) of isotonic sodium chloride after surgical procedure, and each rat was housed in individual cage with free access to food and water. The rats in each group were sacrificed 24 h after surgery.

2.2. Reagents

Sodium taurocholate powder was purchased from Sigma Corporation (purity $\geq 97\%$, 101185852); zinc protoporphyrin IX (ZnPP) was purchased from TocrisBioscience, Britain; TRIzol was purchased from Invitrogen Corporation, USA. RIPA buffer was purchased from Beyotime Institute of Biotechnology. The fluorescence quantitative RT-PCR kit was purchased from TOYOBO. The primers for HO-1, TNF- α , IL-6 and β -actin were synthesized by Invitrogen and the sequences of these primers are presented in Table 1.

2.3. Histomorphologic assessment

The liver, pancreas and intestinal tissues were harvested for morphological studies. The 10% buffered formalin-fixed sample was embedded in paraffin, sectioned at 4 μm thickness, stained using hematoxylin-eosin and then observed under an optical microscope in a blinded manner by two independent pathologists. Three sections from each tissue were randomly selected for histopathology score. The standardized scoring system presented in Table 2 was utilized [28].

2.4. Assessment of amylase, ALT and AST

Blood samples were collected from the abdominal aorta, and centrifuged at 3500 g for 15 min at 4 $^{\circ}\text{C}$. The resulted plasma was kept at -80°C until measurement. The level of serum amylase, ALT and AST was measured using a BECKMAN COULTER UniCel[®] Dx C 800 Synchron[®] Clinical System auto-analyser.

2.5. Analysis of the LPS, DAO and MPO levels via ELISA, the ability to inhibit $\cdot\text{OH}$ via hydroxyl free radical assay kit

Serum was harvested. The expression levels of LPS, DAO and MPO were measured by using an ELISA Kit (Westang, China) according to the manufacturer's protocol. The ability to inhibit $\cdot\text{OH}$ was measured by using an Hydroxyl Free Radical assay kit (Nanjing Jiancheng, China) according to the manufacturer's protocol.

2.6. Assessment of HO-1, TNF- α and IL-6 expression

Total RNA was extracted from homogenized liver, pancreas and intestine samples using TRIzol. The concentration of the total RNA

Table 1
PCR primer sequences.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
β -actin	TGG TGG GTA TGG GTC AGA AG	GAC AAT GCC GTG TTC AAT GG
HO-1	ACC CCA CCA AGT TCA AAC AG	GAG CAG GAA GGC GGT CTT AG
TNF- α	CCC AAT CTG TGT CCT TCT AAC T	CAC TAC TTC AGC GTC TCG TGT
IL-6	AGA GAC TTC CAG CCA GTT GC	AGC CTC CGA CTT GTG AAG TG

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