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Protective effect of ethyl pyruvate on pancreas injury in rats with severe acute pancreatitis

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ABSTRACT

Background: Systemic inflammatory mediators have an important role in the development of acute pancreatitis. In this study, we investigated the effect of ethyl pyruvate (EP) on pancreas injury in rats with severe acute pancreatitis (SAP) and its possible mechanism.

Methods: We randomly allocated rats into the following three experimental groups: control and SAP- and EP-treated. Then, we recorded the mortality rate. We harvested tissue specimens for morphological studies, streptavidin-peroxidase immunohistochemistry examination, and Western blot analysis. We tested the levels of pancreatic tissue malondialdehyde and the activity of serum amylase, myeloperoxidase in the pancreas. In addition, we studied nuclear factor-κB (NF-κB) activation, tumor necrosis factor-α levels, and high mobility group box 1 protein expression levels in the pancreas.

Results: Treatment with EP after SAP was associated with a reduction in the severity of SAP and pancreas injury. Treatment with EP significantly decreased the expression of tumor necrosis factor-α and high mobility group box 1, and ameliorated malondialdehyde concentration and myeloperoxidase activity in the pancreas in SAP rats. Compared with the SAP group, treatment with EP significantly decreased the number of inflammatory cell infiltration, markedly inhibited pancreatic NF-κB DNA binding, and increased the survival rates.

Conclusions: This study demonstrates that preventing the activation of NF-κB by EP ameliorates tissue injury associated with experimental murine acute pancreatitis. This result provides an important insight into the molecular biology of acute pancreatitis.

1. Introduction

Severe acute pancreatitis (SAP) remains a serious clinical problem with significant morbidity and mortality [1,2]. The initial stage of acute pancreatitis is characterized by interstitial edema coupled with infiltration of neutrophils and macrophages in the pancreatic tissue [3]. Such infiltrating inflammatory cells (particularly neutrophils) produce reactive oxygen species (ROS), which subsequently destroy lipid membranes by peroxidation of fatty acids and trigger various

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inflammatory processes [4,5]. The over-release of inflammatory mediators, mainly inflammatory cytokines including tumor necrosis factor-α (TNF-α) and interleukin (IL) 1 is a significant mechanism leading to systemic inflammatory response syndrome and multiple organ system dysfunction. High motility group box 1 (HMGB1) is a large group of low-molecular-weight (nearly 30 KD) nucleoprotein rich in charge, the richest one among nonhistone chromosomal proteins. Studies have shown that HMGB1 is involved in systemic inflammatory response of SAP as a downstream cytokine of early inflammatory factors TNF-α and IL-1β [6–8].

Although the pathogenesis of acute pancreatitis is still not completely understood, the induction of various proinflammatory genes by transcription factor nuclear factor (NF)-κB is thought to be important [9–11]. Activation of transcription factor NF-κB is an early and central phenomenon in the host response to acinar cell injury in acute pancreatitis [12–14].

Recently, our laboratory and others have demonstrated that ethyl pyruvate (EP), a simple aliphatic ester derived from pyruvic acid, is an effective anti-inflammatory agent [15,16]. Prompted by these observations, we hypothesized that treatment with EP might be beneficial in a murine model of acute pancreatitis. Accordingly, the present study was designed to confirm the effects of EP treatment in a murine model of acute pancreatitis caused by taurocholate administration. To gain better insight into the mechanism(s) of action of the observed anti-inflammatory effects of EP, we also investigated the effects of EP on serum amylase activity, lipid peroxidation, neutrophil infiltration, TNF-α production, HMGB1 expression, NF-κB DNA binding activity, and pancreas morphology.

2. Materials and methods

2.1. Animals and reagents

The Experimental Animal Center of China Medical University provided healthy male Wistar rats weighing 250–300 g. Rats were maintained at 23°C on a 12-h light/dark cycle and allowed free access to water and standard laboratory chow. From 12 h before the start of the experiments, we deprived the animals of food but allowed them access to water. This study was conducted with the consent of the Ethics Committee for the Use of Experimental Animals of the Animal Research Center of China Medical University. We purchased the following kits: serum amylase, MDA, and MPO (Jiancheng Company, Nanjing, China); and UltraSensitive SP (Maxim Company, Fuzhou, China). We also purchased goat anti-rat polyclonal anti-HMGB1 antibody (Santa Cruz Biotechnology, Inc, CA); a micro-BCA protein assay kit and enhanced chemiluminescence substrate (Pierce, Rockford, IL); rabbit anti-HMGB1 polyclonal primary antibody (BD Pharmingen, San Jose, CA); rabbit anti-β-actin monoclonal antibody (Invitrogen, Carlsbad, CA); anti-rabbit horseradish peroxidase-coupled secondary antibody (Bio-Rad, Hercules, CA); a TNF-α enzyme-linked immunosorbent assay kit (Sengxiong Biotechnology Co. Shanghai, China); a nuclear and cytoplasmic extraction reagent kit (Pierce Biotechnology, Rockford, IL); and a nonradioactive NF-κB p50/p65 transcription factor assay kit (Chemicon, Temecula, CA). We purchased all other chemicals from Sigma-Aldrich Chemical (St Louis, MO). We prepared EP in solution with sodium (130 mmol/L), potassium (4 mmol/L), calcium (2.7 mmol/L), chloride (139 mmol/), and EP (28 mmol/L) (pH 7.0).

2.2. Animal model

We prepared SAP models according to the method of Aho *et al* [17]. Briefly, we anesthetized rats by intraperitoneal injection of 1% pentobarbital sodium (35 mg/kg body weight) and performed the operation under aseptic conditions. After entering the abdomen via a median epigastrium incision, confirming the bile-pancreatic and hepatic hilus common hepatic ducts, uncovering the pancreas, and identifying the duodenal papilla inside the duodenum duct wall, we used a number 5 needle to drill a hole into the mesenterium avascular area. We inserted a segmental epidural catheter into the duodenum cavity via the hole and in a retrograde direction to the bile-pancreatic duct in the direction of the papilla. We used the microvascular clamp to nip the catheter head temporarily while we employed another microvascular clamp to temporarily occlude the common hepatic duct at the confluence of the hepatic duct. Then, we injected 5% sodium taurocholate (1.5 mL/kg body weight) into the biliopancreatic ducts at an even rate of 0.2 mL/min with a micro-infusion pump. At 5 min after injection, we removed the microvascular clamp and epidural catheter. After checking for bile leakage, we sutured the hole in the duodenum lateral wall. Finally, we closed the abdomen. We housed all rats in individual cages with free access to water.

2.3. Study protocol

We designed the first experiment to determine the effect of treatment with EP (40 mg/kg) after the induction of SAP. We randomly assigned rats to one of three groups. We then randomly divided the groups into 3-, 6-, 12-, and 24-h subgroups, with 12 rats in each subgroup. (1) Control ($n = 48$): in the control group, we injected nothing into the biliopancreatic duct; the remaining procedure was the same as the SAP group. (2) SAP ($n = 48$): this group received an infusion of 5% sodium taurocholate into the pancreatic bile duct. (3) EP-treated ($n = 48$): this group was perfused with EP at a dose of 40 mg/kg body weight through tail vein every 6 h after the induction of SAP (0, 6, 12, and 18 h after SAP). We gave the control group and SAP group rats the same dose of vehicle solution at the same time point. In the second experiment, we randomly divided 45 rats into the control group, SAP group, and EP-treated group, with 15 rats in each group for survival studies. We allowed the rats free access to food and water and recorded the survival time for 48 h.

2.4. Measurement of serum amylase (AMY)

We collected blood samples from the abdominal aorta, conserved them at room temperature for 10 min, and centrifuged them at 3000× *g* for 10 min at 4°C. We kept the serum kept at −70°C until measurement. We determined AMY activity using AMY kits with automated clinical biochemistry analysis equipment (Hitachi Co., Minato-Ku, Tokyo, Japan).