STUDY ON PROTECTING EFFECTS OF PARTHENOLIDE ON HEPATIC INJURY IN RATS WITH SEVERE ACUTE PANCREATITIS

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ABSTRACT

Objective: Our study was designed to investigate the protective effects of parthenolide on hepatic injury in rats with severe acute pancreatitis (SAP).

Methods: The SAP rat models were prepared and randomly divided into five groups: the model control group, parthenolide treated group with different doses of parthenolide, and the sham operated group. The levels of AMY, ALT, AST, IL-6 and TNF-α in serum, NF-κB expression and hepatic pathological changes in all groups were detected.

Results: The levels of AMY, ALT, AST, IL-6 and TNF-α in serum and expression levels of NF-κB were lower in parthenolide treated groups than that in model control group. The model control group showed obviously pathological changes compared with sham group, while the administration of parthenolide dose-dependently inhibited the changes.

Conclusion: Parthenolide demonstrated a well curative capability on rats with SAP.

Keywords: Severe acute pancreatitis; parthenolide; TNF-α; IL-6; NF-κB.

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Introduction

Severe acute pancreatitis (SAP) is one of the most common acute abdomens in clinical practice(1), which can cause systemic inflammatory response syndromes (SIRS) such as effusion of blood vessel, shock and multiple organ functional disturbances or even multiple organ dysfunction syndrome(2-4). SAP generally undergoes two phases. During the first 1 or 2 week, a pro-inflammatory response occurs, which results in systemic inflammatory response syndrome (SIRS), a sterile response in which sepsis or infection rarely occurs.

If the SIRS is severe, then proinflammatory mediators can cause early multiple (respiratory, cardiovascular, renal, and hepatic) organ failure. SAP is often associated with high morbidity and mortality due to the development of pancreatic and extra-pancreatic necrosis, their subsequent infection and multisystem organ failure (MOF)(5-7). Inflammatory mediators is critical for progression of SAP and has been receiving increasing attention in recent years. SAP is a potentially lethal inflammatory condition of the pancreas that involves both peripancreatic tissue and remote organs. Local inflammatory reaction in pancreas leads to systemic SIRS and MOF,
which is believed to be the capital cause of mortality. Inflammatory components include interleukin (IL)-6, tumor necrosis factor (TNF)-α, IL-8, IL-10, and IL-1β are considered to be required for SAP development. A recent study has shown that pro-inflammatory stimuli such as IL-6 and TNF-α play a central role in the initiation and progression of SAP. Despite overall reduced mortality in the last decade, SAP is a devastating disease that is associated with mortality ranging from less than 10% to as high as 85%. Parthenolide is a sesquiterpene lactone that was purified from the shoots of feverfew (Tanacetum parthenium), a traditional herbal medicine that has been used for the treatment of arthritis, fever and migraine in China. The nucleophilic nature of its methylene-γ-lactone ring and epoxide group enables rapid interactions with biological sites. These interactions have been found to be related to its ability to induce oxidative stress, and it shows multiple anti-cancer and pro-apoptotic characteristics. Previous studies have demonstrated that parthenolide has strong anti-inflammatory effects and is a potent nuclear factor kappa-B (NF-κB) inhibitor by specifically inhibiting the IκB kinase complex. Therefore, the present study was focused on the preventive effect of parthenolide in rats model with SAP.

Materials and methods

Chemicals

Parthenolide was purchased from Beijing Institute of Biological Products (Beijing, China) and its purity was determined to be ≥ 98% by HPLC measurement. Sodium taurocholate was purchased from USA Sigma Company. TNF-α and IL-6 enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems, Inc. (Minneapolis, MN, USA). Monoclonal antibodies against NF-κB p65 subunit and β-actin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA).

Animals

Fifty healthy male Sprayer (SD) rats at 250-280 g of body weight (7-8 weeks old) were purchased from the Experimental Animal Center of Suzhou StairMaster technology Co. Ltd. (SPF grade, Certificate No. SCXK20140007). All rats were kept in a regulated environment with a controlled temperature of 22-24°C and humidity of 50-60%, and with free access to food and water. All procedures were in accordance with the Guidelines of the Animal Experiments of Yuhuangding Hospital of Yantai, Affiliated Hospital of Qingdao Medical University.

Model of SAP

The rats were fasted overnight with free access to water 12 h before experiments. Surgical anesthesia was performed by intraperitoneal injection of 10% chloral hydrate. SAP was induced by retrograde infusion of 1% sodium taurocholate (0.1 mL/100 g body weight) into the common pancreatic duct through epidural catheter and duodenal papilla. Five minutes later artery clamp was removed, tube was drawn, the abdominal wall was sutured to establish the animal model of SAP. Duodenum and the pancreas of rats in control group was only injected saline instead of sodium taurocholate then gently put back to the abdomen.

Animal groups

Fifty rats were randomly divided into the sham-operated group, the model control group and parthenolide treated group. In sham-operated group, only exploratory laparotomy was performed, namely after entering abdominal cavity, checking pancreas and duodenum and then closing abdomen. Both sham-operated group and model control group were intraperitoneally infused with tween 80 (0.05%).

ELISA assay

After the completion of treatments, the animals were sacrificed under deep anesthesia with chloral hydrate (300 mg/kg, i.p.) and bloodletting via inferior vena cava. Blood samples were collected and centrifuged (4°C) at 3000 r/min for 15 min. The upper serum was separated immediately and was stored at -20°C for future analysis. ELISA kits (BioSource International, Camarillo, CA, USA) was performed to determine serum levels of TNF-α and IL-6 according to the manufacturer’s instructions. Changes of α-amylase (AMY), alanine aminotransferase (ALT) and aspartate aminotrans-
Ferase (AST) in serum were determined by ELISA kits, respectively.

**Histopathological evaluation**

The hepatic specimens was collected and fixed in 10% formalin for 24 h, embedded in paraffin, and stained with hematoxylin-eosin (HE) and examined by light microscopy.

**Western Blot analysis**

Hepatic tissues were lysed by the nuclear extract kit following the manufacturer’s instructions. SDS-PAGE was performed using 50 or 100 g/l acrylamide gels. Proteins were electrotransfered to polyvinylidene fluoride membranes and probed with primary antibody (anti- NF-κB-p65, 1:500; β-actin, 1:500). The membranes were incubated with corresponding horseradish peroxidase-linked secondary antibody. They were developed by enhanced chemiluminescence (Amersham International, Buckingham, UK), and exposure to X-ray film.

**Statistical analysis**

Data were presented as means ± standard deviation (SD). Statistical comparisons between two groups were analyzed by t test. Values p < 0.05 was considered statistically significant.

**Results**

**Serum AMY, ALT and AST content**

Twelve hours after modeling, the content of AMY, AST and ALT in serum of model control group and parthenolide treated groups were significantly higher than that in sham-operated group (P < 0.05). The parthenolide treated groups were obviously lower than the model control group (P < 0.05), which appeared to be dose-dependent (Figure 1).

**The content of IL-6 and TNF-α in serum**

As shown in Figure 2, 12 h after induction of SAP, the serum IL-6 and TNF-α level in model control group was obviously higher than that in sham-operated group, whereas parthenolide treatment attenuated the increase of serum IL-6 (Figure 2A) and TNF-α (Figure 2B), which appeared to be dose-dependent.

**Pathological findings of liver tissue**

Sham group: Twelve hours after modeling, complete structure of hepatic lobules, occasional inflammatory cell infiltration in portal area were observed. Most liver cells presented normal morphology (Figure 3A). In the model control group, there was obviously damaged hepatic lobule structure, further increased range and area of cell necrosis, residual metamorphic liver cells only at periphery of partial hepatic lobules; relatively large area of inflammatory cell infiltration within lobules or portal area, obvious congestion in sinus hepaticus (Figure 3B). The changes of parthenolide treated group were milder than that of the model control group, which appeared to be dose dependent. The group treated with low or middle dose of parthenolide showed obviously pathological changes than high dose parthenolide treated group (Figure 3C and 3D). The group treated with high dose of parthenolide showed slight swelling of liver cells, mild dilation and hyperemia change of sinus hepaticus.
Punctate necrosis or slight focal necrosis of liver cells, no obvious lamellar necrosis, inflammatory cell infiltration in portal area were also observed (Figure 3E).

Western blot revealed that the expression of NF-κB was significantly (P < 0.05) increased in the nucleus of liver in model control group compared to the sham-operated group. The administration of parthenolide dose-dependently inhibited the elevation of NF-κB in SAP rats (Figure 4).

**Discussion**

Along with the increasing morbidity and mortality of SAP, improving the clinical therapeutic effects on SAP and finding cheap alternates with precise therapeutic effects and fewer side effects have been the hot spots of clinical research. Parthenolide, sesquiterpene lactone existing in the leaves of feverfew, is considered to be the main component with biological activity in the extracts of this perennial plant. Infusions of feverfew containing parthenolide have been applied in patients to prevent migraine and rheumatic pain. Previous studies have demonstrated that parthenolide has strong anti-inflammatory effects and is a potent nuclear factor kappa-B (NF-κB) inhibitor by specifically inhibiting the IκB kinase complex. The present study showed that parthenolide can relieve the severity of hepatic injury in SAP procedure.

Alanine aminotransferase (ALT) is found mainly in liver cells, while aspartate aminotransferase (AST) is found mainly in cardiac muscle, followed by the liver. They are nonspecific intracellular functional enzymes and normally can be found in the serum at very low levels. The membrane permeability of hepatic cells increases when they are damaged. As a result, ALT and AST in cytoplasm are released into the blood, resulting in an increase in the activities and contents of serum ALT and AST. Therefore, serum ALT and AST levels are sensitive parameters for the evaluation of hepatic cell damage. In the present study, the treatment of parthenolide obviously attenuated the content of ALT and AST in serum, which demonstrated the protective effect of parthenolide on hepatic injury in rats with SAP.

A recent study has indicated that pro-inflammatory stimuli such as IL-6 and TNF-α play a central role in the initiation and progression of SAP. The level of IL-6 in serum is a discriminator of SAP, and when accompanied by multi-organ failure could be used as an early marker of a possible fatal outcome. TNF-α can induce plenty of other inflammatory cytokines and activate various immune cells, thus inducing the pro-inflammatory...
response. TNF-α and IL-6 can increase plasma extravasation, induce leukocyte adherence, and then result in SIRS and MODS. Preventing the activity of TNF-α and IL-6 can markedly attenuate the systemic amplification of SAP, and it is responsible for the increased morbidity and mortality associated with SAP. In this experiment, it was found that the content of serum IL-6 and TNF-α was significantly increased in SAP model group, while the administration of parthenolide markedly down-regulated the levels of both IL-6 and TNF-α in serum, which also demonstrated the protective effect of parthenolide on hepatic injury in rats with SAP.

Some studies have found that Nuclear factor kappa-light-chain enhancer of activated B cells (NF-κB) is a key regulator of cytokine induction, which is usually elevated in the pancreas during SAP. Inhibition of NF-κB activation can down-regulate in the release of inflammatory cytokines. NF-κB consists of five major proteins: RelA (p65), RelB, c-Rel, NF-κB1, and NF-κB2. Activated NF-κB is a transcription factor associated with a wide range of cellular responses, including inflammation, immune regulation, survival, and proliferation. The majority of NF-κB activities have connection with TNF-α, foreign antigens, oxidative stress, and exposure to radiation. Phosphorylation of IκB by IκB kinase (IKK) on the cytosolic domain and subsequent ubiquitination and degradation of IκB leads to activation of NF-κB. Interestingly, parthenolide is found to inhibit IKK-mediated phosphorylation of IκB by blocking the activity of IKK, thus leading to suppression of RelA/p65 activity in acute myeloid leukemia cells.

In conclusion, parthenolide can reduce the content of AMY, ALT, AST in serum to improve the survival of rats with SAP. What’s more, it can alleviate the pathological changes of liver. We believe that parthenolide can play certain role in future SAP treatment.

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