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Original article

Early prediction of intestinal mucosal barrier function impairment by elevated serum procalcitonin in rats with severe acute pancreatitis

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ABSTRACT

Objectives: The aim of this study was to evaluate serum procalcitonin (PCT) levels as a prognostic indicator of intestinal barrier function impairment in rats with severe acute pancreatitis (SAP).
Methods: Thirty-six male Sprague Dawley rats were randomly grouped into SAP group (injected sodium taurocholate via biliopancreatic duct), Gln group (gavaged with glutamine after modeling), and control group. Blood, pancreatic, and terminal ileum tissues were obtained from the rats after 6 h of modeling. Serum amylase (Amy) levels were determined using an automatic biochemical detector, while endotoxin (ET), diamine oxidase (DAO), and PCT levels were measured by ELISA test. The pathology of pancreatic and small intestine tissues were observed. PCT protein expression in intestinal tissues were detected by immunohistochemistry and western blot.
Result: Pancreatic and intestinal injuries in Gln group were significantly lower than SAP group. Serum amylase, DAO, and PCT levels in SAP and Gln groups differed greatly and were significantly higher than control group. Immuno-histochemistry and western blot results showed that PCT protein expression levels in small intestine tissues of SAP group were higher than Gln group and control group. Serum PCT levels had a significant correlation with serum endotoxin, DAO levels and intestinal mucosal injury scores.
Conclusion: PCT expression in serum and intestinal tissues in SAP rats increased significantly in the early stages of SAP, and was closely related to the onset and degree of intestinal barrier function impairment. Thus, our results showed that measuring serum PCT can be used to predict intestinal mucosal barrier function impairment in SAP rats.
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In recent years the harmful changes in the standard of living and dietary habits are often accompanied by increasing rates of acute pancreatitis (AP) [1]. Ten to twenty percent of AP cases are diagnosed as SAP, which develops rapidly and is associated with multiple complications and high mortality rates [2]. SAP at an early stage is associated with Systemic Inflammatory Response Syndrome (SIRS) [3,4], and its disease progression can include the development of multiple organ dysfunction syndrome (MODS). A common complication of SAP is bowel function failure, which often precedes an additional organ failure, which serves to promote or aggravate MODS, and influence the development and outcome of SAP [5]. Previous studies have shown that early diagnosis and treatment of intestinal dysfunction protects the intestinal mucosal barrier. It is vital for the treatment of SAP, and can reduce the occurrence of MODS, and improve the prognosis of patients with SAP [6,7]. Currently, there are multiple effective clinical methods to detect bowel dysfunction, whereas the clinical approaches used for detecting SAP have certain limitations. Since SAP is a complex disease with rapid progression, it is important to use a simple effective method to accurately predict the onset and degree of bowel function impairment at an early stage.

Serum PCT has recently gained attention as an effective biomarker for AP that offers high specificity and positive predictive value for SIRS and systemic infection [8,9]. Bacterial endothelin (ET)

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is the major stimulating factor of PCT production [10,11]. Significant increase in serum PCT levels occur in the early stages of SAP and are mainly caused by increased intestinal permeability leading to large volumes of ET release in the blood. In patients with SAP the serum PCT levels are highly correlated with intestinal permeability [12], however the relationships between PCT levels, intestinal function scores and intestinal damage assessment indexes still remains unclear. The utility of PCT detection in assessing bowel function impairment in patients with SAP is not yet well illustrated. In addition, The use of PCT as a specific indicator for predicting SAP-related bowel function impairment requires further investigation.

Materials and methods

Animal experiments

Thirty-six healthy male Sprague–Dawley rats with a body weight of 240 ± 20 g were obtained from Sino-British Sippr/BK Laboratory Animal (Shanghai) Co., LTD (production license: SCXK [Shanghai], 2003–0002) and were housed under pathogen-free conditions in the Putuo Hospital Affiliated to Shanghai University of Traditional Chinese Medicine (animal laboratory license: SYXK [Shanghai], 2005–0008). Rats were randomly divided into 3 groups: the SAP group, the Gln group, and the control group, with 12 rats in each group.

All rats were acclimated in our animal laboratory for more than 2 days. The rats were given nothing but water 12 h prior to the beginning of the experiments. All rats were weighed and anesthetized by intraperitoneal injection with 2.5% sodium pentobarbital (0.2 ml/100 g body weight), after which operations were performed via a midabdominal incision. In the control group, the duodenum and pancreas were observed. In the SAP group, we determined the direction of the biliary and pancreatic ducts and the position of the duodenal orifice. We temporarily occluded the biliopancreatic duct at the level of hepatic hilum with non-invasive clamping and slowly injected 5% sodium taurocholic acid (0.1 ml/100 g body weight) using a trace infusion pump at the speed of 0.1 ml/min via biliopancreatic duct through the duodenal sidewall, and the needle core was kept for 10 min after injection. We pressed the puncture pore gently to check for leakage before the abdomen was closed. For the Gln group, 3% glutamine was administrated by gavage (0.3 g/kg body weight per day) after the SAP model was successfully made [13].

Six hours after the operation, rats from all groups were killed to collect the samples. After sodium pentobarbital anesthesia was administered, the abdominal wall of the rats were opened along the midline incision and abdominal aortic blood was extracted with a 10-ml syringe. Rat blood samples were centrifuged at 3000 rpm for 5 min, and supernatants were aliquoted into 4 separate 1.5 ml Eppendorf tubes and stored at −20 °C for subsequent analysis of serum Amy, ET, diamine oxidase (DAO) and PCT levels. Pancreas and terminal ileum intestinal tissues were washed with normal saline and divided in two equal portions. One half of each tissue section was fixed with formalin for hematoxylin and eosin (H&E) and immunohistochemical staining, and the other half was stored in liquid nitrogen for subsequent western blot analysis.

Serum Amy, DAO, and PCT determinations

Serum Amy determination was performed using a Cobas Integra 400 analyzer (Roche Diagnostics Systems, Switzerland). Serum DAO and PCT determination were performed using ELISA kits purchased from USCN Technology Co., LTD.

Histopathology of the pancreas and small intestine

All tissue biopsies were fixed in 4% formalin for 4 h, dehydrated by conventional gradient alcohol, hyalinized in xylene, embedded with a paraffin wax machine, cut into 4-μm thick sections, attached to slides, and then stained by H&E with an automatic pathological dyeing machine. Next, glass cover slips were added to the specimens with a neutral gum seal. All slides were dried for 4 h in a constant 37 °C temperature oven, and histopathological changes in pancreas and small intestine samples were observed under an optical microscope.

Small intestine histopathologic grading

Small intestinal mucosal injury was scored using Chiu's method [14]. The tissue damage was graded from 0 to 5 according to the following criteria: grade 0, normal structure of villi; grade 2, development of small subepithelial space at the villous apex; grade 2, enlarged subepithelial space but without any change in villous length and width; grade 3, few shortened villi and presence of cells in the lumen; grade 4, the majority of villi are shortened and widened with crypt hyperplasia and presence of cells in the lumen; grade 5, blunting of all villi with elongated crypts and a high number of cells in the lumen.

Detection of PCT protein expression in the small intestine tissue by immunohistochemistry

PCT protein expression in small intestine samples were studied with the ready-to-use immunohistochemical Elivision Plus Kit (KIT 9901, Fuzhou Maxim Company), according to the manufacturer's instructions. The observation of brown, granular staining in the intestinal mucosal layer under an optical microscope was scored as a positive result. Immunohistochemical results were semi-quantitatively analyzed using Image-Pro Plus Medical Image Analysis Software (version 6.0) and 5 visual fields with high intensity of positive expression were selected to calculate the integrated optical density (IOD) of the positive expression areas in each view. The greater the calculated IOD values, the stronger was the protein positive expression. We also determined the average optical density (AOD) values for all tissue sections in each group, where the AOD = IOD/regional tissue area. Data presented as mean ± SD.

Detection of PCT protein expression in small intestine tissue by western blotting

Protein concentrations of tissue specimen lysates were determined using the BCA Protein Quantitative Kit (Beyotime Institute of Biotechnology). Equal amount of protein (50 μg total protein) for each sample was separated by sodium dodecyl sulfate-polyacrylamide gelelectrophoresis (SDS-PAGE) and then transferred to polyvinylidenedifluoride (PVDF) membranes. Nonspecific binding was blocked for 1 h using 5% bovine serum albumin in Tris Buffered Saline with 1% Tween-20 (TBST), PVDF membranes were washed 3 times with TBST and incubated overnight with primary antibodies at 4 °C. Subsequently, the membranes were incubated with secondary antibodies for 1 h and exposed to X-ray film. Images were scanned and bands were quantitated using Image J analysis software. Using this approach, PCT expression was then normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression.

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