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ORIGINAL ARTICLE

Effect of ceramide-1-phosphate transfer protein on intestinal bacterial translocation in severe acute pancreatitis

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Summary
Background and objective: The aim of the study was to investigate the effects of ceramide-1-phosphate transfer protein (CPTP) on the intestinal epithelial tight junction proteins in patients with severe acute pancreatitis (SAP).
Methods: Fifty patients with SAP were classified into two groups according to the presence of bacterial translocation (BT) in the blood. Thirty healthy individuals were included in the control group. The presence of BT was analyzed by polymerase chain reaction. The expression of tight junction proteins and CPTP was determined using immunohistochemistry and western blotting.
Results: Bacterial DNA was detected in the peripheral blood of 62.0% of the patients with SAP. The expression of CPTP and tight junction proteins in SAP patients was lower than that in healthy controls. Among the patients with SAP, those positive for BT(+) showed a lower level of CPTP and occluding (OC) and zonula occludens-1 (ZO-1) expression and a higher level of IVA cPLA2 expression than BT(−) patients. Moreover, the expression of CPTP was significantly associated with ZO-1 and showed a negative correlation with expression of IVA cPLA2 in SAP-BT(+) patients.
Conclusions: CPTP affects the expression of tight junction proteins and may protects the intestinal epithelial barrier by downregulating the expression of IVA cPLA2.

Introduction

Infection and septic complications are the major factors that contribute to the poor outcome in patients with SAP. The annual global incidence of acute pancreatitis is 20–100

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per 100,000 and it has a mortality rate of 20%—30% [1]. The majority of the morbidity and mortality associated with SAP is the result of complications associated primarily with BT [2]. BT from the intestines is thought to be a major source of systemic infection and sepsis in SAP [3,4]. Although the actual route of migration is not known, it has been reported that increased intestinal permeability is involved in the BT associated with SAP [5,6]. BT is defined as the passage of endogenous bacteria that colonize the intestine (or their products) through the intestinal mucosal barrier to the mesenteric lymph nodes and other distant sites [7]. The intestinal mucosal barrier is established by the distribution of microbial flora [8,9] and the tight junctions between intestinal epithelial cells. One of the major factors that promotes BT is damage to the intestinal barrier.

CPTP was discovered and reported in 2013 [10]; it is distributed in the intestines, pancreas and elsewhere. It transports ceramide-1-phosphate (C1P) across membranes. C1P, which is produced by ceramide kinase, is an activator of group IVA cytosolic phospholipase A2 (IVA cPLA2), the rate-limiting releaser of arachidonic acid involved in the production of pro-inflammatory eicosanoids. IVA cPLA2 and eicosanoids are believed to play an important role in inflammation and cell injury [11]. The subsequent increase in intestinal permeability may facilitate bacterial translocation.

Tight junctions are the structural basis of the regulation of intestinal permeability and may play an important role in BT [12,13]. Our preliminary experiment suggested that the expression of CPTP was changed in SAP-associated endogenous infection, but the details of the mechanism are unknown. Therefore, we propose the decreased expression of CPTP affects the function of the intestinal epithelial barrier by increasing expression of IVA cPLA2.

Methods

Patients

The study was performed SAP patients from January 2012 through September 2014 in the Affiliated Hospital of Medical College, Qingdao University and Jinlin Hospital, Nanjing University. Patients with SAP were recruited in accordance with the Consensus of the International Symposium on Acute Pancreatitis (Atlanta definition) [14,15]. Patients with the following criteria were excluded:

- concurrent sepsis or pancreatic infection or peripancreatic infection caused by a second disease;
- patients with acute or chronic gastrointestinal diseases;
- patients sent directly to the intensive care unit for multiorgan failure;
- post-endoscopic retrograde cholangiopancreatography or traumatic or operative pancreatitis;
- pregnancy, malignancy, immunodeficiency or moribund patients regardless of cause within 48 h prior to enrollment.

This study was approved by the Human Subjects Institutional Committee of Affiliated Hospital of Medical College, Qingdao University. All patients provided written informed consent for their participation in this study.

Sample collection

During the first week after the diagnosis of SAP, colonic mucosal tissue was obtained when the SAP patients was treated with colonic irrigation and decompression with endoscopy. Isolation of colonic mucosal cells was performed as described previously [16]. The specimens were placed in RIPA buffer (Tris, NaCl, deoxycholic acid, Triton-X-100, sodium dodecyl sulfate, complete proteinase inhibitor mixture; Roche, Mannheim, Germany) and homogenized. Peripheral venous blood samples were taken on for examination. Samples of colonic mucosal tissue and peripheral venous blood were obtained at the same time for the control group.

DNA extraction and PCR amplification

DNA was extracted using the TIANamp Bacteria DNA Kit (Tiangen, China) according to the manufacturer's instructions. Bacterial DNA was detected as described previously [17]. A broad-range polymerase chain reaction (PCR) for the amplification of a conserved region of the 16S ribosomal RNA prokaryotic gene was carried out using the following universal primers: 5′-AGAGTTTGATCATGGCTCAG-3′ and 5′-ACCGCGACTGCTGCTGGCAC-3′. The primers were located at positions 7-27 and 531-514 (*Escherichia coli* numbering). The total PCR volume was filtered with QIAquick Spin Columns (Qiagen, Hilden, Germany) to remove the remaining primers and analyzed by 2% agarose gel electrophoresis and ultraviolet visualization. The final product was purified by precipitation with ethanol-acetate and analyzed with an ABI PRISM 310 Automated Sequencer (Applied Biosystems, Foster City, CA). Sequences obtained were compared with the database of the National Center for Biotechnology Information (http://www.ncbi.nih.gov). DNA extracted from *E. coli* was used as a positive control, and PCR mixtures (without template) were used as negative control. One colony from a culture of *E. coli* was diluted up to 100,000-fold in sterile water. DNA isolation from 200 μL of each dilution was undertaken.

Measurement of plasma DAO and D(—)-lactate levels

A total of 4 mL of blood was allowed to clot for 20 min at room temperature and centrifuged at 3000 × g for 20 min at 4 °C. Plasma was collected to measure DAO and D(−)-lactate levels by spectrophotometry at 436 nm and enzyme-linked ultraviolet spectrophotometry respectively. The absorbance at 340 nm was recorded.

Immunohistochemical assay

The expression levels of CPTP, IVA cPLA2, OC and ZO-1 were detected by immunohistochemical assays. The sections were dewaxed, hydrated, and then pretreated in a microwave (antigen retrieval). The endogenous peroxidase activity

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