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ORIGINAL ARTICLE

Effect of intestinal epithelial autophagy on bacterial translocation in severe acute pancreatitis

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KEYWORDS
Autophagy;
Bacterial translocation;
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Tight junction proteins

Summary
Background and objective: We examined the impact of autophagy activation on bacterial translocation (BT) and tight junction (TJ) proteins in the intestinal mucosa of patients with severe acute pancreatitis (SAP).
Methods: Thirty-one SAP patients were divided into two groups, BT(+) and BT(−), according to the presence of BT in the blood, as detected by 16S rDNA sequencing. Eight healthy individuals were included in the control group. Serum endotoxin levels were measured by ELISA. Colonic mucosal tissue was obtained by endoscopy, and the TJ proteins and phosphatidylethanolamine-conjugated microtubule-associated protein light chain 3 (LC3-II) were analyzed using immunofluorescence and Western blotting.
Results: The expression of LC3II in patients with SAP was higher than that observed in healthy controls. Patients who tested positive for the presence of BT had a higher level of claudins-2 (CL-2) and a lower level of occludin and Zonula occluden-1 (ZO-1) than BT(−) patients. Moreover, the levels of LC3II in BT(−) patients was higher than that found in BT(+) patients, and occludin and ZO-1 were positively correlated with LC3II.
Conclusions: Autophagy activation in the intestinal epithelial cells of patients with SAP and its effects on BT may act through enhancing para-cellular TJs.

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Introduction

Severe acute pancreatitis (SAP) is defined as a disease due to rapid systemic organ failure and enteric infection, and has a high mortality rate ranging from 10% to 30% [1]. The bacteria that leads to infection and sepsis contributes to most deaths in the later stage of SAP. Bacterial translocation (BT) is defined as the translocation of bacteria (indigenous bacteria within the intestinal lumen, which is adjacent to the epithelial mucosa) to extra-intestinal circumstances. Such bacteria translocate to the mesenteric lymph nodes and other distant sites, which can result in sepsis. A large population of diverse bacteria and toxins derived from the gut are in contact with intestinal mucosa. In this condition, the role of the intestinal barrier is crucial. Moreover, intestinal epithelial cells are essential to the maintenance of immune homeostasis in the gut, as these cells prevent bacterial invasion by the formation of an intestinal mucosal barrier and aid in the production of antimicrobial peptides. Damage to the intestinal mucosal barrier in SAP has been previously demonstrated [2], and has been identified as an important event during the occurrence and progression of SAP. The complications of the infection that are associated with SAP may be a result of BT from the gastrointestinal tract via increased intestinal permeability [3].

Altered tight junction (TJ) proteins contribute to increased intestinal permeability in SAP [4]. Tight junctions are comprised of four types of transmembrane proteins, which include occludin (OC), claudins (CL), junctional adhesion molecules, and tricellulin. Claudins, and OC are the most prominent members among these proteins [5]. Zonula occludens (ZO) proteins are linked with the main transmembrane proteins, and are essential for TJ formation. The TJ barrier is among the innate defense mechanisms of the intestinal mucosa. Moreover, a decreasing TJ barrier is linked with the causation and progression of inflammatory bowel disease by allowing increased antigenic permeation. In our previous study, changes to the TJ, mucosal barrier function and immune defense response may lead to BT [6,7], resulting in subsequent infection and pancreatic necrosis [8]. In a recent study, in vitro experiments were performed using the human colon cancer cell line, Caco-2 cells, and the number of TJs within the mucosal barrier was increased by starvation-induced autophagy [9].

Autophagy is a physiologically and immunologically controlled, ubiquitous, intracellular degradation pathway that sequesters and degrades cytoplasmic targets including macromolecular aggregates, cellular organelles such as damaged mitochondria, and whole microbes or their products [10]. This mechanism is important for the homeostasis and survival of cells. Moreover, autophagy has been suggested to play a role in intestinal cell survival during physiological stress [11]. In addition, previous studies have shown that autophagy is required for resistance to *Citrobacter rodentium* infection in the intestinal epithelium, and plays an important role in host defense against bacterial infection and the regulation of bacterial dissemination [12]. In intestinal epithelial cells, autophagy is essential for host defense against invasive bacteria. The dysfunctional autophagy mechanism leads to chronic intestinal inflammation, such as inflammatory bowel disease. In addition, a number of genome-wide association studies have identified roles for numerous autophagy genes in inflammatory bowel disease [13]. In particular, autophagy has been proposed to be a part of the pathogenesis of enteric bacterial infection [14]. Recent studies have shown the role autophagy plays in epithelial cell interactions, adaptive immune responses, nucleotide-binding oligomerization domain-containing protein 2, also known as NOD2, directed bacterial sensing, and immune-mediated clearance of bacteria in chronic intestinal inflammation [15,16]. In addition, in vitro experiments have been performed using the Caco-2 cells, and the effects of autophagy on the barrier of TJs were found to be linked to the para-cellular permeability for small-sized urea, but not larger molecules [9].

Previous studies have revealed the role of autophagy in host defense against intestinal bacterial infection [17–20] and in para-cellular permeability [9]. However, the role of autophagy in the intestinal epithelium of patients with SAP for the regulation of BT has not yet been described. In this study, we aimed to detect autophagy in intestinal epithelial cells of patients with BT(+) and BT(−) SAP, and its effects on BT and the barrier of TJs.

Methods

Patients

Patients with SAP were admitted to the Affiliated Qingdao Municipal Hospital of Medical College, Qingdao University and Jinlin Hospital, Nanjing University from June 2014 to April 2016. Male and female patients with SAP were included in our study if the onset of upper abdominal pain was detected within 48 h of admission. Severe acute pancreatitis was defined according to the Atlanta classification and definitions by the international consensus criteria on acute pancreatitis, revised in 2012 [21]. During the first 12 h of admission, the diagnosis of acute pancreatitis requires two of the following three features: (1) typical abdominal acute upper epigastric pain; (2) serum amylase and/or lipase levels greater than three times the upper limit of the normal standard; and (3) the results of transabdominal ultrasonography and contrast-enhanced computed tomography [22]. Clinical severity of the disease was assessed by the use of Acute Physiology and Chronic Health Evaluation (APACHE)-II criteria, which is a traditional method of evaluating inflammation or organ failure. Patients were administered according to the standardized protocols of interdisciplinary management, including gastrointestinal decompression, intravenous (iv) fluids, nutritional support and/or organ system support. The patients received antibiotic prophylactic treatment within 48 h after SAP onset that continued until unequivocal clinical improvement was observed. There were eight healthy volunteers (four men and four women, 42.5 ± 12.68 yr) with good health and no history of either pancreatic or gastrointestinal disease, who served as controls. Patients with APACHE-II scores between 8 and 12 points and one of the following clinical features were included in the study: (1) local complications (pancreatic necrosis, pancreatic pseudocyst, pancreatic abscess); (2) organ failure; (3) Balthazar CT grading II or above; and (4) clinical course in the first 3 d showing colonic involvement, severe abdominal distention and colonic irrigation treated by endoscopic decompression.

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