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The gut-liver axis: impact of a mouse model of small-bowel bacterial overgrowth

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ABSTRACT

Background: The mechanisms by which intestinal bacteria impact liver diseases remain poorly understood. The aim of this study was to develop a mouse model of small-bowel bacterial overgrowth and to determine its impact on hepatobiliary injury.

Materials and methods: A jejunal self-filling blind loop (SFBL) was created in C57BL/6 mice. Three weeks after surgery, the mice were euthanized, and bacterial cultures of luminal content of the loop and extraintestinal tissues were performed. Liver and jejunum were collected for histological grading of inflammation and injury. Serum liver biochemistry assays were performed. Hepatobiliary transporter mRNA expression in liver was measured by quantitative real-time polymerase chain reaction. Bile and blood were collected for measurement of total bile acids, phospholipid, and cholesterol. Mice undergoing jejunal transection and reanastomosis and laparotomy only served as control groups.

Results: SFBL induced a dramatic increase in intraluminal bacterial counts, mesenteric lymph node bacterial translocation, and evidence of jejunal and hepatobiliary injury. Significant reductions in hepatic expression of hepatobiliary transporters involved in biliary canalicular export and basolateral uptake were observed in SFBL mice. SFBL resulted in a significant increase in biliary total bile acid concentration, decreases in bile phospholipid and cholesterol output, and an increase in the bile acid/phospholipid ratio.

Conclusions: We have developed a reproducible mouse model of small-bowel bacterial overgrowth with evidence of liver inflammation, altered hepatobiliary transporter expression, and alterations in bile composition. This model may help to elucidate the mechanisms by which gut-derived bacterial factors impact the liver and contribute to the exacerbation of liver diseases and biliary injury.

Introduction

Emerging evidence suggests that intestinal microbiota or bacteria-derived factors play a critical role in the modulation and exacerbation of a number of liver diseases,[1-5] such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), nonalcoholic fatty liver disease (NAFLD), parenteral nutrition-associated liver disease (PNALD), alcoholic liver

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diseases, and cystic fibrosis (CF)—associated liver disease.[6-12] However, the pathogenic link between intestinal microbiota and chronic liver disease requires further elucidation. Furthermore, several studies support the notion that small-bowel bacterial overgrowth (SBBO) and bacterial translocation (BT) may play a crucial role in the progression of liver diseases.[13-16]

The normal human microflora maintains a delicate balance between its constituent parts, numbering 10^{11} bacteria per gram content of the small intestine with over 400 different species.[17] SBBO is defined as an increased number and/or abnormal type of bacteria in the small bowel, and BT is defined as the passage of viable endogenous bacteria or their products from the intestinal tract through the epithelial barrier to the mesenteric lymph nodes (MLNs), systemic circulation, or extraintestinal organs.[18,19] Although studies from both animal models[8,20] and patients with liver disease[13,21] have shown that SBBO and BT are critical in disease progression, the mechanisms by which they modulate intestinal barrier dysfunction and contribute to liver disease are still unclear.

One mechanism by which microbial factors play a role in hepatic function is in the metabolism of bile acids because they participate in the generation of secondary bile acids, which are subsequently taken up by the ileum to enter the enterohepatic circulation.[22] Through modulation of bile, microbiota can interact with the host and impact not only the liver diseases but also other organs and metabolic pathways.[23] This represents one of the crucial manners by which intestinal microbiota communicate with the liver. One such microbe-derived factor, lipopolysaccharide (LPS), present in the outer membrane of gram-negative bacteria, has been demonstrated to be a key mediator in inflammation-induced cholestasis, a process resulting from either a defect in bile formation at the level of hepatocyte or from an impairment of bile secretion and flow at the level of bile ducts.[24,25] LPS and/or LPS-induced proinflammatory cytokines, such as tumor necrosis factor-alpha and various interleukins, mediate the cholestatic effect via inhibition of the expression and function of the hepatobiliary transporter system.[26]

Despite the recognition of the importance of microbial factors, including SBBO and BT in liver disease, there remains a lack of relevant animal models for the investigation of disease pathogenesis. Approximately two decades ago, Lichtman *et al*. surgically created jejunal self-filling blind loops (SFBLs) in rat, and observed bacterial overgrowth in the jejunal loop and mesenteric translocation. Although histologic and biochemical changes were found in rats with SFBL, and antibiotic treatment prevented hepatic injury in SFBL rats, further light could not be shed on the pathogenesis of liver injury associated with SBBO/BT.[27-31] Studying such interactions in a rat model of SBBO is hampering, and genetically-modified mice represent powerful tools to study such interactions. A recent study has described a surgical model of SFBL in mice, using a blind loop created in the distal ileum, to study the role of microbiota in the pathogenesis of ulcerative colitis.[32] In the current study, we describe a mouse model of SBBO/BT using a jejunal blind loop, and we investigate its impact on hepatic injury and function. We hypothesize that SBBO/BT induced by the creation of a jejunal SFBL in mice would result in hepatic injury and alterations in hepatobiliary transporter expression and bile composition.

Materials and methods

Mice

Approximately, 5- to 6-week-old C57BL/6 male mice (weight range, 20-25 gm) were purchased from Charles River (Wilmington, MA). Mice were fed standard chow and had access to tap water *ad libitum*. The mice were maintained in a 12:12-hr day-night rhythm at a constant temperature of 23°C and a relative humidity of 40%-60%. The mice were given liquid diet (microstabilized rodent liquid diet LD101; TestDiet, St. Louis, MO) 24 h before surgery. After surgery, only water was provided *ad libitum* for the first 24 h. Thereafter, all mice were fed liquid diet until euthanasia. The mice's body weights were measured daily. A protocol for this study was approved by the Cincinnati Children's Hospital Medical Center Institutional Animal Care and Use Committee (protocol no. 9D12102).

Experimental design and surgical procedures

The mice were anesthetized with isoflurane. To create SFBL mice, a 2-cm midline laparotomy incision was made, the jejunum was divided 8 cm distal to the ligament of Treitz, and a 3-cm jejunal SFBL was created by an end-to-side anastomosis of the distal jejunal stump to the side of the proximal jejunal limb 5 cm distal to the ligament of Treitz using interrupted 9-0 monofilament suture (Ethicon Endo-Surgery, Cincinnati, OH) (Fig. 1A). For jejunal transection and reanastomosis (JTR) control mice, the jejunum was partially transected at 50% of diameter 5 cm distal to the ligament of Treitz and resutured in interrupted fashion using 9-0 monofilament suture. Sham-operated (Sham) control mice underwent laparotomy without any intestinal transection. We use two cohorts of mice for the study. The first cohort of mice was used for most of the experiments including bacterial cultures, LPS measurement, biochemical study, liver and small-intestine histology, and hepatobiliary transporter expression. For this cohort, we have 11 mice in the Sham group, 13 mice in the JTR group, and 10 mice in the SFBL group, with the unbalanced totals secondary to early postoperative mortality. The second cohort of mice was used for measuring bile flow and composition. We have two groups of mice in this cohort, including eight in the Sham group and eight in the SFBL group.

Bacterial cultures of organs

All mice were euthanized at week 3 after surgery. Mice were anesthetized by triple-mix (12 mL triple-mix solution contained 8.0 mL ketamine at 100 mg/mL, 0.4 mL xylazine at 100 mg/mL, 2 mL acepromazine at 10 mg/mL, and 1.6 mL normal saline), given intramuscularly. Using sterile techniques to expose the abdominal cavity, the skin was opened and then the muscle and peritoneal cavity were opened with a second set of sterile instruments. Peritoneal cultures were performed by swabbing the viscera with sterile cotton-tipped applicator sticks. Portal vein blood of 50 μL was diluted in 500-μl Baltimore Biological Laboratories-enriched thioglycollate medium (BD Biosciences, Sparks,