Video Article

Ileectomy-induced Bile Overaccumulation in Mouse Intestine

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Abstract

Intestinal resection is a common therapeutic approach for human diseases such as obesity, inflammatory bowel disease, Crohn's disease, and colon cancer that often results in severe short bowel syndrome-like adverse effects including bile acid diarrhea, dehydration, electrolyte disturbances, and nutrient malabsorption. Here we introduce a murine ileal resection model, termed ileectomy, to evaluate tissue communication and the maintenance of systemic homeostasis. After ileal resection, circulating blood is permanently devoid of the ileum-specific endocrine hormone fibroblast growth factor 15 (FGF15), which releases its endocrinal inhibition of bile acid synthesis in the liver. In combination with the increased production and abolished reabsorption of bile acids after removing the ileum, mice that underwent surgery suffer from bile salt overaccumulation in the intestine and associated diarrhea, morbidity, and mortality. Novel usage of the surgery model introduced in this study may provide mechanistic and functional insights into ileal control of systemic metabolic regulation in physiology and disease.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55728/>

Introduction

In modern biomedical research, genetically manipulated animal models are widely utilized to glean insights into human diseases. In particular, tissue or cell-specific gain-and-loss functions of genes have been used to study molecular regulation as well as induced biological effects. Despite the advancements in manipulating target genes *in vivo*, there are lingering limitations. First, many cell or tissue specific deletions will affect multiple organs. For example, epithelial gene deletion will eliminate expression in epithelia of multiple tissues. Further, even if deletion is restricted to a specific tissue, spatial control is rarely feasible. For example, in a tissue like the intestine, distinct segments carry out very specific functions that cannot be manipulated with precision *in vivo*. In these situations, resection of the gene-containing tissues is considered to be a more efficient studying approach to determine the mechanistic and functional significance of tissue communication.

Ileectomy is mostly used in patients with Crohn's and inflammatory diseases involving the distal ileum^{1,2,3}. The ileum typically produces several energy storage hormones like fibroblast growth factor 15/19 (FGF15/19), peptide YY (PYY), and glucagon-like peptide 1/2 (GLP1/2); these hormones play important local and endocrine roles in many biological functions^{4,5,6}. Among these hormones, FGF15 has been identified as a robust endocrine inhibitor of bile acid synthesis in the liver. Once reabsorbed into ileal enterocytes, bile acids activate the nuclear receptor farnesoid X receptor (FXR) to stimulate *Fgf15* expression, which subsequently leads to feedback inhibition of hepatic bile acid synthesis⁷. In a recent study, we introduced the mouse ileectomy model in order to study the ileal kruppel-like factor 15 (KLF15)-*Fgf15* signaling axis that regulates circadian bile acid production in the liver⁸. Most importantly, we introduced a novel family, the kruppel-like factors, particularly KLF15, into bile acid biology. Based on functional studies including ileectomy surgery, we determined that KLF15 upregulates bile acid synthesis via an indirect non-hepatic mechanism. Finally, ileal KLF15 is also identified as the first endogenous negative regulator of *Fgf15*.

The intestinal segments descending from proximal to distal regions are responsible for absorption of different nutrients. The ileum is the major segment responsible for bile acid and vitamin B₁₂ (V_{B12}) absorption⁹. An earlier study employed a mouse model of proximal gut resection to study short bowel syndrome; various resection lengths, diets, and suture types were proposed to maintain an optimal post-surgery survival rate¹⁰. Furthermore, a more recent review indicates that ileal resection typically results in more severe disease than other gastrointestinal (GI) segment resections because of the decreased adaptive capacity of the remaining tract¹¹. This topic has gained intensive interests of basic and clinical research groups, whereas the understanding of recovery and the effective therapeutic approaches are still limited.

Bile acid diarrhea results from imbalances in bile acid homeostasis in the enterohepatic circulation^{12,13}. It can be a consequence of ileal resection, gastrointestinal disease, or a result of idiopathic bile acid malabsorption. More than 80% of patients have been found to present with diarrhea after undergoing ileal resection¹⁴. Ileectomy has the potential to be an important surgery model for the investigation of bile acid

diarrhea. In this study, a series of ileal resections provide a gradient assessment of FGF15 deficiency as well as intestinal bile salt malabsorption, overaccumulation, and toxic damages.

Protocol

Animal protocol was reviewed and approved by the Institutional Animal Care and Use Committee at Case Western Reserve University School of Medicine and was conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (8th Edition, 2011). Mice were euthanized by methods compliant with the American Veterinary Medical Association (AVMA) Guidelines for Euthanasia of Animals (2013 Edition). C57BL/6J, male, 8 - 16 week old mice were used in this protocol. Mice were housed in a 12 h dark/light cycle environment.

1. Pre-operative Preparation

1. Transfer mice to a clean cage 24 h before the procedure. Allow animals free access to water. Replace chow food with soft food (gel dietary supplement) 4 - 6 h before the surgery to reduce the contents of the small intestine for surgery.
2. Sterilize all surgical instruments and prepare the single-use sterile surgical materials. Clean the surgery area and the anesthetic nose cone with 70% ethanol.
3. Set up a dissection microscope, isoflurane anesthetic vaporizer, instruments, and a temperature-controlled small animal surgical table for body temperature maintenance. Organize instruments, sutures, and syringes in a location for free access during the surgery.
4. Set up a light source to provide enough light for the surgical area.
5. Prepare sterile 0.9% saline in 5 mL syringe for intestinal and abdominal cleaning.

2. Ileectomy and Anastomosis

1. Anesthetize mice with isoflurane (2 - 3%) in a small animal incubation chamber. Determine adequate anesthetization using the toe pinch technique while the animal is on isoflurane.
2. Remove abdominal hair by applying hair removal product and wipe hair away using surgical sponges while maintaining anesthesia. Place the mouse on a temperature-controlled small animal surgical table (**Figure 1A**) to maintain body temperature at 37 °C. Maintain anesthesia with isoflurane (1 - 2%) through a facemask. Treat the mouse eyes with ocular ointment.
3. Clean the skin using povidone-iodine and 70% alcohol and cover the surgical area of the abdomen with sterile surgical gauze (**Figure 1B**).
4. Make a midline abdominal incision using a surgical scalpel once anesthesia is in effect. Use a cotton-tipped applicator to protect the intestine and pull mouse abdominal muscle with retractors to fully open and expose the abdominal cavity (**Figure 1C**).
5. Locate the cecum. Starting from the cecum, carefully move the connected ileum and part of the jejunum out of abdominal cavity (**Figure 1D**). NOTE: The cecum can be easily identified due to its large size even after fasting.
6. Ligate the upper branch of the superior mesenteric artery with a 7-0 silk suture to occlude the blood supplying the ileal segment that is to be excised. The ileal color changes from pink to dark purple after ligation. (**Figure 1E - F**).
7. Depending on the purpose of the experiment, using scissors, excise and remove 50% or 90% of ileum. NOTE: For sham surgery, do not perform the superior mesenteric artery ligation and do not remove the ileum.
8. Flush the lumen of both ileal ends with 0.9% saline. NOTE: As the remaining intact ileum is still receiving a normal blood supply from the superior mesenteric artery, a small amount of blood will be flushed out during the process. This also indicates that the blood supply to the ileal ends is normal and ensures no ischemia during the anastomosis procedure (**Figure 1G**).
9. Locate the mesenteries on the side of both ileal ends. Align the mesenteries and suture the ileal ends together using 8-0 suture (**Figure 1G - H**).
10. Suture the contralateral side of the ileum to keep the ileum anastomosed in a natural manner (**Figure 1I**).
11. Suture the upper and lower sides between the two original sutures to thoroughly join the two ileal ends together (**Figure 1J**).
12. Confirm that there is no leakage from the anastomosis site after finishing the three-step suturing procedure (**Figure 1G-I**). Return the cecum and the small intestine into the abdominal cavity to the original anatomical location. Wash the surgery area with warm 0.9% saline using a blunt needle. (**Figure 1K**).
13. Close the incision of the abdominal muscle layer with 6-0 suture. Align the abdominal skin incision using forceps and suture the abdominal skin to facilitate optimal wound healing (**Figure 1L**).

3. Post-operative Care

1. Transfer the post-surgery mice to an intensive care unit for recovery. House them in a paper-bedding cage on a temperature-controlled heating pad to continue the post-surgery recovery overnight. Supply mice with soft food in addition to regular food and water.
2. Administrate buprenorphine (0.05 - 0.1 mg/kg) with subcutaneous injection every 8-12 h for analgesia. NOTE: Euthanize mice by CO₂ if severely sick.
3. At the end-point, sacrifice mice using overdosed isoflurane and harvest samples as needed (section 4).

4. Evaluation of Ileectomy-induced Bile Overaccumulation

1. Weigh and dissect the mice one day after a resection of 0% (sham), 50%, or 90% of ileum.
2. Remove the GI tract and weigh it. Calculate the GI weight to body weight ratio to evaluate the severity of bile salt malabsorption and overaccumulation.

3. Transfer the GI tracts into 15 mL conical tubes and cut them into short segments using scissors. After cutting, centrifuge at 3,000 x g for 10 min. Transfer the supernatant (GI fluid containing bile salts) to a clean tube.
4. Measure the total volume and weight of GI fluid and calculate the fluid weight to GI tract weight ratio to further assess bile overaccumulation in the GI tract.
5. Determine the total bile amount in the supernatant by bile acid assay as described in reference ⁸.

Representative Results

The procedures for ileectomy are shown in **Figure 1**. The first step includes preparing mouse abdominal skin, making an abdominal incision, and using retractors to fully expose the intestine (**Figure 1A-C**). Next, the mouse cecum was located (**Figure 1D**); given that its size and shape make it an easily identifiable landmark. The cecum, ileum, and part of the distal jejunum were exposed and removed from the abdominal cavity (**Figure 1D**). Once the resecting ileal segment was determined, the superior mesenteric artery supplying blood to the segment was ligated and the color of the ileum segment rapidly changed to dark purple (**Figure 1E - F**), while the rest of the intestine maintained a normal pink color. The entire ischemic ileal segment was completely resected followed by washing the cut ends with 0.9% saline (**Figure 1G**). The two ileal ends were then sutured together by a three-step suturing process (**Figure 1H-J**). In **Figure 1K-L**, the anastomotic intestine was returned to the original anatomical location in the abdominal cavity and the incised muscle and skin layers were sutured together.

Bile acids produced by the liver are enzymatically synthesized from cholesterol and circulated in the enterohepatic system. They are reabsorbed mostly by the distal ileum and then returned to the liver through the hepatic portal vein. Bile acid circulation between hepatocytes in the liver and enterocytes in the ileum is summarized in **Figure 2A**. In the ileum, bile acids are absorbed by apical sodium dependent bile acid transporter (ASBT) on the luminal side of epithelial enterocytes and transferred by ileal bile acid-binding protein (IBABP) to the basolateral membrane for efflux into the blood via the organic solute transporter (OST). In enterocytes, bile acids also activate FXR to induce the ileum-specific hormone FGF15, which is a potent endocrine inhibitor of bile acid synthesis particularly by downregulating hepatic CYP7A1, the initial and rate-limiting enzyme for bile acid synthesis in the liver. However, *Fgf15* is repressed by ileal KLF15⁸. Our quantitative real-time polymerase chain reaction (RT-qPCR) analysis detected abundant expression of *Fgf15*, *Asbt*, *Ibabbp*, and *Ostb* mRNA in the ileum and less to extremely low expression in the jejunum and rest of the intestine (**Figure 2B-2E**). However, adenomatous polyposis coli (*Apc*) mRNA level (negative control) remained comparable throughout the entire intestine (**Figure 2F**).

These data are consistent with a previous study¹⁵ and further support that the intestinal reabsorption of bile acids occurs primarily in the ileum. These data suggest that bile is critical to nutrient digestion and emulsification for efficient absorption of lipid-soluble nutrients in the proximal small intestine, which biologically fits the primary digestive and absorptive roles of the duodenum and jejunum. Upon arriving at the distal small intestine, the bile has completed its digestive function and is reabsorbed by the ileum to return to the liver and remain part of the enterohepatic circulation. Bile reabsorption also allows bile acids to perform one of their signaling functions by stimulating the ileum to produce the secondary signaling molecule FGF15, which travels to the liver and suppresses bile acid synthesis. In our previous study, we proved the regulatory effect of an ileal KLF15-*Fgf15* axis on hepatic bile acid synthesis by a surgical resection of ~90% of ileum⁸. Bile acid synthesis in the liver loses the inhibitory control by FGF15 after the crucial mechanical removal of ileum, which also causes bile acid malabsorption in the intestine. The large amount of bile is consequently overaccumulated in the intestine causing severe adverse effects including diarrhea, cholestasis, and loss of gastrointestinal activity. To evaluate the effect of resection magnitude and determine a safe ileectomy model, we used C57BL/6J mice to perform 0% (sham), 50%, and 90% resection of the ileum. After 24 h, the intestinal morphology demonstrated characteristic gradient changes (**Figure 3A-3D**). The intestine in sham surgery was normal and comparable to that of non-surgery mice. Removing 50% of ileum caused some bile accumulation, increased intestinal size, and dilation one day after resection. However, the mouse that underwent 90% resection exhibited increased bile overaccumulation, increased intestinal size and severe dilation. To evaluate the ileal resection-induced bile overaccumulation in the intestines, internal fluid from the post-surgery GI tracts were collected. The fluid volumes exhibited a gradient enhancement from 0% to 90% in ileectomy mice (**Figure 4A**). The ratio of GI weight versus body weight was significantly increased only in the 90% ileum-resected mice (**Figure 4B**). The GI fluid collected from the post-surgery GI tracts and the GI fluid weight to GI tract weight ratio demonstrated a gradient increase due to resection (**Figure 4C and 4D**). Finally, the bile acid assay confirmed that the amount of bile acids detected in the supernatant increases with increase in ileal resection (**Figure 4E**). Collectively, the results indicate that the 50% ileum resection is a more applicable mouse ileectomy model with relatively moderate adverse effects such as mild bile acid accumulation in the intestine.

Figure 1: Surgery Procedures of Ileectomy. (A) Maintain body temperature using a temperature-controlled small animal surgical table. (B) Cover the surgery area with sterile gauzes after abdominal skin preparation. (C) Expose the small intestine through a midline abdominal incision. (D) Cecum (highlighted by the white dashed line) is a marked to locate the ileum. Expose the ileum (yellow dashed line) and superior mesenteric artery branches (yellow arrows). (E) Ligate the branches of the superior mesenteric artery, which supply blood to the ileum. Ligation sites are indicated by yellow arrows. The ileal color changes to dark purple or black following the artery ligation. The ischemic segment is highlighted by the yellow dashed line. (F) The dashed line indicates the demarcation of ischemic and normally perfused ileum; the arrow indicates ischemic area of the ileum. (G) Cut and remove the ischemic part of the ileum and flush the ends (yellow dashed lines) with 0.9% saline. The yellow arrows indicate the mesenteric sides. (H) Suture the ileal ends together and keep the mesentery aligned. The first suture on the mesenteric side is indicated by the yellow arrow. (I) Suture the contralateral side of ileum (yellow arrow). (J) Suture the upper and lower sides of ileal ends to completely join the ileum together. The sutures are indicated by the yellow arrow. (K) Return the cecum and the small intestine into the abdominal cavity. The anastomosis area is highlighted by the yellow dashed circle. The pink color indicates no ischemia in the anastomosis area. (L) Close the incision of the abdominal muscle layer and suture the skin incision. [Please click here to view a larger version of this figure.](#)

Figure 2: Bile Acid Synthesis in Hepatocytes and Reabsorption by Enterocytes in the Ileum. (A) Schematic picture of bile acid synthesis in the liver, reabsorption primarily in the ileum, and return to the liver via the hepatic portal vein. Bile acids are synthesized from cholesterol by a series of enzymes including the initial and rate-limiting cytochrome P450 enzyme CYP7A1 in hepatocytes. Most bile acids are reabsorbed by the transporter ASBT on the luminal side of ileal enterocytes. Absorbed bile acids are associated with IBABP and effluxed into the portal vein via the basolateral transporter OST for transport back to the liver. Inside enterocytes, bile acids also activate the nuclear hormone receptor FXR to induce the ileum-specific hormone FGF15, a robust endocrine inhibitor of hepatic bile acid synthesis. In contrast, ileal KLF15 inhibits FGF15 expression. (B-F) Relative expression of *Fgf15*, *Asbt*, *Ibabp*, *Ostf*, and *Apc* mRNA in the whole intestine ranging from the proximal to distal segments including duodenum (D), jejunum (J), ileum (I), and colon (C) (n = 5). Error bars $\pm$ SD. * P < 0.05 vs. duodenum by Student's *t*-test. [Please click here to view a larger version of this figure.](#)

Figure 3: Gradient Resections of Ileum and Cholestasis in Intestine. (A) Mouse small intestine exhibits no dilation after the sham surgery. The inner contents show digestive chyme. (B) After 50% ileal resection, the small intestine shows moderate dilation with significant cholestasis. (C) After 90% ileal resection, the small intestine shows significant amount of bile accumulation and excessive dilation. (D) The GI tract (from the stomach to the rectum) was isolated from the mice with sham surgery (left), 50% ileum resection (middle), and 90% ileum resection (right). [Please click here to view a larger version of this figure.](#)

Figure 4: Bile Overaccumulation Caused by Ileectomy. (A) Internal fluid from mouse intestines 24 h after sham, 50%, and 90% ileectomy. (B) The ratios of the GI weight versus body weight in sham, 50%, and 90% ileectomy surgeries (n = 5). (C) The GI fluid volumes. (D) The ratios of GI fluid weights versus GI weights. (E) Total bile acids in the fluids collected from intestines after sham, 50%, and 90% ileectomy (n = 5). GI: gastrointestinal, GIW: gastrointestinal weight, BW: body weight. Error bars ± SD. * P < 0.05 vs. sham, # P < 0.05 vs. 50% ileectomy by One-way ANOVA. [Please click here to view a larger version of this figure.](#)

Discussion

In order to perform a successful ileectomy, the superior mesenteric artery must be ligated in advance to block the blood supply to the resecting segment. The ischemic ileal segment will turn dark purple after ligation. The ileal segment must then be completely resected. A normal blood supply must be ensured for the retained ends. This is essential to avoid bleeding and improper removal, which can easily cause surgical failure due to ischemic necrosis after suturing. During the anastomosis, it is important to join the ileal ends together in their natural anatomical position. Mesentery is considered the most reliable landmark for the initial suture to avoid the potential occurrence of twisted ends. Following the second suture on the contralateral side of ileal ends, the two ends are positioned in accordance with their natural anatomical structure. After the intestinal anastomosis, the color of the anastomotic area must be checked. The area usually demonstrates a normal pink color. If the area turns purple after the operation, the suture zone may be suffering from ischemia. This may be due to incomplete excision of the ischemic segment or twisting of the two ends while suturing. Under these conditions, the surgery has to be corrected immediately.

The major complications of the ileectomy surgery are bile acid malabsorption-induced toxicity, postoperative intestinal obstructions, and anastomotic leakage. Compared with other parts of the intestinal tract, the ileum contains multiple structural and functional specificities, which are more difficult to be compensated by the rest of the intestine. In comparison to younger mice, the same ileal resection may cause severe damage to older mice, possibly because more bile is produced in older mice. For instance, we noted that bile accumulation was greater in age 16-week old mice than in age 8 week old mice. It is unclear whether gender differences affect ileectomy-induced adverse effects and requires further studies.

The resection of ileal length depends on the purpose of the experiment. In our hands, a removal of 50% ileum is enough to induce significant cholestasis with reduced contractility of the intestine. Too much removal, particularly up to 90% ileum, causes excessive cholestasis and complete loss of intestinal peristalsis. The mice undergoing excessive ileal resection are usually incapable of surviving more than five days. Resecting a shorter segment (e.g. less than 50% ileum) is an effective modification in utilizing the ileectomy as a relatively long-term surgery model. Post-surgery therapy with bile acid sequestrants (e.g. cholestyramine) may be necessary to attenuate bile overaccumulation¹⁶.

We explored a range of ileectomy models with various resection lengths and determined the optimal resection conditions that result in mild bile acid overaccumulation and toxicity in the intestine. Thus, this study provides a mouse model to gain a deeper functional and molecular

understanding of ileectomy-induced diarrhea, lipid-soluble vitamin malabsorption, and steatorrhea. However, the limitation of this study is that we only performed a short-term experiment. A long-term study needs to be further investigated in the future.

FGF15 has been reported to be a long-term insulin regulator important for glucose and protein metabolism. In addition, it plays a role in bile acid homeostasis^{17,18}. The current study may enable us to gain an understanding of FGF15-mediated regulation of glucose and protein metabolism in individuals with ileal resections. Finally, ileal resection also affects the synthesis and circulating levels of other peptide hormones involved in metabolism. For instance, blood concentrations of PYY and GLP, which are synthesized by enteroendocrine L-cells in the distal ileum and inhibit gastric acid secretion and intestinal motility, are usually altered following the surgery^{6,19}.

In conclusion, we have established an acute ileal resection model inducing moderate to severe bile overaccumulation in the mouse intestine. The gradient resections of mouse ilea may be applied to study the complex short bowel syndrome such as bile acid homeostasis and toxicity in the enterohepatic system. In addition, moderate resection of ileum is closely similar to the surgery used for patients with Crohn's disease. Therefore, these models will be beneficial for the study of inflammatory and fibrotic responses in the small bowel with resecting surgical operations. Finally, the mouse surgery models provide a convenient platform for research and clinical assessment of therapeutic treatments for ileectomy-induced adverse effects and diseases.

Disclosures

The authors have nothing to disclose.

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