Video Article

Surgical Models of Gastroesophageal Reflux with Mice

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Abstract

Multiple surgical procedures have been reported to induce gastroesophageal reflux in animals. Herein, we report three surgical models with mice aiming to induce reflux of gastric contents, duodenal contents or mixed contents. Surgical procedures and general principles have been described in detail. A researcher with surgical experience should be able to grasp the technique after a short period of practice. After surgery, most mice can survive and develop reflux esophagitis similar to that in humans. However, it should be noted that histological differences between mouse and human esophagus are the inherent limitations of these surgical models. If used for research on Barrett's esophagus and adenocarcinoma, these procedures may need to be combined with genetic modifications.

Video Link

The video component of this article can be found at http://www.jove.com/video/53012/

Introduction

Gastroesophageal reflux disease (GERD) is a chronic disorder caused by the prolonged exposure of distal esophagus to gastric or gastroduodenal contents[1]. Prolonged exposure to these noxious refluxates impairs the intrinsic defenses within the esophageal epithelium and thus results in esophagitis[2]. Barrett's esophagus arises in the setting of chronic reflux, and is a premalignant lesion with increased risk of esophageal adenocarcinoma[3,4]. Despite the clinical importance, the mechanisms of GERD, Barrett's esophagus and adenocarcinoma have not been well understood.

Animal models are essential for research on etiology, pathology, molecular mechanisms, prevention and treatment of human diseases. Up to date, various animal models of GERD, Barrett's esophagus and adenocarcinoma have been developed using model animals[5,6]. Mouse esophagus is lined with stratified squamous epithelium which is histologically similar to that in human esophagus. Although a mouse esophagus is different from human esophagus in terms of keratinization and the absence of submucosal glands, the mouse is still an appealing model animal because of its relatively low cost of maintenance and its potential of sophisticated genetic modifications. Two approaches are commonly used to model GERD, Barrett's esophagus and adenocarcinoma in mice: reflux surgery and genetic modification. Reflux surgery is the best way to induce reflux and genetic modifications mimics molecular alterations[5,7]. Reflux surgery can be combined with genetic modifications to further understand disease mechanisms[8].

Many surgical procedures have been reported by us and others[6,9]: (1) gastric reflux: pyloric ligation, pyloric constriction with forestomach ligation, Wendel cardioplasty, and esophagogastric anastomosis; (2) mixed reflux: esophagogastroduodenal anastomosis, esophagoduodenostomy (or esophagojejunostomy); (3) duodenal reflux: esophagogastroduodenal anastomosis plus gastrectomy; (4) reflux of chemical components: bilious reflux, pancreatic reflux, esophageal perfusion; and (5) esophageal transplantation[5]. Recently a microsurgical mouse model was reported to produce jejunal reflux via an esophagojejunostomy with magnets[10]. These surgical models have advantages over *in vitro* cell culture or organotypic culture models. *In vitro*, esophageal cells cannot tolerate a medium with high acidity or high concentrations of bile acids. Unconjugated bile acids which are commonly used to produce changes in esophageal epithelial cells *in vitro* are usually not present in the duodenal refluxate *in vivo*. Thus conclusions drawn from such *in vitro* studies should be taken with caution.

Surgery on the mouse esophagus remains a technical challenge because of its small size. A low rate of postoperative survival does not allow experiments which require certain sample size to reach statistically sound conclusions. In the past we have successfully developed and characterized surgical models of gastric reflux, mixed reflux, duodenal reflux with mice in long-term experiments[9,11,12]. We have also provided consultation to several other groups in their mouse surgery. Herein, we describe three surgical procedures in mice in order to help the community to establish these models in their labs.

Protocol

All the animal experiments have been approved by the Institutional Animal Care and Use Committee.

1. Mouse Preparation

1. Use mice over ~20 g in body weight or above ~6 weeks in age for surgery.
2. Before surgery, give mice a laboratory chow and water *ad libitum* and maintain on a 12:12 hr light-dark cycle.
3. Shave the surgical area with a hair clipper. Anesthetize mice by intraperitoneal injection of 80 mg/kg ketamine and 12 mg/kg xylazine. This dose puts mice into sleep in a few minutes and provides sufficient anesthesia for the following surgical procedures.
4. Confirm proper anesthetization based upon the absence of corneal reflex and limb retraction when the footpad is pinched. Apply lubricating ophthalmic ointment on eyes to prevent dryness.
5. Sterilize skin with betadine solution.

2. Gastric Reflux Model (Figure 1B)

1. Make an upper midline incision of ~2 cm starting from the xiphoid pointing to the anus.
2. Open up the abdominal cavity through the midline. Remove the xiphoid to enhance exposure with a pair of scissors.
3. Separate and cut the connective tissues between the liver and the stomach. Ligate and cut the vessel bundle between the spleen and the fundus in order to completely free up the fundus.
4. Turn the fundus slightly to the left and expose the left side of the gastroesophageal junction in the field. Make a 5 mm longitudinal incision on the muscle along the distal esophagus using a pair of sharp scissors to expose the epithelium.
5. Cut open the epithelium along the same direction (incision 1, **Figure 1B**). Make three incisions on the forestomach (incision 2, 3 and 4, **Figure 1B**) to cut off most of the forestomach with sharp operating scissors. If the stomach is not empty, carefully remove gastric contents.
6. Place an 8-0 prolene suture (with a taper point needle) through Point a (on the esophagus) and Point a' (on the forestomach) with accurate mucosal to mucosal opposition. Likewise place sutures through Point b and Point b', and other pairs of Points, respectively. Evenly space these sutures 3-4 mm from each other.
7. Wash the abdominal cavity with normal saline to clean up blood and gastric contents. Close the abdominal wall with silk sutures and the skin with metal clips.

3. Mixed Reflux Model (Figure 1C)

1. Follow steps 2.1 to 2.4 to expose the gastroesophageal junction.
2. Gently separate the dorsal side of the esophagus from the blood vessels behind the esophagus. Pass a small cotton tip between the esophagus and blood vessels.
   Note: Cotton may be partially removed from the tip to reduce its size. This cotton tip serves two purposes, lifting up the esophagus and protecting the blood vessels.
3. Make two 5 mm longitudinal incisions each on the gastroesophageal junction and the proximal end of duodenum adjacent to the pylorus with sharp operating scissors. For the incision on the duodenum, avoid blood vessels and place on the anti-mesenteric border.
4. Anastomose the incisions with accurate mucosal to mucosal opposition with interrupted 8-0 Prolene sutures. Usually place 3-4 sutures on the dorsal side and 2-3 sutures on the front side.
5. Remove the cotton tip. Wash the abdominal cavity with normal saline and close the abdominal wall and the skin.

4. Duodenal Reflux Model (Figure 1D)

1. Follow step 3.1 to 3.4 to generate mixed reflux.
2. Carefully lift up the stomach to expose its back side. A lobe of the liver may be caught by connective tissues between the liver and the back of the stomach. Carefully cut the connective tissues, properly protect the liver, and expose the blood vessels on the dorsal side of the esophagus.
3. Ligate and cut the blood vessels. Ligate and cut the esophagus at the gastroesophageal junction.
4. Ligate the duodenum at the pylorus. Ligate and cut the mesenterium. Remove the whole stomach.
5. Wash the abdominal cavity with normal saline. Close the abdominal wall and the skin.

5. Post-surgical Treatment

1. After surgery maintain body temperature with a heating pad. Only after regaining sternal recumbency, place the mice back with other animals.
2. Give antibiotics and analgesic to prevent infection and alleviate pain. Inject Baytril (10 mg/kg, *i.p., q.d*, for 3 days) to prevent infection, and Buprenorphine hydrochloride (0.05 mg/kg, *s.c., b.i.d*. for two days) as painkiller. Fasting is not necessary after surgery.
   A liquid or soft diet may be given.
3. Assess the general health condition on daily basis.If the mice show the following signs (dramatic weight loss >15%, lack of appetite, vocalization, discharge from the mouth, nose or eyes, hunched gesture, inactivity, abnormal grooming activity), euthanize the mice.

Representative Results

Most mice (>95%) can survive the surgery. During the perioperative period, the leading causes of death include overdose of anesthetics, bleeding and unknown reasons.

Four weeks after surgery, >90% mice with gastric reflux or mixed reflux and >80% mice with duodenal reflux can survive. During this period, mice primarily die of esophageal stricture and inability to eat. These mice show signs of severe stress (hunched posture, inactivity, vomiting, sunken eyes, vocalization, *etc.*) and need to be euthanized. Medical treatment with antibiotics and infusion usually cannot save these mice from dying. Mice usually lose their body weight by a couple of grams after surgery, especially those with duodenal reflux. Nevertheless body weight will recover and increase later on.

Forty weeks after surgery, >80% mice with gastric and mixed reflux and >70% mice with duodenal reflux can survive. During this period, some mice may die from malnutrition or unknown reasons. We have kept mice for 80 weeks after surgery. In general, they are still quite active and healthy[9].

At four weeks after surgery, the architecture of esophageal epithelium will be damaged more or less by reflux. Under microscope, the epithelium becomes thicker and the papilla elongates. Hyperproliferation of epithelial cells and infiltration of inflammatory cells (*e.g.*, neutrophils, mast cells and eosinophils) are obvious signs of esophagitis (**Figure 2**). Cytokines in esophageal epithelium (*e.g.*, IL1β, IL6 and IL8) are elevated particularly in the presence of duodenal reflux[11]. Under transmission electron microscope, dilated intercellular space reflects the impairment of epithelial barrier function[12]. Function-wise, trans-epithelial electrical resistance is dramatically decreased, especially in the presence of duodenal reflux.

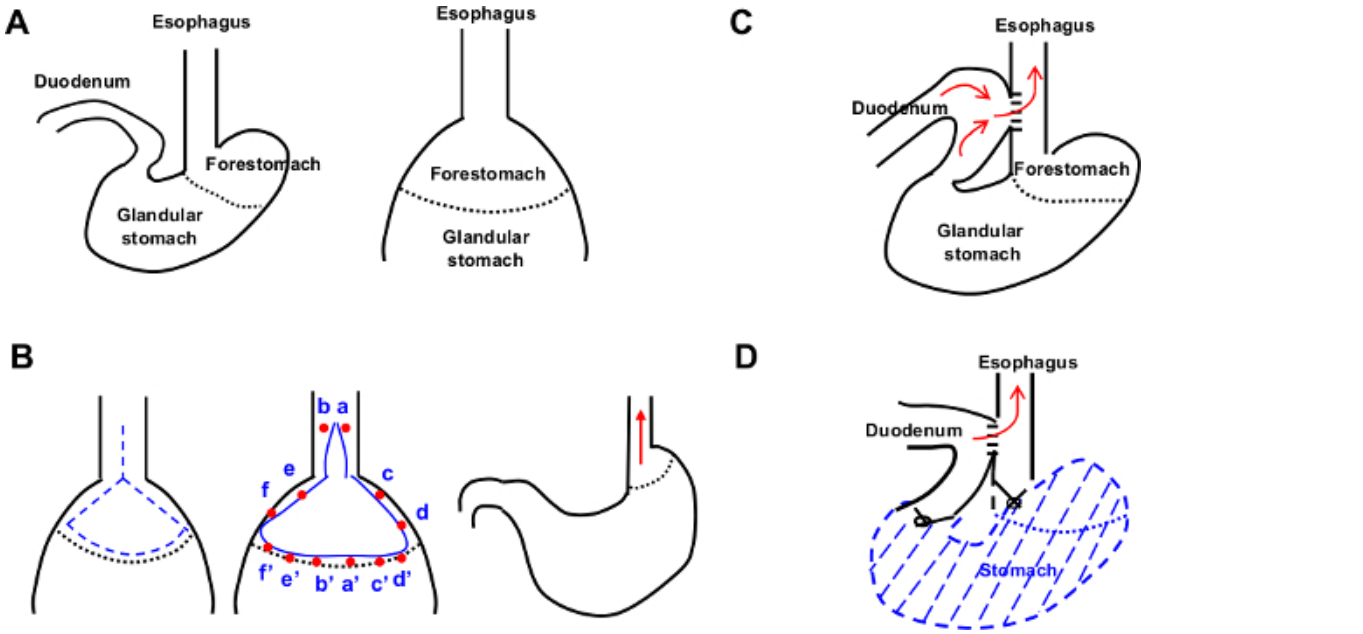

Figure 1: (**A**) Anatomy of the upper digestive tract in mice (front view and side view); (**B**) Gastric reflux (side view); (**C**) Mixed reflux (front view); (**D**) Duodenal reflux (front view).

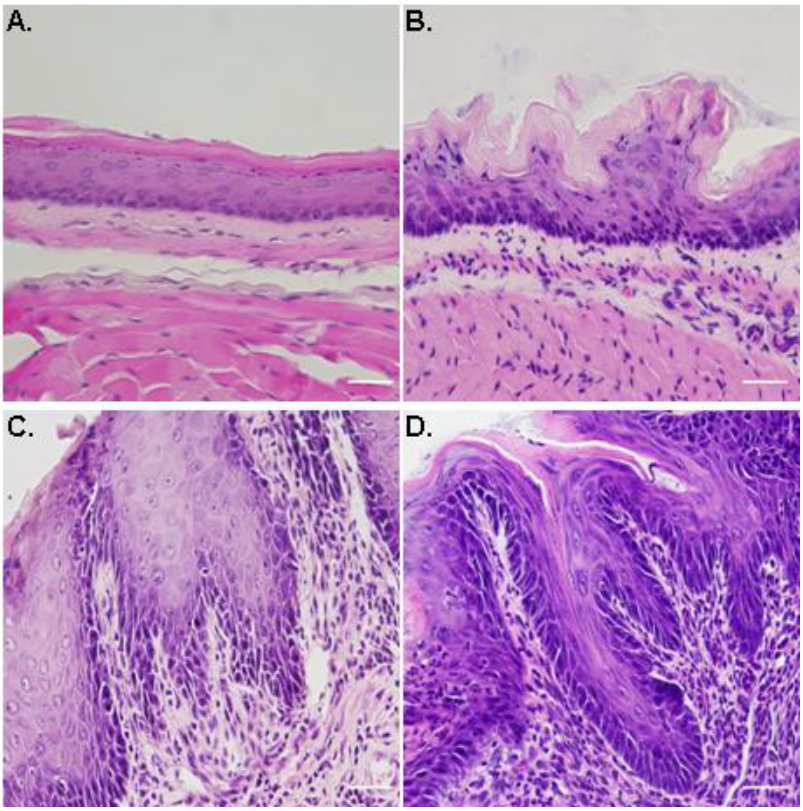

Figure 2: Histology of mouse esophageal epithelium without reflux (**A**), or with gastric reflux (**B**), mixed reflux (**C**), duodenal reflux (**D**). Scale bar = 50 µm.

Discussion

Various surgical models have been established to mimic gastric, duodenal and mixed reflux in rodents. These three procedures described here are suitable for long-term experiments with reasonable rates of postoperative survival. A researcher with surgical experience should be able to grasp the technique after a short period of practice.

Bleeding may result from intraperitoneal injection of anesthetics before surgery, laceration of the liver during separation of the connective tissues between the liver and the stomach, and inadvertent damage of the blood vessels. This should be avoided as much as possible. Battery-operated mini-cautery may be used to stop bleeding if appropriate. Excessive stretching should be avoided as much as possible when organs and tissues are manipulated. The tips of the tweezers may be wrapped with cotton, or cotton tips may be used like chopsticks to replace tweezers. The mouse esophagus has two layers or two “tubes”, outer reddish “muscle tube” and inner whitish “epithelium tube”. When an incision is made on the esophagus, both layers need to be cut open. Sutures should be evenly spaced with accurate mucosal to mucosal opposition. Leakage may result from a wide distance between the sutures, whereas stricture may develop due to a narrow distance between the sutures. However, stricture may block the passage of food and cause postoperative death, whereas slight leakage may be fixed by local adhesion and fibrotic reaction and thus is usually not life-threatening.

There are several critically general principles to follow within the protocol. The duration of surgery should be shortened if possible (about 20-30 min in the lab). Fasting before and after surgery is unnecessary. In fact, prolonged fasting (>1 day) may be harmful. Surgery is best performed by one surgeon. An assistant may help anesthetize the mice and supply surgical materials. A microscope is not necessary unless the surgeon has been properly trained in microsurgery. Otherwise the duration of surgery tends to be longer than necessary and thus postoperative survival may be potentially worse.

These procedures have been used in previous publications and have generated satisfactory results. Using wild-type and genetically modified mice, we demonstrated that NFκB and Nrf2 pathways regulated the barrier function of esophageal epithelium during gastroesophageal reflux[11,12]. However, we were not able to produce full-blown Barrett’s esophagus or adenocarcinoma in these mice, instead, scattered mucinous cells and squamous cell carcinoma were induced[9]. Histological differences between mouse esophagus and human esophagus may be the inherent limitations of these surgical models in research on Barrett’s esophagus and esophageal adenocarcinoma.

We believe these surgical models are useful for studies on GERD. Functions of genes and molecular pathways can be elucidated to understand the molecular mechanisms of GERD and develop mechanism-based therapy. When used for producing Barrett’s esophagus and esophageal adenocarcinoma, genetic modifications will be needed in combination with reflux surgery.

Disclosures

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