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Changes in morphology and function in small intestinal mucosa after Roux-en-Y surgery in a rat model

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ABSTRACT

Background: Currently there is no an appropriate model to study intestinal mucosal atrophy *in vivo* that preserves the nutritional status of the organism.
Materials and methods: We created a defunctionalized segment of jejunum via a dead-end Roux-en-Y anastomosis in rats. We compared tissue morphometric parameters in the intestinal mucosa of the defunctionalized bowel with that of the mucosa proximal and distal to the anastomosis. We further measured extracellular signal-regulated kinase (ERK) activation within the mucosa as well as sucrase-isomaltase and dipeptidyl peptidase-4 levels as markers of intestinal mucosal differentiation by Western blotting of mucosal scrapings.
Results: Three days after anastomosis, the defunctionalized bowel exhibited decreased diameter and thickness of both the mucosa and the fibromuscular layer compared with adjacent bowel in continuity for luminal nutrient flow or with bowel from control animals. Sucrase-isomaltase and dipeptidyl peptidase-4 levels also were decreased. Furthermore, mucosal ERK activation, assessed as the ratio of phosphorylated to total ERK, also was reduced. Animal weights did not differ between bypassed and control animals.
Conclusions: Deprivation of nutrient flow in a segment of bowel by defunctionalizing Roux-en-anastomosis produces mucosal atrophy as indicated by altered histology, differentiation marker expression, and ERK signaling, in animals that are otherwise able to maintain enteral nutrition.

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1. Introduction

The intestinal mucosa atrophies during fasting and in patients with ileus or sepsis who are not eating. Total parenteral nutrition (TPN) of laboratory animals has been used as a model to study mucosal atrophic processes [1,2]. Although TPN induces intestinal atrophy [3], it also has profound effects on intraepithelial cytokine signaling [4] and mucosal immune defense [5,6] that may be independent of the mucosal atrophy itself. TPN also promotes ion transfer and permeability across the intestine in experimental animals [7,8] that may change the pharmacokinetics or effects of drugs being tested for use in patients with mucosal intestinal pathology.

In addition to growth factors and intraluminal nutrients, repetitive mechanical deformation engendered by peristalsis or villous motility also modulates intestinal epithelial biology

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[9–11]. *In vitro*, repetitive deformation stimulates the proliferation of intestinal epithelial cells on collagen substrates, modulates differentiation marker expression, and activates p38 and c-Jun N-terminal kinase (JNK) signaling in intestinal Caco-2 cells in response to repetitive deformation [12]. *In vivo*, repetitive deformation alone stimulates intracellular signaling within small intestinal or colonic mucosa in anesthetized rats [13]. Suppressed contractility and diminished luminal nutrient flow reduce metabolism and initiate mucosal atrophy [1]. *In vitro*, extracellular signal-regulated kinase (ERK) signaling critically mediates the effects of repetitive deformation on human Caco-2 intestinal epithelial cells [14]. However, the signaling mechanisms involved in mucosal atrophy have not been characterized *in vivo*. We hypothesized that creating a blind Roux-en-Y anastomosis in rat jejunum will lead to mucosal atrophy and reduction of function in the defunctionalized intestinal segment, which is deprived of luminal flow, while still permitting normal enteral nutrition and preserving normal biology in other segments of the intestine in the same animal.

2. Methods

2.1. *Animals and surgical procedures information*

All experiments were approved by the University Laboratory Animal Resources at Michigan State University. Three-month old female Wistar rats were purchased from Charles River (Wilmington, MA) and were housed using a 12 h light/12 h dark cycle. Diets and water were provided *ad libitum*. Control animals were maintained on regular diet until one day before harvest. Sham- and anastomosis-operated rats were kept on liquid diets for 2 d before surgery and killed 3 d postoperatively. After surgery, each animal was maintained on liquid diet for 3 d. An additional group of animals was kept for 30 d after defunctionalizing anastomosis, and samples were then analyzed morphologically. Sham-operated animals served as controls.

2.1.1. *Roux-en-Y anastomosis*

To create a defunctionalized segment of jejunum in continuity with the remainder of the bowel, we performed a defunctionalizing Roux-en-Y anastomosis in rats through a midline laparotomy. The jejunum was divided 1 cm from the ligament of Treitz using 5–0 silk sutures (Ethicon, Inc, Somerville, NJ). The proximal jejunum (proximal limb) was anastomosed to the distal jejunum (distal limb) 3 cm more distally than the original transection with a side-to-side anastomosis using running 7–0 vicryl sutures (Fig. 1A and B). In control-operated animals, the abdominal wall was opened and the intestine was manipulated and measured. In sham-operated animals, the small intestine was transected twice in the jejunum at sites 4 cm apart, at sites equivalent to those in which the experimental defunctionalizing Roux-en-Y anastomosis transections were performed. However, the bowel was then reconstructed in continuity with two end-to-end anastomoses constructed with 7–0 vicryl suture. The abdominal cavity was closed using a running 5–0 vicryl suture. The skin was closed by using surgical staples.

2.2. *Morphometric analysis*

Samples of the intestinal segments from the proximal, distal, and Roux limbs were fixed in 10% formalin for 24 h and embedded in paraffin. Step sections (4.0 μm thick) were prepared from all the blocks and stained with hematoxylin and eosin (H&E). Sections were visualized and photographed on a Nikon Microphot-FXA (Nikon, Tokio, Japan). Villous height and thickness of the fibromuscular layer was measured on transverse sections stained with H&E using ImageJ software (NIH, Bethesda, MD) each compared with bowel proximal and distal to the defunctionalized segment from the same animal and with specimens from sham-operated animals at the same sites.

2.2.1. *Protein isolation and Western blotting analysis*

Mucosal scrapings from target intestinal segments after sacrifice and harvest were immediately immersed in ice-cold

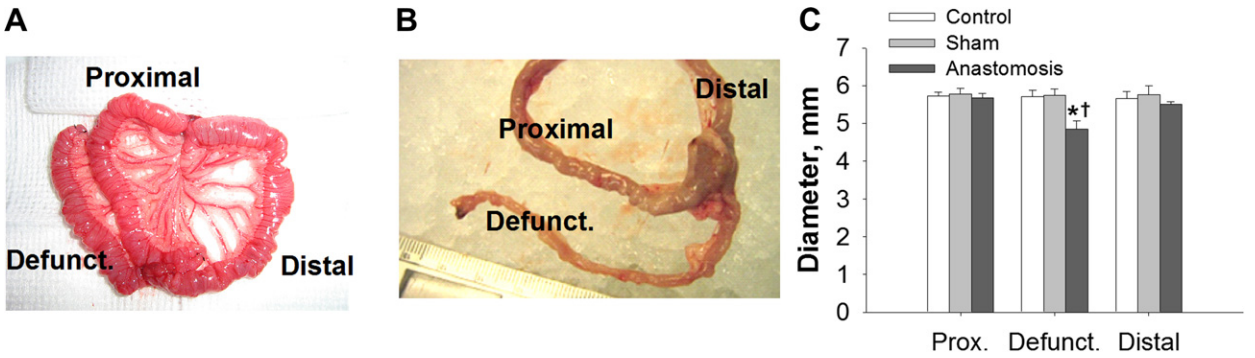

Fig. 1 – Defunctionalized intestine changes morphology within 3 d after surgery. (A) General view of the small intestine of rat after creation of defunctionalized limb in small intestine of rat. Jejunum was divided and was transposed to the distal part of the jejunum approximately 3 cm from the transaction point, where it was anastomosed in a Roux-en-Y end-to-site fashion, thereby creating a defunctionalized section of jejunum. (B) General view of intestine at the time of harvest. Dissected intestinal segments 3 d after anastomosis surgery. (C) Diameters of intestinal segments of defunctionalized (Defunct.) Roux-en-Y anastomosis- *versus* sham-operated animals or controls ($n = 8$, $P < 0.05$). *Significantly different from proximal and distal limb of same group; †significantly different from area representing defunctionalized region of intestine in control- or sham-operated animals. (Color version of figure is available online.)