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Intestinal lengthening in an innovative rodent surgical model

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ABSTRACT

Purpose: Current animal models of mechanical lengthening separate intestinal segments from enteric continuity. Such models are difficult to use for repeated lengthening procedures and result in intestinal tissue loss during restoration into continuity. We sought to create a novel surgical model to allow multiple lengthening procedures for the purpose of maximizing the net increase in tissue after intestinal lengthening.
Methods: A Roux-en-y jejunojejunostomy with a 6-cm blind-ended Roux limb was created in the proximal jejunum of rats. Encapsulated 1-cm polycaprolactone springs were placed into the closed end of the roux limb and secured with a vessel loop. After 4 weeks, lengthened segments and normal jejunum were retrieved for histologic analysis.
Results: Jejunal segments were lengthened from 1.0 cm to 3.0 cm. Lengthened segments had increased smooth muscle thickness, fewer submucosal ganglia, and similar numbers of myenteric ganglia compared to normal intestine. When compared to normal jejunal mucosa, lengthened segments demonstrated unchanged villus height and increased crypt depth.
Conclusions: We created an innovative surgical model for intestinal lengthening and successfully lengthened jejunal segments with a degradable spring. The Roux-en-y model may allow the use of a degradable spring for the treatment of short bowel syndrome.

Short bowel syndrome (SBS) is a disorder of malabsorption due to inadequate intestinal length. Neonatal diagnoses such as necrotizing enterocolitis, intestinal atresias, midgut volvulus, abdominal wall defects, complicated meconium ileus, and aganglionosis lead to SBS and mortality has remained 20%–40% [1–3]. Despite medical and surgical advancements, patients with SBS suffer significant long-term morbidity [4,5]. Surgical treatment options are limited to bowel lengthening and transit slowing procedures and many patients are not optimal candidates [4,6,7].

Limitations in treatment options for SBS have led researchers to focus on lengthening existing intestine using mechanical force, or distraction enterogenesis. Strategies of intestinal lengthening include the use of hydraulic pistons, saline distension, tissue expanders, implanted screws, and spring devices [8–13]. All current models isolate intestinal segments from continuity during lengthening, which is associated with loss of tissue upon restoration and does not allow repeated lengthening of the isolated segment. We therefore sought to create a surgical model for intestinal lengthening that may serve as a platform for repeat lengthening procedures to maximize the net increase in tissue length.

1. Materials and methods

The use of animals was approved by the Animal Research Committee (Institutional Review Board Number 2002-037-22). All materials were FDA approved for use in humans. Intestinal lengthening was achieved using springs made from polycaprolactone (PCL), a biodegradable polymer used in absorbable sutures and other medical devices [14]. PCL springs were fabricated as previously described [13], placed into size 5 gelatin capsules (Torpac Inc., Fairfield, NJ) and coated with cellulose acetate pthalate (Eastman Chemicals, Kingsport, TN) for delayed expansion.

1.1. Surgical procedure

Adult female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing > 250 g were anesthetized with inhaled oxygen and isoflurane (n = 8). The abdomen was entered through a midline laparotomy incision and the jejunum was located and transected approximately 10 cm from the ligament of Treitz. To create a defunctionalized Roux limb, a longitudinal, antimesenteric enterotomy was made 6 cm from the open proximal end of the distal segment. The proximal jejunal segment was then anastomosed in an end-to-side configuration to the distal enterotomy with 6-0 Prolene (Ethicon, Johnson & Johnson; Somerville, NJ) in a simple interrupted fashion to restore enteric continuity. A small opening was created in the mesentery 1 cm from the end of the Roux limb and a vessel loop

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was placed around the jejunum. Coated and encapsulated PCL springs with spring constants between 1 and 2 N/m were placed into the Roux limb, and the end was closed with 6-0 Prolene suture (Ethicon, Johnson & Johnson; Somerville, NJ) in a simple interrupted fashion. The vessel loop was tied loosely and secured with surgical clips, positioning the spring between the end of the defunctionalized limb and the vessel loop (Fig. 1). The bowel was carefully placed back into the abdomen to ensure that there was no twisting of the mesentery, and the abdominal wall was closed in layers. Encapsulated 1-cm PCL tubes were placed into defunctionalized Roux limbs to serve as controls ($n = 3$). Lengthened segments were retrieved after 4–6 weeks and measured for length. Animal weights were recorded weekly.

1.2. Histologic analysis

Mechanically lengthened and normal jejunal tissues were fixed in 10% buffered formalin overnight followed by embedding in paraffin. Care was taken to align tissue cross sections perpendicularly in order to obtain accurate histologic measurements. Tissue blocks were cut into 5-μm sections then stained with hematoxylin and eosin. Sections were examined and recorded at 40× and 100× magnification using light microscopy (Leica Microsystems). Muscularis propria thickness, villus height, crypt depth and circumference were measured for each specimen. Adjacent unstained tissue sections were prepared and stained for S100-positive glial cells as previously described [15]. Using fluorescent light microscopy, the number of ganglia was assessed at 100× magnification in submucosal and myenteric plexuses and expressed as total number of ganglia per cross section. The density of ganglia was calculated and expressed as number of ganglia per mm circumference.

1.3. Statistical analysis

Data were expressed as mean values ± standard deviations. Two-tailed, paired and unpaired Student's *t*-tests were used for statistical analyses where appropriate.

2. Results

Experimental segments were lengthened from 1.0 to 3.0 ± 0.5 cm ($p < 0.0001$). In the control segments there was a slight increase in length from 1.0 to 1.4 ± 0.1 cm ($p < 0.05$). The increase in length between the experimental and control segments was statistically significant (195 ± 47% versus 40 ± 10%, $p < 0.0001$). PCL spring devices had either partially degraded or were not present in the lengthened segment at the time of exploration. Rats demonstrated a mean weight gain of 39 ± 21 g at the time of sacrifice.

2.1. Histologic analysis

Lengthened jejunum had a greater circumference (1.51 ± 0.32 cm versus 1.02 ± 0.14 cm, $p = 0.01$) and thicker muscularis propria (297 ± 58 versus 104 ± 28 μm, $p < 0.0001$) when compared to normal jejunum (Fig. 2). Examination of the mucosa revealed increased crypt depth (270 ± 77 versus 162 ± 27 μm, $p = 0.02$) in the lengthened jejunum. Differences in villus height were not statistically significant (404 ± 96 versus 475 ± 123 μm, $p = 0.34$). The total number of ganglia in lengthened jejunum was decreased in the submucosa (14.5 ± 5.7 versus 29.6 ± 2.3 ganglia, $p < 0.001$) and was unchanged in the myenteric plexus (45.5 ± 7.5 versus 42.8 ± 6.7 ganglia, $p = 0.55$) (Fig. 3). When comparing ganglion density, lengthened jejunum had decreased density of both submucosal ganglia (1.2 ± 0.7 versus 3.1 ± 0.3 ganglia per mm, $p < 0.001$) and myenteric ganglia (3.3 ± 0.6 versus 4.2 ± 0.4 ganglia per mm, $p < 0.05$).

3. Discussion

Multiple devices designed for distraction enterogenesis have shown promising results. However, all models require that a segment of intestine be separated from enteric continuity. We previously showed that an isolated jejunal segment could be lengthened nearly 3-fold with a degradable PCL spring [13]. We then restored the lengthened segment into enteric continuity and demonstrated regained motor and absorptive function [16]. While the success of restoring lengthened jejunum is encouraging, its clinical impact is diminished by the loss of tissue that occurs when intestinal anastomoses are created. Size constraints

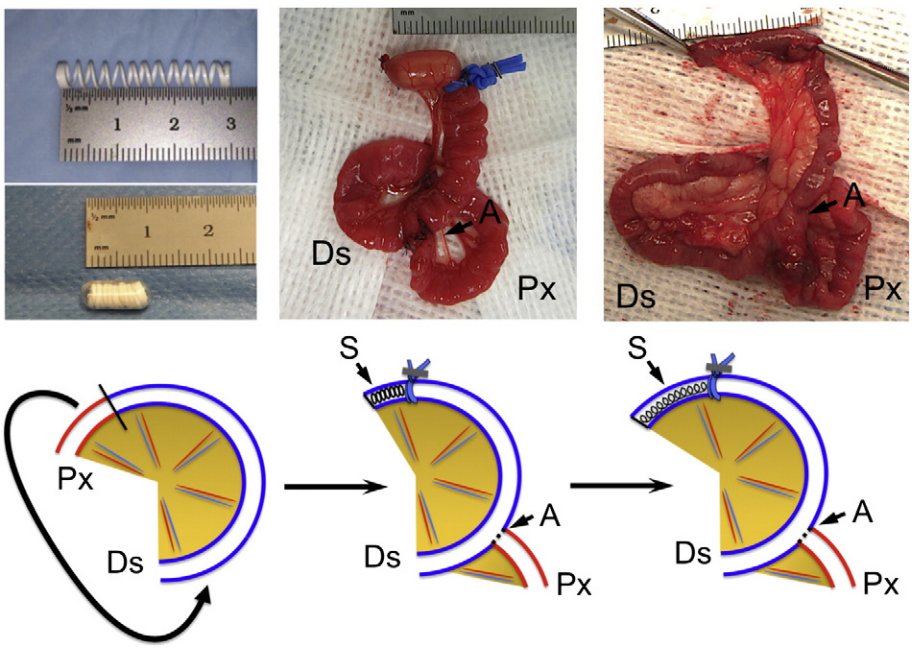

Fig. 1. Photographs and diagram of the Roux-en-Y procedure. The top photographs show the spring device, the bowel configuration at device placement, and lengthened bowel. The bottom diagram is a schematic representation of the lengthening model. Px = proximal, Ds = distal, S = spring, A = anastomosis.