Journal of Pediatric Surgery 49 (2014) 971–975

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

journal homepage: www.elsevier.com/locate/jpedsurg

Function of mechanically lengthened jejunum after restoration into continuity

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ARTICLE INFO

Article history:
Received 23 January 2014
Accepted 27 January 2014

Key words:
Mechanical enterogenesis
Bowel lengthening
Distraction enterogenesis
Short bowel syndrome
Degradable lengthening device
Glucose absorption

ABSTRACT

Purpose: Distraction enterogenesis is a potential treatment for patients with short bowel syndrome. We previously demonstrated successful lengthening of jejunum using a degradable spring device in rats. Absorptive function of the lengthened jejunum after restoration into intestinal continuity needs to be determined.
Methods: Encapsulated polycaprolactone springs were placed into isolated jejunal segments in rats for four weeks. Lengthened segments of jejunum were subsequently restored into intestinal continuity. Absorption studies were performed by placing a mixture of a non-absorbable substrate and glucose into the lumen of the restored jejunum.
Results: Restored jejunal segments demonstrated visible peristalsis at specimen retrieval. Compared to normal jejunal controls, restored segments demonstrated equal water absorption and greater glucose absorption. Restored segments had thicker smooth muscle, increased villus height, increased crypt depth, and decreased sucrase activity compared to normal jejunum. The density of enteric ganglia increased after restoration to near normal levels in the submucosa and to normal levels in the myenteric plexus.
Conclusion: Jejunum lengthened with a degradable device demonstrates peristaltic and enzymatic activity as well as glucose and water absorption after restoration into intestinal continuity. Our findings further demonstrate the therapeutic potential of a degradable device.

Short bowel syndrome (SBS) is a highly morbid disease caused by congenital or acquired loss of significant intestinal length causing insufficient absorption of nutrients. Although rare in the neonatal population at an estimated 24.5 per 100,000 live births, mortality has remained 20–40% [1,2]. The most common etiologies of SBS include necrotizing enterocolitis, complicated meconium ileus, midgut volvulus, intestinal atresia, abdominal wall defects, and aganglionosis [1]. The accompanying malnutrition, malabsorption, dependence on parenteral nutrition, and secondary hepatic dysfunction make it one of the most challenging and costly diseases to manage. Despite its relatively low incidence, health care costs in the pediatric population are estimated at $1.6 million dollars per patient [2]. The most severe cases may progress to liver failure necessitating intestinal or multivisceral transplantation. Currently, the 5-year survival rate after small bowel transplantation for SBS is 55% [3]. Surgical therapies include lengthening and transit-slowing procedures; however, patient selection is limited and the long-term benefits of these procedures have not been clearly demonstrated [4–6].

Distraction enterogenesis, the use of mechanical force to lengthen intestinal tissue, has recently been described as a potential treatment for SBS. Similar mechanical distracting devices have been clinically successful in expanding bone, breast, esophagus, and aorta [7–10]. To lengthen intestinal tissue, several different devices have been tested in animal models [11–15]. We previously used a Nitinol spring device to lengthen rat jejunum, which was successfully restored into intestinal continuity [16,17]. However, this device was limited by a tendency to buckle or perforate the jejunum. We improved upon this device by developing a more structurally stable, degradable polycaprolactone (PCL) spring that successfully lengthened rat jejunum nearly three-fold [18]. Here we report histologic, functional, enzymatic, and absorptive analyses of jejunum lengthened with a degradable spring device after the segment was restored back into intestinal continuity.

1. Materials and methods

Adult female Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 250–350 g were used after approval from 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 PCL springs compressed into cellulose acetate pthalate-coated (Eastman Chemicals, Kingsport,

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http://dx.doi.org/10.1016/j.jpedsurg.2014.01.033
0022-3468/

TN), size 5 gelatin capsules (Torpac Inc., Fairfield, NJ). Springs deployed upon degradation of the capsules [16].

1.1. Surgical procedure

Each animal underwent two surgical procedures. After anesthetizing rats with inhaled oxygen and isoflurane, encapsulated PCL spring devices were placed into isolated 1-cm segments of proximal jejunum located approximately 10 cm from the ligament of Treitz as described previously ($n = 11$) [16]. After device insertion, the ends of the isolated jejunal segments were closed with interrupted non-absorbable sutures. A jejunojejunostomy was then created to maintain continuity of the remaining intestine. A second procedure was performed after 4 weeks. Lengthened segments were measured, trimmed, and restored into continuity for another 4 weeks as previously described [17]. Lengthened segments were restored just distal to the previous anastomosis. Animal weights were recorded weekly.

1.2. Functional evaluation

Prior to euthanasia, an upper gastrointestinal contrast study with small bowel follow through using Omnipaque (GE Healthcare, Waukesha, WI) was performed, and gastrocecal transit times were recorded. The restored jejunal segment was visualized by identifying surgical clips placed during the first procedure. Four weeks postoperatively, under general anesthesia, restored segments were dissected free from surrounding adhesions and were observed grossly for peristaltic activity (Fig. 1).

1.3. Histologic analysis

Normal and restored jejunal segments were retrieved and divided into two halves. One half was stored in a −80 °C freezer for future enzymatic assays. The other half was fixed in 10% buffered formalin overnight and was embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin, and images were recorded on a light microscope at 40× and 100× magnification. Muscularis propria thickness, circumference, villus height, and crypt depth were measured. Unstained sections were immunostained for S-100 to identify ganglia as previously described [19]. The number of ganglia per mm circumference in both submucosal and myenteric plexuses was assessed at 100× magnification. Normal rat jejunum was identically processed and examined at the same magnification.

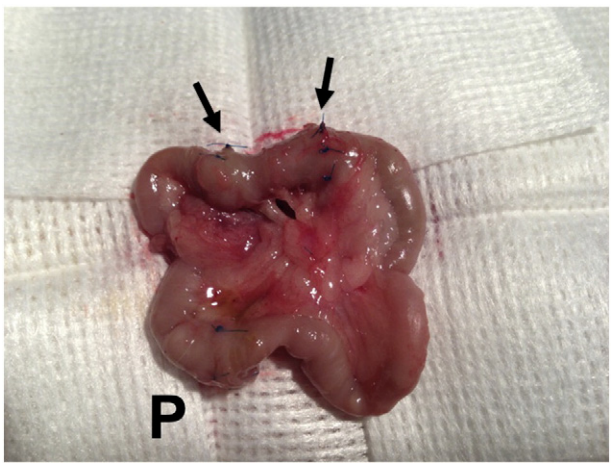

Fig. 1. Photograph of the lengthened then restored segment of jejunum. Arrows mark restored segment. P marks the proximal anastomosis where the isolated segment was removed. *Ex vivo* peristalsis was seen throughout the restored segment and adjacent bowel.

1.4. Absorption analysis

Sucrase activity assays were performed per previously established protocols on normal and restored jejunal segments [20,21]. After homogenizing the mucosa and incubating with sucrose substrate-buffer at 37 °C, glucose oxidase/peroxidase reagent (Sigma, St. Louis, MO) was used to quantify glucose produced by sucrose hydrolysis. Samples were analyzed spectrophotometrically against standard glucose curves. Protein concentrations were assessed using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA) and quantified using standard protein curves. Sucrase-specific activity for each sample was determined and expressed as the total enzyme activity per unit protein (μmol sucrose hydrolyzed/min/mg protein). Prior to euthanasia, *in vivo* absorption studies were performed. Under general anesthesia, restored jejunal segments were dissected free from adhesions and flushed with saline while maintaining perfusion. A 1:1 mixture of glucose (1 mg/mL) and phenol red (50 mg/L; Sigma), a non-absorbable substrate, was injected into a 1-cm isolated section of the restored segment for 15 minutes. Contents were collected and centrifuged. Glucose concentration was quantified as described above. Phenol red concentration was determined spectrophotometrically by measuring absorbance at 560 nm against standard curves to determine the volume of water absorbed. Final glucose concentration was adjusted according to the calculated volume of water absorbed. Glucose absorption was expressed as rate of glucose absorbed per unit length (μmol/min/cm). Absorption in restored lengthened segments was compared to normal jejunal controls.

1.5. Statistical analysis

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

2. Results

2.1. Functional evaluation

After mechanically lengthened jejunal segments were restored into continuity, all animals survived, and all but one demonstrated weight gain (mean 20 ± 17 g). Fluoroscopy demonstrated normal gastrocecal transit times without bowel dilation, stricture, or delay in passage of contrast in restored segments. At fluoroscopy, peristalsis was evident throughout the restored segments and in proximal and distal adjacent jejunum [18]. The same peristaltic movement was directly observed throughout the restored segments and adjacent bowel at the time of euthanasia.

2.2. Histology

Jejunal segments were lengthened from 1.0 cm to 2.7 cm prior to restoration into intestinal continuity. On histologic analysis, restored jejunal segments had thicker muscularis propria compared to normal jejunum (278 ± 107 versus 93 ± 30 μm, $p < 0.005$). There was an increase in villus height (360 ± 34 versus 339 ± 9 μm, $p = 0.037$), and crypt depth was significantly greater compared to normal jejunum (322 ± 5 versus 183 ± 18 μm, $p < 0.005$). The density of ganglia increased after restoration to near normal levels in the submucosa (2.2 ± 0.5 versus 3.0 ± 0.4 ganglia/mm, $p = 0.03$) and to normal levels in the myenteric plexus (3.0 ± 0.8 versus 3.4 ± 0.9 ganglia/mm, $p = 0.46$) (Fig. 2).

2.3. Absorption and enzymatic analysis

Sucrase activity was lower in the mucosa of restored jejunal segments ($n = 4$, 1.13 ± 0.82 versus 3.25 ± 1.49 μmol/min/mg