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Basic fibroblast growth factor eluting microspheres enhance distraction enterogenesis

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ABSTRACT

Purpose: The purpose of this study was to determine if distraction enterogenesis using self-expanding polycaprolactone (PCL) springs is a potential therapy for short bowel syndrome. Sustained release basic fibroblast growth factor (bFGF) microspheres have been shown to induce angiogenesis and intestinal regeneration in tissue engineered scaffolds. We hypothesized that the provision of bFGF-loaded microspheres would increase angiogenesis and thereby enhance the process of enterogenesis.
Methods: A 10-mm segment of rodent jejunum was isolated and an encapsulated PCL spring inserted. Blank or bFGF-loaded microspheres were delivered to the segment. After 4 weeks, jejunal segments were assessed for lengthening, morphology, quantification of blood vessels, and ganglia.
Results: Lengthened intestinal segments receiving bFGF microspheres demonstrated significantly increased microvascular density compared to those with blank microspheres. There were also significantly more submucosal and myenteric ganglia in the segments that received bFGF microspheres. Segments achieved similar lengthening and final muscular thickness in both blank and bFGF groups, but the bFGF microsphere caused a significant increase in luminal diameter of the jejunal segment.
Conclusion: Sustained release bFGF microspheres enhanced distraction enterogenesis through improved vascularity. The synergy of growth factors such as bFGF with distraction enterogenesis may yield improved results for the future treatment of patients with short bowel syndrome.

Short Bowel Syndrome (SBS) is a congenital or acquired condition characterized by the loss of intestinal absorptive capacity resulting in malnutrition, malabsorption and dehydration. This condition affects roughly 3–5 per 100,000 births per year [1,2]. Common etiologies resulting in SBS in children include intestinal atresias, abdominal wall defects, necrotizing enterocolitis, volvulus, and extensive aganglionosis [1]. Current treatment strategies for SBS involve parenteral nutrition and small bowel transplantation; various transit slowing and bowel lengthening procedures have been employed in highly selected subpopulations [3]. However, these therapies have shown limited success and are associated with high rates of sepsis, intestinal failure-associated liver disease, and mortality [3]. As such, there is great opportunity for the development of alternate treatment options for the children and adults who suffer from this debilitating and highly morbid disease.

The individual concepts of distraction-induced tissue growth and pharmaceutical proliferative signaling have been separately established as treatment strategies for SBS [4–6]. Most recently, we have successfully performed the technique of distraction enterogenesis using biodegradable, FDA-approved polycaprolactone (PCL) springs [7]. One limitation of this technique has been possible tissue ischemia resulting in loss of submucosal ganglia [8].

Basic FGF is a protein that has been shown not only to promote angiogenesis, but also to stimulate proliferation of many components of intestinal tissue including endothelial cells, fibroblasts, and smooth muscle cells [9,10]. Combining the modalities of biochemical enhancement with mechanical force is a novel research direction in the quest to treat short bowel syndrome. To this date, only one study has combined the use of mechanical force with biochemical supplementation [11].

We aimed to combine distractive forces with pharmacologic signaling to enhance the biochemical milieu in favor of bowel lengthening and tissue proliferation by using bFGF growth factor eluting microspheres.

Abbreviations: SBS, short bowel syndrome; bFGF, basic fibroblast growth factor; PCL, polycaprolactone; PLGA, poly(D,L-lactic-co-glycolic acid); vWF, von Willebrand factor.

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We hypothesized that bFGF would promote angiogenesis within our model and thereby enhance mechanical enterogenesis. Furthermore, we hypothesized that this increased vascularity would help limit the relative loss of ganglia in our lengthened segments.

1. Methods

The use of all animals was approved by the Animal Research Committee (Institutional Review Board no. 2005-052-33C).

1.1. Spring and microsphere creation

Intestinal lengthening springs were made from electro-spun PCL as previously described, and all had spring constants from 1 to 2 N/m [12]. The springs were compressed into size 5 gelatin capsules (Torpac Inc., Fairfield, NJ) and coated three times with cellulose acetate phthalate (Eastman Chemicals, Kingsport, TN).

Basic-FGF protein-loaded PLGA microspheres were prepared by a double emulsion solvent evaporation method as previously described [13]. Briefly, 100 mL of bFGF (10 μg) was used with 500 mL of 0.5% solution of 85/15 PLGA (intrinsic viscosity 0.61 dL/g; Birmingham Polymers). From our previous studies, the efficiency of proteins encapsulation within microspheres is assumed to be 50% [13].

1.2. Surgical procedure

Adult female Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA) weighing 200–250 g were anesthetized with inhaled oxygen and vaporized isoflurane ($n = 10$). Rats underwent a midline laparotomy, a jejunal segment 10 cm from the ligament of Treitz was isolated, and springs were implanted. Blank microspheres ($n = 4$) and bFGF microspheres ($n = 4$) were both injected into the lumen of the isolated segment and spread across the serosal surface. Two rats did not receive any microspheres.

Animals were euthanized at postoperative day 30, and lengthened and nonlengthened segments were measured and submitted for histologic analysis.

1.3. Histology

Isolated jejunal segments were prepared in 10% buffered formalin fixative for 24 h and embedded in paraffin blocks. Serial 4-μm sections were taken, and one slide per specimen was stained with hematoxylin and eosin to assess morphology. Unstained sections were prepared for immunostaining to identify S100 positive glial cells and blood vessels positive for von Willebrand Factor (vWF) as previously described [8,13]. Microvascular density was normalized to tissue area and represented as a percentage (%). S100 ganglia were manually counted and expressed relative to submucosal or myenteric circumference. Sections were examined in a blinded fashion using light microscopy and epifluorescence imaging at 40× and 100× magnification (Leica Microsystems, Buffalo Grove, IL).

1.4. Statistical analysis

Data were expressed as mean values ± standard deviations. Two-tailed and paired Student t-tests were used for statistical analysis as appropriate.

2. Results

All intestinal segments with implanted springs expanded roughly 3-fold; Basic-FGF microsphere, blank microsphere, and no microsphere control groups lengthened to 31 ± 3, 29 ± 5, and 26 ± 1 mm respectively ($p = 0.31$) (Fig. 1). These measurements are consistent with previous 3-fold lengthening [8,14].

2.1. Histologic analysis

The luminal diameter of the lengthened segments was significantly greater in the bFGF microsphere group compared to the blank microsphere control, 3.6 ± 1.0 vs. 2.9 ± 0.4 mm ($p = 0.01$). Similar to our previous work, muscularis propria thickness was significantly greater in lengthened segments compared to nonlengthened bowel, 237 ± 62 vs. 47 ± 8.7 μm ($p = 0.02$) [7]. However, there was no difference in muscularis propria thickness between bFGF microsphere and blank microsphere lengthened segments, 230 ± 78 vs. 244 ± 46 μm ($p = 0.55$) (Fig. 2).

Crypt depth was significantly increased in the bFGF microsphere groups in both lengthened and nonlengthened segments. Lengthened spring segments showed a difference in crypt depth of 172 ± 24 vs. 119 ± 39 μm in bFGF microsphere groups compared to blank microsphere groups ($p < 0.01$). Even when assessing nonlengthened, normal bowel, those in the bFGF microsphere group exhibited increased crypt depth as compared to those receiving blank microspheres, 120 ± 39 vs. 74 ± 12 μm ($p < 0.01$). Consistent with our prior work, there was increased crypt depth in lengthened segments compared to nonlengthened segments in both bFGF and blank microsphere groups [12].

The villous height in lengthened and nonlengthened segments was unchanged between groups. Among lengthened segments, bFGF microsphere group was not significantly different than the blank microsphere group, 277 ± 108 vs. 264 ± 64 μm ($p = 0.64$). These were also similar to normal bowel controls in each of the bFGF and blank microsphere groups, 246 ± 56 vs. 249 ± 49 μm ($p = 0.88$).

Microvascular density in each of the segments was assessed using vWF immunostaining (Fig. 3). Because of circumference differences in lengthened vs. nonlengthened segments, as well as differences between bFGF and blank microsphere groups, microvascular density was normalized to cross-sectional area and displayed as a percentage. Within nonlengthened segments, microvascular density was not significantly different between bFGF and blank microsphere groups, 0.93 ± 0.31 vs. 0.81 ± 0.08% ($p = 0.56$). However, there was a marked significant increase in the bFGF microsphere group above that of blank microspheres, 2.16 ± 0.86 vs. 0.48 ± 0.19% ($p = 0.001$). Interestingly, the combination of bFGF microspheres and mechanical stretch caused the greatest significant increase in microvascular density.

Submucosal and myenteric enteric plexuses were assessed with S100 immunostaining (Fig. 4). We normalized these values to the submucosal and myenteric bowel circumferences to account for differences between the sizes of each cross section. Overall, there was continued S100 ganglia loss in lengthened segments, however this loss was attenuated by the presence of bFGF microspheres, which demonstrated increased ganglia presence compared to blank microsphere groups. Basic FGF microsphere groups in comparison to blank microsphere groups showed 5.7 ± 2.9 vs. 2.4 ± 0.5 submucosal ganglia per mm circumference ($p = 0.01$), 7.4 ± 3.5 vs. 4.4 ± 1.1 myenteric ganglia per mm circumference ($p = 0.05$), and 13.1 ± 6.3 vs. 6.8 ± 1.6 total ganglia per mm circumference ($p = 0.03$). Normal, nonlengthened segments had significantly more overall ganglia than lengthened segments, but no differences between nonlengthened bFGF and blank microsphere groups: 14.0 ± 2.0 vs. 12.1 ± 2.2 submucosal ganglia per mm circumference ($p = 0.31$), 20.1 ± 3.1 vs. 21.4 ± 1.8 myenteric ganglia per mm circumference ($p = 0.54$), and 33.5 ± 3.7 vs. 34.1 ± 3.4 total ganglia per mm circumference ($p = 0.82$).

3. Discussion

This study clearly demonstrates that the combination of distractive forces and biochemical modulation in the form of sustained release bFGF enhances the process of mechanical enterogenesis.

Both distraction-induced tissue growth and biochemical proliferative signaling have been explored as treatment strategies for SBS, but