Journal of Pediatric Surgery 50 (2015) 573–580

Contents lists available at ScienceDirect

Journal of Pediatric Surgery

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

Proposal of intestinal tissue engineering combined with Bianchi's procedure☆

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ARTICLE INFO

Article history:
Received 27 August 2013
Received in revised form 2 October 2014
Accepted 5 November 2014

Key words:
Small intestinal tissue engineering
Small intestinal submucosa
Short bowel syndrome
Bianchi's procedure

ABSTRACT

Aim: The aim of this study is to examine the feasibility of the small intestinal submucosa (SIS) when the longitudinal staples during Bianchi's procedure are replaced with SIS graft.
Methods: The mesentery of the bowel was separated based on the bifurcated vessels in five beagles. A 2 × 7-cm longitudinal half of the bowel was excised and the defect was repaired using SIS with similar blood supply in Bianchi's operation. Six months later, intestinal motility in the SIS-grafted area was recorded. Tissue preparations were obtained from the reorganized area. An organ bath technique with electrical field stimulation was applied. Both the native small intestine and grafted area were morphologically investigated using immunohistochemistry.
Main results: All dogs survived and thrived with no anastomotic leakage. Isoperistaltic migrating contractility during fasting was observed through the grafted segment including the reorganized area. The SIS-reorganized tissue contracted in response to an acetylcholine agonist and electrical field stimulation. The mucosa was covered with normal epithelium. Reorganization of neural and smooth muscle cells was observed.
Conclusions: SIS has the potential for use as a scaffold that promotes the formation of a physical and physiological neointestine. Our present proposal approaches a novel surgical treatment in patients with short bowel syndrome.

More than 30 years have passed since Bianchi [1] published the first report on a novel surgical treatment for patients with short bowel syndrome (SBS), in which the severely dilated bowel is longitudinally divided into two tubes that are reconnected in series with the rest of the small intestine. Several modifications were made during these three decades with modest results [2–5]. The aim of such intestinal lengthening and/or tapering operations for patients with SBS with a dilated small intestine is to decelerate the intestinal transit time and/or increase the intestinal absorptive capacity in expectation of intestinal adaptation.

In 1996, Saday and Mir [6] described an interesting surgical model that incorporated intestinal lengthening with neomucosal growth by serosal patching. However, follow-up clinical studies were unable to be performed. Recent technological advancements in the field of tissue engineering have allowed a variety of tissues and organs to be regenerated. Intestinal tissue engineering is expected to introduce potential therapy for patients with SBS. Various researchers have developed the technique of scaffold-based tissue engineering. Porcine-derived small intestinal submucosa (SIS) is one such decellularized, collagen-rich bioscaffold. It has been reported to contain functional growth factors considered vital for tissue restoration [7–9] and to preserve a mixture of bioactive factors including cell adhesion factors, mitogenic factors, chemotactic cytokines, and angiogenic factors [8,10]. Several reports have described the utility of SIS in intestinal tissue engineering using animal models [11–16]. More than a few authors have reported that tissue engineering using SIS can be used to create a neointestine with structural features of the normal intestine [11–14]. Chung et al [17] also studied intestinal engineering using SIS in a dog model. They succeeded in creating a remodeled wall that contained mucosal epithelial, smooth muscle, and serosal layers by patch grafting with SIS for a substantial defect created in the small intestine, while a tubular replacement of SIS interposed between small intestinal segments showed significant morbidity [11]. Therefore, it is essential to maintain the blood supply to the midportion of the graft, even when using SIS as a bioscaffold.

We conceived an innovative design concept beneficial in the treatment of patients with SBS irrespective of the diameter of the small intestine using a small intestinal tissue engineering technique. In brief, the bowel is divided as described by Bianchi [1] without the use of surgical staplers. Rather than sewing the edges of the bowel to itself to form two separate conduits, the defect is closed with interpositioning of the SIS sutured to the edges of the defect. Next, the two separate conduits that were integrated with SIS are moved in the opposite direction with the mesentery, which contains a single-blood supply

☆ This research was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI) from the Japan Society for the Promotion of Science (grant no. 22591488 to T.U.).
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http://dx.doi.org/10.1016/j.jpedsurg.2014.11.035

from one of the bifurcated vessels, and are reconnected in series with the rest of the small intestine, as in Bianchi's operation. This technique theoretically increases the absorptive area of the small intestine to an area twice as large as that of the original bowel without changing the caliber of the initial lumen, assuming local intestinal regeneration is induced by the SIS graft. The aim of this preliminary study was to test a novel and original concept of intestinal tissue engineering combined with Bianchi's vessel treatment and to evaluate the feasibility of the SIS graft when the blood supply to the graft is delivered in the same way as in Bianchi's procedure.

1. Materials and methods

1.1. Initial surgical manipulation

This experiment was reviewed by the Committee of Ethics on Animal Experiments at Yamaguchi University School of Medicine and performed in accordance with the Guideline for Animal Experiments in Yamaguchi University School of Medicine and the Law (no. 105) and Notification (no. 6) from the Japanese Government.

Five female beagle dogs (TOYO beagles; Kitayama Labes, Nagano, Japan) weighing 11.1–16.4 kg were used in this study. Food was withheld for 12 h prior to surgery; water was available *ad libitum*. Anesthesia was induced with a single intravenous injection of 5 mg/kg of propofol (Diprivan; AstraZeneca, Tokyo, Japan) with 0.1 mg/kg of pancuronium bromide (Mioblock, MSD, Tokyo, Japan). General anesthesia was maintained by intratracheal inhalation of isoflurane in oxygen. The abdomen was aseptically prepped for a 10-cm ventral midline laparotomy. Under visualization with a surgical binocular loupe at 2.5× magnification, initial surgical manipulation was performed as follows (Fig. 1). A 7-cm length of mesenterium containing one side of the straight arteries and veins that are distributed separately to the small intestine from the anastomotic arcades (bifurcated vessels) was transected 40 cm distal to the ligament of Treitz. In other words, one channel of the two blood flows to the jejunum including the straight artery and vein in the mesenterium was cut off without affecting the residual blood flow. An enterotomy was created longitudinally on the antimesenteric wall of the jejunum. Continuity was cut off at both ends of the longitudinal half that had mesenterium manipulation. A 2- × 7-cm longitudinal, semicircular, full-thickness section from half of the jejunum was then excised. The defect was repaired using a 2- × 7-cm 8-ply SIS (Biodesign, Surgisis; Cook Biotech, West LaFayette, IN), which was equivalent to the defect in the area, after rehydration for 15 min in normal saline solution. The SIS was secured to the naked bowel edge with an absorbable 5–0 polydioxanone suture (PDS-II; Ethicon, Tokyo, Japan) in a continuous manner. Additional nonabsorbable polypropylene sutures (Prolene; Ethicon, Tokyo, Japan) were placed at the four outer corners of the rectangle-shaped SIS in an interrupted pattern to ensure future identification of the SIS site because the SIS is an absorbable material. Adequate hemostasis was maintained throughout the procedure, and the wound was closed with interrupted 2–0 nylon sutures (Ethilon; Ethicon, Tokyo, Japan) for the peritoneum, muscular, and fascial layers. The skin was closed with a skin stapler (3 M Precise Vista Disposable Skin Stapler; Sumitomo-3 M, Tokyo, Japan). An antibiotic (flomoxef sodium; Shionogi & Co., Ltd., Osaka, Japan) was administered once immediately after the operation. The animals were given a solid diet immediately after the surgery.

1.2. Measurement of muscle motility in vivo

Six months postoperatively, the abdomen was reentered under general anesthesia. The reorganized area of the small intestine was easily identified by the polypropylene markers. Four strain gauge force transducers (Model F12IS, 8 × 14 mm; Star Medical Inc., Tokyo, Japan) were implanted on the seromuscular layer to record the autonomous motility of the small intestine through the regenerated area (Fig. 2). Two transducers were sutured to the jejunum, one of which was 10 cm and the other 5 cm proximal to the reorganized area (transducers 1 and 2, respectively). One transducer was applied in the center of the reorganized segment (transducer 3), and another was sutured to the jejunum 5 cm distal to transducer 3 (transducer 4). Cables were led out through a skin incision made between the scapulae via a subcutaneous tunnel in the costal flank, and the end of the cables was stored in a jacket protector. The dogs were allowed to recover from the surgery for 1 week. The end of the cables was connected to a transmitter of a telemetry system (Star Medical Inc., Tokyo, Japan). Small intestinal motility during fasting in the awake state was observed and analyzed with a computer-assisted system (PowerLab; ADInstruments, Castle Hill, Australia).

1.3. Measurement of muscle motility in vitro

After measurement of the motor activity *in vivo*, the animals were sacrificed and the small intestine including the reorganized area was adequately excised. Two muscle strips along with their longitudinal muscle fibers were taken from the middle of the four nonabsorbable sutures to prevent contamination of the *de novo* bowel tissue that was unrelated to the reorganized area. Two additional muscle strips were also harvested from the untreated conduit of the small intestine as control specimens. Tissue-organ baths filled with buffer were used to investigate the physiology and pharmacology of the tissue specimens *in vitro*. Dose- or frequency-response studies were conducted to assess the tissue response to a certain drug dosage or stimulus potency in isolated tissue preparations. The lengths and widths of the tissue preparations were trimmed to 10.0 × 5.0 mm. Both ends of the tissue preparations were suspended between two platinum electrodes in 10-mL tissue-organ baths (ADInstruments). Krebs–Henseleit buffer (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, and 11 mM glucose) was used to maintain the integrity of the tissues for several hours in a temperature-controlled environment at 36 °C while physiological measurements were performed. Continuous bubbling with 95% O_2 and 5% CO_2 was performed throughout the experiment. Tissue preparations were adjusted at a resting load of 0.5 g and equilibrated for 1 h. Changes in mechanical contractility *in vitro* were recorded on a polygraph using isometric transducers (Nihon Kohden, Tokyo, Japan) and analyzed with a computer-assisted system (PowerLab; ADInstruments). A dose-response curve was obtained for each tissue preparation with a muscarinic receptor agonist (carbachol hydrochloride, CCH; 1×10^{-8} to 10^{-4} M). Activation of intrinsic nerves was achieved by electrical field stimulation (EFS; 50 mV, 1.0-ms duration, and 10-s trains at 2.5, 5, 10, 20, and 40 Hz) induced by an electric stimulator (Nihon Kohden, Tokyo, Japan). All chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Fresh stock solutions of CCH were routinely prepared in saline. Each stock solution was then further diluted to the appropriate concentration. An area under the curve during 4 min or 30 s of each stimulation with CCH or EFS was calculated using the computer-assisted system and expressed as the motility index (MI).

1.4. Immunohistological evaluation

Several intestinal specimens including circular muscle fibers and the reorganized area were obtained for immunohistological analysis. The specimens were fixed with 4% buffered paraformaldehyde (Wako Pure Chemical Industries), embedded in paraffin, and sectioned along the circular muscle. Hematoxylin and eosin staining, periodic acid-Schiff staining, and elastica van Gieson staining were performed according to conventional methods. Immunohistochemistry was used to confirm the regrowth of smooth muscle and neural fibers using antibodies to α-smooth muscle actin (Abcam, Tokyo, Japan), desmin (Abcam, Tokyo, Japan), and S-100 protein (MBL, Nagoya, Japan). The sections were incubated with primary antibodies (anti-α-SMA, 1:400; anti-desmin, 1:100; anti-S-100 protein,