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豬隻小腸移植中腸道神經再生機制之探討

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前言、研究目的

INTRODUCTION

Intestinal transplantation (ITx) is a life-saving therapy for patients with intestinal failure who have failed total parenteral nutrition (TPN) therapy and those with a life-threatening abdominal pathology.[1-3] In clinical practice, however, ITx has not yet reached the level of success seen with kidney or liver transplantation. Postoperative rejection and infection are common causes of graft loss in ITx.[4] There are many underlying reasons for the poor outcome of ITx, e.g., the widely distributed lymphoid tissue in the gut, the strong expression of antigen, and the inherent microflora within the bowel lumen.

In order to improve the clinical outcome of ITx, further clinical and basic research is warranted. Here we report on the feasibility and efficacy of a modified Paul-Mikulicz ileostomy procedure. This method was intended to facilitate sampling of both the graft and the native tissue without interrupting the bowel conduit. We used a double-barrel ileostomy with proximal side-to-side anastomosis (modified Paul-Mikulicz ileostomy) as the distal gastrointestinal anastomosis in a swine model of ITx. .

MATERIALS AND METHODS

Twenty male Taiwan Lan-Yu piglets weighing 15-20 kg were used in this study. All animals were allowed free access to food and water until 1 day before the experiment and were housed alone in an animal room on a 12-h light/dark cycle. The animal experiments were conducted according to the *Guide for the Care and Use of Laboratory Animals* and were approved by the Animal Care and Use Committee of the National Taiwan University.

Anesthesia

The animals were generally anesthetized according to the following protocol. Anesthesia was induced by intramuscular injection of ketamine HCl (10 mg/kg) and atropine (0.2 mg/kg). The animals were then intubated with a 6.5-7 Fr. endotracheal tube with the assistance of a laryngoscope and were mechanically ventilated. Anesthesia was maintained with 0.5% to 2% isoflurane inhalation. During the surgery, lactated Ringer solution was infused through the cannulated jugular vein.

In the recipient, a venous catheter was inserted into the external jugular vein for monitoring of central venous pressure and perfusion before the abdominal incision. Arterial blood pressure was monitored through an arterial catheter inserted into the carotid artery. Heart rate and body temperature were monitored continuously throughout the surgery.

Small Bowel Graft Procurement

After a midline laparotomy, the superior mesenteric artery (SMA) and vein (SMV) were

dissected and then looped at the level just below the pancreas (Fig 1A). At that level, the trunk of the mesenteric artery could be seen at the left border of the SMV, and 1 or 2 colonic arteries were visible at the right border of the SMV, accompanied by corresponding venous branches to the SMV. The first jejunal and the colonic branches were divided respectively to gain enough length for anastomosis. After the initial dissection of the vascular root, the jejunum was divided along the corresponding vessels and the ileum was transected 10 cm proximal to the ileocecal valve, at the top of the curve formed by the end of the mesenteric artery. The corresponding mesentery was transected. At this time point, the small bowel graft was attached to the donor by only the vascular pedicles (Fig 1B).

Systemic heparinization was achieved by intravenous injection of 5,000 IU heparin. The graft was subsequently removed by dividing the vascular pedicles and was immediately immersed in iced lactated Ringer solution. The arterial stump was then cannulated carefully and perfused with 500 ml of iced lactated Ringer solution. Special attention was paid to not cause any injury to the arterial intima during cannulation. Good blanching of the graft and smooth venous outflow indicated the efficacy of arterial perfusion. If necessary, we removed the parts that did not blanch well after perfusion. Otherwise, no further modification of the graft was done in the back table. We adapted this ex vivo instead of in vivo cooling and perfusion technique with the aim of developing human living-related small bowel transplantation in the future.

Implantation of the Graft

The recipient pig underwent nearly total enterectomy with meticulous dissection of the main SMA and SMV at the same time as graft procurement. The iced lactated Ringer solution-perfused

graft was brought to the recipient. We started vascular anastomosis with intermittent 8-0 prolene sutures with the assistance of a surgical microscope. After the completion of arterial and venous anastomosis, the vascular clamps were released and reperfusion of the graft began. In the meantime, attention was paid to any changes in graft color and potential bleeding from the vascular anastomosis (Fig 2).

In cases without vascular occlusion or bleeding, the operation proceeded into the intestinal anastomosis. Primary end-to-end jejuno-jejunal anastomosis was performed as the proximal anastomosis. The distal anastomosis was done in a fashion of side-to-side ileo-ileo anastomosis, approximately 10 cm proximal to the distal ends (Fig 3). Then, both the native and the graft ileal ends were exteriorized as the ileostomy, which we refer to as the "modified Paul-Mikulicz ileostomy."

Induction, Immunosuppressant Regimen, and Postoperative Management

The induction regimen comprised 500 mg of methylprednisolone administered intravenously just before reperfusion. Immediately after reperfusion, continuous infusion of 1 mg tacrolimus (Prograf, Fujisawa Pharmaceutical Co., Osaka, Japan) for 24 hours was started. At the end of surgery, animals were extubated and placed in metabolic cages with heat lamps. Intravenous cefmetazole (25 mg/kg) was given twice daily as a prophylaxis. However, no antifungal or antiviral agents were administrated.

The animals were kept fasting until 5 days after transplantation. During the period of fasting, parenteral nutrition was given with Nutriflex solution (60 ml/kg daily, B. Braun Melsungen AG, Bethlehem, PA) and lipid emulsion via the central venous catheter left in the external jugular vein.

Oral feeding was resumed with water for 2 days, followed by commercial feed. At that time, tacrolimus was changed to the oral form, 0.1 mg/kg twice daily.

Data and Tissue Collection

The animals were kept alive until they died naturally. Body weight and bowel movements were recorded daily. Until the animal's death, biopsy of both ends of the ileostomy was done twice weekly under pentobarbiturate anesthesia, along with blood sampling for blood cell count, chemistry profile, and tacrolimus level.

Tissue specimens were fixed in 4% buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin for histopathological examination of potential rejection. Some tissue specimens were stored at -80℃ and in liquid nitrogen for further proteomic and genomic studies, respectively.

結果

RESULTS

All procedures were completed as planned. The mean operative time for procurement was 114±25.2 minutes (range: 80-160 minutes). None of the donors experienced massive bleeding or unstable hemodynamics during or after procurement. No animals died in related to the operation or systemic heparinization.

On the back table, perfusion was satisfactory in all animals, as indicated by the smooth outflow through the SMV branch. Good blanching was observed in more than 90% of the grafts. We performed partial resection of the bowel graft in 5 animals.

The mean animal weight of the recipients at the time of surgery was 15.8±2.8 kg (range:11.1-21.8 kg). All animals lost less than 10% of their initial body weight at 1 week after surgery. One animal surviving for 6 months gained weight after TPN cessation (Fig 4).

The mean duration of the recipient surgery was 410±50 min (range: 345-515 min). The mean warm ischemia time was 56±15 min (range: 42–70 min). No significantly unstable hemodynamics occurred after reperfusion. During surgery, the maximum systolic arterial blood pressure was 120 mm Hg, and the minimum systolic blood pressure was 60 mmHg. Three animals experienced immediate venous thrombosis after reperfusion, and therefore removal of the graft was necessary. All animals could be extubated after the operation. All but three survived the procedure for at least 1 week. All animals started spontaneous feeding the fifth day after surgery. The first stool passage via the native anus occurred between the second and third days after normal feeding. The tacrolimus level was maintained at 11.2±3.8 ng/ml (range: 3.7-30.1 ng/ml)

Whole-layer biopsies of both graft and native tissues were all done with surgical scissors at

the modified Paul-Mikulicz ileostomy smoothly under ketamine anesthesia (1 mg/kg intravenously). Hemostasis was satisfactorily achieved by putting horizontal mattress sutures around the intestinal wound. The average time necessary for biopsy was 10±2.5 minutes. Microscopically, the native bowel had intact intestinal villi and did not show any lymphocyte infiltration. (Fig 5A) On the contrary, there were marked sloughing of the epithelial villi, mucosal ulceration and numerous lymphocytes infiltration in the rejected graft. (Fig 5B)

The length of survival and the causes of death are summarized in Table 1. At autopsy, no leakage was noted in any bowel anastomosis, nor any intraabdominal infection.