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Surgical experience of refined 3-cuff technique for orthotopic small-bowel transplantation in rat: a report of 270 cases

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Abstract

BACKGROUND: Establishment of an instant, reproducible, and reliable rat model of a refined 3-cuff technique for performing orthotopic intestinal transplantation is reported, and the surgical skills required to perform modified surgical procedure are discussed.

METHODS: A retrospective analysis was used to study 270 rat cases subject to orthotopic intestinal transplantation (OIT) performed in our transplantation center from March 2006 to March 2008. After establishing the portal vein cuff method, a conventional hand-sewn anastomosis method combination, with porto-to-portal re-establishment by cuffed anastomosis technique, was used in group 1 (n = 140), and the modified 3-cuff anastomosis method was applied in group 2 (n = 130). Statistical comparison was made between the 2 groups.

RESULTS: In group 1, 97 of 140 (69.3%) recipients survived >7 days, and 69 (49.3%) survived >30 days, whereas in group 2, respective survival was 110 of 130 (84.6%) and 86 of 130 (66.2%). Average cold ischemic times in the 2 groups were 48.5 ± 5.1 minutes and 31 ± 3.0 minutes, respectively. There was a significant difference between the 2 groups ($P < .05$). In most cases, the average volume of bleeding during recipient surgery was <1 mL using the simplified 3-cuff anastomosis technique. There was shorter graft revascularization time with the new model of sutureless microanastomosis using cuff apparatus for OIT in rats compared with the control group. The method adopted in group 2 was much easier, more stable, and more feasible than that in group 1. Sixty-three rats died in 7 days, and autopsy verified the causative factors leading to death, which are summarized in the text. The results obtained were acceptable and satisfactory. Overall, there was a comparative lower incidence of complications associated with the procedure used in group 2.

CONCLUSIONS: The modified 3-cuff anastomosis technique for rat OIT models has several obvious advantages, which can be summarized as follows: vascular anastomosis is stable and simplified, and blood loss is significantly decreased; natural anatomic physiologic portal graft drainage is maintained; and intraoperative mortality and postsurgical morbidity are minimized. Furthermore, technical refinement of rat OIT models established by our research team can be carried out without a microscope and can be easily implemented in the laboratory by 1 trainee with acceptable success after a short period of training. We regard it as one of the best available orthotopic small-bowel transplantation methods in rat.

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Patients with intestinal failure can obtain long-term survival with the support of total parenteral nutrition (TPN). However, sustained TPN may lead to serious complications, such as TPN-related end-stage liver disease, dysfunction of central venous access, and multiple episodes of sepsis. Moreover, some patients with permanent intestinal failure cannot tolerate sustained parenteral nutrition at all. For these patients, small-intestine transplantation (SIT) may be the only choice for survival. Because of unique immunologic and physiologic characteristics, postsurgical complications after intestinal transplantation occur more often and are more serious than those after liver and kidney transplantation. The perioperative mortality of intestinal transplantation is consequently higher due to the onset of acute and chronic rejection, graft-versus-host disease, and infection. Therefore, many efforts have been made to understand the immunologic mechanism involved in SIT, for which a suitable and stable animal model is the basic requirement.

In 1971, Monichick and Russell[1] first established a heterotopic small-bowel transplantation model, which was then further developed by Kort et al.[2] Simplicities in establishing, breeding, and handling have made the *Rattus* model the most optimal and widely used model in this field. More importantly, the well-defined histocompatibility of various inbred strains is the preferred choice for immunologic and physiologic studies.[3]

Several different methods exist by which to establish models. In the conventional method, the portal vein (PV) of the graft is introduced directly into the inferior vena cava (IVC) from the renal vein. However, normal functional anatomy and physiologic retrograde inflow will consequently be disrupted. Meanwhile, the entire SIT procedure from the Treitz ligament to the ileocecal valve cannot be accomplished by the conventional method because of the limitation in blood supply from the renal vessels. Therefore, the conventional method is not appropriate for nutritional or functional studies, which require the entire SIT.[4,5]

The traditional suture for vascular construction requires a complex microsurgical technique for microvascular anastomosis. As a result, complications are usually inevitable, and mortality is high. In 1988, Wallander et al[6] first reported an alternative method, ie, the cuff technique, as an optimal and easier vascular anastomosis method. Many researchers have achieved a high success rate in the establishment of the SIT model using the cuff technique. Furthermore, histopathologic study confirmed a mild and acceptable foreign-body reaction of the graft and polyethylene cuff on the vessel anastomotic site.[7]

Although a polyethylene cuff may the best choice for short-term study and an alternative method for long-term research, no significant benefits have been shown even with the recent strategic advancement in cuff methods. Implantation of the graft through an end-to-end aorto–aorta handsewn anastomosis,[5,8] with its high rate of technical failure, restricts its acceptance among surgeons.[9] Such limitations of the model necessitate conversion to a much safer, feasible, valid, and reliable model, the establishment of which has challenged surgeons and researchers in the field of intestinal transplantation for many years. Exploration and further innovation of the conventional method is undoubtedly necessary.

In this study, we introduced a new means of performing microsurgical vascular anastomosis using porto-portal cuff anastomosis without a microscope and traditional suture for vascular construction. The basic components established here are modified cuffs used to unite the vessel walls in a rat model of SIT by applying a specially designed vascular anastomosis technique, we accumulated first-hand experience and made satisfactory progress in the investigation of orthotopic intestinal transplantation (OIT). Given its decreased rate of complications and increased survival rate, the feasibility and possible advantages of this technique were further analyzed.

Materials and Methods

Animals and stages

From March 2006 to March 2008, 270 contiguous OITs in rats were performed using adult male Lewis (RT11) rats weighing between 200 and 280 g (Vitalriver, Beijing, China) as donors and recipients (isografts). Donor and recipient were paired according to similar body weight. Donor rats were fasted for 48 hours, and recipient rats were fasted for 24 hours (only water ad libitum allowed during fast) before the study. All rats underwent laparotomy using a midline incision. The research team involved in the surgeries of both groups included junior and senior doctors. This study was approved by our institution's ethics committee, and all procedures were carried out in accordance with institutional norms for laboratory animal care.

The exploratory process of the research work was divided into 3 stages:

1. The aim of the first stage (n = 140) was to establish porto-to-portal anastomosis in reconstitution of the natural physiologic portal graft drainage by the cuffed superior mesenteric vein (SMV) method because this cuff was fixed on the recipient's SMV first.
2. In the second stage (n = 140), in addition to the porto-portal anastomosis, the graft was revascularized by an end-to-side anastomosis between the donor's superior mesenteric artery (SMA) and the recipient's aorta using continuous 9-0 monofilament suture (aorta to aorta) followed by cuffed end-to-end anastomosis between the donor's PV and the recipient's SMV.
3. In the third stage, 130 rats (group 2) were included to establish the models of modified 3-cuff anastomosis in revascularization. Average donor weight was 30 to 70 g, which was less than that of the average recipient. The technique involved in OIT procedure was the main con-