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• CLINICAL RESEARCH •

Modified techniques of heterotopic total small intestinal transplantation in rats

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Abstract

AIM: **To establish a successful model of heterotopic total small intestinal transplantation (SIT) in rats in order to reduce the complications and increase the survival rate.**

METHODS: **A total of 196 Wistar rats underwent heterotopic SIT with microsurgical technique. Technical modifications included shortening fasting time and supplying energy before surgery, administering optimal volume of crystalloid fluid to the donor and recipient during surgical procedures, reducing mechanical and ischemic injuries to donor intestine, revascularizing small intestinal graft with a combination of conventional aorta to aorta anastomosis and a cuffed portal vein to left renal vein anastomosis which resulted in an acceptably short warm ischemic time, and also an adequate blood supply and drainage of the graft.**

RESULTS: **The average time for the donor surgery was 86min±20min, the mean operative time for the recipient was 115min±20min and warm ischemia time was shortened to 40min±5min. There was a shorter revascularizing time of the graft, the abdominal aorta (AA) to AA anastomosis being 21min±10min, and the cuffed portal vein (PV) to the renal vein anastomosis being 5min±5min.The one-week survival rate of 98 rats with SIT was 88.78% (87/98), without thrombosis and stenosis of anastomosis. The longest survival time of recipient rats was more than 389 days after SIT, the rats were maintaining normal weight, with perfect intestinal function and intact intestinal histology.**

CONCLUSION: **These modified techniques for SIT would remarkably reduce the complications and improve survival rate in rats, which provided a potentially more consistent and practical model for experimental and clinical studies.**

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INTRODUCTION

Since Monchick and Russel[1] established the model of small intestinal transplantation (SIT) in 1971, much modification and development have been achieved[2-11]. However, the technical complexity and high mortality have hindered the wide use of this valuable model[12-24]. Parallel to our clinical SIT practice, we have successfully established a stable and practical model of heterotopic SIT with fewer complications and higher survival rate using the modified techniques.

MATERIALS AND METHODS

Animals

One hundred and ninety-six male adult Wistar inbred strain rats weighing between 180g and 310g (Shanghai Laboratory Animal Center of Academy of Sciences of China) were used as donors and recipients. Housed and fed at the Animal Center of Nanjing University, the rats were put accustomed to the environment for at least 7 days before surgery. The donor and recipient were paired according to the similar body weight.

Preoperational care and anesthesia

All the donor and recipient rats stayed fasting in metabolic cages with no access to waster but allowed to drink 5% glucose normal saline added with 160 000U/L gentamycin ad libitum for 10 h-12 h. The rats were anesthetized with an intraperitoneal injection of 1% ketamine (1mL/100g) supplemented with the 1/4 primitive dose of ketamine as required.

Donor operation

Lactated Ringer's solution with 2.5g/L Cefazomelin was infused via the penile vein by micropump (Perfusor Secura FT, B. Braun Melsunge AF, Germany) at 4 ml/h. The abdomen was opened using a "⊥" -shaped incision, and the jejunum was cut at 1cm away from the Treitz's ligament and ileum at 2 cm proximal to ileocecal valve. The entire colon was removed. The portal vein (PV) was separated from pancreas. The segment of abdominal aorta (AA) containing the superior mesenteric artery (SMA) was mobilized by ligating and dividing the lumbar artery. The lumbar arteries from the AA were meticulously ligated with 8-0 nylon sutures to minimize bleeding between the celiac and left renal artery. The left renal vessels were then ligated. The dissected AA was ligated below the left renal artery. The celiac artery was ligated, followed by the ligation of the pyloric vein and splenic vein. Five to eight ml 2.5g/L Cefazomelin in saline was injected into the small intestine through the upper end of the jejunum. The AA was cannulated with a fine polyethylene catheter and the PV was cut off near hepatic hilum. The graft was perfused in situ with 2-3 ml 4 ℃ lactate Ringer's solution containing 125 000U/L heparin by micropump at 40 ml/h until the graft intestine and mesentery turned pale, and the fluid in the PV became clear. At last, the intestine and its vascular supply including a part of AA were removed en bloc. Under operational microscope and

in lactated Ringer's solution ice-water bath, the PV end was placed into a polyethylene cuff tube and its end part of endothelium was turned over to cover the end of cuff tube. The PV end and cuff tube were fixed with 6-0 silk sutures. Hence, the round orifice of the PV was exactly in the center of the cuff tube (Figure 1). The small intestinal graft was stored in lactated Ringer's solution at 4 ℃[25-27].

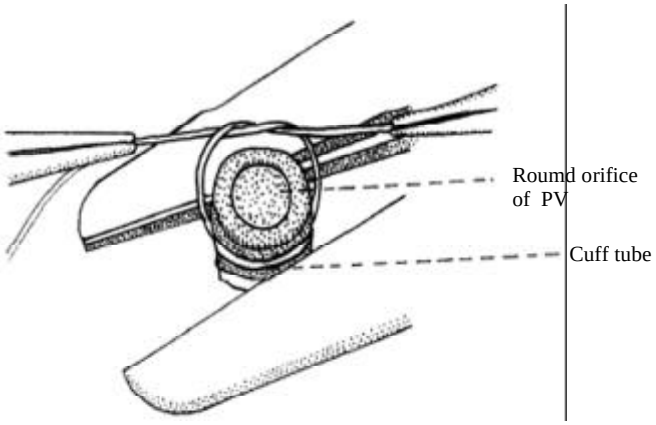

Figure 1 Fix cuff tub of PV

Recipient operation

Anesthesia and intravenous infusion for the recipient were the same as for the donors. The abdomen was opened via a midline incision from the ensisternum to the bladder level. The left ureter and renal atery were ligated. The left renal vein was dissected. The pedicle near renal helium was ligated and the ligating suture was left as a tractor after removal of the left kidney. Segment of the recipient's abdominal AA (0.6-1.0 cm) was mobilized below the vessels to the left kidney. Under operating microscope (×10 amplification), the AA of the adventitia membrance of the anterior wall was removed and opened via a longitudinal arteriotomy. The lumen was flushed with low molecular dextran solution. The donor's small intestine was picked up from the ice water, surrounded by a gauze sponge packed with ice crystals, and then placed onto the right flank of the rats. The arterial anastomosis was performed first. After ensuring that the artery was not twisted, an end-to-side anastomosis was performed using continuous 9-0 non-traumatic nylon suture. The posterior wall was anastomosed from the inside of the vessels. The anterior wall of the arterial anastomosis was sutured externally. Each lateral wall of the artery was sutured with 8 -10 sutures. The end of the left renal vein of the recipient was opened with a longitudinal incision. Two 9-0 nylon stay sutures were placed at the lateral sides of the anastomosis as a self-retaining retractor. The upper and lower sides of the incision were hauled by the pedicle ligating suture and microtweezer respectively. The cuffed PV of the small intestinal graft was inserted into the left renal vein of the recipient to revascularize the heterotopic small intestinal graft. The anastomosis was fixed with 5-0 silk suture (Figure 2). The left renal venous clamp was released first, followed by the clamps over and beneath the AA anastomosis, and the blood supply of the small intestinal graft was recovered. The arterial anastomosis was compressed lightly with a dry sponge for 1 to 2 min after reperfusion and then usually the oozing blood could be easily stopped. If blood was spouting from the arterial anastomosis, it should be quickly repaired with interruptive sutures. For the purpose of warm and flush, 20 mL warm saline was instilled in the peritoneal cavity. The small intestinal graft was put in order, and placed onto the left flank of the rats. Both ends of the graft were exteriorized as stomas. The stomas were sutured with four 7-0 silk sutures between the host perttoneum and the seromuscular layer of the graft and four 5-0 silk sutures between the skin and the everted mucosa of the graft (Figure 3). The abdomen was closed using two layers of 1-0 silk continuous sutures.

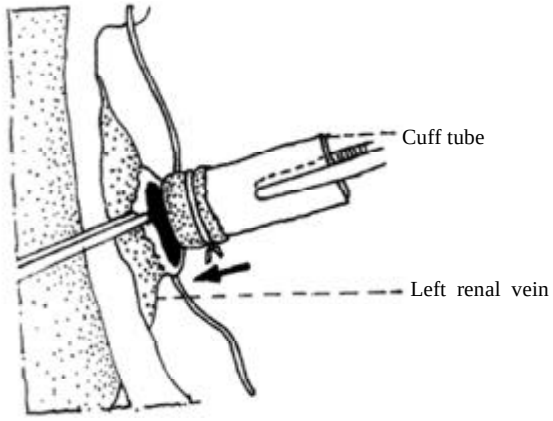

Figure 2 PV to left renal vein anastomosis

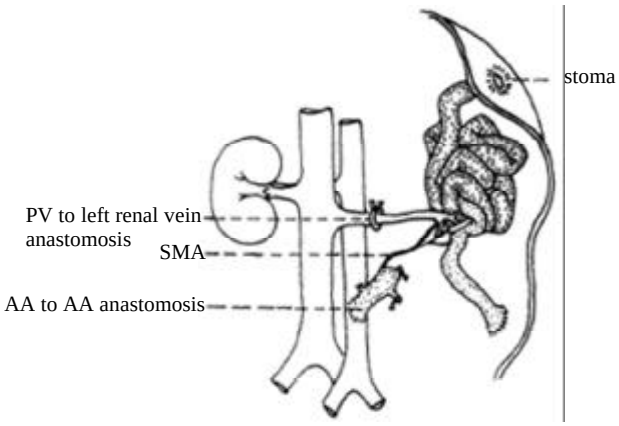

Figure 3 Heterotopic total small intestinal transplantation in rats

RESULTS

Operated by one person, the average time for the donor surgery was 86±20 min, and 115±20 min for the recipient and the average warm ischemic time being 40±5 min. There was a shorter revascularization time of the graft, the AA to AA anastomosis was 21±10 min, and the cuffed PV to the renal vein anastomosis was 5±5 min. Sixteen rats which died from anesthetic accidents and hemorrhage during operation were not included in the statistical data. Among the 98 heterotopic whole small intestinal transplantation, 11 rats died in 6 days, the autopsy verified 2 cases of arterial anastomotic hemorrhage, 3 cases of the native small intestinal dysfunction, 4 cases of infection of abdominal cavity, and 2 cases of the pulmonary complications (Figure 4). There was no gross or microscopic evidence of either vascular occlusion in any of the grafts or stoma-related complications. The one-week survival rate was 88.7% (87/98). The rats recovered vigor and vitality after the operative day. The shape and color of the transplanted small intestines were nearly the same as the native intestines from the tenth day. The longest survival time of recipient rats was more than 389 days after SIT when the data were collected. They maintained normal weight, perfect intestinal function and intact intestinal histology.

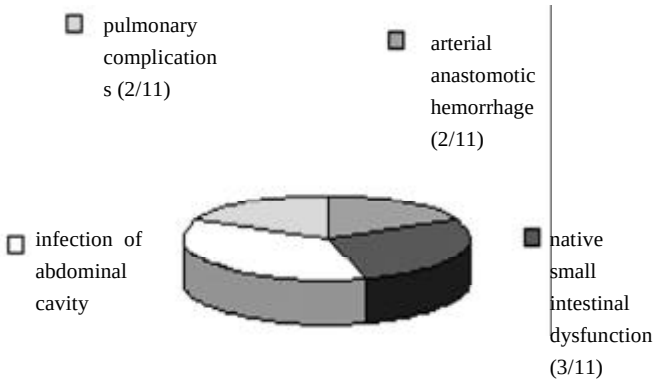

Figure 4 Cause of death in SIT rats

DISCUSSION

SIT of the rat remains a microsurgical technique which is difficult to manage. According to the need of clinical SIT practice, we used Wistar inbred strain rats as donors and recipients to practise heterotopic SIT, so that the model would not be affected by immune responses[28-40]. Following the modified methods of Zhong *et al*[2] and Kiyozaki *et al*[3], we have accumulated a great and original experience with reducing complications and increasing survival rate in rats.

Enhancement of operative tolerance

Shortening fasting time Both donor and recipient rats were placed in metabolic cages and kept fasting before surgery, without eatable cushion and dirt. The fasting time was shortened from 48 h (donors) and 24 h (recipients) respectively to less than 12 h, a satisfying result could be achieved through this management, only a little bile and bowel fluid were found in the small intestinal graft by surgery. The rats received 5% glucose normal saline ad libitum before surgery for a supplement of water, salt and energy.

Intravenous infusion Hypovolemic shock was the most common cause of postoperative death in SIT rats because of hemorrhage, evaporation and loss of bowel fluid[15].The total blood volume of rats was about 5.5-6.5 ml/100 g body weight, if the loss of blood exceeded 3 mL, ischemic damage of small intestinal graft would occur in donors, and death would happen in recipients. Besides improving dissecting and anastomotic techniques to reduce bleeding, continuous infusion with lactated Ringer' s solution via penile vein could keep blood pressure stable during surgery and increase survival rate. If there was a mass bleeding or blockage in intravenous infusion during surgical procedure, rats were given 4-5 ml (2 ml/100 g body weight) of 5 % glucose normal saline via back subcutaneous injections after SIT. Fatal pulmonary edema and heart failure could be caused by overdose solution or fast infusion, so the infusion should be controled to an optimum volume, never excessive.

Improvement in the vitality of small intestinal graft The quality of donor organ affected directly the result of transplantation. This is especially true for the small intestinal graft, which is much liable to mechanical and ischemic injuries during the procedure. The recipient rat "stupor" or "no vitality" or failure to death within 1 d-2d after SIT mostly occurred due to the quality of small intestinal graft. During the whole harvest procedure, the non-traumatic techniques should be adopted, a gauze sponge with saline was used to mobilize the graft gently instead of holding or clamping with hand and microtweezer, and not to toss and turn the graft repeatedly so as to avoid damage. Because of the "⊥" –shaped incision, the small intestinal graft dissected from the colon could be easily placed into abdominal cavity to reduce the exposure and vaporization damage. As vigorous intra-luminal irrigation and graft perfusion directly damaged the microcirculation of the graft[41-44], we changed the method of perfusion from parting body to in situ graft perfusion in living donors and significantly reduced the volume of intra-luminal irrigation from 50-70 ml[1] to 5-8 ml. The small intestine was put in order before graft perfusion, then the solution in the gut lumen could easily flowed out. The speed of irrigation was not quickened till the intra-luminal solution flowed out, avoiding over-distention of the donor small intestine. The volume of graft perfusion *in situ* was reduced from 12-20 ml[1] to 3-5 ml. The speed and volume of perfusion were accurately controlled by micropump instead of gravity, the graft perfusion was complete and the damage was reduced to minimum. Ischemic injury due to hypovolemic shock during the harvesting was often neglected by surgeons. For example, early ligation of the pyloric and splenic vein rapidly caused splanchnic venous congestion leading to shock[2].Therefore, we performed this procedure just prior to perfusion and after ligation of the celiac artery to avoid graft blood flow reduction. We also minimized ischemic injury to the donor small intestine by meticulous ligation of the lumbar vessels to avoid unnecessary blood loss, early ligation of the distal AA to improve perfusion of the graft and intravenous administration of 6-8 ml of lactated Ringer' s solution to supply blood volume and improve blood circulation of the small intestinal graft during the surgery. A shorter warm ischemic time was very important for the improvement of graft vitality, the warm ischemic time in our experiment was almost controlled in 40 min. If the time was too long, the normal blood circulation could not be recovered, the result of transplantation would be seriously affected, which appeared as the graft edema, segmental venous stasis, congestion and extremely thin enteric fluid.

Improvement of recipient surgical procedure

It was critical that there was an adequate blood flow from the SMA to the graft and out through the PV smoothly. Based on Zhong[2] and Kiyozak[3] surgery procedure, we refined some techniques. The blood flow of the inferior vena cava(IVC) was not blocked during surgery, so the blood circulation was not interrupted. The cuff technique was used with a cuffed anastomosis of the donor PV to the recipient left renal vein, the whole procedure of anastomosis spent about 5 min. Since the cuff tube was sculped from a polyethylene tube with 2.3 mm in outer diameter and 1.8mm in inner diameter, there was a larger and standard anastomosis. Furthermore, the endangium of the cuffed PV was well overturned, there was no any exposed anastomostic material in the venous lumen. So the unobstructed rate was much higher, none of rats died of the venous anastomostic complication. The AA column with SMA of the graft was anastomosed end to side to the AA of the recipient, the anastomotic bore was bigger and shapeable, so the anastomosis could be in progress smoothly without any tension. We used low molecular dextran solution without heparin to rinse the anastomosis, the damage to the endothelium was slighter[45-46], and the chance of anastomotic stenosis and thrombosis was greatly reduced[47-50]. These improved techniques resulted in an adequate blood flow to the graft without acute and chronic graft ischemia and the survival rate of the transplanted rat was obviously increased. It was true

that removal of one kidney did not increase the mortality in our experiment yet. After removal of one kidney, the remained kidney usually has a capacity to compensate. The adaptation may take place within 12-24 h and reach the largest degree during 1-2 wk. There was no an obvious disadvantage effect on physiological function and some experimental researches such as the absorptive function and permeability of transplanted small intestine could be studied on the model without any inconvenient.

In conclusion, our results suggested that applying these modified techniques would remarkably reduce the complications and improve survival rate in rats, the transplanted small intestine had a long-term fine function, this provided a potentially more consistent and practical model meeting the need of experimental and clinical studies.

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