Journal of Investigative Surgery, Volume 1, pp. 193–200
Printed in the USA.

0894-1939/88 $3.00 + .00

Orthotopic Nonauxiliary Total Bowel Transplantation Using Rats: Technical Procedure

RYO SHIMAZU, MD
JAMES B. GROGAN, PhD
SESHADRI RAJU, MD

The University of Mississippi Medical Center
Department of Surgery–Transplantation
2500 North State Street
Jackson, MS 39216-4505

Abstract *A procedure for nonauxiliary orthotopic total bowel transplantation is described, in which the graft from jejunum to distal colon is transplanted with portal venous drainage in end-to-end fashion in a one-stage procedure. The early postoperative mortality (within 4 days) was 23.8% in this study and presently is approximately 20% in our laboratory. It is noteworthy that the incidence of venous thrombosis is very low in this model. Continuous venous infusion, vascular perfusion, and luminal irrigation with Euro-Collins solution were employed in this preparation. The technique described is expected to prove useful for orthotopic nonauxiliary bowel transplant investigation because of its technical simplicity, low early postoperative mortality, and low incidence of vascular complications.*

Keywords: Total bowel transplantation, rat, orthotopic, nonauxiliary.

Clinical small bowel transplantation has yielded poor results with conventional immunosuppression. The advent of cyclosporine, however, has stimulated renewed interest in bowel transplant studies.

To resolve some of the complex immunological reactions associated with bowel allografts, inbred rats have been employed. Monchik and Russell[1] developed the heterotopic auxiliary model, in which the total small bowel graft is placed as a Thiry-Vella loop and its artery and vein are anastomosed to the recipient's aorta and inferior vena cava, respectively, in side-to-end fashion. Because of the technical simplicity and lower incidence of technical failure (10%), this model has been used frequently to study bowel transplantation in rats.[2–4] In some studies,[5,6] this graft was fashioned with the reestablishment of intestinal continuity. From the clinical viewpoint, the small intestine should naturally have portal venous drainage, but the reconstruction of the portal vein is difficult. Kort et al. developed the orthotopic model, in which the graft's vein is anastomosed to the recipient's portal vein in end-to-side fashion and the intestinal continuity is restored nonauxiliarily; the incidence of portal venous thrombosis was over 30%.[7] No studies using this model had been performed for years until the studies by

Schraut et al.,[8] probably because of the technical difficulties and high incidence of complications associated with venous anastamoses.

We have perfected a rat model of orthotopic nonauxiliary bowel transplantation in which the entire bowel, including the colon and the small intestine, is transplanted with portal venous drainage. This segment can be isolated very easily with a single vascular pedicle and implanted into the recipient without disturbing the anatomical relationship.

Materials and Methods

Animals

Commercially obtained Lewis rats (RT1^{l}; Le), ACI rats (RT1^{a}; ACI), and F_1 hybrid rats (ACI × Lewis; RT1^{la}; F_1) bred in our laboratory were used in the study. Recipients and donors weighing 250–300 g were chosen for ease of vascular anastomosis. Lewis rats were always used as recipients. The donors were fasted for 24–48 h and the recipients for 24 h preoperatively, but allowed water containing glucose or sucrose (3–4%) ad libitum. Pentobarbital (45 mg/kg) containing a small amount of atropine sulfate was given intraperitoneally for the initial anesthesia. When necessary, an additional 2–3 mg of this agent was administered intraperitoneally during the procedure.

Operative Procedure

Graft Preparation. A midline incision was employed. The right colon was retracted out of the abdominal cavity to the left and wrapped with gauze sponges moistened with lactated Ringer's solution. The rest of the bowel was placed in the abdominal cavity to avoid unnecessary manipulation. The omentum and the pancreas were removed from the colonic edge and the mesentery with sharp and blunt dissection, and the anterior side of the portal vein was easily exposed (Fig. 1). Following division of the ligament of Treitz, the section of the portal vein between the splenic and inferior mesenteric veins was freed from surrounding tissue and a few inferior pancreatic dudodenal vessels supplying the pancreatic head were ligated and divided. The distal centimeter of colon was resected and the remaining left colon freed from the retroperitoneum. At this point, the superior mesenteric artery was freed from surrounding tissue. Heparin (100 units) was administered intravenously via the inferior vena cava. The vascular bed of the graft was flushed in situ (5–6 mL of cold (4 °C) Euro-Collins solution containing heparin (1.5 units/mL)) via a catheter placed in the distal aorta after clamping the proximal aorta at the diaphragmatic level. After resection, the graft lumen was irrigated gently through both ends of the graft and a hole made in the cecum, initially with cold (4 °C) lactated Ringer's solution containing 0.1% kanamycin sulfate and then with cold (4 °C) Euro-Collins solution. The graft was preserved in cold lactated Ringer's solution until transplantation.

Recipient Operation. A thin silicone catheter (0.3 mm i.d. and 0.625 mm o.d.) was inserted into the right epigastric vein before laparatomy. Lactated Ringer's solution was infused continuously (1 mL/h) via the catheter during and after the

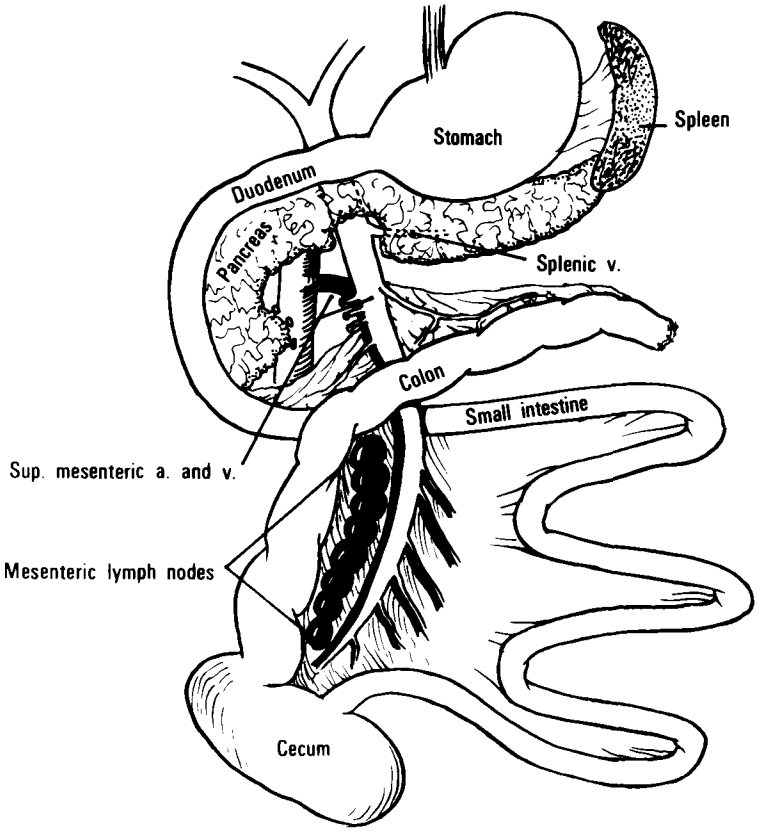

Figure 1. Schematic anatomy.

procedure. The rate of infusion was increased during vascular declamping because of the irregular precipitous fall in blood pressure that accompanies this maneuver. As in the donor, a long midline incision was used. After the entire intestine was retracted, the distal colon was ligated, resected, and left in the pelvic cavity. The superior mesenteric artery and the segment of the portal vein between the splenic and inferior mesenteric veins were freed from the surrounding tissue. The duodenum was resected at a point 5 mm distal to the entry of the bile duct. Along the line from this point to the freed portal vein, the pancreatic tissue was ligated and cut, and the intestine of the recipient was removed. Thus, the portion of the pancreas corresponding to the uncinate process of the human pancreas was sacrificed with the intestine. The superior mesenteric artery and vein were clamped and cut, avoiding interruption of the splenic vein. Retention of the splenic vein was found to be important in preventing postoperative pancreatitis in our preliminary studies utilizing outbred rats (unpublished data). If the segment of superior mesenteric vein was not long enough for the anastomosis, the inferior mesenteric vein was ligated and cut and the distal portion of the superior mesenteric vein was used. Reconstruction of the artery and vein was accomplished using end-to-end anastomoses with interrupted sutures (10-O nylon) under microscopic magnification. During the vascular anastomoses, the graft was kept cold by application of gauze sponges that were moistened with 4 °C lactated

Ringer's solution. The duodenum was anastomosed with 6-O silk. The schema of the reconstruction is shown in Figure 2. The hole in the cecum was closed with 6-O silk. The end of the colon was exteriorized on the left flank as a colostomy to permit examination of the mucosa. The abdominal wall was closed with two-layer closure (2-O nylon).

Postoperative Care

Continuous intravenous fluid infusion was maintained as long as possible after the procedure. The recipients were warmed under a heat lamp overnight. Ten milliliters of lactated Ringer's–5% dextrose solution was given subcutaneously twice a day for 3 days after surgery. Cefazolin sodium (20 mg) was given subcutaneously twice a day for 5 days beginning with the day of operation. Water was permitted ad libitum from the first postoperative day, and normal diet was resumed on the third postoperative day.

Experimental Groups

Group 1: Isogeneic transplantation. Eleven Le grafts were transplanted.

Group 2: Semiallogeneic transplantation. Ten F_1 grafts were transplanted. Only the rejection reaction was expected to occur.

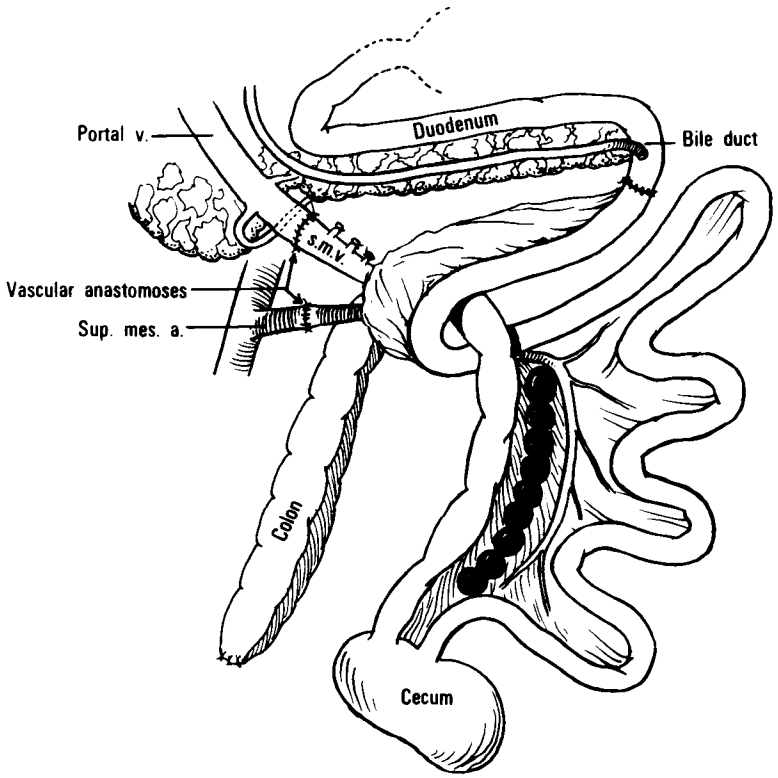

Figure 2. Schema of reconstruction.

The recipients were weighed, the abdomens palpated, and the colostomies inspected daily. When the recipients became moribund, the patency of the vascular anastomoses was examined by exploratory laparotomy under deep anesthesia and then sacrificed.

Results

Operating time was approximately 3 h, from the induction of anesthesia to the closure of the recipient's abdomen. Cold ischemic time of the graft was approximately 60 min.

Clinical Course and Body Weight Change

All recipients of either isografts or allografts lost 20–30% of their preoperative body weight within 7 days. Allotransplanted animals were sacrificed without having recovered their body weight, while isotransplanted animals slowly regained their body weight. A relatively long period was required for attaining the preoperative weight (45.3 ± 8.7 days; mean ± SE).

The allotransplanted recipients that survived the early postoperative period usually drank water well on the first postoperative day (POD) and ate normal chow well on the third POD. As soon as oral intake began, they had loose (but not watery) stools. They ate well for several days, but suddenly stopped eating, appeared dehydrated, and assumed a posture of sitting on their haunches with their backs humped. These developments were concurrent with a dried and ischemic mucosa of the colostomy on the day before sacrifice.

Survival (Table 1)

Three (27.3%) of 11 isotransplanted animals died within 4 days from the complications accompanying the operative procedure: one from venous thrombosis, which was certified macroscopically by exploratory laparotomy before sacrifice, one from graft bleeding, and one from a retroperitoneal abscess related to the cut end of the colonic remnant. One death (19th POD) was attributed to emaciation

Table 1
Cause of Death and Mean Survival Time

	Cause of death	Mortality (within 4 days)	Mean Survival (days)
Isograft (Le → Le) $n = 11$	Vascular thrombosis (2) Retroperitoneal absess (1) Emaciation (1)[a]	3/11 (27.3%)	>200 ($n = 7$)
Allograft (F_1 → Le) $n = 10$	Rejection (8) Bleeding from arterial anastomosis (1) Graft bleeding (1)	2/10 (20%)	9.8 ± 1.5 ($n = 8$; mean ± SD)
	Total	5/21 (23.8%)	

[a] On the 19th postoperative day.

and dehydration, suggesting the possibility that nonimmunological concerns also affect the recipient's survival after small bowel transplantation. The remaining 7 have survived 200 days or longer.

In the allotransplant group, two recipients (20%) died within 4 days from postoperative complications: one from graft bleeding and the other from bleeding from the arterial anastomosis. Overall, 5 of 21 (23.8%) died from technical failure within 4 days. With no immunosuppressive regimen, the remaining 8 died of rejection or rejection-related complications. Mucosal change or destruction and lymphoid cell infiltration in the bowel wall and mesentery were consistent with rejection, although the rejection reaction is not always apparent in small bowel transplantation. After excluding the animals that died within 4 days, the mean survival time was 9.8 ± 0.5 days (mean ± SE).

Discussion

In our preliminary studies utilizing outbred rats (unpublished data), two major problems were apparent: (1) the instability of the cardiovascular system after reperfusion and (2) the difficulty of preservation of a sizable graft. Arterial blood pressure was monitored in rats in which the superior mesenteric artery was isolated in the same way as in the current model, but without actual division and without flushing the vascular bed. The intestine was cooled inside and out with cold saline and left in situ for 60 min after the vessels had been clamped under heparinization. When the clamps were removed, arterial blood pressure dropped markedly and remained low, although it rose temporarily after a bolus infusion of lactated Ringer's solution (Fig. 3). At times, marked bradycardia developed, apparently caused by the influx of cold blood from the graft; this arrhythmia often reversed spontaneously. Lactated Ringer's infusion, during operation and for as long as feasible thereafter, markedly reduced the incidence of these abnormalities. Without constant infusion, the animal went into irreversible shock, especially when significant blood loss during the procedure contributed to the so-called "declamping shock." A large amount of fluid is lost into "third space" after reperfusion.

Euro-Collins solution was used for vascular perfusion of the graft. This solution appeared to be superior to lactated Ringer's solution or saline in clearing the graft of blood elements. The rats had frequently exsanguinated after transplanta-

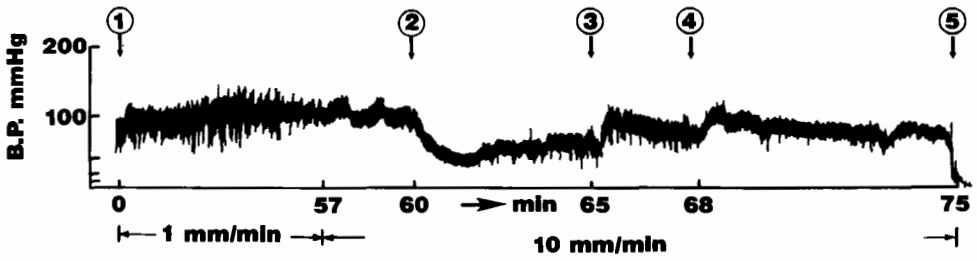

Figure 3. The arterial blood pressure after declamping the superior mesenteric artery and vein. 1, Clamp; 2, declamp; 3, infusion (3 mL of lactated Ringer's solution); 4, reinfusion; 5, sacrifice.

tion before Euro-Collins solution was used for luminal irrigation. In our preliminary in vitro study of graft preservation, irrigation with lactated Ringer's solution produced graft edema and destruction more readily than did Euro-Collins solution (unpublished data). After Euro-Collins solution was used for the last step of the luminal irrigation of the graft, the incidence of graft bleeding was markedly reduced.

In all reports of orthotopic bowel transplantation, the investigators mentioned that the most frequent cause for early postoperative mortality was most frequently attributable to vascular thrombosis, in both the rat model and canine models. According to Kort et al., 13 of 40 recipients (32.5%) died within 48 h and most of these deaths were due to thrombosis of the portal vein. In addition to this early postoperative mortality, among the isogeneic group, one recipient died from portal venous thrombosis within 3 days and two from infection within 8 days, demonstrating that even survival of the early postoperative period did not assure protection from nonimmunological factors (40% mortality). It was certainly true that vascular thrombosis was a major problem in the preliminary studies in outbred rats. In this study, however, the incidence of vascular thrombosis was extremely low (1/21 (4.8%)) and the early postoperative death attributable to some other cause. Consequently, the early postoperative mortality is at present about 20%. Possible explanations for this include (1) the major vessels, the superior mesenteric artery and vein, are anastomosed in end-to-end fashion because of anatomic simplicity in our model, while the drainage vein of the graft and the portal vein of the recipient were anastomosed in end-to-side fashion in the Kort model; (2) the graft is placed in the exact orthotopic position with no changes in the anatomical relationship in our model; (3) the recipient portal vein is not completely occluded during anastomoses, in that the splenic venous outflow is retained; and (4) the cardiovascular system is well controlled after operation, especially just after reperfusion, by using continuous venous infusion.

Although orthotopic bowel transplantation in the canine appears technically easier than in the rat, the reported mortality in the early postoperative period is 30–50%.[9,10] The 23.8% mortality in our rat model is more acceptable. In addition, immunological investigations, the most important elements of bowel transplantation, are not as easily accomplished in canine models.

The technique described herein promises to be useful for a variety of immunological and nonimmunological investigations in bowel transplantation.

Acknowledgment

Support by NIH Grant 5 RO1 DK32249-02.

References

1. Monchik GJ, Russell PS: Transplantation of small bowel in the rat: Technical and immunological considerations. *Surgery* 1971;70:693–702.
2. Telford GL, Corry RJ: Immunological enhancement of rat small intestinal allografts. *Arch Surg* 1978;113:615–617.
3. Kirkman RL, Lear PA, Madara JL, Tilney NL: Small intestine transplantation in the rat: Immunology and function. *Surgery* 1984;96:280–287.
4. Hatcher PA, Deaton DH, Bollinger RR: Transplantation of the entire small bowel in inbred rats using cyclosporine. *Transplantation* 1987;43:478–484.

5. Lee KKW, Schraut WH: Structure and function of orthotopic bowel allografts in rats treated with cyclosporine. *Am J Surg* 1986;151:55–60.
6. Kaplan S, Racelis D, Martinelli GP, Schanzer H, Aufses AH Jr: Orthotopic, segmental small-bowel transplantation in the rat. *Mt Sinai J Med* 1986;53:94–98.
7. Kort WJ, Westbrock DL, MacDicken I, Lameijer LDF: Orthotopic total small bowel transplantation in the rat. *Eur Surg Res* 1973;5:81–89.
8. Schraut WH, Abraham VS, Lee KKW: Portal versus caval venous drainage of small bowel allografts: Technical and metabolic consequences. *Surgery* 1986;99:193–198.
9. Craddock GN, Nordgren SR, Reznick RK, Gilas T, Lossing AG, Cohen Z, Stiller CR, Cullen JB, Langer B: Small bowel transplantation in the dog using cylosporine. *Transplantation* 1983;35:284–288.
10. Fujiwara H, Grogan JB, Raju S: Total orthotopic small bowel transplantation with cyclosporine. *Transplantation* 1987;44:469–474.