Journal of Investigative Surgery, Volume 2, pp. 107–114
Printed in the USA.

0894-1939/89 $3.00 + .00

A Successful Model of Small Bowel Autotransplantation in the Dog

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Abstract *Experimental small bowel transplantation has continued to be a complex procedure with high mortality. We investigated the technical aspects of small bowel transplantation in an effort to define a procedure that would result in an improved survival rate. Three methods of graft harvesting were examined in a model of canine small bowel autotransplantation. Harvesting the graft by first flushing with room-temperature lactated Ringer's followed by iced lactated Ringer's resulted in the best preservation and subsequently the best survival rate (71%). Flushing with iced lactated Ringer's alone resulted in survival rates of 22 and 50% in two additional groups. We also investigated two methods of graft reanastomosis. Although either venous drainage regimen appears to be suitable, graft venous reanastomosis to the host portal vein resulted in a slightly higher postoperative weight than reanastomosis to the host inferior vena cava. A model of small bowel transplantation with a high long-term survival rate has been developed. This model can now be applied to studies of the various physiological aspects of small bowel transplantation.*

Keywords: small bowel transplantation, autotransplantation, dog, organ preservation.

Transplantation of the kidney, heart, liver, and lung has become somewhat commonplace in the last decade. While great strides have been made in the transplantation of these organs, little progress has been made in small bowel transplantation. This is due in part to the complex immunology of the small bowel and the subsequent phenomenon of rejection and graft versus host disease seen with these transplantation attempts. With the advent of the immunosuppressive agent Cyclosporine, there has been a resurgence of interest in small bowel transplantation. However, a successful model of small bowel transplantation with a high, long-term survival rate has remained elusive. A successful, reproducible model of small bowel transplantation is critical if further investigations of both the immunology and physiology of the transplanted gut are to be possible.

The present study was initiated to investigate the technical difficulties associated with small bowel transplantation. A canine autotransplantation model was

used to minimize immune complications so that efforts could be concentrated on the technical aspects of the procedure. Three methods of graft harvesting and two methods of graft reimplantation were examined. The results of our investigations follow.

Methods

Anesthesia and Surgical Isolation of the Graft

Adult mongrel dogs of either sex (approximately 18 kg body wt), certified free of intestinal parasites and in general good health, were used throughout. Animals were anesthetized with pentobarbital sodium (30 mg/kg iv). An endotracheal tube was positioned and the animals were subsequently ventilated with a Byrd respirator throughout the procedure. Through a midline abdominal incision, the duodenum was mobilized to expose the superior mesenteric artery and vein. The mesentery was dissected carefully in a caudal direction, such that preservation of the mesenteric arcades was possible. The line of mesenteric dissection was continued to the left side of the animal along the lower border of the pancreas. The mesenteric dissection proceeded so that all but approximately 2 cm of the terminal ileum proximal to the ileocecal valve was left viable for later anastomosis. Care was taken throughout the mesenteric dissection to minimize handling of the intestine and thereby avoid possible spasm of the smooth muscle. Heparin (4000 U) was administered just prior to division of the superior mesenteric artery and vein. Sectioning of these vessels took place just distal to the pancreaticoduodenal artery. The small bowel itself was resected proximally at the fourth portion of the duodenum and distally 2 cm proximal to the ileocecal valve.

Graft Harvesting

To examine the possible effects of different harvesting methods on operative mortality, the animals were divided into three groups. In group I animals ($n = 9$), the superior mesenteric artery was flushed with 500 mL of cold (4 °C) heparinized lactated Ringer's. The small bowel was subsequently immersed in lactated Ringer's solution at 4 °C immediately after harvest. Group II animals ($n = 16$) were pretreated with 1 g/day of Neomycin for two days prior to the surgical procedure. The superior mesenteric artery was flushed with 500 mL of cold heparinized lactated Ringer's (4 °C) and the small bowel was subsequently immersed in lactated Ringer's at 4 °C immediately after harvest. Group III animals ($n = 14$) were also administered Neomycin 1 g/day for two days before the autotransplantation procedure. However, in group III animals, the superior mesenteric artery was first flushed with heparinized lactated Ringer's at room temperature (25 °C) until the venous effluent was almost clear (approximately 200–300 mL). At this point, 500 mL of cold heparinized lactated Ringer's solution (4 °C) was infused into the superior mesenteric artery and the graft was subsequently immersed in cold lactated Ringer's. In all three groups, the small bowel graft was irrigated intraluminally with 500 mL of cold lactated Ringer's containing 500 mg of Kanamycin. The grafts from the three groups were carefully inspected and the inade-

quately flushed portions were excised. About 15 to 20 cm of the graft at either end was resected for this reason (Fig. 1*A*).

Graft Reimplantation

The small bowel grafts were orthotopically autotransplanted back into the donor animal. Two different venous reconnection procedures were examined. The first procedure involved reanastomosis of the superior mesenteric vein of the graft to the portal vein of the donor. The second procedure involved reanastomosis of the superior mesenteric vein of the graft to the vena cava of the recipient. All small bowel grafts in this portion of the study were harvested in an identical manner as

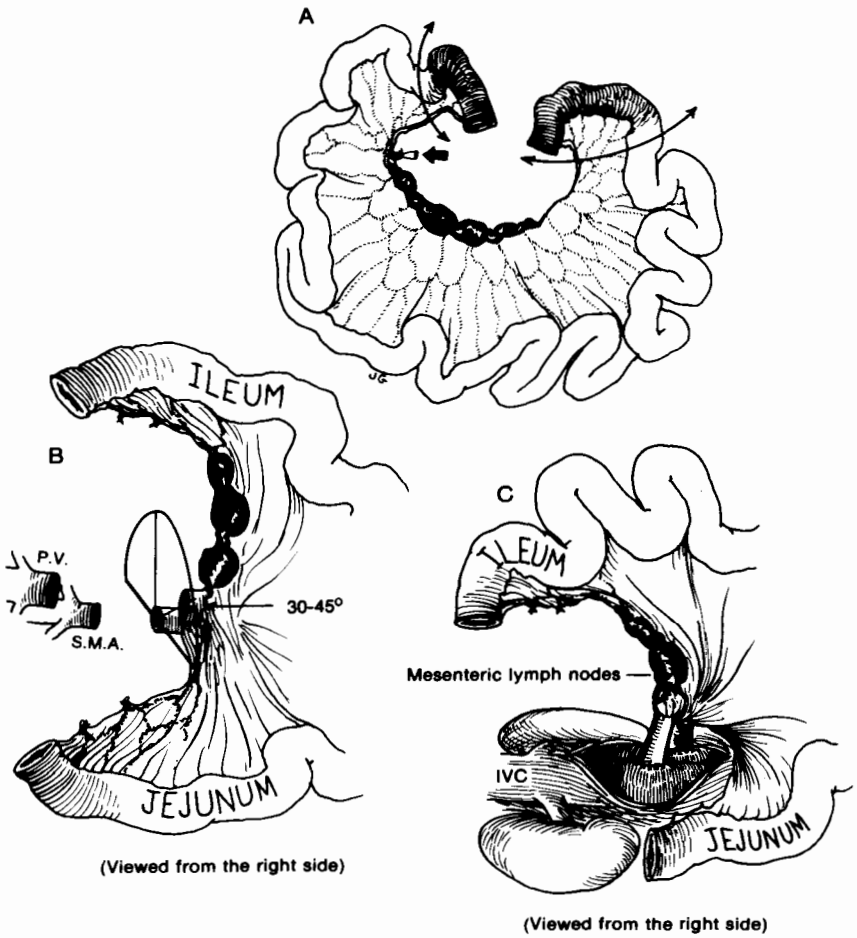

Figure 1. Technique of small bowel transplantation. (*A*) Underperfused ends of the transplant are resected. The arrow points to the infusion cannula. (*B*) Portal venous drainage technique. The mesenteric vein should be sewn with a slight rotation to the right so that the anastomosis will not kink when the bowel is placed in the abdominal cavity. (*C*) Vena caval drainage technique.

in group III animals. The superior mesenteric artery was reanastomosed to the abdominal aorta near the stump of the recipient superior mesenteric artery in both procedures. When the superior mesenteric vein was sewn back to the portal vein, the anastomosis was situated somewhat to the right of the arterial anastomosis at a 30–45° angle (Fig. 1*B*). When the superior mesenteric vein was sewn to the vena cava, the venous anastomosis was placed a few centimeters cranial to the reanastomosis of the superior mesenteric artery to the aorta (Fig. 1*C*).

The arterial and venous anastomoses were accomplished with the small bowel wrapped in a cool, moist towel. Interrupted sutures (6-O Prolene) were used. The small bowel graft itself was reconnected to the recipient small bowel using a single layer 3-O chromic catgut anastomosis both proximally and distally. Any mesenteric defects were closed at this time. During the anastomosis, particular care was taken to orient the mesentery to avoid kinking or twisting of the vascular reconnections. Before closure of the abdomen, the peritoneal cavity was irrigated with large volumes of warm saline solution to alleviate any hypothermia that may have occurred during the autotransplantation procedure. These maneuvers resulted in the orthotopic autotransplantation of approximately 90% of the originally transsected small bowel.

Postoperative Care

During the closure of the abdomen, the animals received large volumes (2–3 L) of intravenous fluids to compensate for the fluid loss during gut transplantation. Animals were maintained on intravenous hyperalimentation for 2 days following the autotransplant. On the third postoperative day, a fluid diet was begun. By the fifth postoperative day, animals were switched to a solid food diet. A combination of cephalosporin and aminoglycoside antibiotics was administered parenterally during this time. Neomycin (2 g/day) was given orally for 7 days following the initiation of the solid food diet.

Statistics

Differences between treatment groups were compared using chi square with Yates correction. A p value of less than 0.05 was determined to be significant.

Results

Survival Rate of Various Harvesting Techniques

The results of the various graft harvesting procedures are shown in Table 1. The overall operative success rate in group I was 22%. The majority of animals succumbed to arterial thrombosis of the graft. The overall operative success rate in group II improved to 50%. However, the most common cause of death in these animals also was arterial thrombosis of the graft. Group III had the best postoperative survival rate, with 71% of the animals surviving the procedure.

Table 1
Orthotopic Small Bowel Autotransplantation: Progressive Modifications and Results

	Number	Pretreatment	Preservation[a]	60-Day Survival (%)	Cause of Failure	
Group I	9	NPO for 24 h	(1) Flush graft with 500 mL cold LR. Immersion in cold (4 °C) LR		Arterial thrombosis	4
					Venous thrombosis	2
				2 (22)	Unknown	1
			(2) Intraluminal irrigation with 500 mL cold KM-LR			
Group II	16	NPO for 24 h	(1) Flush graft with 500 mL cold LR. Immersion in cold (4 °C) LR		Arterial thrombosis	5
					Venous thrombosis	1
		Neomycin PO,		8 (50)	Hemorrhage	1
		1 g × 2/day for 3 days	(2) Intraluminal irrigation with 500 mL KM-LR		Unknown	1
Group III	14	NPO for 24 h	(1) Flush graft with 200–300 mL warm (25 °C) LR followed by 500 mL cold (4 °C) LR. Immersion in cold LR (4 °C).		Arterial thrombosis	4
		Neomycin PO		10 (71)*		
		1 g × 2/day for 3 days	(2) Intraluminal irrigation with 500 mL KM-LR			

[a] LR, lactated Ringer's solution; KM-LR, LR with 500 mg Kanamycin.
* $p < 0.05$.

Portal vs. Vena Caval Venous Anastomosis

Animals having superior mesenteric venous anastomosis to the inferior vena cava had a lower postoperative weight than those having the mesenteric venous anastomosis to the recipient portal vein (Table 2). The animals with portal venous drainage from the graft appeared to recover and to exhibit a healthier manner than those animals with vena caval graft drainage. The differences between the portal vs. vena caval drainage groups were not dramatic, and both types of venous drainage appear to be usable in the model.

Postoperative Complications

Thrombosis of the arterial anastomosis was responsible for every mortality in group III animals. Antiplatelet agents were used in six dogs in an attempt to circumvent this problem. Fifty milligrams of aspirin and dipyridamole were given for 3 days prior to surgery and for 7 days postoperatively. Two of the six dogs so treated had arterial thrombosis despite this measure. A third dog died of a lethal postoperative hemorrhage. Therefore, antiplatelet agents were determined to be of limited benefit in resolving the problem and were discontinued in subsequent studies.

Watery diarrhea began when the intravenous hyperalimentation was discontinued and oral alimentation was initiated. In the majority of the animals, the diarrhea improved over a period of several weeks. However, in several animals diarrhea recurred and persisted for prolonged periods. During this period, antidiarrheal agents were successfully utilized. Neomycin sulfate 20 mg/kg, 1.5 mg/kg methscopolamine nitrate, kaolin and pectin, and Lomotil (2.5 mg of diphenoxylate hydrochloride and 25 μg atropine sulfate) were effective in controlling diarrhea and in maintaining satisfactory postoperative weight of the transplanted animals.

Discussion

The mortality rate of animals undergoing small bowel transplantation has remained high[1-4] even in more recent attempts of allotransplantation in which Cyclosporine was used for immunosuppression.[5] Kirkman[6] noted that recipient deaths occurred as a result of improper or inadequate bowel preservation. Rapid cooling of the intestinal transplant often induced vasospasm, which resulted in an

Table 2
Body Weight Changes[a] in Experimental Animals with Portal vs Vena Caval Drainage of the Small Bowel Graft

		Weeks Post-transplant			
Method	Number	1	2	3	4
Portal venous drainage	8	93.0 ± 2.4	94.4 ± 2.4	93.8 ± 3.2	94.4 ± 3.2
Vena caval drainage	5	93.0 ± 2.6	89.6 ± 4.1	87.7 ± 3.9	90.0 ± 3.8
Student's *t* test		NS	$p < 0.025$	$p < 0.10$	$p < 0.05$

[a] Percentage of preoperative weight.

inadequate clearance of the corpuscular elements and thus poor graft preservation.

We examined three methods of graft harvesting. Initial graft flushing with iced lactated Ringer's solution (groups I and II) led to an inferior harvest and a poor operative success rate. Gradual cooling of the graft by initial flushing with room-temperature lactated Ringer's solution, followed by iced lactated Ringer's when the graft appeared mostly clear of blood elements, resulted in a superior harvest and a markedly improved survival rate (group III). In addition, intraluminal irrigation of the graft was instituted as early as possible to help cool the organ rapidly and also to remove the enzyme-rich solutions. Evidence indicates that pancreatic enzymes may injure the enterocytes of the unperfused gut.[7]

We have noted that operative manipulation itself can cause a poor flushout. This was particularly evident at the proximal and distal ends of the transplant. To overcome these difficulties, unnecessary manipulation of the gut was avoided during the autotransplantation.

Considerable fluid loss occurs into the wall into the lumen of the transplanted gut following reperfusion. This phenomenon further exacerbates any hypovolemia already present due to the blood loss inherent in massive small bowel resection. Therefore, transplanted animals required large volumes of fluid for hemodynamic support in the immediate postoperative period. Fluid volumes often approached 3 L in 12–18 h.

In our early experience, several animals were lost to irreversible hypothermia. The small bowel graft, due to its large surface area, represents a large mass of cold tissue which acts as a cooling radiator. Considerable heat loss during reperfusion can be compounded by any existing hypothermia that may follow a long surgical procedure in an anesthetized animal. Irrigation of the peritoneum and the transplanted graft with warm saline solution following reperfusion is useful in minimizing the heat loss.

Diarrhea is a troublesome postoperative complication of small bowel transplantation. The accompanying weight loss resulting from diarrhea and subsequent poor absorption may be as much as 25% of the preoperative weight.[8] Both Ballinger et al[8] and Ruiz et al[3] noted diarrhea following simple denervation of the gut. Schiller et al[9] provided electrophysiological evidence of abnormal motility in the denervated gut following transplantation. Reznick et al[10] observed accelerated transit time in denervated allotransplants as well. Thus, there are considerable data to suggest that denervation itself results in abnormal bowel motility.

Malabsorption from disrupted lymphatic connections at the root of the mesentery can be expected to exacerbate the already poor absorption resulting from diarrhea. The presence of a protein-losing enteropathy[5] can be an additional factor responsible for postoperative diarrhea. Therefore, vigorous efforts should be made to minimize postoperative fluid and weight loss from this complication. The two antidiarrheal agents we employed were quite useful in alleviating this postoperative complication.

We also investigated the merit of portal versus vena caval drainage of the transplanted small bowel. The body weights of the animals that had vena caval drainage were slightly lower than those with portal venous drainage. In our experience, there was no difference in the mortality rate among the animals with portal versus vena caval drainage.

Early vascular thrombosis is among the more common complications in small

bowel transplantation.[3,8,10,11] Purely technical causes such as excessive skeletonization of vessels, inadequate suture technique or torsion of the mesenteric root are undoubtedly responsible for some instances of this complication. However, even when these incriminating factors were apparently eliminated, there has continued to be an irreducible minimum incidence of vascular thrombosis. Presently, mortality in our autotransplanted animals is attributed almost exclusively to this complication. A low-flow state resulting from postoperative fluid loss and diarrhea, vasospasm from abnormal sensitivity of the denervated gut to circulating catecholamines, edema of the graft from lymphatic interruption, secondary thrombosis, and endotoxemia could all be cited as possible mechanisms.

In summary, we have demonstrated that small bowel autotransplantation can be accomplished with an acceptable incidence of mortality. Proper graft preparation and a technically competent reimplantation are the paramount considerations when attempting this procedure. Also important are the proper management of postoperative fluid balance and the nutritional state of the animal. A successful autotransplantation model is an essential initial step in the investigation of nontechnical aspects of small bowel transplantation, such as post-transplant absorptive function and immunologic complications in an allotransplanted small bowel.

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