Video Article

Small Bowel Transplantation In Mice

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Abstract

Since 1990, the development of tacrolimus-based immunosuppression and improved surgical techniques, the increased array of potent immunosuppressive medications, infection prophylaxis, and suitable patient selection helped improve actuarial graft and patient survival rates for all types of intestine transplantation. Patients with irreversible intestinal failure and complications of parenteral nutrition should now be routinely considered for small intestine transplantation. However, Survival rates for small intestinal transplantation have been slow to improve compares increasingly favorably with renal, liver, heart and lung. The small bowel transplantation is still unsatisfactory compared with other organs. Further progress may depend on better understanding of immunology and physiology of the graft and can be greatly facilitated by animal models. A wider use of mouse small bowel transplantation model is needed in the study of immunology and physiology of the transplantation gut as well as efficient methods in diagnosing early rejection. However, this model is limited to use because the techniques involved is an extremely technically challenging. We have developed a modified technique. When making anastomosis of portal vein and inferior vena cava, two stay sutures are made at the proximal apex and distal apex of the recipient's inferior vena cava with the donor's portal vein. The left wall of the inferior vena cava and donor's portal vein is closed with continuing sutures in the inside of the inferior vena cava after, after one knot with the proximal apex stay suture the right wall of the inferior vena cava and the donor's portal vein are closed with continuing sutures outside the inferior vena cava with 10-0 sutures. This method is easier to perform because anastomosis is made just on the one side of the inferior vena cava and 10-0 sutures is the right size to avoid bleeding and thrombosis. In this article, we provide details of the technique to supplement the video.

Protocol

Donor preparation and small bowel harvest:

1. The mouse is anesthetized with an intraperitoneal injection of pentobarbital and placed supine on the operation field.
2. A long midline abdominal incision is made. The proximal of jejunum is identified and ligated with 6-0 sutures. Attached mesenteric vessels distal to the jejunum tie are ligated with 8-0 sutures.
3. The distal of ileum is identified and ligated with 6-0 sutures. The attached mesenteric vessels distal to the ileum are ligated with 8-0 sutures.
4. The portal is exposed from the surrounding fat and attached tissues, ligated and divided the pancreaticoduodenal and splenic veins.
5. The abdominal aorta is exposed and exposed the superior mesenteric artery from the surrounding tissues.
6. 1 ml of heparin (10u/ml) is injected into the inferior vena cava for heparinization.
7. The portal vein is divided at the hilum of the liver, and superior mesenteric artery is divided from aorta, including a Carrel patch of aorta.
8. The jejunum and ileum are divided distal to the ties. The entire small bowel is removed, then stored at 4°C in saline solution.

Recipient preparation and transplantation:

1. The mouse is anesthetized with an intraperitoneal injection of pentobarbital and placed supine on the operation field.
2. A long midline abdominal incision is made. Abdominal contents are retracted outside the abdominal with gauze to expose the abdominal aorta and the inferior vena cava.
3. The branches of the abdominal aorta and the inferior vena cava are exposed and ligated with 10-0 sutures.
4. The proximal and distal ligature is placed around the aorta and inferior vena cava respectively.
5. The venotomy is made in the inferior vena cava of the recipient with a 30 gauge needle. The opening is then extended to a length of equal to the donor's portal vein with micro-scissors.
6. The aortotomy is made in the abdominal aorta of recipient with a 30 gauge needle. The opening is then extended to a length of equal to the donor's superior mesenteric artery Carrel patch with micro-scissors.
7. The incisions of the abdominal aorta and the inferior vena cava are perfused with saline solution.
8. The donor's small bowel is placed on the left side of the recipient's abdomen covering with gauze.
9. The stay sutures are placed at the proximal apex and distal apex of recipient abdominal aorta with donor's superior mesenteric artery Carrel patch.
10. The anastomosis of the right side of the recipient's abdominal aorta and donor's superior mesenteric artery Carrel patch are completed first with continuing sutures.
11. Two stay sutures are made first at the proximal apex and distal apex of the recipient's inferior vena cava with the donor's portal for the anastomosis of the donor's portal vein and the recipient's inferior vena cava.
12. The left wall of the inferior vena cava and donor's portal vein is closed with continuing sutures in the inside the inferior vena cava.
13. After one knot with the proximal apex stay suture, the right wall of the inferior vena cava and the donor's portal vein is closed with continuing sutures outside the inferior vena cava.
14. The donor's small bowel is turned over to the right side of the recipient's abdomen. The right wall of the recipient's abdominal aorta and the donor's superior mesenteric artery anastomosis is closed with continue sutures.
15. After all anastomosis is made, the distal ligature is removed first to check the bleeding from the anastomosis.
16. If there is little or no bleeding from the anastomosis the proximal ligature is removed. The donor's small bowel is filled with blood immediately; the color of the small bowel becomes red.

17. The distal end of graft ileum is anastomosed end-to-side to the proximal part of recipient's jejunum with a continuous suture of 8-0 sutures.
18. The proximal jejunum of the donor is exteriorized as a stoma and secured to the skin on the right side of the abdominal wall with four sutures each side of 8-0 sutures.
19. The intestines are returned to the abdominal cavity and closed with 6-0 continue sutures.
20. The recipient mouse is placed on warm area. After one hour, the recipient recovers.

Discussion

The unique immune response after small bowel transplantation has been the subject of extensive research using small bowel transplantation model in mice. The basic immunological reactions, such as graft-versus-reactions, host-versus-graft-reaction, a combination of both reactions, chronic rejection and tolerance have been involved. Almost all immunosuppressive agents of proven or potential clinical relevance have been tested for their efficacy in small bowel transplantation model.

We modified the mouse small bowel transplant technique in several ways to improve efficiency and success rates:

1. The inside of donor's small bowel should be keeping clear and no any feces left.
2. The edge of Carrel patch of aorta of superior mesenteric artery should be divided smooth and equal size with the opening of the recipient's abdominal aorta.
3. Do not twist the portal vein with the superior mesenteric artery when anastomoses the portal vein with recipient's inferior vena cava. Because the portal is vein longer than the superior mesenteric artery.
4. The small bowel should be placed on the middle of the abdomen and keep the superior mesenteric artery and portal vein in proper tension when rebuild blood circulation to avoid thrombosis.
5. The superior mesenteric artery should be keep proper tension when the host small bowel returned into the abdomen cavity to avoid thrombosis after closing the abdomen.
6. 0.1 ml cefuroxime (20mg/kg) are left in the abdomen cavity to avoid infection before closing the abdomen.
7. To be sure do not let the feces flow into the abdomen activity when performing the small bowel anastomosis to avoid infection after operation.
8. Do not damage the mesenteric vessels when harvesting the small bowel to avoid small bowel necrosis after operation.

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