Video Article

Murine Renal Transplantation Procedure

Jiao-Jing Wang[1], Sara Hockenheimer[2], Alice A. Bickerstaff[1], Gregg A. Hadley[1]

[1]Department of Surgery, The Ohio State University

[2]Integrated Biomedical Sciences Graduate Program, The Ohio State University

Correspondence to: Jiao-Jing Wang at Jiao-jing.wang@osumc.edu

URL: http://www.jove.com/video/1150
DOI: doi:10.3791/1150

Keywords: immunology, Issue 29, mouse, kidney, renal, transplantation, procedure

Date Published: 7/10/2009

Citation: Wang, J.J., Hockenheimer, S., Bickerstaff, A.A., Hadley, G.A. Murine Renal Transplantation Procedure. *J. Vis. Exp.* (29), e1150, doi:10.3791/1150 (2009).

Abstract

Renal orthotopic transplantation in mice is a technically challenging procedure. Although the first kidney transplants in mice were performed by Russell et al over 30 years ago (1) and refined by Zhang et al years later (2), few people in the world have mastered this procedure. In our laboratory we have successfully performed 1200 orthotopic kidney transplantations with > 90% survival rate. The key points for success include stringent control of reperfusion injury, bleeding and thrombosis, both during the procedure and post-transplantation, and use of 10-0 instead of 11-0 suture for anastomoses.

Post-operative care and treatment of the recipient is extremely important to transplant success and evaluation. All renal graft recipients receive antibiotics in the form of an injection of penicillin immediately post-transplant and sulfatrim in the drinking water continually. Overall animal health is evaluated daily and whole blood creatinine analyses are performed routinely with a portable I-STAT machine to assess graft function.

Video Link

The video component of this article can be found at http://www.jove.com/video/1150/

Protocol

Donor Organ Harvest (2):

1. Anesthetize the donor using 65mg/kg pentobarbital IP; anesthesia is maintained by Isoflurane (2-3%).
2. Make a midline incision from sternum to pubis to enter the donor abdomen and expose the left kidney by moving the intestine laterally to the right side.
3. Isolate the left kidney by ligating and dividing the adrenal and testicular vessels with an 8-0 silk suture.
4. Mobilize the aorta above its junction with the renal artery and vein.
5. Mobilize the inferior vena cava (IVC) above and below its junction with the renal artery and vein.
6. Ligate the aorta below the renal artery and vein with an 8-0 silk suture to minimize bleeding.
7. Dissect the left ureter from the renal hilum to the bladder.
8. Expose the ureterovesical junction using caudad retraction on the bladder dome.
9. Excise a small, elliptical patch of bladder containing the left ureterovesical junction, used for urinary reconstruction via a bladder-to-bladder anastomosis. The right ureter is not included with the bladder.
10. Ligate the IVC below the renal artery and vein with an 8-0 silk suture.
11. Ligate the aorta above the renal artery and vein, and slowly perfuse the graft in situ with 0.2-0.4ml cold, heparinized Ringer's lactate solution via the infrarenal aorta.
12. Ligate the IVC above the renal artery and vein with an 8-0 silk suture.
13. Transect the renal vein at its junction with the IVC.
14. Divide the aorta obliquely, approximately 2mm below the renal artery.
15. Remove the kidney and its vascular supply with ureter attached to the bladder patch en bloc. Store in Ringer's lactate solution on ice.
16. Euthanize the donor mouse by cervical dislocation under anesthesia.

Recipient Transplantation (2):

1. Anesthetize the recipient with 65 mg/kg pentobarbital IP and maintain the anesthesia by Isoflurane (2-3%).
2. Make a midline incision, cover the intestine with wet gauze and carefully retract it to the left side.
3. Ligate the right ureter, renal artery and vein, and remove the right kidney.
4. Carefully isolate and cross-clamp the infrarenal aorta and IVC with two 4-mm microvasular clamps after ligating the lumbar branches.

5. Place a 10-0 nylon suture through a full thickness of aorta and retract to make an elliptical aortaotomy by a single cut (about one-fifth of the diameter of the vessel).
6. Make a longitudinal venotomy by piercing the IVC with a 30 gauge needle and extend appropriately using microscissors.
7. Irrigate both the aorta and IVC with heparinized saline to clear intraluminal blood or clots.
8. Remove the donor kidney from the ice and place it intra-abdominally in the right flank of the mouse. The donor kidney is kept moist with a cold saline-soaked gauze pad.
9. Place two stay sutures at the proximal and distal apex of the recipient's aortaotomy with the donor's aortic cuff.
10. Make an end-to-side anastomosis between the donor aortic cuff and the anterior wall of the recipient's aortaotomy, using 2 ~ 3 continuous 10-0 nylon sutures in the outside of the aorta.
11. Turn the donor kidney graft over to the left flank of the recipient. repeat the previous procedure between the donor aortic cuff and the posterior wall of the recipient's aortaotomy
12. Perform an end-to-side anastomosis between the donor renal vein and the posterior wall of the recipient's IVC, using 4 to 5 continuous 10-0 nylon sutures in the inside of the IVC. and then the anterior wall of IVC and donor's renal vein are closed with 4 ~ 5 continuous sutures outside the IVC.
13. The anastomosis of both vein and artery must be finished within 20 minutes.
14. Rinse the graft with cold saline several times during anastomosis.
15. Divide the dome of the recipient bladder, and stop bleeding with cautery.
16. Make a small cut on the dome.
17. Place two stay sutures of 10-0 nylon to anastomize the donor's small bladder patch with the recipient's bladder dome. Each side of anastomosis is sutured with 8 ~ 10 continuous stitches.
18. Close the abdomen in two layers with continuous 4-0 sterile, synthetic absorbable vicryl suture.
19. Give recipient mice 0.4ml warm saline during surgery by intermittent injection IV.

Post-operative Care of Recipient

1. After closing the abdomen, a single intramuscular injection of penicillin (500U/10g) is administered (2).
2. In addition, recipients receive 0.05 mg/kg buprenorphine intramuscularly on day 0 and day 1 post surgery for pain. Following surgery, transplant recipients receive water containing sulfatrim (100mg/kg) until the experiment is terminated.
3. For the first 24h postoperatively mice are kept in a temperature controlled incubator.
4. Mice usually recover from anesthesia within 1 hour of the operation and are given regular food and water ad libitum.
5. Skin sutures are removed 2 weeks after the surgery.

Recipient Nephrectomy

1. Anesthetize the recipient 4 ~ 7 days post kidney transplantation with 65 mg/kg pentobarbital IP; anesthesia is maintained by Isoflurane (2-3%).
2. Open the abdomen via a midline incision.
3. Cover the intestine with saline-soaked gauze and carefully retract it to the right side.
4. Remove the left kidney after ligating the left ureter, kidney vein and artery.
5. Close the abdomen in two layers with continuous 4-0 synthetic absorbable vicryl suture.

Whole Blood Creatinine Measurements

1. Weekly to biweekly blood collection of less than 0.2 cc is performed either submandibularly without anesthesia or retro-orbitally under Isoflurane anesthesia.
2. Quantitative whole blood creatinine levels are determined using an I-Stat Portable Clinical Analyzer (Heska Corp., Fort Collins, CO).
3. Conventional units (mg/dl) are converted to SI units of micromol/L by multiplying the conventional units by 88.4.

Discussion

We have performed approximately 1200 cases of renal transplantation in mice with a technical success rate of >90%. Compared to heart transplantation, the success rate of kidney transplantation is usually low. However, our laboratory has maintained a high success rate (> 90%) of kidney transplantation. The key points for success are to reduce reperfusion injuries to both donor kidney and recipients, and minimize bleeding and thrombosis throughout the procedure and post-operatively. The following factors may be most important: (a) reduce bleeding in the recipient during surgery as much as possible through careful and gentle operation; (b) minimize warm ischemic and cross-clamping times during donor kidney preparation and recipient transplantation; (c) use a small donor bladder patch to prevent necrosis, insufficient blood supply and bladder leakage post-operation; (d) delay nephrectomy of the native kidney until 4 ~ 7 days post-transplantation to allow the transplanted kidney to recover function following surgery; (e) perform anastomoses (between the donor aortic cuff and the anterior wall and between the donor aortic cuff and the posterior wall of the recipient's aorta) outside of the aorta to reduce the incidence of thrombosis; (f) keep recipient mice warm for at least for 24 hours following surgery; (g) give antibiotics to recipient mice post-operatively to prevent potential infections.

In this protocol, we use 10-0 instead of 11-0 suture without affecting survival rate. Although the 11-0 suture is ideal and used in many transplantation laboratories, we feel that 10-0 suture is sufficient for use in kidney transplantation as long as the factors described above are well controlled.

The removal of the remaining native kidney is essential so that decline in kidney graft function can be monitored by serum creatinine measurements. All kidney graft recipients undergo nephrectomy of the left native kidney between 4 and 7 days post kidney transplantation.

Nephrectomy is not performed at the time of kidney transplantation because recipients characteristically suffer ill health or do not survive when both procedures are conducted at the same time. A 4 to 7 day lag between procedures permits the kidney graft to recover from ischemia and reperfusion injury before the native kidney is removed. Kidney graft function is assessed by daily examinations of overall animal health and blood creatinine levels. For reference, a non-transplanted, normal mouse has a creatinine level of about 20 micromol/L. Renal allograft rejection is suspected when the recipient mouse shows signs of illness and the creatinine level are elevated near 100 micromol/L at which time grafts are harvested for histopathological analyses.

Disclosures

Experiments on animals were performed in accordance with the guidelines and regulations set forth by The Ohio State University IACUC.

Acknowledgements

These studies were supported in part by funding by NIH R01 AI36532 to GAH. We would like to acknowledge the outstanding support in transplant pathology provided by Dr. Tibor Nadasdy, and the invaluable guidance and foresight provided by the late Dr. Charles G. Orosz in initiating this research effort.

References

1. Russell, PS., Chase, CM., Colvin, RB., Plate, JMD. Kidney transplants in mice. An analysis of the immune status of mice bearing long-term, H-2 incompatible transplants. *JExpMed.* 147:1449-1468. (1978).
2. Zhang, Z., Schlachta, C., Duff, J., Stiller, C., Grant, D., Zhong, R. Improved techniques for kidney transplantation in mice. *Microsurgery.* 16(2):103-109. (1995).

