Video Article

Orthotopic Aortic Transplantation: A Rat Model to Study the Development of Chronic Vasculopathy

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Abstract

Research models of chronic rejection are essential to investigate pathobiological and pathophysiological processes during the development of transplant vasculopathy (TVP).

The commonly used animal model for cardiovascular chronic rejection studies is the heterotopic heart transplant model performed in laboratory rodents. This model is used widely in experiments since Ono and Lindsey (3) published their technique. To analyze the findings in the blood vessels, the heart has to be sectioned and all vessels have to be measured.

Another method to investigate chronic rejection in cardiovascular questionings is the aortic transplant model (1, 2). In the orthotopic aortic transplant model, the aorta can easily be histologically evaluated (2). The PVG-to-ACI model is especially useful for CAV studies, since acute vascular rejection is not a major confounding factor and Cyclosporin A (CsA) treatment does not prevent the development of CAV, similar to what we find in the clinical setting (4). A7-day period of CsA is required in this model to prevent acute rejection and to achieve long-term survival with the development of TVP.

This model can also be used to investigate acute cellular rejection and media necrosis in xenogeneic models (5).

Video Link

The video component of this article can be found at http://www.jove.com/video/1989/

Protocol

Rats weighing approximately 250-300g are purchased from Charles River (Sandhofer Weg 7, D-97633 Sulzfeld).

Transplants are performed using PVG rats as donors, and ACI rats as recipients.

Rats are housed under conventional conditions, fed standard rat chow and water ad libidum.

All surgical instruments are sterilized prior to use.

Donor preparation:

Anesthetize rat with isoflurane (2,5-3%) using an induction chamber.

1. Shave the abdominal and thoracic hair and place the rat on its back and place a facemask over its nose and mouth to keep up the anesthesia.
2. Disinfect the abdominal and thoracic area widely using Provo-Iodine, next use 80% ethanol, repeat this step three times.
3. Check reflexes pinching the hind feet to be sure that the rat is sufficient anesthesized.
4. Exsanguinate the rat by opening the abdomen and cutting a hole into the abdominal aorta.
5. Carefully open the thorax to find the descending thoracic aorta.
6. Dissect the thoracic aorta from surrounding tissues like fat, nerves and the oesophagus.
7. Withdraw a 1.5cm piece of the aorta using a couter, without tissue damage of the aorta.
8. Perfuse the aortic graft throughly with cold sterile saline and store the graft at 4°C.

Recipient:

Anesthetize rat with isoflurane (2,5-3%) using an induction chamber. Body temperature is maintained during the surgical procedure.

1. Shave abdominal area and apply eye ointment to prevent the eyes from drying during anesthesia.
2. Place the rat on its back and place a facemask over its nose and mouth to keep up the anesthesia.
3. Disinfect the abdominal area using Provo-Iodine, next use 80% ethanol, repeat this step three times.
4. Check reflexes pinching the hind feet to be sure that the rat is sufficient anesthesized.
5. Perform a midline abdominal incision separating the skin and muscle in two steps to open the abdomen.
6. Place the intestines in a warmed saline moisturized powder free glove. Fold the glove around the intestines to prevent loss of moisture.
7. Remove the fatty tissue covering the abdominal aorta.
8. Dissect the aorta form the infrarenal region to the bifurcation, careful not to cause damage on the branches of the vessels.
9. If necessary ligate branches of the aorta.
10. First use a microsurgical clamp on the infrarenal part of the dissected aorta to stop the blood flow.
11. Next, place a second clamp close to the bifurcation of the aorta.
12. Now that the blood flow is stopped, remove a short segment of the aorta.
13. Take the donor aortic graft, that has been kept in 0,9% sterile saline on ice, shorten it to the adequate length and position it in the gap.
14. Connect the donor aorta to the recipient aorta, performing running sutures using 8-0 prolene suture (Ethicon, Norderstedt, Germany). Start with the cranial end-to-end anastomosis.
15. When the distal end-to-end anastomosis is finished, carefully open the distal clamp.
16. When there is no bleeding after opening the distal clamp, carefully open the cranial clamp. In case of bleeding at the sutures, close the clamp again, locate the bleeding and stop the bleeding using one single stitch.
17. There should be a visible pulse at the distal end of the aorta.
18. Next move the intestines back into the abdomen.
19. Flush the abdomen with prewarmed sterile saline.
20. Close the muscle layer of the abdominal wall using 6-0 prolene running sutures (Ethicon, Norderstedt, Germany).
21. Use 5-0 prolene (Ethicon, Norderstedt, Germany) running sutures to close the skin.
22. While the rat is still in anesthesia, inject 4-5mg/kg Carprofen subcutaneously.
23. To provide sufficient analgesia for this type of procedure, metamizol is added to the drinking water (50mg Metamizol per 100ml) for pain medication for 3 days post transplantation.

Discussion

For several reasons we found the orthotopic aortic transplantation model a more accurate way to investigate the development of TVP compared to the heterotopic heart transplantation model:

Regarding the surgical technique, the aortic transplant model embodies an easily feasible method, requiring solely the accomplishment of end-to-end-anastomosis, whereas the heterotopic heart transplant model may be considered a more complex and error-prone challenge to the surgeon s skills, requiring end-to-side anastomosis on two major vessels.

Analysis and measurement of histological specimens is drastically facilitated since transplant vasculopathy may be examined in one single vessel of a sole defined diameter instead of the exploration of numerous small cardiac vessels showing a vast variety in size and constitution which could handicap the investigator in gaining objective and credible results. The thoracic aorta of the recipient may serve as normal control, offering an easy and reliable object of comparison in diameter and morphological appearance, while in the heterotopic heart transplantation model it is difficult to compare peri- or myocardial vessels underlying a great interindividual variability.

In addition to these findings, aortic allografts are not as prone to disruptive histological alterations that may result from the applied surgical procedures. Fibrosis or infarctions may occur in and possibly affect heterotopic heart transplant tissue and embedded vessels, rendering them unsuitable for evaluation.

Due to its size and constitution, the aorta is an ideal subject to simple and exact investigation of morphological, biochemical and molecular remodeling within each one of the three layers of the vascular wall, such as the degree of concentric intimal thickening, myocyte necrosis in the media or increased synthesis of cytokines and growth factors.

The immune response generated by an aortic allograft is indeed sufficient to trigger chronic alterations in the transplant, but was found insufficient to induce acute rejection episodes (2). Thanks to this intermediate immunogenicity of the graft, no transplant is lost to acute rejection and no initial immunosuppressive treatment with CsA is required. The model thus enables to investigate the initial effects of drugs on chronic allograft vasculopathy without the influence or adulteration by prophylactic immunosuppression often seen in heterotopic heart transplants (2, 4).

Disclosures

No conflicts of interest declared.

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