Video Article

Heterotopic Heart Transplantation in Mice

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Abstract

The mouse heterotopic heart transplantation has been used widely since it was introduced by Drs. Corry and Russell in 1973. It is particularly valuable for studying rejection and immune response now that newer transgenic and gene knockout mice are available, and a large number of immunologic reagents have been developed. The heart transplant model is less stringent than the skin transplant models, although technically more challenging. We have developed a modified technique and have completed over 1000 successful cases of heterotopic heart transplantation in mice. When making anastomosis of the ascending aorta and abdominal aorta, two stay sutures are placed at the proximal and distal apexes of recipient abdominal aorta with the donor s ascending aorta, then using 11-0 suture for anastomosis on both side of aorta with continuing sutures. The stay sutures make the anastomosis easier and 11-0 is an ideal suture size to avoid bleeding and thrombosis.

When making anastomosis of pulmonary artery 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 pulmonary artery. The left wall of the inferior vena cava and donor s pulmonary artery is closed with continuing sutures in the inside of the inferior vena cava after, one knot with the proximal apex stay suture the right wall of the inferior vena cava and the donor s pulmonary artery are closed with continuing sutures outside the inferior vena cave 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 Heart Harvest:

1. The mouse is anesthetized with an intraperitoneal injection of pentobarbital and placed supine on the operative field.
2. A long midline abdominal incision is made. Abdominal contents are retracted with gauze to left in order to expose the abdominal aorta and the inferior vena cava.
3. 1 ml of heparin (10u/ml) is injected into the inferior vena cava for heparinization.
4. The thoracotomy is made: the ribs are divided on both sides of the spine and the anterior chest wall is levered up to expose the heart.
5. The inferior vena cava, the superior vena cava and azygous vein are ligated with 6-0 silk and divided superior to the ligatures.
6. The ascending aorta and pulmonary artery are separated and divided as far distally as possible.
7. The pulmonary veins are ligated and divided distal to the ties.
8. The heart is perfused with 4 C^o saline solution.
9. The heart is stored in ice cold saline solution.

Recipient Preparation and Transplantation

1. The mouse is anesthetized with an intraperitoneal injection of pentobarbital and placed supine on the operative field.
2. A long midline abdominal incision is made. Abdominal contents are retracted outside the abdomen 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 ligatures are 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 pulmonary aorta with micro-scissors.
6. The aortotomy is made in the abdominal aorta of recipient with a 30 gauge needle.
7. The opened abdominal aorta and the inferior vena cava are irrigated with saline solution.
8. The donor's heart is placed on the left side of the recipient's abdomen and is covered with gauze.
9. The stay sutures are placed at the proximal and distal apexes of recipient abdominal aorta with the donor's ascending aorta.
10. The anastomosis of the right side of the recipient's abdominal aorta and the donor's ascending aorta are completed 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 pulmonary artery for the anastomosis of the donor's pulmonary artery and the recipient's inferior vena cava.
12. The left wall of the inferior vena cava and donor's pulmonary artery 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 pulmonary artery are closed with continuing sutures outside the inferior vena cave.
14. The donor's heart 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 ascending aorta anastomosis are closed with continue sutures.
15. After anastomosis is made, the distal ligature is removed to check the bleeding from the anastomosis.
16. If there is little to no bleeding from the anastomosis, the proximal ligature is removed. The donor's heart fills with blood immediately; the color of the heart becomes red and it begins to beat again.
17. The intestines are returned to the abdomen, which is closed with 6-0 continuous sutures.
18. The recipient mouse is placed on a warm area. After one hour the recipient recovers.

Discussion

Results

We have successfully performed more than 1000 cases of heterotopic heart transplantation in mice and achieved over 90% success rate in different strains of mice, including wild type, transgenic and gene knockout mice.

Discussion

Since Drs. Corry and Russell introduced heterotopic heart transplantation in mice in 1973, this model has proven to be a particularly valuable for studying immune rejection response and developing novel immunosuppressive strategies. This model can be used more broadly now because the emergence of numerous transgenic and gene knockout mice has provided new ways to study the mechanisms of rejection/tolerance. The vascularized heart transplant model may be more relevant to clinical solid organ transplantation. Although skin transplants or subcutaneous transplants of neonatal hearts are technically simpler, the neovascularization that is required for graft survival in these transplants are particularly sensitive to allogenic responses. Thus, skin and subcutaneous hear transplants can be useful for a stringent test of tolerance, but not for the development or optimization of immunosuppressive or tolerance inducing protocols.

We modified the vascular heart transplant technique in several ways to improve efficiency and success rates:

1. The donor's heart should be irrigated with saline solution from the ascending aorta until the heart become light color after harvesting.
2. The branches of the abdominal aorta and inferior vena cava of the recipient should be ligated with 10-0 sutures, preventing bleeding during the operation and thrombosis after operation.
3. The lumen of the abdominal aorta and the inferior vena cava of the recipient should be irrigated with saline solution until no blood is left. This can prevent thrombosis after the operation.
4. Air bubbles inside the vessels should be avoided when making anastomoses.
5. The artery anastomoses should use 11-0 sutures, to help prevent bleeding from the anastomoses.
6. The incision of the abdominal aorta and inferior vena cava of the recipient should be equal to the opening of the pulmonary artery and ascending aorta of the donor’s heart.
7. It is important to have a clear and expansive operating field, therefore use 6-0 suture instead of the clamps to temporarily occlude the aorta and inferior vena cava.

Conclusion

Although the technique of heterotopic heart transplantation in mice is technically challenging, it is an important technique for studying alloresponses. Practice and technical modifications have resulted in a successful rate of over 90% in our laboratory. We believe that the video and the accompanying manuscript will help to lessen the learning curve for laboratories that wish to use this technique.

References

1. Robert J. Corry, Henry J. Winn, and Paul S. Russell. Primarily vascularized allografts of heart in mice. Transplantation. Vol. 16, No4, 343-350(1973).

