Video Article

Hamster-to-Rat Heterotopic Cardiac Xenotransplantation Surgical Technique

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Abstract

Hamster-to-rat heterotopic cardiac xenotransplantation is a concordant difficult species combination model widely used to study acute vascular rejection[1], delayed xenograft rejection in long-term xenograft survival[2], accommodation and tolerance[3-5], as well as to develop novel immunosuppressive strategies in the field of transplantation[6-8]. In this species combination, the cardiac surgical model offers several advantages over the renal model. In particular, the heart is easier to harvest and implant, and it can be followed up and monitored postoperatively much more easily. Previous papers on the surgery of this species combination usually adopt the rat-to-rat cardiac heterotopic transplantation technique. In other cases, modifications to the original model have been described, but there is still no detailed video description in the literature of a proper technique in the case of hamster-to-rat xenotransplantation. The surgical model used at our Centre[9] is presented here with a detailed and improved up-to-date description. Efforts have gone into developing a reliable and easily reproducible experimental procedure. The description is enriched with “tips” from our experience with this model. In conclusion, we believe that the described technique is a valid model for xenotransplantation studies on rodents. We also believe that the video and the accompanying manuscript will help to reduce the learning curve for laboratories that wish to adopt this technique.

Video Link

The video component of this article can be found at http://www.jove.com/video/3693/

Protocol

1. Recipient preparation

1. General maintenance anesthesia with Sevorane at 2.5% with oxygen at 1 liter per minute is provided with a Fluovac Halotane Scavanger unit. Anesthesia is potentiated with 5 mg/kg of intraperitoneal tramadol.
2. The recipient is positioned in a supine position on a cork tray with an incorporated heating plate.
3. The abdomen is shaved, disinfected and covered with kitchen film.
4. A midline xiphopubic incision is performed and home-made mini retractors are used to keep the abdomen open.
5. A gauze soaked with warm saline is used to cover the intestines which are pulled upward.
6. Retroperitoneum and big vessels are then exposed.
7. A loop of 0 silk is passed behind to abdominal aorta and the inferior vena cava.
8. The graft is placed halfway between the renal vessels and the iliac bifurcation.
9. Lumbar vases of the area of interest for the procedure are ligated.
10. To reduce the cold ischemia time, the surgery is interrupted and the recipient is left under anesthesia on the back table.

2. Donor preparation and heart harvesting

1. Surgery now commences with the hamster donor. Terminal anesthesia with Zoletil and Xylazine is provided.
2. Donor is laid in a supine position. A xiphopubic incision with lateral openings is performed.
3. A volume of 1 ml of cold saline containing 500 IU of heparin is administered through the inferior vena cava.
4. After 1 min. the diaphragm is cut and the thorax fully opened.
5. The beating heart is cooled by dripping over cardioplegic preservation solution at 4°C.
6. Pericardium and thymus are removed.
7. An opening is made in the thoracic vena cava to pour in the preservation solution.

8. The aorta is freed from the surrounding tissue and cut at the arch, between the origin of the brachiocephalic artery and the left carotid artery.
9. The thoracic inferior vena cava, the superior vena cava and the anonymous vein are ligated with 5/0 silk and cut.
10. The pulmonary artery is divided, to ensure maximum vascular length.
11. An *en bloc* ligature with 4/0 silk is then performed.
12. Pulling on the ligature, the heart is separated from the surrounding tissues and immediately dipped in cardioplegic solution.
13. The heart is placed on a gauze moistened with cold cardioplegic solution and its vessels are inspected on the back table. Abundant surrounding tissue is cut and the aorta is shortened below the level of the brachiocephalic artery.

3. Back with the recipient: cardiac xenotransplantation

1. With the aid of the silk loop, the big abdominal vessels are lifted and a small curved "bulldog" clamp is inserted.
2. The wall of the abdominal aorta taken between the jaws of the clamp is punctured with a needle and the aortotomy is extended using fine tip microscissors.
3. To remove any clots, the aorta opening is flushed with saline solution.
4. The graft is inserted into the recipient's abdomen, put *in loco* and left to fall on the right side of the aorta and inferior vena cava.
5. Two corner stitches are applied first caudally, then cranially.
6. The animal is then rotated 90° clockwise with head to the operator's right.
7. A 9/0 Prolene running suture is used to perform the end-to-side anastomosis between the aorta of the graft and the recipient's abdominal aorta.
8. To complete the other side of the anastomosis the graft is moved to the left, while the recipient is rotated 180° counterclockwise with head to the operator's left.
9. This side of the anastomosis is completed in the same way as above.
10. Without changing the recipient's position, the inferior vena cava portion included in the clamp's jaws is grasped gently and a small, elliptical venotomy is performed. Prolene 10/0 is used to perform the end-to-side anastomosis.
11. Two corner stitches are inserted.
12. Now the needle is passed inside the venous lumen posteriorly to the caudal (right-hand) corner of the cava, running from right to left.
13. The last stitch is passed outside the pulmonary artery and tied with the cranial (left-hand) corner stitch.
14. The anterior side of the venous anastomosis is thus completed.

4. Declamping and animal closure

1. Some pressure is exercised for a few seconds with two cotton buds at the site of the anastomosis.
2. The intestines are returned to their proper place, while a window is opened in the omentum, so that it lies more superficially, facilitating palpation during the follow-up.
3. The abdomen is closed in 2 layers.

5. Follow up

1. The animal is placed under a heating lamp and monitored visually until it is fully awake.
2. The analgesic tramadol (5 mg/kg), is used subcutaneously twice a day for the first 48 hrs after surgery.
3. The cardiac xenografts are monitored by direct palpation through the abdominal wall.

Discussion

The video that we have shown has been produced not for its own sake, but as part of a specific study in which we have used this surgical model[10]. The procedure has been performed respecting all guidelines and regulations, after approval on the Local Ethic Committee and according to the Italian Law on the use of experimental animals (DL n. 16/92 art. 5). The hamster-to-rat model, especially with the heterotopic heart as a graft, is widely used and considered a concordant difficult species combination, as far as xenotransplantation is concerned. The model resembles the current immunological situation observed when transplanting genetically-engineered porcine organs into primates[11]. The cardiac surgical model is especially used to study acute vascular rejection, delayed xenograft rejection and is considered also a key tool for immunological and accommodation/tolerance studies[1-5]. The rejection pattern in this model is likely to be T-cell independent since both normal and nude (T cell deficient) rats reject the hamster hearts in a similar manner[12]. The cardiac surgical model offers several advantages over the renal model in the same species combination. In particular, the heart is easier to harvest and implant, and it can be followed up and monitored postoperatively much more easily. Previous papers on the surgery of this species combination usually refer to the rat-to-rat report, firstly described by Abbott *et al.*[13-14]. In other cases, modifications to the original model have been described in detail, but there is still no detailed video description in the literature of a proper technique in the case of hamster-to-rat xenotransplantation. This video provides such a description, going through all the necessary steps to facilitate the procedure. The many details provided here should also be of assistance to both beginners and experienced surgeons, helping them to improve their performance. The surgical technique reported is safe and an excellent opportunity for gaining familiarity with microvascular and experimental surgery.

In conclusion, we believe that the above-described technique is a valid model for xenotransplantation studies on rodents. The contents of this video should contribute to the development of a successful hamster-to-rat cardiac xenotransplantation model refining the experimental procedure with this species combination. Also, a reduction of the overall number of animals used in experimental practice has been observed, since the success rate of the surgery in our lab is over 90%. We believe that the video and the accompanying manuscript will help to reduce the learning curve for laboratories that wish to adopt this technique.

Disclosures

Experiments on animals were performed in accordance with the guidelines and regulations set forth by the local Ethic Committee and according to the Italian Law on the use of experimental animals (DL n. 16/92 art. 5).

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References

1. Lin, Y., Vandeputte, M., & Waer, M. Natural killer cell and macrophage-mediated rejection of concordant xenografts in the absence of T and B cell responses. *J. Immunol.* **158**, 5658-5667 (1997).
2. Ginesta, M.M., Ribas, Y., Mollevi, D.G., Vidal, A., Manez, R., Figueras, J., & Jaurrieta, E. Acute xenograft rejection, late xenograft rejection and long term survival xenografts in the hamster-to-rat heart transplantation model: histological characterization under low-dose of FK506. *APMIS.* **110**, 737-745 (2002).
3. Lin, Y., Soares, M.P., Sato, K., Csizmadia, E., Robson, S.C., Smith, N., & Bach F.H. Long-term survival of hamster hearts in presensitized rats. *J Immunol.* **164**, 4883-4892 (2000).
4. Komori, K., Fuchimoto, Y., Morikawa, Y., Obara, H., Kawachi, S., Tanabe, M., Hoshino, K., Shimazu, M., Matsuzaki, Y., & Kitajima, M. The role of graft and host accommodation in a hamster-to-rat cardiac transplantation model. *Transplantation.* **85**, 112-117 (2008).
5. Suhr, B.D., Black, S.M., Guzman-Paz, M., Matas, A.J., & Dalmasso, A.P. Inhibition of the membrane attack complex of complement for induction of accommodation in the hamster-to-rat heart transplant model. *Xenotransplantation.* **14**, 572-579 (2007).
6. Zhu, T., Bradley, A., White, D., & Chen, Z.K. Effect of ERL080A, RAD, and Neoral on hamster-to-rat heart xenograft survival. *Transplant Proc.* **33**, 3871-3872 (2001).
7. Brouard, S., Cuturi, M.C., Pignon, P., Buelow, R., Loth, P., Moreau, A., & Soulillou, J.P. Prolongation of heart xenograft survival in a hamster-to-rat model after therapy with a rationally designed immunosuppressive peptide. *Transplantation.* **67**, 1614-1618 (1999).
8. Schrepfer, S., Deuse, T., Koch-Nolte, F., Krieger, T., Haddad, M., Schäfer, H., Pelletier, M.P., Robbins, R.C., & Reichenspurner, H. FK778 in experimental xenotransplantation: a detailed analysis of drug efficacy. *J Heart Lung Transplant.* **26**, 70-77 (2007).
9. Dedja, A., Dall'Olmo, L., Cadrobbi, R., Baldan, N., Fante, F., Calabrese, F., Rigotti, P., Ferraresso, M., Delriviere, L., Cozzi, E., & Ancona, E. Heterotopic cardiac xenotransplantation in rodents: report of a refined technique in a hamster-to-rat model. *Microsurgery*. **25**, 227-234 (2005).
10. Dedja, A., Zaglia, T., Dall'Olmo, L., Chioato, T., Thiene, G., Fabris, L., Ancona, E., Schiaffino, S., Ausoni, S., & Cozzi, E. Hybrid cardiomyocytes derived by cell fusion in heterotopic cardiac xenografts. *FASEB J.* **20**, 2534-2536 (2006).
11. White, D.J. Transplantation of organs between species. *Int Arch Allergy Immunol.* **98**, 1-5 (1992).
12. Zhang, Z., Bédard, E., Luo, Y., Wang, H., Deng, S., Kelvin, D., & Zhong, R. Animal models in xenotransplantation. *Expert Opin Investig Drugs.* **9**, 2051-2068 (2000).
13. Abbott, C.P., Lindsey, E.S., Creech, O. Jr., & Dewitt, C.W. A technique for heart transplantation in the rat. *Arch Surg.* **89**, 645-52 (1964).
14. Ono, K., & Lindsey, E.S. Improved technique of heart transplantation in rats. *J Thorac Cardiovasc Surg.* **57**, 225-259 (1969).

