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Modified Suture Technique in a Mouse Heart Transplant Model

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BACKGROUND: The mouse abdominal heart transplantation model is a basic and important immunological research model. We developed a technique for placing entire everting sutures instead of half inverting and half everting sutures for anastomosis between donor and recipients' caval veins. The purpose of this study was to evaluate this modified method.

METHODS: Each technique was used in 25 mice subjected to isogenic abdominal heart transplantation. Recipient operation time, graft warm ischaemia time, time of caval anastomosis, and re-beating time were recorded. After transplantation, the heartbeat was palpated through the abdominal wall once a day for 100 days.

RESULTS: Recipient operation time (40.7 ± 2.5 min *vs.* 44.3 ± 2.3 min, $p < 0.01$), cava-caval anastomosis time (8.4 ± 1.3 min *vs.* 12.1 ± 1.2 min, $p < 0.01$), and warm ischaemia time were significantly shorter (23.4 ± 1.7 min *vs.* 27.2 ± 1.6 min, $p < 0.01$) with the modified technique. Re-beating time was 1.2 ± 0.4 minutes with the modified technique *vs.* 1.5 ± 0.5 minutes ($p = 0.04$). There was a tendency for less surgical complications in the modified group, but there were no differences in survival rates.

CONCLUSION: The new suturing technique for mouse cardiac transplantation facilitates easier anastomosis of the outflow tract, thereby reducing operation, warm ischaemia, and re-beating times. [*Asian J Surg* 2011;34(2):86–91]

Key Words: heart transplantation model, heterotopic transplant, mouse

Introduction

With the development of microsurgery and molecular biology, the mouse model for organ transplants has become increasingly popular. The mouse heart transplantation model is frequently used because of its convenience for the examination of graft function by palpation of cardiac contraction.

Since the first heterotopic heart transplantation in the mouse reported by Corry et al,[1] many subsequent modifications have contributed to the development of this animal model.[2–4] We previously developed a novel technique in the mouse heart transplantation model that adopted the donor's thoracic inferior vena cava instead of the pulmonary artery as the outflow tract.[5] Compared with traditional methods, this novel technique facilitates

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vessel anastomosis and therefore shortens the graft warm ischaemia time.

In this paper, we report a further modification of our technique and first results using this modified technique. We placed entire everting sutures instead of half inverting and half everting sutures for the anastomosis between donor and recipients' caval veins to decrease the technical difficulty, shorten the operation time, and decrease postoperative complications.

Materials and methods

Animals

Male C57BL/6J (H-2^b) inbred mice (aged 8–12 weeks and weighing 25–30 g) were used for isogeneic transplantation. Mice were obtained from the Animal Centre of Essen University Hospital (Essen, Germany). All of them were housed under standard conditions at constant room temperature and humidity and subjected to regular 12-hour light/dark cycles. Food and water were supplied *ad libitum*. All animals received care in compliance with the Principles of Laboratory Animal Care, and the protocol was approved by the local Animal Care and Research Committee.

Instruments

A basic set of microsurgical instruments from Aesculap (Tuttlingen, Germany) was used, including one straight fine-tip forceps (Catalogue number: BD331R), one straight spring-type microscissors (OC498R), one curved spring-type microscissors (OC499R), a Barraquer micro needle holder (FD284R), and two 5-mm microvascular bulldogs. A microsurgical microscope (OPMI 1 FR, Carl Zeiss, Germany) was used with 10, 15, and 25 in-procedure interchangeable magnifications. The sutures for anastomosis of vessels were made of nonabsorbable nylon (10-0) and placed with a needle (RESORBA, Nürnberg, Germany). Silk (7-0) was used for ligation in donor procurement.[5]

Anaesthesia

Mice were anaesthetised with a mixed solution of 2% xylazine hydrochloride (Rompun, Bayer Animal Health, Germany) and 10% ketamine hydrochloride (Dopalen, Agribrands Brasil, Ltd.) diluted to 2 mg/mL and 10 mg/mL, respectively, in 0.9% saline solution. The solution was injected intraperitoneally at a dosage of 0.1 mL for each 10 g body weight.

Study protocol

Fifty mice were divided into two groups. Twenty-five underwent the modified anastomosis technique (modified method group), and the other 25 underwent the standard method that we previously introduced (standard method group).[5]

Surgery

All operations were performed under clean, but not sterile, conditions. The surgical and other *in vivo* procedures described in this paper were performed by a single surgeon. Mice were shaved and cleansed with 70% alcohol before surgery. All surgical instruments and suture materials were the same as those previously described.[5]

Donor operation

The donor operation was performed as previously described.[4,5] Briefly, bilateral thoracotomy was performed under deep anaesthesia. The mouse was then heparinised with a 0.1-mL injection of saline solution containing 50 IU heparin intravenously via the inferior vena cava. The heart was then flushed with 2 mL ice-cold preservation solution (Custodiol, Köhler GmbH, Hamburg, Germany) via the descending aorta until complete cardioplegia. The ascending aorta was dissected from the surrounding tissue and transected proximal to the innominate artery. The thoracic inferior vena cava was cut as close as possible to the diaphragm to maximise the diameter of the vessel. Next, the heart was lifted up and a 7-0 silk ligature was placed around the heart to ligate all other vessels. The graft heart was subsequently resected and stored in preservation solution at 4°C.

Recipient operation

Under deep anaesthesia, the recipient was placed in the supine position on the operating board with the caudal part of its body toward the surgeon. The following procedures from vessel preparation to aorta anastomosis were performed as previously described.[5] Briefly, a median transverse abdominal incision was made. The intestines were pushed toward the left side of the abdomen to expose the abdominal aorta and inferior vena cava. The two vessels were dissected from the surrounding tissues. Next, an approximately 4-mm section of the two vessels was prepared for the later anastomosis. Two microvascular bulldogs were placed to block the blood flow in both vessels. Next, two optimal openings were made in the

aorta and caval vein, respectively. The opening in the caval vein was approximately 2 mm cephalic to the opening in the aorta. The heart graft was then removed from the preservation solution, and the heart graft's aorta was anastomosed end-to-side with the recipient's aorta as previously reported.[1,2,4,5]

After anastomosis of the aorta, the recipient was positioned with the caudal part of its body toward the operator's right side, and the heart graft was placed at the left side of the abdominal cavity. At this time, great attention was paid to confirm the correct direction of the donor vena cava to avoid twisting. A simple and convenient method was used to accomplish this procedure: flushing the donor's vena cava with cold preservation solution to engorge and distend it. Thus, the opening of the donor's caval vein was quite clearly distinguished. Next, a cranial corner stitch was inserted through the opening of the donor's and recipient's venae cavae and tied. A running everting suture (out/in-in/out stitch pattern) was adopted on the anterior side of the veins from cranial end to caudal end (usually 5–6 stitches). The heart graft was subsequently turned to the right side of the abdomen, and the mouse was turned 180° clockwise to restore the same position as that at the beginning of the graft aorta-recipient aorta anastomosis procedure. The suture needle was then passed through the triangle-like field enclosed by the donor's aorta, donor's vena cava, and recipient's vessels (Figure 1A and 1B). Another running everting suture (out/in-in/out stitch pattern) was continued from the caudal corner to the cranial corner. With the last stitch, a knot was made with the first stay stitch.

After completion of the reconstruction of the graft's inflow and outflow tracts, the two microvascular clamps were opened to re-perfuse the heart transplant. The graft warm ischaemia time (time between the end of cold storage to re-perfusion) and the re-beating time (time to restoration of heartbeat) were recorded. The intestines were restored to their suitable place. The abdomen was closed in two layers with a continuous 5-0 silk suture. The recipient operational time was recorded as well. Finally, the animal was placed on a heating pad and monitored visually until it was completely awake. It was left to recover at room temperature with immediate and unrestricted access to food and water.

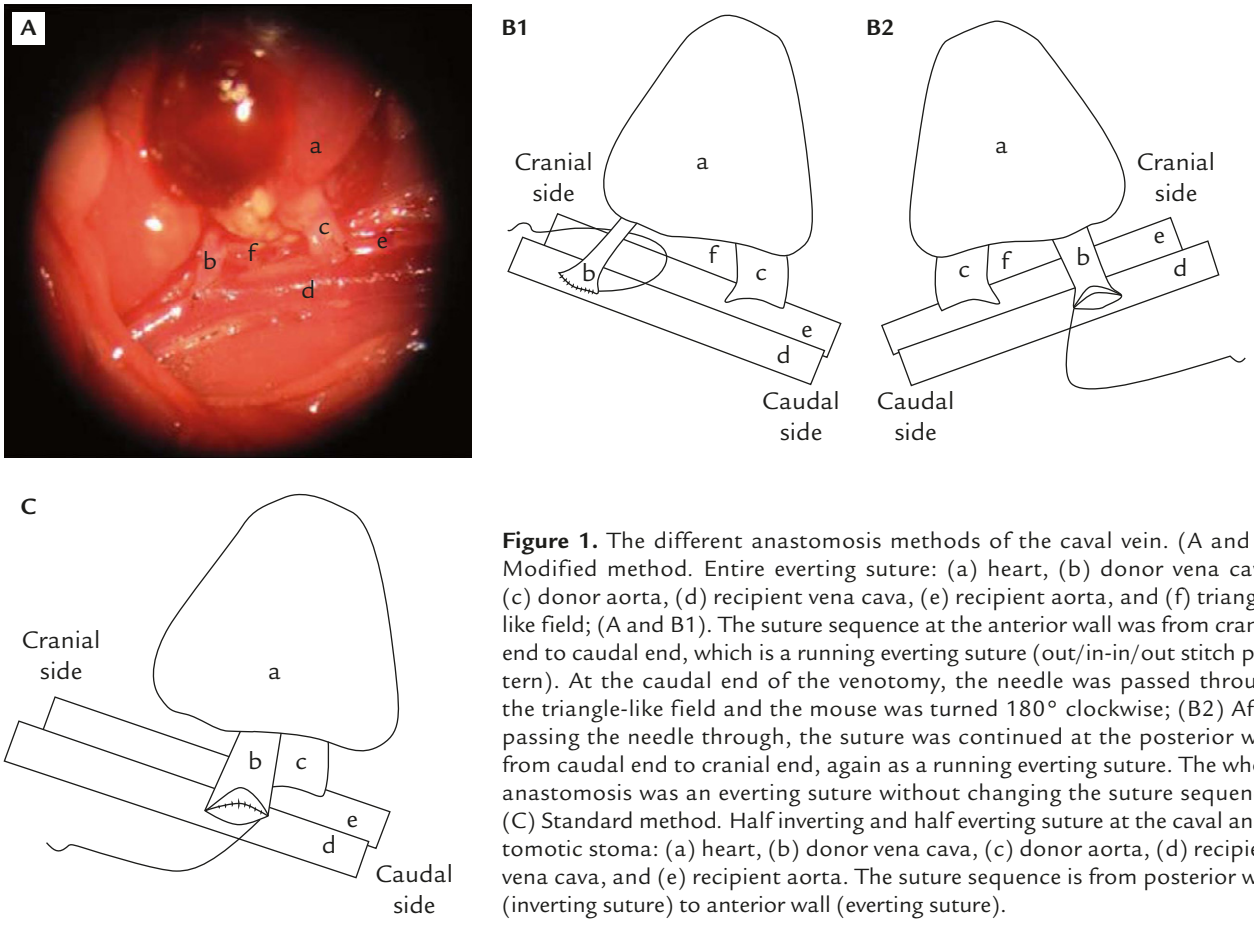

Figure 1. The different anastomosis methods of the caval vein. (A and B) Modified method. Entire everting suture: (a) heart, (b) donor vena cava, (c) donor aorta, (d) recipient vena cava, (e) recipient aorta, and (f) triangle-like field; (A and B1). The suture sequence at the anterior wall was from cranial end to caudal end, which is a running everting suture (out/in-in/out stitch pattern). At the caudal end of the venotomy, the needle was passed through the triangle-like field and the mouse was turned 180° clockwise; (B2) After passing the needle through, the suture was continued at the posterior wall from caudal end to cranial end, again as a running everting suture. The whole anastomosis was an everting suture without changing the suture sequence; (C) Standard method. Half inverting and half everting suture at the caval anastomotic stoma: (a) heart, (b) donor vena cava, (c) donor aorta, (d) recipient vena cava, and (e) recipient aorta. The suture sequence is from posterior wall (inverting suture) to anterior wall (everting suture).

Further observations and measurements

The heartbeat was palpated through the abdominal wall once a day until 100 days after surgery. The function of the donor heart was assessed using a subjective score of 0–3 (0 for no beating, 0.5 for very weak beating, 1 for weak beating, 2 for moderate beating, and 3 for full beating).[6] In addition, an electrocardiogram (Bio Amp, ADInstruments, Australia) was employed to determine the graft function in the modified method group at 30 days postoperation.

Statistical analysis

The results are presented as mean ± standard deviation. Statistical analysis was performed by Student's *t* test. A value of $p < 0.05$ was considered significant. For statistical analysis, SPSS 13.0 (SPSS, Inc., Chicago, IL, USA) was used.

Results

The cava-cava anastomosis time was significantly shorter with the modified technique (8.4 ± 1.3 minutes) than with the standard technique (12.1 ± 1.2 minutes, $p < 0.01$). Recipient operation time was significantly shorter with the modified technique than with the standard method (40.7 ± 2.5 minutes *vs.* 44.3 ± 2.3 minutes, $p < 0.01$). Similarly, warm ischaemia time was significantly shorter with the modified technique (23.4 ± 1.7 minutes *vs.* 27.2 ± 1.6 minutes, $p < 0.01$). Re-beating time was 1.2 ± 0.4 minutes with the modified technique and 1.5 ± 0.5 minutes with the standard technique ($p = 0.04$; Table 1). One month postoperation, grafts of the modified method group showed regular heart function in the electrocardiogram.

Complications

In the standard method group, one mouse died of bleeding and two grafts stopped beating because of venous stenosis at the vena cava's anastomotic stoma (survival rate: 88%). In the modified method group, only one mouse died of bleeding and another died of ileus without any vascular complication (survival rate: 92%). No statistical difference was found between the two groups. The comparison of the function of donor hearts evaluated with the palpation score also showed no differences between the two groups.

Table 1. Comparison between modified method and standard method ($n = 25$ per group)

	Modified method	Standard method	*p*
Recipient operation (min)	40.7 ± 2.5	44.3 ± 2.3	< 0.001
Warm ischaemia time (min)	23.4 ± 1.7	27.2 ± 1.6	< 0.001
Time for cava-caval anastomosis (min)	8.4 ± 1.3	12.1 ± 1.2	< 0.001
Re-beating time (min)	1.2 ± 0.4	1.5 ± 0.5	0.04

Discussion

The abdominal-heterotopic heart transplant in mice is a basic and important model for transplant research, but it is difficult to master within a short period because it involves an advanced technique. Traditionally, an anastomosis between the donor's pulmonary artery and the recipient's abdominal vena cava is sutured to reconstruct the outflow tract of the graft.[1] The pulmonary artery of the donor heart is adjacent to and shorter than the ascending aorta. The ascending aorta is adopted to reconstruct the inflow tract of the graft. Consequently, if we complete the anastomosis between the donor's and the recipient's aorta, the donor's aorta will partly occupy the surgical field for donor's pulmonary artery-recipient's vena cava anastomosis, especially the posterior side of the veins. This increases the technical difficulty of the operation and prolongs the recipient operation and graft warm ischaemia time. Moreover, in the traditional abdominal transplantation model, two anastomotic stomas are adjacent to each other and the available segment length of the donor pulmonary artery is shorter than that of the aorta; thus, the graft heart is prone to rotate around the vessel-formed axle, which easily leads to blocking of the outflow tract and congestion of the graft.

In general, placement of interrupted sutures is a common method for vessel anastomosis[7]; it results in less strictures/stenoses of the anastomosis than do running sutures. However, interrupted sutures usually require more time for knotting. The additional time required during transplantation aggravates the ischaemia injury of the graft. Parachuting anastomosis is a posterior-wall-first continuous suturing technique for anastomosis of vessels. This technique is commonly performed in a setting with limited space when the posterior wall of the vessel is difficult to expose. Some groups may use it in the mouse model. However, it is difficult for new microsurgical trainees to master within a short time because of its demanding suturing technique in the posterior wall,

especially if the posterior wall is difficult to visualise.[8,9] Furthermore, the creation of a bevel at the donor's vascular end is a common way to enlarge an anastomotic stoma. This technique is used in murine transplantation, e.g. when the diameter of the donor's vein is a bit smaller than that of the recipient. The difficulty of this method is in control of the angle while cutting the donor's vessel because the resultant diameter of the donor's end depends on the cutting angle. If the cut is not at exactly the same angle, the orifice will not be nicely round/oval, but angular. In our model, the donor's vein was cut as close as possible to the diaphragm to enlarge the anastomotic stoma and to achieve a similar diameter at the outflow tract in all operations.

We previously employed the thoracic caval vein of the donor heart for the anastomosis with the recipient caval vein to reconstruct the outflow tract of the graft heart.[5] This shortened both the operation time and the warm ischaemia time, and it reduced the incidence of complications such as vessel twisting or graft heart congestion. However, in our standard method model, the suture pattern of the anastomotic stoma on the cava vein did not change; we still used the traditional suture pattern previously described (performing the anastomosis of the posterior wall with inside to outside stitches) (Figure 1C).[1,8] With this method, the suture at the posterior wall follows an in/out-out/in stitch pattern, which actually is the inverting suture and could result in the involvement of the outer wall as well as outer tissues of veins into the anastomotic stoma. Therefore, we still experienced the complication of venous stenosis with the standard method. An entire everting suture without any outer blood vessel walls involved in the anastomotic stoma can further reduce the possibility of vascular endothelium injury[6] and involvement of outside tissues at the anastomotic stoma. This is the ideal pattern for vascular anastomosis.

We designed this new anastomotic pattern in our modified model on the basis of our standard method model. Our model preserves the advantage of the standard method in that a segment of the donor's vena cava of adequate length can be supplied for anastomosis, making a completely running-everting suture for outflow tract available. On the other hand, completing the anastomosis of the posterior side of the veins was technically the most difficult part of the recipient operation because the posterior suture line must be within view but was always very difficult to expose.[8] In addition, the suture sequence had to be changed at the caudal corner (from an in-out/out-in stitch pattern on the posterior side to an out-in/in-out on the anterior side) in the traditional anastomosis, which resulted in additional technical difficulty. Using our modified anastomosis pattern, exposing the posterior side of the vein and changing the needle suture sequence do not require long periods of time because only one kind of suture pattern needs to be performed. This simplifies the performance of the surgery. New surgeons may more quickly master this easier method.

The comparison between the outcomes of the standard and modified methods showed that shorter operation and warm ischaemia times were achieved in the modified group and were associated with shortened graft re-beating times after re-perfusion. However, there were no significant differences in the comparisons of long-term survival rate and cardiac function. This is probably due to the very short cold ischaemia time; the donor heart did not suffer severe ischaemia injury. In research involving experiments on preservation or cardioplegic solutions, the donor is usually subjected to prolonged cold storage or perfusion, which leads to more severe damage to the donor heart. In this case, the modified technique could be the better choice for these experiments because of the more standardised warm ischaemia times. In addition, no venous stenosis occurred in our modified method group. We attributed this finding to the complete everting suture pattern, which reduced the potential for vascular endothelium injury and involvement of the outer walls of blood vessels at the anastomotic stoma.

In conclusion, on the basis of our novel heart transplant model in the mouse, the modified method further facilitates the anastomosis of the outflow tract in recipient operations. This modified method can more easily be learned and mastered by novice microvascular surgeons.

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