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Cervical heterotopic kidney transplantation in rats using non-suturing and preserving-bag techniques

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Abstract

Background. This study describes a simple and stable cervical heterotopic kidney transplantation method in rats that uses artery sleeve anastomosis, vein cuff anastomosis and preserving-bag techniques.

Methods. The donor graft, consisting of kidney, renal vein (RV), renal artery (RA) and a ureterocystic flap, was removed en bloc and perfused in situ. The donor RA was end-to-end anastomosed to the recipient left common carotid artery (CCA) using a sleeve anastomosis, and the donor RV was connected to the recipient right external jugular vein (EJV) using a cuff technique. During the vascular anastomosis, the kidney graft was placed in a lactated Ringer’s solution ice-water preserving bag. The donor bladder patch was exteriorized to form cervical cutaneous stoma.

Results. A total of 104 heterotopic renal transplantations were performed, which included pre-experimental (62 operations) and experimental stages (42 operations). The success rates of the two stages were 80.6% and 95.2%, respectively. The time for surgery was 40 ± 6 min, the average time for donor surgery was 20 ± 5 min, the preparation time for the graft was 8 ± 2 min, the operative time for the recipient was 18 ± 3 min that included the time for the arterial anastomosis (5 ± 2 min) and venous anastomosis (2 ± 1 min), the cold ischaemic time of the graft was 15 ± 3 min and the warm ischaemic time of the graft was 2 ± 1 min.

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**Introduction**

Kidney transplantation in the rat is a classic experimental model used for transplantation-related research, and has been used in organ preservation, ischaemia-reperfusion injury, graft rejection, tolerance and immunosuppression studies [1–3]. Since the first description of an abdominal kidney transplantation in rats by Fisher and Lee in 1965 [4], numerous modifications have been reported over the last four decades [5–9]. However, technical complexity and a high mortality have inhibited wide use of this valuable model. In contrast, cervical heterotopic kidney implantation requires less surgical interventions by avoiding laparotomy and by preserving the recipient kidney, which together may contribute to a high success rate. The cervical heterotopic transplantation technique has been widely used in rat transplantation models such as liver, intestine and heart transplantations [10–12]. Herein, we describe the establishment of a reliable and practical model of cervical heterotopic kidney transplantation. This technique is less difficult to perform, includes shorter operation and warm ischaemic times, and produces a high success rate (95.2%) using a combination of nonsuture, preserving bag and other modified techniques. Technical details of these procedures are given in this paper.

**Subjects and methods**

**Animals**

Male inbred Wister rats weighing 220–260 g were purchased from the Shanghai Animal Center (China Academy of Science, Shanghai, China) and maintained at the Laboratory Animal Center of Dalian University (Dalian, China). They were used as donors and recipients. Animals were housed in individual cages and maintained on a 12:12-h light–dark cycle in a temperature-controlled room (22 °C) with access to chow and water *ad libitum* for at least 1 week before the study. All procedures were carried out in accordance with the Principles of Laboratory Animal Care (NIH publication, vol 25, no. 28, revised 1996). The animal protocol was approved by the Animal Committee of Dalian Medical University (DMU).

**Donor operation**

For both donor and recipient operations, rats were anaesthetized by intraperitoneal injections of 40 mg/kg body weight pentobarbital sodium. The abdomen was opened by a midline incision from the xyphoid to symphysis pubis. The bowel was covered with moist gauze and retracted to the right. The aorta and vena cava were cross-clamped close to the bifurcation of the iliac arteries to optimize blood supply to the abdominal viscera. The left adrenal and testicular vessels were exposed, ligated and cut. The left ureter was identified and dissected free from the retroperitoneum distally to the ureterovesicular junction. The ureter and its connection to the bladder, including a 3 mm diameter intramural section of the bladder (bladder patch), were cut. The left kidney was completely mobilized from its retroperitoneal attachments. The left renal vein and artery were identified and separated from each other. The proximal aorta was then clamped distal to the right renal artery but proximal to the left renal artery. The inferior vena cava was clamped and transected just distal to the right renal vein (RV). This allowed for the egress of blood and perfusate from the left kidney. A 22-gauge needle was inserted into the infrarenal aorta. The left kidney was perfused with a 10 ml 4 °C lactated Ringer’s solution containing heparin (25 U/ml), which caused recovery of blood supply to the kidney (Figure 3). After flushing of the right EJV, two stay sutures were placed at the previous suture. The U-shaped suture on each side of the donor RA was carefully pulled out, such that the left recipient CCA was ligated distally and the proximal portion of the artery was clamped. It was then flushed with a low molecular weight dextran solution to clean the blood. The procedure was begun by using two sets of 7-0 monofilament nylon that contained two needles at either end. An initial suture was passed through the lateral side adventitia of the left CCA at 0.2 mm from the artery end (Figure 2A). This needle and the opposite end needle were manipulated using a pin holder (Figure 2B), and were together placed inside the lumen of the donor RA. The needles were then inserted through the lateral side of the RA at a distance equal to 1.2–1.5 times the right CCA diameter (Figure 2C). The same procedure was repeated at 180° from the previous suture. The U-shaped suture on each side of the donor RA was carefully pulled out, such that the left recipient CCA was inserted into the donor RA, and the sutures were tied (Figure 2D). At the time of pulling, care was taken to avoid folding or wrinkling.

After flushing of the right EJV, two stay sutures were placed at the lateral sides of the anastomosis as a self-retaining retractor. Theuffed RV of the kidney graft was inserted into the right EJV of the recipient. A 6-0 tie was used to fix the cuff into the right EJV of the recipient (Figure 3). The proximal EJV clamp was released first, followed by release of the left CCA clamp, which caused recovery of blood supply to the kidney graft. The area was warmed and flushed by irrigation with 20 ml warm saline into the cervical cavity. The arterial anastomosis was compressed lightly with a dry sponge for 1–2 min after reperfusion, and this usually stopped the oozing of blood. Once the graft was reperfused, the kidney promptly became hyperaemic (Figure 4). The graft was positioned into the subcutaneous space while avoiding kinking of the vessels. The patch of the donor bladder was brought out to form a cervical cutaneous stoma. The stoma was formed using four 6-0 nylon sutures that connected the skin to the bladder patch of the graft, and it involved only the bladder peritoneum and outer layer muscle (Figure 5). Finally, the neck incision was closed with 1-0 silk continuous sutures to avoid excessive tension. The recipient was kept under a heating lamp until recovery from anaesthesia and then given water and food *ad libitum*. Neither subcutaneous nor intravenous fluids or antibiotics were given. On the 120th postoperative day, surviving animals were euthanized with CO₂ at an initial concentration of 30%, as suggested by the American Veterinary Medical Association on Euthanasia.

**Results**

Death within 5 days was considered a technical failure. A total of 104 heterotopic renal transplantations were performed that included pre-experimental (62 operations)
Fig. 1. The donor kidney graft was placed into a preserving bag filled with a lactated Ringer’s ice-water solution. The renal artery (RA), cuffed renal vein (RV) and ureterocystic flap were left out of the bag in order to perform the anastomosis.

Fig. 2. A guide suture was placed in the adventitia at 0.2 mm from the artery end on the lateral side of the recipient CCA. Both needles were placed in the lumen of the donor RA and were passed through the lateral side at a distance equal to 1.2–1.5 times the recipient CCA diameter (A–C). The same procedure was repeated at 180° from the previous suture. The U-shaped sutures on each side of the artery were carefully pulled out, so that the recipient CCA was inserted into donor RA, and the sutures were tied (D).

and experimental stages (42 operations). The success rates of the two stages were 80.6% and 95.2%, respectively. The causes of failure in the pre-experimental stage included anaesthesia accidents, thrombosis of the arterial anastomosis, massive haemorrhage, air embolism and phlebectomy. In the experimental stage, two rats died due to late anastomotic haemorrhage (1) and thrombosis (1), and 40 survived more than 2 months. The incidence of vascular complications was 5%. The surgery time was 40 ± 6 min, the average time for donor surgery was 20 ± 5 min, the preparation time for the graft was 8 ± 2 min, the operative time for the recipient was 18 ± 3 min that included the time for the arterial anastomosis (5 ± 2 min) and venous anastomosis (2 ± 1 min), the cold ischaemia time of the graft was 15 ± 3 min and the warm ischaemia time of the graft was 2 ± 1 min.

At 120 days following transplantation, the 40 surviving rats were killed and a blind histological examination was performed by a skilled transplant pathologist. Three rats revealed mild to moderate hydrenephrosis, and two rats showed proximal ureteral dilatation during the experimental stage. The rate of urinary complications was 12.5%. Of 35 kidney grafts that appeared normal macroscopically, none showed signs of focal necrosis, peritubular oedema, tubular dropout, inflammatory infiltration or histological evidence of ischaemic injury (Figure 6).
Discussion

During the last four decades, various kidney transplantation techniques in rats have been reported to shorten cold and warm ischaemic times, to simplify procedures for investigators, to improve the success rate and to reduce preventable complications [1–9].

The warm ischaemic time has been the most critical factor for successful rat kidney transplantation procedures [14,15]. In previous [1–9,14–27] rat kidney transplantation studies, the warm ischaemic time began at the moment of placing the donor kidney into the recipient abdomen and ended when circulation to the graft had been established. During this phase, proper intra-abdominal cooling of the graft by using ice-cold solution-soaked gauze pads was nearly impossible. When renal temperature and metabolism begin to rise, perfusion must be quickly re-established to reduce the risk of irreversible damage to the nephrons. It has been generally advocated that the warm ischaemic time must be <30 min [15–17]. In this paper, we describe a new technique wherein the kidney graft was placed in an ice-water lactated Ringer’s solution preserving bag during vascular anastomosis, rather than ice gauze, to maintain a low temperature environment for the graft (Figures 1 and 3). The preserving-bag technique described in this paper offers several advantages over other previously reported surgical techniques [1–9,14–27]. Briefly, these advantages include (1) significantly reduced warm ischaemic time (mean 2.5 min) compared with all previous reports; (2) mobilization of the graft using nontouch techniques; (3) a prevention of vascular pedicle twisting and (4) a lack of direct contact between the ice gauze and the body of recipient that prevents a decrease in recipient body temperature. This preserving-bag technique may have potential application for clinical organ transplantation.

According to previous studies, methods of vascular anastomosis during kidney transplantation have included the following: (1) the end-to-end technique where graft vessels were end-to-end anastomosed to recipient vessels with hand sutures [5,15,18–20]; (2) the end-to-side technique where graft vessels were end-to-side anastomosed to

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**Fig. 4.** The kidney promptly became hyperaemic, and the arterial anastomosis (black arrow) and the venous anastomosis (white arrow) did not bleed.

**Fig. 5.** The kidney graft (yellow arrow) was transplanted into a subcutaneous pocket in the cervical region of the recipient. The donor RA was end-to-end anastomosed to the recipient left common carotid artery (CCA) using a sleeve anastomosis (blue arrow), and the donor RV was connected to the recipient right external jugular vein (EJV) using a cuff technique (black arrow). The patch of the donor bladder was brought out to form a cervical cutaneous stoma. The white arrow shows the ureter, the green arrow shows the donor head and the red arrow shows the left forelimb.
recipient vessels with hand sutures [4,14,21]; and (3) the end-in-end technique that is performed either with the ‘sleeve’ or with the ‘cuff’ procedure. The sleeve procedure is performed by inserting the recipient vessels into the lumen of graft vessels [19,22–25]. The cuff procedure is performed by connecting graft vessels to recipient vessels using small polyethylene cuffs [25,26]. The end-to-end and end-to-side methods required complex microvascular hand-suture anastomosis techniques. It takes much time to reach the level of microsurgical skill needed to perform microvascular anastomoses. Compared with hand-suture techniques, the end-in-end methods (sleeve and cuff techniques) for microvascular anastomosis were easier to perform and the anastomosis time is greatly shortened. Moreover, we found that a minimal training period was sufficient for the microsurgeon to become proficient and to initiate experimentation. We applied the sleeve anastomosis technique for artery anastomosis and the cuff technique for venous anastomosis, which together greatly shortened the warm ischaemic time [27,28]. The arterial sleeve anastomosis required ∼5 min, which is similar to previous reports [23,24], whereas the inserting and fixing procedures were greatly simplified (Figure 2). Our cervical cuff technique (Figure 3) was easier to perform than the abdominal cuff methods, and the entire anastomosis procedure lasted ∼2.5 min shorter than that reported by Savas et al. [25] (mean 6 min) and Lopez-Neblina et al. [26] (mean 3.5 min). Unlike the method of Savas et al., our procedure preserves the recipient kidney. Our cuff tube construction, sculpted from a polyethylene tube having a 2.3 mm outer diameter and a 1.8 mm inner diameter, created an ideal anastomosis having a wide diameter to avoid any outflow obstruction that might cause severe graft dysfunction (Figure 4). Furthermore, because the endangium of the cuffed RV was well overturned, there was no exposed anastomotic material in the venous lumen. Since the rate of obstruction was much lower, none of the rats died of venous anastomotic complications.

Ureter anastomosis is possibly the most delicate and difficult surgical step of kidney transplantation in rats because of the very small ureter diameter [16,17]. In our study, we exteriorized the donor bladder patch to form a cervical cutaneous stoma, which was sutured using only four interrupted sutures between the skin and the bladder patch of the graft and included only the bladder peritoneum and the outer layer muscle. Our bladder-patch-to-cutaneousomy technique can easily be performed without special suturing skills, and allowed us to avoid lethal urinary complications that occur during abdominal kidney transplantation such as abdominal urinary leakage and necrosis. Our reported rate of urinary complications within the first 120 postoperative days was 12.5%, which is consistent with other studies using bladder-to-bladder anastomosis (Lee et al. [21], 15%), ureroneocystostomy (Engelbrecht et al. [23], 12.5%) or ureteroureterostomy (Pietsch et al. [29], 10%).

In conclusion, we developed a useful, easy, stable and practical method for cervical heterotopic kidney transplantation that included a greatly shortened warm ischaemic time and high success rate by using artery sleeve anastomosis, vein cuff anastomosis and preserving-bag techniques. Our model minimized surgical intervention during the kidney transplantation procedure by avoiding laparotomy and by preserving the recipient kidney. It can be performed without a high level of microsurgical skill and can be applied to preservation, reperfusion injury, as well as transplantation rejection and retransplantation pathology studies.

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Conflict of interest statement. L.Z. designed the study, performed the study and wrote paper. D.G. performed the study and analysed data. Y.Z. corrected and revised the paper. L.W. and F.L. corrected and revised the paper. Y.W., L.G., Q.W. and B.Y. collected and analysed the data. Y.Z. designed the study and corrected and revised the paper. Y.Z. collected and analysed the data. L.W. and F.L. corrected and revised the paper.

References

Fig. 6. Photomicrographs of rat kidney isografts at 120 days posttransplantation using HE staining (original magnification, A×40, B×100 and C×400). Black squares in panels A and B were enlarged for details in panels B and C, respectively.
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