

Surgical Technique in the Rat Model of Kidney Transplantation

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ABSTRACT

Today successful kidney transplantation procedures, techniques and immunosuppression protocols are a consequence of extensive research on animal models. During every transplantation surgery there are two crucial points for the success of the entire procedure: vascular (arterial end venous) and ureteral or ureterovesical anastomosis. Renal artery and vein of the donor kidney can be anastomosed end-to-side to the abdominal aorta and vena cava of the recipient (heterotopic transplantation), or end-to-end to the remains of renal artery and vein of the recipient (orthotopic transplantation) after nephrectomy. The ureter can be anastomosed also end-to-end or we can connect it directly to the urinary bladder (ureterocystoneostomy). The aim of this study was to elucidate which technique has better results according to: animal survival, reperfusion and perfusion of the transplanted kidney, elimination of the urine from the transplanted kidney and procedure costs. The study included 240 (120 donors and 120 recipients) male Wistar rats (3 months old; weight 250–300 g). Our results are clearly showing that the end-to-end vascular anastomosis, and Paquins ureterovesical anastomosis have better results in transplanted rat kidneys survival and urine drainage compared to end-to-side vascular anastomosis and end-to-end ureteral anastomosis. Based on our experience we can conclude that described methods of end-to-end vascular anastomosis and Paquins ureterovesical anastomosis are less technically demanding and have a shorter learning curve. Therefore, we can recommend the use of described methods in kidney transplantation related researches.

Key words: kidney transplantation, rat model, urine drainage, vascular anastomosis

Introduction

Renal transplantation is a gold standard in long term treatment of chronic renal failure. Today successful transplantation procedures, techniques and immunosuppression protocols are a consequence of extensive research on animal models^{1–5}. Do to modern immunosuppressant the rejection, especially acute, of transplanted kidney is rarely seen in everyday practice^{3–12}. The incidence of acute tubular necrosis and impaired early graft function caused by cold and warm ischemia is rising^{5–16}. Up to 18% of the grafts from cadavers and 8% from living donors cease to function within the first year⁵. A variety of immunologic and nonimmunologic factors seems to be responsible for these events⁵. So, the importance of animal models in transplantation research remains high. Especially, the rat model of kidney transplantation, do to abundance of antibodies for immunological research, inexpensive breed-

ing and care, pliancy to surgical interventions using microsurgical techniques, resistance to infections etc., is very popular among scientists^{1–16}. During every transplantation surgery there are two crucial points for the success of the entire procedure: vascular (arterial end venous) and ureteral or ureterovesical anastomosis. In the recent literature there are two main techniques for vascular and ureteral anastomosis in rat model of kidney transplantation^{1–16}. Renal artery and vein of the donor kidney can be anastomosed end-to-side to the abdominal aorta and vena cava of the recipient (heterotopic transplantation), or end-to-end to the remains of renal artery and vein of the recipient (orthotopic transplantation) after nephrectomy. The ureter can be anastomosed also end-to-end or we can connect it directly to the urinary bladder (ureterocystoneostomy)^{17–21}.

The aim of this study was to elucidate which technique has better results according to: animal survival, reperfusion and perfusion of the transplanted kidney, elimination of the urine from the transplanted kidney and procedure costs.

Materials and Methods

This study was performed at the Laboratory for animal research (Department of Anatomy, Medical Faculty, University of Rijeka, Rijeka, Croatia). The study included 240 (120 donors and 120 recipients) male Wistar rats (3 months old; weight 250–300 g). Rats were supplied by the Institute for Medical Research and Occupational Health (Zagreb, Croatia). Animals were housed in a climate controlled facility with a constant 12h light – dark cycle, had free access to water and were fasted 12h before operation. The experimental protocol of this study was approved by the institutional ethical committee, and all experiments were performed in accordance with Croatian legislation on Laboratory Animal Experiments. All animals were anesthetized with 3.6% chloral hydrate (10 µL/g). Also, all rats received heparin (0.3 IU/g) in the penile vein at the beginning of the surgical procedure. During the procedure in recipient rats their body temperature was maintained constant. In donor rats, laparotomy incision was performed in the midline. Viscera were exteriorized to expose both kidneys. Perirenal fat tissue was dissected and both kidneys with their ureters and vascular pedicles, attached to aorta and vena cava, were isolated, harvested and placed in small container with cold saline. The suprarenal end of the abdominal aorta was cannulated, while the infrarenal end of abdominal aorta was clamped. Vena cava inferior was clamped at the infrarenal end, and the suprarenal part was left opened. Both kidneys were flushed with 15 mL of University of Wisconsin solution (Viaspan, DuPont Pharma, Wilmington, USA). The arterial patch was made separating the left renal artery from the aorta. The renal vein was separated from the vena cava and the left kidney was stored at 4°C for 30 minutes (Figure 1). The donor rats were sacrificed after nephrectomy. Recipients were divided in 3 groups. In recipient rats, laparotomy incision was also performed in the midline. Viscera were moved to the right side and covered with saline soaked gauze to expose the left kidney in the second (40 recipient rats) and third group (40 recipient rats). Perirenal fat tissue was dissected and the left kidney was removed leaving

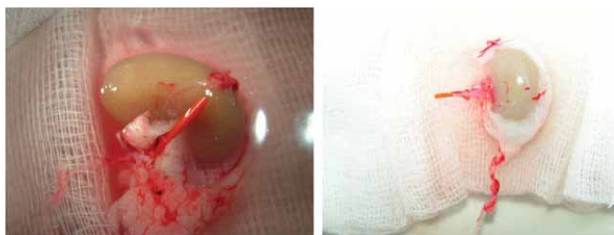


Fig. 1. Left kidney after donor's nephrectomy and perfusion.

the renal artery and vein clamped with microclamps. In the first group (40 recipient rats) we didn't expose the left kidney but we exposed the infrarenal part of the abdominal aorta and vena cava. The abdominal aorta and vena cava were clamped proximally and distally to the incision spot. The aorta and vena cava were incised and their lumen was flushed with saline. The vascular and ureteral anastomoses were performed under biomicroscope magnification of 20–30× using 10.0 nonresorbable Premilene suture (BBraun AG, Melsungen, Germany). Group 1 consisted of 40 rats in which vascular anastomoses were performed end-to-side. The renal artery was anastomosed end-to-side to the abdominal aorta distal to renal arteries, and proximal to the bifurcation (Figure 2). The renal vein was anastomosed end-to-side to the vena cava (Figure 2). We used the continuous suture with two anchorage sutures at the proximal and distal end of the arterial patch and renal vein end. The ureterovesical anastomosis was performed as described by Paquin in humans¹⁷. In 40 rats of the Group 2 the vascular anastomoses were performed end-to-end (Figure 3). The donor's renal artery and vein was anastomosed end-to-end to the recipient's left renal artery and vein by 4–6



Fig. 2. End-to-side vascular anastomosis. The renal artery and vein of the graft are anastomosed respectively to the abdominal aorta and vena cava.

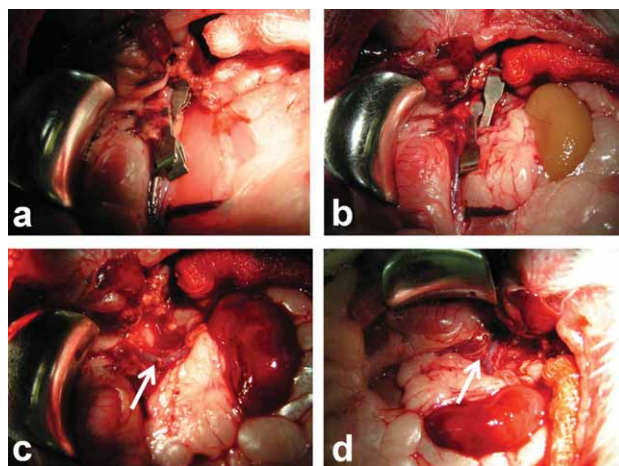


Fig. 3. End-to-end vascular anastomosis. Situation after donor's nephrectomy (a). Situation after vascular anastomoses are finished (b). Situation after removal of vascular microclamps (c,d).

interrupted sutures, after left nephrectomy was performed. The ureterovesical anastomosis was performed as described by Paquin in humans¹⁷. Group 3 consisted of 40 rats in which vascular anastomoses were performed as in Group 2. The ureteral anastomosis was performed end-to-end by 3–4 interrupted sutures. After vascular anastomoses were finished the vascular microclamps were removed. Eventual bleeding from anastomoses was controlled and solved by slight compression of the bleeding spot. The duration of cold ischemia was in all cases approximately 30 minutes. The duration of warm ischemia was in all cases from 25 to 35 minutes. Immediately after the microclamps were removed and the haemostasis was acceptable we assessed the reperfusion of the graft. The reperfusion in all cases was excellent.

The recipient rats were sacrificed after 3 weeks. And the transplanted kidneys were removed and analyzed macroscopically and microscopically for evidence of bad perfusion and hydronephrosis. For pathohistological (microscopical) analysis transplanted kidney tissue specimens were fixed in 10% buffered formalin and embedded in paraffin wax. Serial sections at 3 µm thickness were made, mounted on glass slides and kept at room temperature until use. The first of sequential sections from each specimen was stained with hematoxylin and eosin (H&E). The diagnosis of ischemic necrosis was made by an experienced pathologist. In all cases the macroscopical findings correlated with pathohistological diagnosis.

Statistic analysis

The statistic analysis was performed using Fisher’s two-tailed exact test. The p<0.05 was considered statistically significant.

Results

After 3 weeks the number of grafts with no blood circulation was significantly higher in the first group in which the vascular anastomosis was performed by end-to-side method compared to the second group in which the vascular anastomosis was performed end-to-end (Table 1). The absence of blood circulation was confirmed both macroscopically and microscopically. There was no significant difference in blood circulation between the first and the third group (p=0.44).

The number of grafts with hydronephrosis was significantly higher in the third group in which the ureteral anastomosis was performed end-to-end (Table 2).

TABLE 1
ASSESSMENT OF GRAFT BLOOD CIRCULATION AFTER 3 WEEKS FROM TRANSPLANTATION IN GROUP 1 (VASCULAR ANASTOMOSIS PERFORMED END-TO-SIDE) AND GROUP 2 (VASCULAR ANASTOMOSIS PERFORMED END-TO-END)

	Blood circulation in the graft preserved	No blood circulation in the graft
Group 1	18	22
Group 2	32	8

TABLE 2
PRESENCE OF HYDRONEPHROSIS IN THE GRAFT. GROUP 2 (VASCULAR ANASTOMOSIS PERFORMED END-TO-END AND PAQUIN’S URETEROVESICAL ANASTOMOSIS), GROUP 3 (VASCULAR ANASTOMOSIS PERFORMED END-TO-END AND URETEROURETERAL END-TO-END ANASTOMOSIS)

	Evident hydronephrosis	No hydronephrosis
Group 2	11	29
Group 3	23	17

Discussion and Conclusion

Our results are clearly showing that the end-to-end vascular anastomosis and Paquins ureterovesical anastomosis have better results in transplanted rat kidneys survival and urine drainage compared to end-to-side vascular anastomosis and end-to-end ureteral anastomosis.

As seen in previous studies, the importance of animal models in transplantation research remains high^{1–16}. Especially, the rat model of kidney transplantation, do to availability of antibodies for immunological research, inexpensive breeding and care, pliancy to surgical interventions using microsurgical techniques, resistance to infections etc., is very suitable for research^{1–16}. During every transplantation surgery there are two crucial points for the success of the entire procedure: vascular (arterial end venous) and ureteral or ureterovesical anastomosis.

There are many studies and articles discussing the adequate technique of vascular and ureteral anastomosis in human kidney transplantation. According to those researches the best results are obtained by anastomosing end-to-side renal artery and vein to external iliac artery and vein^{6–9}. The best results of urine drainage are obtained by antireflux ureterovesical anastomosis. Based on our experience the diameter of rat’s ureter, renal artery and vein prevents the application of surgical techniques used in humans. The microsurgical technique therefore, must be modified and possibly simplified. Our results with end-to-side vascular anastomosis are poor. More animals have ischemic grafts after 3 weeks and require a higher quantity of suturing material per operation. So, the method is more expensive. Also the results of end-to-end uretero-ureteral anastomosis are poor resulting often with hydronephrosis do probably to stenosis at the site of anastomosis. The end-to-end ureteral anastomosis also, raises the research expenses. Our results are showing that the end-to-end vascular anastomosis, and Paquins uretero-vesical anastomosis in the rat model of kidney transplantation are more effective with less complications, they require less suturing material and therefore are less expensive.

Based on our experience we can conclude that described methods of end-to-end vascular anastomosis and Paquins ureterovesical anastomosis are less technically demanding and have a shorter learning curve. Therefore, we can recommend the use of described methods in kidney transplantation related researches.

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KIRURŠKA TEHNIKA U ŠTAKORSKOM MODELU TRANSPLANTACIJE BUBREGA

SAŽETAK

Uspješna transplantacija bubrega, postupci, tehnike i protokoli imunosupresije posljedica opsežnog istraživanja na životinjskim modelima. Tijekom svake operacije transplantacije postoje dva ključna trenutka za uspjeh cijelog postupka: vaskularna (arterijska i venska) i ureteralna ili ureterovezikalna anastomoza. Bubrežna arterija i vena donorskog bubrega može biti anastomozirana postranično na trbušnu aortu i šuplju venu primatelja (heterotopna transplantacija), ili na kraj ostataka bubrežne arterije i vene primatelja (ortotropna transplantacija) nakon nefrektomije. Mokraćovod se može anastomozirati s primateljevim termino-terminalno ili se spaja s mokraćnim mjehurom (ureterocistoneostomija). Cilj ovog istraživanja bio je razjasniti koja tehnika ima bolje rezultate po preživljenje životinja, reperfuziju i perfuziju transplantiranog bubrega, drenažu urina te troškove. U istraživanju je korišteno 240 (120 davatelja i 120 primatelja) muških Wistar štakora (3 mjeseca starosti, težine 250–300 g). Naši rezultati su pokazali kako termino-terminalne vaskularne anastomoze i Paquinova ureterovezikalna anastomoza imaju bolje rezultate u transplantaciji štakorskih bubrega od termino-lateralnih vaskularnih anastomoza i terminoterminalnih uretero-ureteralnih anastomoza. Na temelju naših iskustava možemo zaključiti kako su opisane metode termino-terminalnih vaskularnih anastomoza i Paquinove uretero-vezikalne anastomoze tehnički manje zahtjevne i imaju kraće razdoblje učenja. Stoga, možemo preporučiti korištenje opisanih metoda transplantacije bubrega u transplantacijskim istraživanjima.