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Original article

Short- and long-term outcomes of kidney transplants with kidneys lavaged by retrograde perfusion technique

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Abstract

Objective: To evaluate the clinical safety and efficacy of the retrograde perfusion technique in kidney transplantation.

Methods: Between January 2001 and June 2011, 24 cases of kidney transplantation with kidneys perfused using the retrograde perfusion technique due to renal artery variations or injury were selected as the observation group (retrograde perfussion group, RP group). Twenty-two cases of kidney transplantation via conventional perfusion were chosen as the control group (antegrade perfussion group, AP group). There were no statistically significant differences in donor data between the two groups. Cold ischemia time, warm ischemia time, renal perfusion time, amount of perfusion fluid, acute renal tubular necrosis, wound infection, urinary fistula, graft kidney function, and the 1-year, 3-year, and 5-year survival rates for the grafted kidney in both groups were observed and recorded.

Results: The kidney perfusion time was shorter in the RP group than that in the AP group (3.14 ± 1.00 vs. 5.02 ± 1.15 min, $P = 0.030$). There were 10 cases of acute renal tubule necrosis in the RP group and 5 in the AP group. The length of hospital stay was 40 ± 14 d in the RP group and 25 ± 12 d in the AP group. The follow-up time was 3.5−8.5 years (mean 6.25 years). The 1-, 3-, and 5-year survival rates for the grafted kidney were 95.8%, 75.5%, and 65.5% in the RP group and 97.1%, 82.5%, and 68.4% in the AP group, respectively ($P>0.05$).

Conclusions: This study indicates that retrograde perfusion is safe and practicable for cadaveric kidney harvesting and can be regarded as a better alternative or remedial measure for a poorly perfused kidney due to vascular deformity or injury.

Keywords: Retrograde perfusion; Allograft vascular deformity; Allograft vascular injury; Kidney procurement

Background

Kidney transplantation is now well recognized as the best treatment method for end-stage renal disease. With the development of clinical transplantation immunology, the continuous advancement of new immunosuppressive

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agents and the improvement in kidney preservation solutions, great progress has been made in clinical kidney transplantation. However, there are still some problems existing, like a shortage of kidneys, how to fully take advantage of precious donor kidneys and expand the kidney donor pool, whether to further improve all aspects of surgical procedures to enhance long-term survival after kidney transplantation, and etc.

In the process of removing donor kidneys we had encountered renal artery injury, anatomic variations, and malformations of the renal artery and we were unable to perfuse. These affected the transplantation outcome and even led to the discarding of donor kidneys. Occasionally, we found that perfusion beginning from veins could quickly and fully irrigate donor kidneys. This brought us to the clinical hypothesis that donor kidneys irrigated by this method could be used for transplantation. Therefore, we carried out a serial experimental and clinical study on retrograde perfusion of donor kidneys for kidney transplantation. In this study we introduced the modified retrograde perfusion technique. This article is to evaluate the long-term clinical safety and efficacy of the retrograde perfusion technique in kidney transplantation.

Materials and methods

During January 2001−June 2011 we had 24 cases with kidney transplantation using retrograde perfusion kidneys due to renal artery variations or injury when we were unable to perfuse or achieve a good perfusion of donor kidneys with conventional perfusion procedure. These patients were selected as the observation group (retrograde perfusion group, RP group). All 24 donor kidneys, taken from 23 donors, were perfused via retrograde perfusion. Six donors had variations of the single side renal artery and 12 donors had single side renal artery injury, whereas there were the healthy contralateral renal vessels. Five donors had both side renal artery variations, among which, four were managed to be perfused with the antegrade method in one side kidneys (with two arteries) and had to be perfused with a retrograde technique in the other kidney (with three arteries). One of them was perfused with the retrograde technique on both side kidneys because of artery variations. Twenty-two cases of kidney transplantation using the other side kidney from the same group of donors via conventional perfusion were chosen as the control group (antegrade perfusion group, AP group). All donors were healthy (22 males, one female) age 22−44 years old (median age 35.5 years). Data from donors are shown in Table 1. The two groups of donors were identical with a special one whose both kidneys were servered as in observation group. The procurement and perfusion of donor kidneys were accomplished by the same team in our hospitals. Donors had no history of hepatitis, tuberculosis, cardiovascular diseases, hypertension, or diabetes. Donors were tested negative for hepatitis and human immunodeficiency virus, as well as testing negative with the syphilis antibody test.

Twenty-four cases of kidney transplantation using retrograde perfused kidneys were performed in our hospitals by the same surgery team. There were no statistical differences between the two groups of

Table 1
Demographic of donor characteristics in both groups.

Characteristics	RP group	AP group	*P*
Number (*n*)	24	22	−
Gender (male/female)	23/1	21/1	−
Age, years, range	34.5 ± 13.5 (22−44)	34.2 ± 13.6 (22−44)	−
Left/right kidney, *n* (%)	11/13	12/10	−
Renal artery injury, *n* (%)	12 (50)	0	0.000
Renal artery variations, *n* (%)	12 (50)	4 (18)	0.032
Angioplasty operations, *n* (%)	18 (75)	4 (18)	0.000

RP group: retrograde perfussion group; AP group: antegrade perfussion group.

Table 2
Demographic of recipients' characteristics in two groups.

Characteristics	RP group	AP group	*P*
Number (*n*)	24	22	
Gender (male/female)	16/8	15/7	NS
Age, years, range	35.5 ± 12.5 (20−56)	34.3 ± 12.0 (20−53)	NS
Primary cause of uremia, *n* (%)			
Chronic glomerulonephritis	16 (67)	16 (73)	NS
Polycystic disease	1 (4.1)	1 (4.5)	NS
Hypertension/nephrosclerosis	2 (8.3)	2 (9.0)	NS
Diabetes mellitus	1 (4.1)	1 (4.5)	NS
IgA nephropathy	2 (8.3)	1 (4.5)	NS
Other causes	2 (8.3)	1 (4.5)	NS
Dialysis duration, months	15.8 ± 13.5	15.0 ± 12.5	NS
Type of dialysis (HD/PD/ND)	20/1/3	18/2/2	NS
Mean PRA, %	7.8 ± 2.6	7.5 ± 2.1	NS
HLA-MM(mean ± SD)	2.16 ± 1.35	2.13 ± 1.24	NS
Lymphocytotoxicity test, %	6.4 ± 3.5	6.3 ± 3.2	NS
Initial treatment, *n* (%)			
CyA + Aza + S	6 (25)	6 (27)	NS
CyA + MMF + S	8 (33)	7 (32)	NS
FK + MMF + S	10 (42)	9 (41)	NS

RP group: retrograde perfussion group; AP group: antegrade perfussion group; HLA-MM: human leukocyte antigen-mismatch; HD: hemodialysis; PD: peritoneal dialysis; ND: no dialysis performed; SD: standard deviation; PRA: panel-reactive antibody; ATG: antithymocyte globulin; CyA: cyclosporine; Aza: azathioprine; S: steroid; MMF: mycophenolate mofetil; FK: tacrolimus: NS: no significance.

recipients for sex, age, primary disease, dialysis duration, loci of HLA mismatch, panel reactive antibody, reception of left/right donor kidney, transplant method, and postoperative immunosuppression regimens (Table 2).

Retrograde perfusion procedure

During the procurement operation, we splitted the abdominal aorta between both renal arteries to find the opening − after harvesting the cadaveric donor kidneys by an en bloc resection technique before January 2005. Once renal artery variations or injuries were found that we were unable to perfuse, we immediately identified the inferior vena cave along the abdominal aorta, retracted symmetrically and splitted it to find the opening of the renal veins. Then a catheter was inserted into the renal vein and perfusion was performed with perfusion fluid at 4 °C (manufactured by Shanghai Changzheng Hospital) (Fig. 1). The perfusion was interrupted after 30−40 ml was perfused to let the perfusate backflow out of kidney through the renal vein. The kidney graft was completely perfused to pale after this maneuver was repeated 3−4 times. It was also evident that the outflow at the arterial end was clear. The gravity filling height was 60−80 cm. Since January 2005, we began en bloc harvesting the liver-kidney using *in situ* perfusion through the abdominal aorta. If renal artery variations, injury, or spasm resulted in poor perfusion, then, the-retrograde perfusion procedure was promptly carried out. Antegrade perfusion was performed in the control group from renal arteries to the renal vein with the same perfusion fluid. The perfusion was continuous until the return flow at the venous end was clear. The gravity filling height was 80−100 cm. All donor kidneys in both groups were perfused with 20−30 ml 4 °C perfusion fluid again in the operating room as part of kidney trimming procedures before transplantation.

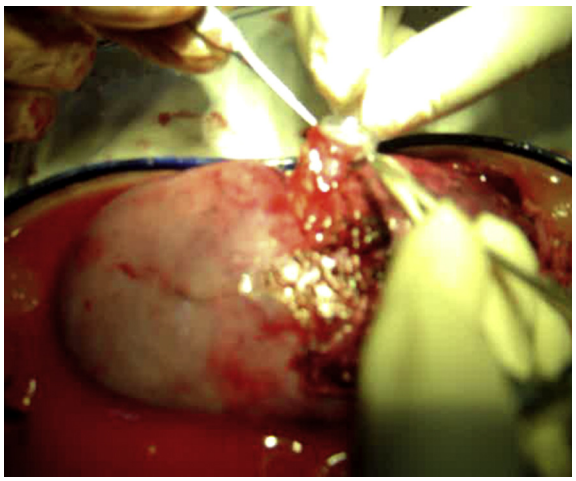

Fig. 1. A catheter was inserted into the renal vein and a perfusion was performed with perfusion fluid from renal vein to renal artery. The perfusion was interrupted after 30−40 ml was perfused to let perfusate backflow out of kidney through the renal vein.

Clinical variables

The following variables were evaluated in each case; cold/warm ischemia time, renal perfusion time, and the amount of perfusion fluid. After surgery the acute rejection reaction, acute renal tubular necrosis, wound infection, urinary fistula, the duration of hospital stay, graft kidney function, and the 1-year, 3-year, and 5-year survival rates of the grafted kidney in both groups were observed and recorded. The renal perfusion time was calculated from the start of perfusion using either arteries or veins until donor kidneys turned white and the lavage fluid became clear. According to this the amount of perfusion fluid was calculated. Since January 2005, en bloc harvesting of the liver-kidney and *in situ* perfusion through the abdominal aorta was applied. With this technique the calculation of the amount of perfusion fluid, the perfusion time, cold ischemia time, and warm ischemia time became difficult, and values from a few cases were not included when the final values were calculated. Variations or injury of renal donor kidneys were often observed in the retrograde group. We performed delicate angioplasty operations. Kidney transplantation surgeries were completed as a routine procedure in both groups. Allografts managed through the two-flush perfusion technique went to the two groups of recipients randomly. Both subjects signed uniform informed consent forms in an attempt to achieve single-blind conditions. The study was approved by the hospital ethics committee.

After discharge from the hospital, transplant recipients were closely monitored in our outpatient clinic. In patients with a 20% rise in serum creatinine, a biopsy was performed to rule out rejection. Biopsy proven rejection was treated with pulse steroids or antilymphocyte agents. Transplant nephrectomy or return to dialysis was defined as graft loss.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). The analysis of variance (ANOVA) was used to compare the difference between the two groups.

Nominal data were compared using the chi-square test or Fisher's exact test. Survival rate was calculated with Kaplan–Meier and the Log-rank test was used to compare the survival curves. $P < 0.05$ was considered as statistically significant.

Results

Details of the post-operation data of both groups' recipients are shown in Table 3 and Table 4. The kidney perfusion time in the RP group was significantly shorter than in the AP group ($P<0.05$), while there was no significant difference between the two groups in terms of the amount of perfusion fluid ($P>0.05$). The warm ischemia time was longer in the RP group than that in the AP group ($P<0.05$). The number of acute renal tubule necrosis were 10 and length of hospital stay was 40 ± 14 d in the RP group and that were 5 and 25 ± 12 d in the AP group. In the RP group, one patient lost the donor kidney due to acute rejection combined with rupture of the transplanted kidney nine days after surgery. One case had a delayed graft function and began to urinate nine weeks after surgery; at 12 weeks post operation he still had good graft function. The surgical complications of the two groups of recipients are shown in Table 4. One case in the AP group lost the donor kidney because of acute rejection.

Follow-up time was 3.5–8.5 years (mean 6.25 years). Three cases were lost during the follow up in the retrograde group and two in the control group. The 1-year, 3-year, and 5-year survival rates for the grafted kidneys were 95.8%, 75.5%, and 65.5% respectively in the RP group, whereas they were 97.1%, 82.5%, and 68.4% in the AP group (Fig. 2). The difference was not statistically significant between the two groups (log rank test, $P=0.663$).

Table 3
The parameters of perfusion and transplantation in two groups.

Variables	RP group	AP group	*P*
Number (*n*)	24	22	
Perfusion time, min	3.14 ± 1.00	5.02 ± 1.15	0.030
Amount of perfusion fluid, ml	230 ± 37	246 ± 38	0.209
Cold ischemia time, h	11.9 ± 4.5	12.1 ± 4.5	0.912
Warm ischemia time, min	9.5 ± 3.5	4.5 ± 2.3	0.000
Hospital stay, d	40 ± 14	25 ± 12	0.006
Delayed graft function, *n* (%)	12 (50)	6 (27)	0.140
AR, *n* (%)	6 (25)	5 (23)	1.000
ATN, *n* (%)	10 (42)	5 (23)	0.217
Serum creatinine at discharge, mg/dl	2.8 ± 0.6	2.6 ± 0.6	0.324

RP group: retrograde perfussion group; AP group: antegrade perfussion group; AR: acute rejection; ATN: acute tubular necrosis; With exclusion of 3 cases in both groups before calculation of the amount of perfusion fluid, perfusion time, cold ischemia time and warm ischemia time because of *en bloc* harvesting of liver-kidney using *in situ* perfusion through abdominal aorta. In retrograde group, warm ischemia time included time spent attempting antegrade perfusion.

Table 4
The surgical complications in two groups (n).

Variables	RP group	AP group	*P*
Number	24	22	
Perinephric effusion	3	1	0.609
Urinary fistula	4	1	0.349
Wound infection	2	1	1.000
Graft rupture	1	0	1.000

RP group: retrograde perfussion group; AP group: antegrade perfussion group.

Discussion

Traditionally, the initial perfusion of donor kidneys begins from artery to vein during the cadaveric kidneys harvesting. However, it is very difficult to perfuse the donor kidneys with vascular variants or intra-operative vascular damage in a timely and effective manner. In this case retrograde perfusion could produce unexpected perfusion results.

From an anatomical point of view, retrograde perfusion is feasible for cadaveric kidney harvesting flush perfusion. The reasons are as follows: 1) The renal veins shows less variation than the renal arteries, 2) The diameters are greater in the renal veins than in the renal arteries, 3) Renal veins have extensive communicating branches in the kidney, 4) There are no venous valves in the renal venous system.[1–3] It has been reported that retrograde perfusion was carried out from efferent arterioles to afferent arterioles in the study of the prostaglandin effect on renal blood flow.[4] This also confirms that renal retrograde perfusion is feasible from an ultrastructure point of view. Thus it seems to be possible to perfuse from veins to arteries, and this may be superior for the purpose of allograft perfusion.

Through an extensive literature search and review, we found that some investigators infused oxygen into donor kidneys by the retrograde route from veins to arteries for the preservation of donor kidneys in order to extend the storage time and improve transplantation outcome.[1,5] One of the reason for the use of retrograde oxygen persufflation is that high concentrations of oxygen does damage to glomerular arteries. In these studies, more than eight pores, about 1.0 cm deep, were bored on both surfaces of donor kidneys by fine

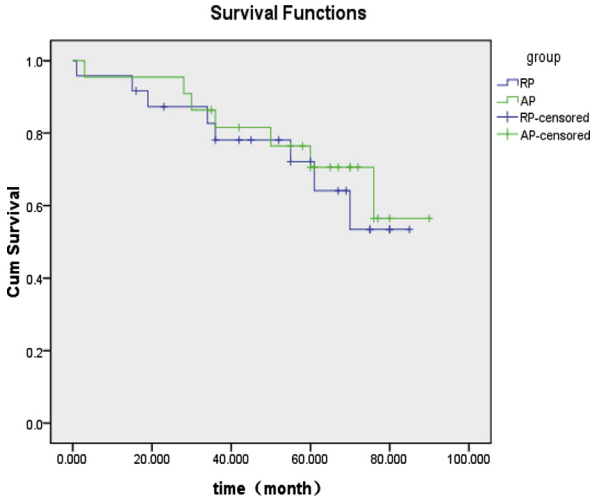

Fig. 2. Kaplan–Meir survival curve of renal allograft of two groups.

needles. Oxygen entered from renal veins, passed through the puncture holes and escaped via the surface perforation on the kidneys. This could alleviate renal hypoxia and reduce ischemia-reperfusion injury. In a similar study oxidative perfusion fluid was administered by retrograde perfusion in the control group.[1]

We also found that retrograde perfusion via the inferior vena cava (IVC) had been carried out in some cardiothoracic operations in order to protect abdominal viscera, including kidneys, which offered good protection.[2,3] All these studies have shown the feasibility and safety of retrograde perfusion for kidney transplantation.

We had conducted an animal experiment on retrograde perfusion of donor kidneys prior to clinical trials. After retrograde perfusion of rabbit kidneys and retaining them by simple ice storage for 2 h, 12 h, or 24 h, the morphological changes were observed under a light microscope and electron microscope. The results still showed good morphologic characteristics 24 h after retrograde perfusion.[6] In our animal trials we compared retrograde perfusion with antegrade perfusion in six pairs of sheep, and a faster and more efficient perfusion was seen with the retrograde perfusion technique as monitored by X-ray imaging.[6] We have also published an initial clinical study on the retrograde perfusion technique for kidney transplantation. In that study, transplantation using kidneys with renal artery injuries and variations were perfused by a retrograde technique on the spot. They had a better utilization rate as a donor kidney and better clinical results, compared with using kidneys with the same problems that were perfused by the antegrade route after a period of time in a bench surgery.[7]

A clinical study on retrograde perfusion of donor lungs for lung transplantation has been published.[8] The retrograde perfusion of donor livers has also been described in clinical study reports of liver transplantation.[9,10] The clinical study report on kidney transplantation after initial retrograde perfusion has not been available so far, except for our initial clinical study on this subject. We found retrograde perfusion was used in some basic research and a few clinical studies. However, most of these studies used retrograde perfusion to continuously provide oxygen for the preservation of the donor kidneys to prolong the storage time and enhance graft acceptance.[1,5] The difference between this study and other published studies are as follows: 1) The retrograde perfusion of donor kidneys has only been described in the preservation of donor kidneys perfused with the conventional antegrade method as reported in the literature. No other study, other than the one we reported, has been conducted with initial retrograde perfusion of donor kidneys during the procurement operation. 2) Most previous study results came from animal studies, whereas this is a clinical study with a large number cases and long-term observation data. 3) Most previous studies investigated the effect of retrograde perfusion with oxygen by a continuous persuffflation, while our study was to investigate the effect of retrograde perfusion of donor kidneys with perfusate by intermittent lavage.

Perfusion pressure of the graft kidney is also an important factor in the graft kidney viability that needs to be considered. Some investigators believe that the lower the better for perfusion pressure, otherwise it will result in perfusion nephropathy.[1,11,12] It has been reported that the optimal perfusion pressure by the traditional method in animal experiments should be 20–30 mmHg.[1,13,14] In order to reduce the incidence of perfusion nephropathy we used a lower perfusion pressure (maximum of 60–80 cm) via retrograde perfusion than was used in the AP group (maximum of 80–100 cm). Moreover, we also performed RP in an interrupted fashion, rather than continuously, in an attempt to lessen hydrostatic mechanical damage. This is also the main difference of the technique from that previously used in our initial study. And this may be another factor in retrograde perfusion protection of donor kidneys.

When performing clinical and statistical analysis we noted that the warm ischemia time was longer in the retrograde group than in the control group. This is an undoubtedly important factor influencing graft function and may be the main reason for acute tubular necrosis and surgical complications after surgery seen in the RP group. The reason for a longer warm

ischemia time is mainly because the retrograde perfusion method was mostly used after the conventional perfusion failed during kidney harvesting. But if we did not use this method time would be even longer, and even then the donor kidneys would be poorly perfused, or actually unable to be perfused. All donor kidneys after retrograde perfusion had vascular malformation or vascular damage, while less are found in the AP group. So this might cause bias in the clinical observations. All these might also be possible reasons for more acute tubular necrosis and early postoperative complications in the RP group than in the AP group.

Retrograde perfusion also avoids excessive or blind operations to find the injured or the deformed renal artery during kidney harvesting and reduces additional injury to the kidney and ureter. This method can improve donor kidney perfusion, enlarge the marginal donor kidney, and enhance the utilization rate of donor kidneys. This technique is becoming more meaningful and valuable because of the donor kidney shortage. This study confirms that retrograde perfusion is safe and practicable for cadaveric kidney harvesting regarded as a better alternative or remedial measures for a poorly perfused kidney due to vascular deformity or injury, and may have potential medical value.

Conflicts of interest

The author declare that they have no conflicts of interest.

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