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Remote ischemic conditioning enhanced the early recovery of renal function in recipients after kidney transplantation: a randomized controlled trial

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ABSTRACT

Background: To investigate whether remote ischemic conditioning (RIC) can attenuate ischemic reperfusion injury (IRI) in recipients after kidney transplantation using donation after cardiac death.

Methods: Forty-eight recipients referred for kidney transplantation were recruited. The paired recipients who received the kidneys from the same donor were randomly assigned (one received RIC and the other did not). RIC was induced by three 5-min cycles of brief repetitive ischemia and reperfusion by clamping the exposed external iliac artery. Blood samples were withdrawn at hour 2, hour 12, days 1–7, day 14, and day 30 to measure serum creatinine level and estimated glomerular filtration rate after transplantation. Urine samples were collected at hours 2, 12, 24, and 48 to measure urine neutrophil gelatinase–associated lipocalin after transplantation. Renal tissues were obtained at 30 min for histologic changes after transplantation.

Results: There were no significant differences in clinical characteristics of the recipients and donors between RIC and control groups. The serum creatinine level was lower in the RIC group compared with that of the control group (12 h, days 1–14, $P < 0.05$; other $P > 0.05$); the estimated glomerular filtration rate was higher in the RIC group compared with that of the control group (12 h, days 1–14, $P < 0.05$; other $P > 0.05$); urine neutrophil gelatinase–associated lipocalin, an early marker of IRI, was lower in the RIC group at hours 2, 12, 24, and 48 (2 h, 48 h, $P > 0.05$; 12 h, 24 h, $P < 0.05$) compared with that of the control group. The graft pathology showed no differences between RIC and control groups.

Conclusions: RIC enhanced the early recovery of renal function in recipients after kidney transplantation. Our results provide a novel potential approach to attenuate transplantation-associated IRI.

1. Introduction

Kidney transplantation is the most effective therapy for end-stage renal disease, but the shortage of organs limits its clinical application. To expand the organ pool, organ donation after cardiac death (DCD) was suggested in many countries [1,2], including China [3]. Currently, ischemic reperfusion injury (IRI) is an inevitable event accompanying kidney

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transplantation. The severity of IRI correlates with the delayed graft function (DGF) and acute rejection (AR) [4], as well as chronic fibrosis and graft loss [5]. Kidneys from DCD donors retain a long duration of warm ischemia from the cardiocirculatory arrest of donors to the cold preservation of the donated organs. Therefore, prevention of the IRI holds the potential to improve renal function and make full use of DCD.

Remote ischemic conditioning (RIC) is induced by several periods of ischemia on a tissue (arm or leg) to produce systemic protection against IRI in distant organs [6]. Several clinical trials of RIC have found protective effects against IRI in cardiac surgery [7,8]. Similarly, protective effects of RIC on IRI in kidney have been demonstrated in cardiac and vascular surgeries [9,10]. A study in Sprague–Dawley rats found RIC to attenuate IRI in renal IRI model [11]. Soendergaard *et al.* [12] also demonstrated that RIC improved glomerular filtration rate (GFR) and renal plasma reperfusion in a porcine kidney transplantation model. However, there is no clinical trial of RIC in recipients undergoing kidney transplantation.

The present study was to investigate whether RIC performed in recipients during kidney transplantation can attenuate transplantation-associated IRI using DCD.

2. Materials and methods

2.1. Patient population and study design

From February 2012 to July 2012, 48 recipients from The Kidney Disease Center, The First Affiliated Hospital, Medical College of Zhejiang University referred for kidney transplantation were recruited. Forty-eight renal grafts were obtained from 24 donors. The forty-eight recipients were primary transplants. The forty-eight recipients and the 24 donors were Chinese. The paired recipients who received the kidneys from the same donor were randomly assigned (one received RIC and the other did not). The 48 recipients were divided into two groups: RIC ($n = 24$) and control ($n = 24$). Panel reactive antibody titers of 48 recipients were <10%. The blood type was compatible between the recipients and the donors. DGF was defined by the need for dialysis during the first week after transplantation; AR was defined according to the clinical criteria including oliguria after transplantation, serum creatinine (Cr) level increase ≥25% above baseline, fever and graft swelling, and pathologic findings, as reported in our previous study [13].

According to their own choices before transplantation, 44 of 48 recipients (92%) received immunosuppressive induction therapy (simulect or thymoglobulin). All recipients received basic triple immunosuppressive therapy, consisting of corticosteroids, mycophenolate mofetil or rapamycin, and cyclosporine or tacrolimus. The detailed usage was described in our previous reports [14]. Forty-eight recipients received ganciclovir for 2 wk (serum Cr level <300 μmol/L) and sulfamethoxazole for 6 mo to prevent cytomegalovirus and *Pneumocystis* infection after transplantation, respectively. Written informed consent was obtained from all recipients enrolled in this trial. The protocol was approved by the Ethics Committee.

2.2. The RIC protocol

RIC was performed by three 5-min cycles of external iliac artery ischemia after anesthesia. Ischemia was achieved in unilateral lower limb of the recipients by clamping the exposed external iliac artery during transplantation. The control group had external iliac artery exposed as a sham procedure of equal length but without clamping. Each cycle was separated by a 5-min period of reperfusion, during which the artery clamp was removed. The RIC protocol was induced in recipients during kidney transplantation. During the surgery, external iliac artery was exposed. Two 5-min cycles were completed, during which the end-to-side anastomosis of the graft vein to the external iliac vein was performed. The graft vein was clamped, and the external iliac vein was opened. The third cycle was completed, during which the end-to-side anastomosis of the graft artery to the external iliac artery was performed. The graft artery was clamped, and the external iliac artery was opened. After 10-min blood flow recovery of external iliac artery, the graft vein and artery were opened, and the time was counted as 0. The ureter was sutured with the bladder by modified Joho method.

2.3. Postoperative assessment

Blood samples were withdrawn at hour 2, hour 12, days 1–7, day 14, and day 30 after transplantation. Urine samples were collected at hours 2, 12, 24, and 48 after transplantation. Renal tissues were obtained at 30 min after transplantation by Fine Core Biopsy Needle (FC16G*150 mm; Doctor Japan Co, Ltd, Tokyo, JN). The serum and urine samples were stored at −80°C until use.

The blood samples were used to measure serum Cr level. Estimated GFR (eGFR) was calculated by the following formula: eGFR (mL/min/1.73 m^2) = 186 × (serum Cr level [mg/dL])$^{-1.154}$ × (age [y])$^{-0.203}$ × 0.742 (if female) × 1.212 (if black). Urine samples were used to measure urine neutrophil gelatinase–associated lipocalin (uNGAL) concentration. uNGAL was assayed quantitatively by ELISA kit (DLCN20; R&D Systems, Minneapolis, MN). The renal tissues were analyzed and graded for histologic changes by a blinded kidney pathologist with 20 y of experience.

2.4. Statistics

Numerical variables were expressed as mean ± standard deviation. Numerical variables were tested by paired Student t-test or Wilcoxon test according to the result of normal distribution test (Shapiro–Wilk test). Categorical variables were tested with chi-square or Fisher exact test. A P value of <0.05 was considered as significant. All statistical analyses were performed with SPSS 17.0 software (SPSS Inc, Chicago, IL).

3. Results

The clinical characteristics of the recipients and their immunotherapy were listed in Table 1; the donor characteristics

Table 1 – Clinical characteristics of 48 recipients and their immunotherapy.

Characteristics	RIC ($n = 24$)	Control ($n = 24$)	P
Age (y)	40.6 ± 11.6	39.7 ± 10.2	0.688
Gender (*n*)			0.383
Male	12	15	
Female	12	9	
Weight ratio (graft-to-recipient; g/kg)	2.8 ± 0.8	2.7 ± 0.5	0.304
Underlying kidney disease (*n*)			0.932
Glomerulonephritis	17	17	
Polycystic kidney	2	3	
Diabetes	1	1	
Alport syndrome	1	1	
Hypertension	0	1	
Other	3	1	
Mode of pretransplantation dialysis (*n*)			0.745
Hemodialysis	18	17	
Peritoneal dialysis	6	7	
Meantime of pretransplantation dialysis (d)	1139.5 ± 995.6	1090.3 ± 632.0	0.815
Recipients of dialysis on the day of transplantation (*n*)			1
Yes	10	10	
No	14	14	
Mean serum Cr concentrations on the day of transplantation (μmol/L)	911.1 ± 344.0	1032.0 ± 327.3	0.161
HLA mismatches	2.8 ± 1.1	3.0 ± 1.2	0.149
CMV antigen (*n*)			0.768
Negative	14	15	
Positive	10	9	
Immunotherapy (induced therapy, *n*)			1
Simulect	14	14	
Thymoglobulin	8	8	
No induced therapy	2	2	
Immunotherapy (basic therapy, *n*)			0.491
Pred + MMF + FK506	16	15	
Pred + MMF + CsA	5	3	
Pred + Rapa + CsA	3	6	

CMV = cytomegalovirus; CsA = cyclosporine A; FK506 = tacrolimus; HLA = human leukocyte antigen; MMF = mycophenolate mofetil; Pred = prednisolone; Rapa = rapamycin.
Data are presented as mean and standard deviation unless otherwise specified.

were listed in Table 2. There were no significant differences in clinical features such as age, weight ratio (graft-to-recipient), gender, and so forth in recipients between RIC and control groups.

The procedure was performed by four transplant surgeons randomly, and there was no significant difference between them ($P = 0.183$). After kidney transplantation, no AR occurred in 48 recipients within 1 mo; no DGF occurred in the RIC group and 3 of 24 DGF (13%) occurred in the control group. One of 24 recipients (4%) in the RIC group had serum Cr level rebound from 149 μmol/L on day 4 to 321 μmol/L on day 5 after transplantation. AR was excluded by allograft biopsy. We speculated that cyclosporine toxicity might be the reason and then substituted cyclosporine by tacrolimus; the serum Cr level was dropped to 127 μmol/L on day 15 after transplantation. One of 24 recipients (4%) in the RIC group had satisfactory renal recovery (serum Cr level dropped to 80 μmol/L on day 10) but had renal artery rupture caused by fungal infection on day 11, and the graft was removed.

Figure A shows serum Cr concentrations of recipients before and after transplantation. The RIC group demonstrated lower serum Cr concentrations compared with the control group from hour 2 to day 30, but serum Cr concentrations were significantly lower at hour 12, days 1–7, and day 14 after transplantation compared with the control group.

Figure B shows eGFR of recipients after transplantation. The RIC group demonstrated higher eGFR levels compared with the control group from hour 2 to day 30, but eGFR was significantly higher at hour 12, days 1–7, and day 14 after transplantation compared with the control group.

Figure C shows the uNGAL secretion. The RIC group demonstrated lower uNGAL secretion compared with the control group from hours 2–48; at hours 12 and 24 after transplantation, uNGAL secretion was significantly lower.

The graft pathology shows no differences between RIC and control groups at 30 min after transplantation by Banff [15] and Rabb scorings [16]; glomerulitis, tubulitis, interstitial inflammation, intimal arteritis, peritubular capillaritis, interstitial fibrosis, glomerulosclerosis, and tubular injury were observed in both groups.

4. Discussion

To our knowledge, this is the first clinical study on the protective effects of RIC on renal graft IRI after kidney transplantation using DCD. Chen *et al.* [17] failed to improve early

Table 2 – Clinical characteristics of 24 donors.

Characteristics	Statistics
Age (y)	31.9 ± 8.4
Gender (*n*)	
Male	22
Female	2
Mean serum Cr concentrations on the day of transplantation (μmol/L)	90.6 ± 40.9
Mean eGFR levels on the day of transplantation (mL/min/1.73 m^2)	102.2 ± 66.9
Mean warm ischemia time (min)	8.8 ± 3.8
Mean cold ischemia time (h)*	8.4 ± 2.9
Cause of death (*n*)	
Traumatic brain injury	22
Drowning	1
Ganglia glioma	1
Cytomegalovirus antigen (*n*)	
Negative	10
Positive	8
No	6

Data are presented as mean and standard deviation unless otherwise specified.
No significant differences were observed between the two groups.
*RIC group, 8.6 ± 2.9; control group, 8.3 ± 2.9 ($P = 0.235$).

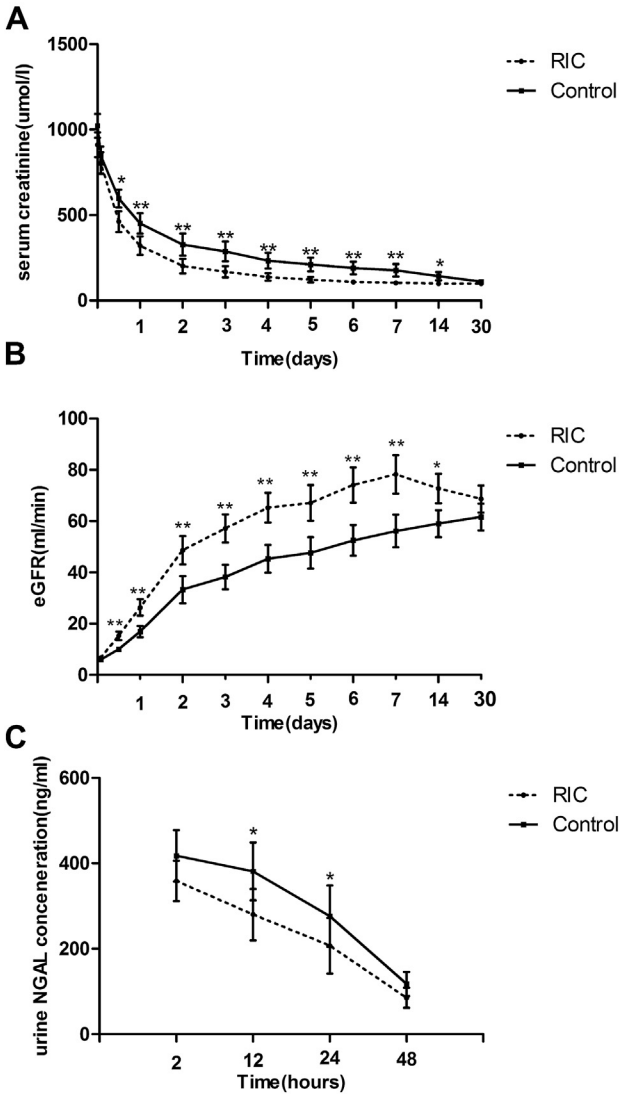

Fig. – (A) Comparison of serum Cr level between RIC ($n = 24$) and control ($n = 24$) groups before and after kidney transplantation. *$P < 0.05$; **$P < 0.01$. (B) Comparison of eGFR between RIC ($n = 24$) and control ($n = 24$) groups after kidney transplantation. *$P < 0.05$; **$P < 0.01$. (C) Comparison of uNGAL between RIC ($n = 24$) and control ($n = 24$) groups after kidney transplantation. *$P < 0.01$.

renal function of patients undergoing living-donor renal transplantation. It is possible that living donors have less IRI than DCD donors or need more sensitive indicators to detect the protective effects.

We found that the serum Cr level was lower and eGFR was higher in the RIC group compared with those of the control group (Fig. A and B) from hour 2 to day 30. We demonstrated that RIC enhanced the early recovery of renal function in recipients after kidney transplantation.

We found that there were no differences about graft pathology between RIC and control groups at 30 min after transplantation. This is consistent with the study by Soendergaard in porcine kidney transplantation. However, Soendergaard *et al.* [12] found that the RIC group had less glomerulitis at 10 h after kidney transplantation compared with the control group ($P < 0.05$). Our study also found that serum Cr level and uNGAL were significantly lower and eGFR was significantly higher in the RIC group compared with those of the control group 12 h after kidney transplantation.

NGAL is a 25-kDa protein bound to gelatinase from neutrophils and normally expressed at very low levels in human tissues including the kidney. NGAL is a new sensitive marker of IRI in renal allograft recipients [18]. uNGAL secretion can predict graft recovery after kidney transplantation [19,20]. In our study, uNGAL secretion was lower in RIC recipients compared with that of control recipients after kidney transplantation (Fig.C). Our result may suggest a possible protective effect of the graft from IRI.

The organ-protecting mechanisms behind RIC remain unclear. Three main mechanisms were proposed, such as humoral, neural, and systemic and interactions among them [21,22]. Czeiger *et al.* [23] demonstrated that the number of $CD34^+$ cells increased after lower limb ischemia in healthy volunteers.

IRI is an inevitable event accompanying kidney transplantation. Kidneys from DCD donors retain a long duration of ischemia. The severity of IRI correlates with DGF, AR, chronic fibrosis, and graft loss [4,5]. Therefore, prevention of the IRI is important. Until now, studies of donor protection were reported, focusing on hemodynamic and fluid management, immunosuppressants, preconditioning, preservation fluids, and pulsatile perfusion [24,25]. Studies of improving storage solutions or machine perfusion also were reported [26,27]. Compared with other methods, RIC—which is a powerful innate mechanism of multiorgan protection that can be induced by transient occlusion of blood flow to a limb—could

become a clinical technique [28]. RIC provides a new idea and method to attenuate transplantation-associated IRI. IRI after kidney transplantation depletes the finite ability of the tissue to repair and triggers inflammation, which may further stress the parenchyma and vessels, increase immune recognition, and promote fibrosis of chronic allograft nephropathy [29,30]. Pascual *et al.* [31] also reported that IRI has a role in the pathogenesis of chronic allograft nephropathy. We speculate that RIC possibly affects long-term survival of the graft and the recipients, whether RIC affects long-term survival of the graft and recipients is being followed up.

The limitation of the present study is that the third 5-min cycle of external iliac artery ischemia is inaccurate. Because, the third 5-min cycle of external iliac artery ischemia was performed during anastomosis of the graft artery to the external iliac artery. The time of anastomosis of the graft artery to the external iliac artery is 10.4 ± 1.4 min, so the third external iliac artery ischemia is 10.4 ± 1.4 min. In the subsequent study, kidney transplantation can be performed by end-to-side anastomosis of the graft artery to the internal iliac artery, or RIC is induced by blood pressure cuff or tourniquet on extremities.

5. Conclusions

RIC enhanced the early recovery of renal function in recipients after kidney transplantation using DCD. Our results provide a novel potential approach to attenuate transplantation-associated IRI. The present study is an initial report, whether RIC affects long-term survival of the grafts and the recipients is being followed up.

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