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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 Blood samples were withdrawn at hour 2, hour 12, days 1–7, day 14, and day 30 to measure serum creatinine level and estimated glomerular filtration rate after transplantation.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