Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–10, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2016.01.028

● *Original Contribution*

USE OF CONTRAST-ENHANCED ULTRASONOGRAPHY TO EVALUATE CHRONIC ALLOGRAFT NEPHROPATHY IN RATS AND CORRELATIONS BETWEEN TIME-INTENSITY CURVE PARAMETERS AND ALLOGRAFT FIBROSIS

QIANG ZHANG,* ZEXING YU,† YUE XU,* SONG ZENG,* ZIJIAN ZHANG,* WENRUI XUE,‡ WEI WANG,* XIAODONG ZHANG,* and XIAOPENG HU*

*Department of Urology, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China; †Department of Ultrasonography, Beijing Chao-Yang Hospital, Capital Medical University, Beijing, China; and ‡Department of Urology, Beijing YouAn Hospital, Capital Medical University, Beijing, China

(*Received* 31 *July* 2015; *revised* 28 *January* 2016; *in final form* 30 *January* 2016)

Abstract—This study quantitatively analyzed changes in the hemodynamic characteristics of renal allografts at different stages in a rat chronic allograft nephropathy (CAN) model as well as the relationship between hemodynamic parameters and renal allograft fibrosis using contrast-enhanced ultrasonography (CEUS). The experimental group used a CAN rat model (n = 30), and the control group used an orthotopic syngeneic renal transplant model (n = 30). After surgery, creatinine clearance rates were regularly monitored every 2 wk. The checking times were set at 4, 12 and 24 wk after surgery, which represent early, middle and late stage of CAN, respectively. At different stages of CAN, eight rats from each group were randomly selected for CEUS examination. Time-intensity curve (TIC) parameters, including rise time, peak intensity, mean transit time, area under the curve, wash-in slope, time-to-peak and α-smooth muscle actin (α-SMA) expression; Vimentin expression; and chronic allograft damage index scores were evaluated by linear correlation analysis. Before the creatinine clearance rate showed significant abnormalities, the renal allografts in the experimental group had already presented pathologic changes associated with CAN. In the early stage after surgery, compared to the TIC curve of the control group, the experimental group showed increased rise time, mean transit time, area under the curve and time-to-peak, and decreased wash-in slope ($p < 0.05$). Chronic allograft damage index scores and the expression levels of α-SMA and Vimentin proteins in renal allografts were correlated with TIC parameters ($p < 0.05$). Compared to creatinine clearance rate, CEUS can detect CAN at earlier stages. The correlations between TIC-related parameters and the expression levels of α-SMA and Vimentin in renal allografts indicate that CEUS is a feasible way to assess the degree of renal allograft fibrosis quantitatively. (E-mail: xiaopeng_hu@sina.com) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Chronic allograft nephropathy, Contrast-enhanced ultrasonography, Time-intensity curve, Allograft fibrosis.**

INTRODUCTION

Kidney transplantation is widely recognized as the most effective treatment for end-stage renal diseases. Over the past 20 y, along with the application of calcineurin inhibitor–based immunosuppressive regimens and advancements in tissue typing and organ preservation technology, the 1-y renal allograft survival rate has increased to 90% or greater. However, there have been no detectable changes in long-term survival, with a 10-y renal allograft survival rate of less than 50% (Lamb et al. 2011; McDonald et al. 2007). Chronic allograft nephropathy (CAN) is the leading cause of long-term graft dysfunction. Clear and effective prevention and treatment methods are currently lacking in clinical practice (Li and Yang, 2009; Nankivell and Kuypers, 2011). The gradual progression of CAN will eventually lead to renal failure in transplanted kidneys. A significant increase in the number of renal failure patients with CAN will lead to an exponential growth in the patient population waiting for kidney transplantation; therefore,

Address correspondence to: Xiaopeng Hu, Department of Urology, Beijing Chao-Yang Hospital, Capital Medical University, No.8 GongTi South Road, Beijing, 100020, China. E-mail: xiaopeng_hu@sina.com

the current, serious situation of the shortage of organs from donors will become even more severe. In addition, the difficulties associated with multiple surgeries will increase the surgical risk and economic burden for patients. Therefore, the early prevention and early treatment of CAN has become a consensus in the organ transplantation community.

Ultrasonography has the advantages of being non-invasive, simple, economical and reproducible. The clinical value of ultrasonography has been widely recognized by the medical profession after years of clinical practice. Color Doppler ultrasonography has become a routine method for examination and monitoring after renal transplantation (Radermacher et al., 2003; Sommerer et al. 2002). Clinicians can conduct real-time observations of blood flow distribution in the relatively large arterial and venous branches within renal allografts as well as measure blood flow hemodynamic parameters in different arterial branches using a pulsed Doppler system. The resistance index and derived peak intensity are important indicators that assess renal transplantations (Nezami et al. 2008). However, these two indicators lack specificity in evaluating the state of renal allografts (Heine et al. 2005). In addition, for CAN patients, traditional ultrasonography can only reveal meaningful changes in indicators that often occur only at advanced disease stages. Therefore, it is urgently necessary to identify a new technology that will solve these problems.

Contrast-enhanced ultrasonography (CEUS) is an excellent method for the quantitative evaluation of parenchymal organ blood perfusion. This study aimed to investigate and analyze a CAN rat model using the quantitative technique of CEUS. By comparing the CEUS results to the laboratory tests, pathology, immunofluorescence and western blotting, we will explore the value of using CEUS for the early diagnosis of CAN and the evaluation of the degree of renal allograft fibrosis.

MATERIALS AND METHODS

Animals and reagents

Procedures involving animals and their care were conducted in accordance with the Principles of Laboratory Animal Care and were approved by the Capital Medical University Animal Studies Committee. Specific-pathogen-free–grade male inbred Fisher and Lewis rats, aged 8–12 wk and weighing 200–250 g, were used (Beijing Vital River Laboratory Animal Technology Co, Beijing, China). The rats were maintained in an environment with a temperature of 22 ± 1°C, a relative humidity of 55 ± 5% and a 12-h dark/12-h light cycle. The rats were fed standard rat chow. The ultrasound contrast agent used was SonoVue (Bracco Suisse SA, Manno, Switzerland). Histologic staining was performed using a hematoxylin-eosin (HE) staining kit, Periodic Schiff-Methenamine Silver staining solution for basement membrane detection and Masson three-color staining solution (TechLab Biotechnology Co, Beijing, China). For immunofluorescence and western blotting, we used anti-alpha smooth muscle actin antibody and anti-Vimentin antibody (Abcam, Cambridge, UK) as well as Alexa Fluor 568 Donkey Anti-Rabbit IgG antibody and Alexa Fluor 488 Donkey Anti-Mouse IgG antibody (Life Technologies, Eugene, OR, USA).

Animal model establishment (Lutz et al. 2007)

Thirty CAN model rats were established as the experimental group. The left kidneys from healthy Fisher rats were used as donor organs. After the animals were anesthetized, the left kidneys were removed from the Lewis rats and the donor kidneys from Fisher rats were orthotopically transplanted at the same time. On the day of surgery and 1–10 d after the day of surgery, the rats received sub-cutaneous injections of low-dose cyclosporine at 1.5 mg/kg/d, and the original right kidneys of the recipients were removed 7 d after the surgery. The control group consisted of 30 Lewis rats that received syngeneic orthotopic renal transplantation using the same procedure as mentioned for the experimental group. Littermate Lewis male rats were used as donors and recipients, and the post-surgery treatments were the same as those of the experimental group.

Determination of renal allograft function

For all of the rats, serum and urine samples were collected beginning from 14 d post-surgery to conduct tests of renal allograft function, which were performed once every 2 wk. The recipients were placed in metabolic cages with access to water *ad libitum*, and 24-h urine samples were collected in containers under the cages. Blood and urine creatinine concentrations (mmol/L) were determined by using the picric acid method, and a Dimension Rxl Max automatic biochemical analyzer (Siemens, Newark, DE, US) was used. The creatinine clearance rate (CCR) was calculated according to the following formula: CCR = urine creatinine (mmol/L) · urine volume (mL)/creatinine (mmol/L) · 1440 (min)/weight (kg).

CEUS of renal allografts

At 4 wk (early stage), 12 wk (mid-stage) and 24 wk (late stage) after surgery, we randomly selected eight rats from the experimental group and eight rats from the control group on which to conduct CEUS using a Philips iU22 ultrasound diagnostic apparatus (Philips, Eindhoven, The Netherlands). The L9-3 probe was used for 2-D scanning and contrast enhancement, with a frequency of 3.0–5.0 MHz. The ultrasound contrast agent used was SonoVue, which has a chemical composition of sulfur hexafluoride microbubbles. Before CEUS,