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● *Original Contribution*

ULTRASOUND-MEDIATED MICROBUBBLE DESTRUCTION INCREASES RENAL INTERSTITIAL CAPILLARY PERMEABILITY IN EARLY DIABETIC NEPHROPATHY RATS

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Abstract—Diabetic nephropathy (DN) is defined as persistent proteinuria corresponding to a urinary albumin excretion rate >300 μg/mg in the absence of other non-diabetic renal diseases. The aim of this study was to determine if ultrasound (US)-mediated microbubble (MB) destruction could increase renal interstitial capillary permeability in early DN rats. Diabetes was induced with streptozotocin. DN rats presented with mild micro-albuminuria 30 d after onset of diabetes. DN rats (N = 120) were divided into four groups that received Evans blue (EB) followed by: (i) no treatment (control group); (ii) continuous ultrasonic irradiation for 5 min (frequency = 7.00 MHz, mechanical index = 0.9, peak rarefactional pressure = 2.38 MPa: US group); (iii) microbubble injection (0.05 mL/kg: MB group); and (iv) both ultrasound and microbubble injection (US + MB group). Another 8 DN rats were subjected to ultrasound and microbubbles and then injected with EB after 24 h (recovery group). EB content, EB extravasation and E-selectin mRNA and protein expression significantly increased, and interstitial capillary walls became discontinuous in the US + MB group. Neither hemorrhage nor necrosis was observed on renal histology. Urine samples were collected 24 h post-treatment. There was no hematuria, and the urinary albumin excretion rate did not increase after ultrasound-microbubble interaction detected by urinalysis. EB content returned to the control group level after 24 h, as assessed for the recovery group. In conclusion, ultrasound-mediated microbubble destruction locally increased renal interstitial capillary permeability in DN rats, and should be considered a therapy for enhancing drug and gene delivery to the kidney in the future. (E-mail: gyh755631@163.com) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Diabetic nephropathy, Ultrasound, Microbubble, Capillary permeability.**

INTRODUCTION

As one of the most detrimental long-term complications of diabetes mellitus (DM), diabetic nephropathy (DN) has evolved as a leading cause of end-stage renal disease worldwide and is highly associated with the premature morbidity and mortality of diabetic patients (Ezquer et al. 2009). Traditionally, DN is considered one of the microvascular complications of DM; advanced DN is also characterized by tubulo-interstitial fibrosis (Bonegio and Susztak 2012). It is widely accepted that tubulo-interstitial damage correlates with the degree of renal dysfunction and is a reliable predictor of end-stage renal disease (Lindenmeyer et al. 2007). DN has been subdivided into two stages based on the urinary albumin excretion rate (UAER): micro-albuminuria (30–299 μg/mg creatinine) and macro-albuminuria (≥300 μg/mg creatinine) (Maahs et al. 2007). Micro-albuminuria is an early sign of diabetic nephropathy.

Kidney transplantation is the preferred cell replacement therapy for DN. However, the scarcity of transplantable donors and the need for lifelong immunosuppression limit widespread use of this curative therapy. New antifibrotic therapies aimed at inhibition of profibrotic mediators are in development, but require large amounts of antibodies and need to be tested on top of the existing therapy of renin-angiotensin system inhibition (Deelman et al. 2009, 2010). The sustained hyperglycemic state accelerates the progress of early diabetic nephropathy in both type 1 and type 2 diabetic patients. Meticulous control of blood glucose decreases the risk of developing

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nephropathy, but is not always feasible because of the limitations of currently available drugs and therapeutic techniques (Babaei-Jadidi et al. 2003). There is now strong evidence that early intervention and tight control have the potential to slow, or perhaps even reverse, the progression of early diabetic nephropathy. Consequently, early prevention of DN is an urgent medical issue at present.

Ultrasound microbubble contrast agents have been developed to enhance conventional ultrasound imaging in clinical ultrasonography, by destroying intravascular microbubbles to characterize refill kinetics. Furthermore, ultrasound-mediated microbubble destruction is a new and promising technique applied in many fields and has been used to deliver drugs and genes to the cardiovascular system (Ferrara et al. 2007; Mayer and Bekeredjian 2008), blood-brain barrier and blood-tumor barrier (Vlachos et al. 2011; Wang et al. 2011); to enhance capillary and vascular permeability enhancement (Bekeredjian et al. 2007; Stieger et al. 2007); and to home stem cells (Xu et al. 2010). *In vitro*, 1–2 $\times$ 10^7 microbubbles bearing 100 pg small inhibitory RNA were added to a 60-mm dish seeded with 1 $\times$ 10^5 mouse squamous cell carcinoma cells (SCC-VII). The dish was inverted to permit microbubble-cell contact *via* microbubble buoyancy and continuously insonified at 1.3 MHz with a mechanical index (MI) of 1.6 for 2 min. Epidermal growth factor receptor expression in SCC-VII cells and epidermal growth factor-dependent growth were significantly reduced. The rupture of microbubbles *via* ultrasonic irradiation resulted in the deposition of microbubble shell components and increased cell membrane permeability localized to the site of microbubble-ultrasound interaction (Carson et al. 2012). *In vivo*, transforming growth factor β signaling and renal fibrosis were blocked in a rat ureteral obstruction model by transferring a doxycycline-regulated Smad7 gene using an ultrasound-microbubble-mediated system (continuous-wave output of 1-MHz ultrasound at 5% power output, for a total of 60 s with thirty 30-s intervals). The mechanism may be ultrasound-mediated microbubble cavitation, which increases the permeability of capillary and tubular basement membranes, allowing local release of DNA across the capillary (and tubular) basement membrane and its entry onto into glomerular, interstitial and tubular epithelial cells (Lan et al. 2003). However, no studies have reported whether ultrasound-mediated microbubble destruction is feasible for increasing renal interstitial capillary permeability in DN. On the basis of these previous findings, we explored whether this technique can be implemented to increase interstitial capillary permeability in DN because, in theory, most drugs and antifibrotic genes would benefit from increased capillary permeability. It is possible to take advantage of this technique to selectively increase drug and gene delivery to the tubulo-interstitial area, to enhance the therapeutic effect, to slow disease progression or, perhaps in the future, even to reverse the progression of DN.

Previous studies on ultrasound-mediated microbubble destruction in the kidney focused mostly on the bio-effect with a combination of low-frequency, high-power and interval irradiation (Johnson et al. 2012). Miller et al. (2009) found that glomerular capillary hemorrhage, intratubular obstruction and tubular injury contribute to peritubular fibrosis in rats kidneys exposed to diagnostic ultrasound (1.5 MHz, MI = 1.9, intermittent image exposure, peak rarefactional pressure amplitude = 2.3 MPa) for 5 min. However, other researchers did not observe the same phenomenon under different conditions. Wible et al. (2002) reported that higher-frequency (>5 MHz), low-output-power ultrasound causes minimal biological alterations, and there was little no hemorrhage on the renal surface during continuous ultrasound exposure at higher frequencies (4 and 6 MHz). Jiménez et al. (2008) confirmed that there was no evidence of renal tissue damage and no capillary bleeding in porcine kidneys exposed to ultrasonic irradiation with a high MI (1.9), PPRA of 2.1 MPa and spatial-peak temporal-average intensity of 607 mW/cm^2. These studies indicated that microbubble insonation and inertial cavitation may be tolerated by the kidney. Hence, our objective in the present study was to investigate whether ultrasound-mediated microbubble destruction increases renal interstitial capillary permeability in early diabetic nephropathy rats that presented with microalbuminuria but no renal histopathological changes. If it does, the technique might be exploited to promote higher concentrations of drugs, genes or antifibrotic mediators in the tubulo-interstitial area and improve the therapeutic effect. In addition, tubular epithelial cells express a variety of renal transporter proteins, which are localized mainly in the kidney proximal tubules. We studied mainly ultrasound-microbubble interaction and its impact on renal peritubular capillaries.

On the basis of existing information, we optimized the parameters by evaluating the variables (MI, frequency, irradiation duration and dosage of microbubbles), and then used a combination of temperate MI (0.9), high frequency (7 MHz), peak rarefactional pressure (PRP) of 2.38 MPa and continuous irradiation. In particular, E-selectin is found only in endothelium. So, changes of state of endothelial cells after ultrasonic irradiation were detected by E-selectin mRNA and protein expression. Renal interstitial capillary permeability was assessed by Evans blue (EB) content assay, confocal laser scanning microscopy and transmission electron microscopy (TEM). Possible injury to the renal structure was evaluated by renal histology. Capillary permeability recovery was assessed by EB content assay.