Ultrasound in Med. & Biol., Vol. 41, No. 11, pp. 2977–2989, 2015

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2015.07.002

● *Original Contribution*

ENHANCED HOMING ABILITY AND RETENTION OF BONE MARROW STROMAL CELLS TO DIABETIC NEPHROPATHY BY MICROBUBBLE-MEDIATED DIAGNOSTIC ULTRASOUND IRRADIATION

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(*Received* 22 *January* 2015; *revised* 22 *June* 2015; *in final form* 6 *July* 2015)

Abstract—Bone marrow stromal cell (BMSC) transplantation can successfully treat diabetic nephropathy (DN), but the lack of a specific homing place for intravenously injected cells limits the effective implementation of stem cell therapies. The migration and survival of transplanted BMSCs are determined by inflammatory reactions in the local kidney micro-environment. We tested the hypothesis that microbubble-mediated diagnostic ultrasound irradiation could provide a suitable micro-environment for BMSC delivery and retention in DN therapy. In this study, red fluorescent protein–labeled BMSCs were administered combined with microbubbles to streptozotocin-induced DN rats 4 wk after diabetes onset. We observed enhanced BMSC homing and retention in microbubble-mediated diagnostic ultrasound-irradiated kidneys compared with the contralateral kidneys on days 1 and 3 post-treatment. The results from immunohistochemical analysis, Western blot and enzyme-linked immunosorbent assay indicated that the local and transient expression of various chemo-attractants (*i.e.,* cytokines, integrins and trophic factors) found to promote BMSC homing was much higher than observed in non-treated kidneys. The local capillary endothelium rupture observed by transmission electron microscopy may account for local micro-environment changes. Histopathologic analysis revealed no signs of kidney damage. These results confirmed that renal micro-environment changes caused by appropriate microbubble-mediated diagnostic ultrasound irradiation may promote BMSC homing ability to the diabetic kidney without renal toxicity and cell damage. This non-invasive and effective technique may be a promising method for BMSC transplantation therapy. (E-mail: Xqgaoyh@outlook.com) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Bone marrow stromal cells, Homing, Ultrasound, Microbubble, Diabetic nephropathy.**

INTRODUCTION

Diabetic nephropathy (DN) is a major complication in patients with diabetes and represents the leading cause of end-stage renal disease (Sahay et al. 2014; Zhou et al. 2013). Currently, there is no cure for DN. Palliative therapeutic strategies include medical interventions for tight glycemic and hypertension control and blockage of the renin–angiotensin system to delay the onset of DN (Choudhury et al. 2010; Collins et al. 2013). Thus, a novel therapeutic strategy should be developed to manage DN onset and progression.

In recent years, bone marrow stromal cell (BMSC) transplantation has shown promise as a strategy because of their renoprotective potential through paracrine secretion and transdifferentiation, though there was no improvement in hyperglycemia levels (Li et al. 2013; Morigi and De Coppi 2014). Intravenous injection is the most convenient and least invasive method of stem cell administration and is frequently used in clinical and animal trials (Harting et al. 2009). However, many studies have reported that specific BMSCs home to kidney tissues at very low levels after intravenous transplantation, which has limited the effective implementation of BMSC-based therapies (Lee 2009; Schrepfer et al. 2007). Cheng et al. (2013) reported that most intravenously administered BMSCs were localized in pulmonary capillaries; only a small fraction could enter and survive in the targeted kidney. Therefore, developing a method to improve BMSC homing to tissue impaired by DN is essential for clinical application.

The underlying mechanisms by which intravenously injected BMSCs home to the site of damaged or inflamed parenchyma are not well understood, but their homing

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behavior pathology is thought to be analogous to leukocyte recruitment to a site of inflammation (Kavanagh et al. 2014; Sohni and Verfaillie 2013). Numerous studies have suggested that the release of some cytokines, adhesion molecules and trophic factors associated with inflammation and injury is of great importance for BMSC adhesion and the subsequent transmigration from the vasculature into inflamed tissues (Karp and Teol 2009; Wang et al. 2012). Because BMSC engraftment to the diabetic kidney may be the key for improving therapeutic application, developing a non-invasive modality to trigger chemoattractant release in the local kidney micro-environment would be valuable for stem cell therapy.

Recently, studies have begun to test the feasibility of using microbubble mediated ultrasound irradiation to increase non-invasively the ability of BMSCs to home to targeted tissues (Tong et al. 2013). Tang et al. (2012) reported that microbubble destruction by 1.0 W/cm^2 ultrasound could promote BMSC homing to acute kidney injuries. Zhong et al. (2012) also proved that therapeutic ultrasound-mediated stimulation of microbubbles enhances BMSC migration across the ischemic myocardium *in vivo*. These effects may be the result of the changes in the micro-environment induced by local inflammatory reactions after microbubble-mediated ultrasound irradiation treatment. A mild inflammatory response may be helpful for BMSC homing and survival, whereas the harsh environment may decrease the efficiency of stem cell therapy (Greco and Rameshwar 2008). Compared with the therapeutic ultrasound apparatus used in many studies, diagnostic ultrasound devices have less output and an appropriate image monitoring system, which is more convenient for mediating targeted microbubble (MB) destruction in the diabetic kidney (Wang et al. 2013). We intended to study whether microbubble-mediated diagnostic ultrasound irradiation could mildly alter the kidney micro-environment, thereby providing suitable conditions for DN therapy.

In this study, we investigated the local and transient micro-environmental (cytokines, trophic factors and integrins) changes induced by microbubble-mediated diagnostic ultrasound irradiation, which is known to increase the tropism of systemically administered BMSCs. Kidneys are good models given that less than 0.1% of the intravenously injected stem cells could engraft in this tissue, and the contralateral kidney could serve as internal control (Deak et al. 2010). Our study indicated that the combination of BMSCs with microbubble-mediated diagnostic ultrasound irradiation is a feasible strategy for promoting stem cell homing and retention in rat models of streptozotocin-induced DN. It may provide a valuable therapeutic strategy for clinical application.

METHODS

Diabetic nephropathy models

Twenty-four adult male Sprague–Dawley (SD) rats were provided by the Center for Experimental Animals of Xinqiao Hospital. The experimental protocol conformed to the *Guidelines for the Care and Use of Laboratory Animals* (NIH Publication No. 85–23, revised 1996). All experiments were approved by the Animal Care and Use Committee of the Third Military Medical University. To establish the early DN rat model, 24 adult male SD rats (weighed 190–220 g) fasted overnight without water deprivation received a single intraperitoneal injection of 40 mg/kg streptozotocin (STZ, Sigma, St. Louis, MO, USA) that had been dissolved in 0.1 M citrate buffer at pH 4.5 for 5 consecutive days.

Three days after STZ injection, we began to collect blood from the tail vein and test blood glucose with the Contour TS Blood Glucose Meter (Bayer HealthCare LLC, Mishiwaka, IN, USA). After 3 d of feeding, rats were considered to have developed diabetes when their blood glucose increased to a stable level 16.7 mmol/L for 3 consecutive days (Ezquer et al. 2008), at which point they were used for the subsequent study. Four weeks after the confirmation of diabetes, the early DN rat model was established on the emergence of mild micro-albuminuria, which is an early sign of DN. Micro-albuminuria levels were measured in the Xinqiao Hospital clinical laboratory using an automatic biochemical analyzer (Hitachi, Tokyo, Japan).

Ultrasound apparatus and microbubbles

We used a commercial clinical S2000 Dimension ultrasound device (S2000, Siemens, Munich, Germany) to obtain the echographic kidney images and perform microbubble-mediated diagnostic ultrasound irradiation. Lipid-coated microbubbles filled with perfluoropropane were prepared at our department, as previously described (Liu et al. 2011). The mean diameter of the microbubble is 2 μm, and 98% of the particles have diameters <5 μm. The bubbles were diluted with saline to a concentration of 7×10^9 bubbles/mL before use. Liu et al. (2011) reported that this kind of microbubble has no significant effect on blood pressure and kidney function.

Rat BMSC culture and labeling

Twenty-four male SD rats (4-wk-old, weighing 65–80 g) were sacrificed by a chloral hydrate overdose and then placed in ethanol (70% v/v). BMSCs were obtained by flushing the femur and tibia and pooled into one single-cell suspension with sterile phosphate-buffered saline. After centrifugation, cells were cultured (37°C, 5% CO_2) with α-MEM (Hyclone, Logan, UT, USA) medium