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Beneficial effects of urine-derived stem cells on fibrosis and apoptosis of myocardial, glomerular and bladder cells

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ABSTRACT

Urine-derived stem cells (USCs) are isolated from voided urine and display high proliferative activity and multiple differentiation potentials. The applicability of USCs in the treatment of bladder dysfunction and in cell-based urological tissue engineering has been demonstrated. Whether they could serve as a potential stem cell source for the treatment of diabetes mellitus (DM) and its complications has not been investigated. Here, we report the repairing and protective effects of USCs on pancreatic islets, the myocardium, the renal glomerulus and the bladder detrusor in diabetic rat models. Type 2 diabetic rat models were induced by means of a high fat diet and intraperitoneal injection with streptozotocin. USCs isolated from voided urine were administered via tail veins. The functional changes of pancreatic islets, left ventricle, glomerulus and bladder micturition were assessed by means of insulin tolerance tests, echocardiography, urine biochemical indexes and cystometry. The histologic changes were evaluated by hematoxylin and eosin staining, Masson's trichrome staining and TUNEL staining. Treatment with USCs significantly alleviated the histological destruction and functional decline. Although the USC treatment did not decrease fasting blood glucose to a significantly different level, the fibrosis and apoptosis of the myocardium, glomerulus and detrusor were significantly inhibited. This study indicates that administration of USCs may be useful for the treatment of the complications of DM.

1. Introduction

Diabetes mellitus (DM) is a chronic, systematic metabolic condition and is a global health problem. The number of people suffering from diabetes has significantly increased over the last half-century despite rapid advances in the understanding of the disorder (Ammar et al., 2015). Both type 1 and type 2 diabetes mellitus involve the destruction and dysfunction of pancreatic islet beta cells (Tsai et al., 2014). The main and long-term complications are diabetic cardiomyopathy, nephropathy, neuropathy, retinopathy and cystopathy (Psutka et al., 2014). Exogenous drug therapy is the only option to lower blood glucose for enhancing quality of life and preventing or delaying chronic complications of diabetes. Although drug therapy is helpful for controlling of blood glucose level, it is not successful enough in preventing chronic complications (de Boer et al., 2011; Unsal et al., 2015). Stem cell-based therapy has become a new strategy for DM (Godfrey et al., 2012). Stem cells, such as embryonic (Soria et al., 2000; Liang et al., 2015), bone marrow derived mesenchymal (Bhansali et al., 2014; Fotino et al., 2010) and adipose derived types (Ni et al., 2015; Bassi et al., 2012), have been shown to have the potential to differentiate into pancreatic beta cells for the treatment of DM. In diabetes-damaged organ tissues, stem cells are not only classically thought to

Abbreviations: AGEs, Advanced glycation end products; α-SMA, Alpha smooth muscle action; BC, Bladder capability; Ccr, Creatinine clearance rate; Co, Cardiac output; DCM, Diabetic cardiomyopathy; DCP, Diabetic cystopathy; LVPWd, Diastolic left ventricular posterior wall; DM, Diabetes mellitus; DN, Diabetic nephropathy; EF, Ejection fractions; eGFP, Enhanced green fluorescent protein; FBG, Fasting blood glucose; FS, Fractional shortening; IPGTT, Intraperitoneal glucose tolerance test; ITT, Insulin tolerance test; MBP, Maximum bladder pressure; MI, Micturition internal; RUV, Residual urine volume; LVPWs, Systolic left ventricular posterior wall; SYN, Synaptopodin; T2D, Type 2 diabetes; USCs, Urine-derived stem cells.

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differentiate and generate new functional cells via multi-lineage differentiation and self-renewal (Candiello et al., 2011; Bose et al., 2012), but also exert a therapeutic effect via the secretion of bioactive factors that have anti-apoptotic, anti-scarring, neovascularization, and immunomodulatory effects in the injured area (Sindberg et al., 2014; Aali et al., 2014). However, these types of stem cell are relatively rare, and their procurement is invasive and painful, requires anesthesia, and potentially produces a low yield. Investigators continue to identify novel, less-invasive cell sources for application.

In recent studies, we have reported the successful isolation and expansion of stem cells from voided human urine (Bharadwaj et al., 2011; Zhang, He, Ni et al., 2013; Zhang et al., 2014). The urine-derived stem cells (USCs) can be easily isolated from human voided urine, possess progenitor features and are convenient to obtain. These cells display many characteristics of stem cells and are capable of differentiating into multiple cell lines, including those of osteoblasts, chondrocytes, adipocytes, skeletal myocytes and smooth muscle cells. USCs hold several advantages as a cell source for tissue repair and disease treatment. They are easily harvested without any need for invasive surgical techniques. Furthermore, there is a significant financial advantage. It costs US$50 to obtain cells from urine and approximately US$5000 to obtain cells by means of a biopsy procedure. Finally, USCs can be used autologously, obviating potential ethical issues and the potential for adverse immune reactions (Tran and Damaser, 2015). In this study, we aimed to systematically evaluate the therapeutic effects of USCs transplantation on DM and diabetes-related complications, such as cardiomyopathy, nephropathy and cystopathy. Our results provide the first evidence that USC therapy may constitute a new therapeutic intervention in type 2 diabetes (T2D) and its complications.

2. Materials and methods

2.1. Animal models

All Sprague–Dawley rats were purchased from the Center for Experimental Animals of the Third Military Medical University. The experiments were approved by the Animal Care and Use Committee of Third Military Medical University, and comply with the Guide for the care and use of laboratory animals, Eighth edition (2011). All rats weighting approximately 150–170 g were used in this study and housed at 20–25 °C under a standard 12 h/12 h light–dark cycle. All rats were randomly allocated into three groups: a normal control group (NC rats, n = 24), a T2D group (T2D rats, n = 24) and a T2D plus USCs group (T2D + USCs rats, n = 24). The T2D rat models were induced as previously described (Zhang et al., 2008). The T2D and T2D + USCs rats were fed with a high-fat diet (consisted of 22% fat, 48% carbohydrate, and 20% protein with total calorific value 44.3 kJ/kg), while NC rats received a standard diet. After feeding with a high-fat diet for 4 weeks, T2D rats were injected intraperitoneally twice with streptozotocin (Sigma–Aldrich, USA) at a dose of 35 mg/kg body weight with a 1 week internal. After 3 days of streptozotocin injection, the rats with fasting blood glucose (FBG) > 16.7 mmol/L were confirmed as being the T2D rats. After 16 weeks of streptozotocin injection, the rats were sacrificed for experiment.

2.2. Cell procedure

As we previously reported (Ouyang et al., 2014), sterile urine samples were obtained from 4 healthy donors (male; 25–27 years old). After urine samples were centrifuged, cell pellets were resuspended and plated in 24-well tissue culture plates. A mixed medium, composed of keratinocyte serum-free medium and progenitor cell medium in a 1:1 ratio, was used to culture USCs. Only cells that attached to culture wells by day 2 after plating were used. Enhanced green fluorescent protein (eGFP) was used as a cell tracker in the experiments assessing the localizations of USCs. Cells were transfected with lentiviral-eGFP (Genechem, Shanghai, China) according to the manufacturer's protocol.

2.3. USCs differentiation in vitro

The endothelial and smooth muscle differentiation of USCs were assessed as we previously reported (Bharadwaj et al., 2013). For myogenic differentiation, equal volumes of DMEM (containing 10% FBS) and embryonic fibroblast medium (containing 2.5 ng/mL TGF-β1, 5.0 ng/mL PDGF-BB, and 10% FBS) were used. For endothelial differentiation, DMEM containing 10% FBS was mixed with KSFM at a 1:4 ratio, and 30 ng/mL epidermal growth factor was added into the mixture. The differentiation medium was replaced every third day. The specific protein makers, CD31 and desmin (Abcam, Cambridge, UK) were applied to stain the differentiated endothelial cells and smooth muscle cells by immunofluorescence assay.

2.4. Cell implantation in vivo

Two weeks after streptozotocin injection, T2D + USCs rats received an injection of USCs (2×10^6 suspended in 0.2 ml PBS) for six times every other week. Rats was anesthetized with chloral hydrate (10% in PBS, 0.3 ml/kg), and the cell suspensions were injected into tail veins.

2.5. Physical and biochemical analysis

The body weights and fasting blood glucose (FBG) levels of rats were measured every 4 weeks, and the weights of hearts, kidneys, bladders were measured at the end of the experiment. After rats fasted overnight, blood samples were obtained from the retro-orbital sinus at the time of sacrifice. The serum creatinine, triglycerides and total cholesterol were determined by means of an automatic analyzer (Roche, Basel, Switzerland) (Ouyang et al., 2014; Wang, Li, Zhao et al., 2013). The levels of serum insulin and advanced glycation end products (AGEs) levels were quantified by an insulin ELISA Kit (Merk Millipore Corp., Billerica, USA) and an AGEs ELISA Kit (Cloud-Clone Corp., Houston, USA) according to the manufacturer's protocol.

2.6. Urinalysis

Twenty-four hours urine samples from rats in metabolic cages were collected and measured for red blood cells, albumin, nitrogen and creatinine. The urinary albumin, nitrogen and creatinine concentrations were determined in the clinical laboratory of Xinqiao Hospital using an automatic biochemical analyzer (Hitachi, Tokyo, Japan). The creatinine clearance rate (Ccr) was calculated as (urinary creatinine × urine volume)/serum creatinine and expressed as milliliters per minute (Ouyang et al., 2014).

2.7. Intraperitoneal glucose tolerance test and insulin tolerance test

After a 12 h overnight fast, the rats were intraperitoneally injected with 40% glucose (2 g/kg body weight). Blood samples were collected from the tail veins at 0, 30, 60, 90 and 120 min to measure the glucose levels. Insulin (0.75 IU/kg) was injected intraperitoneally and blood samples were collected from tail veins at 0, 30, 60, 90 and 120 min for the measurement of the plasma glucose levels. Values are presented as the percentage of the initial plasma glucose level (Zhang et al., 2008).