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Intracavernous Injection of Human Umbilical Cord Blood Mononuclear Cells Improves Erectile Dysfunction in Streptozotocin-Induced Diabetic Rats

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ABSTRACT

Introduction: Erectile dysfunction (ED) worsens in men with diabetes. Human umbilical cord blood (HUCB), because of its widespread availability and low immunogenicity, is a valuable source for stem cell-based therapies.

Aim: To determine the effect of intracavernous injection of HUCB mononuclear cells (MNCs) on ED in rats with diabetes induced by streptozotocin.

Methods: Thirty adult male Sprague-Dawley rats were equally divided into three groups: (i) control, (ii) diabetes induced by streptozotocin (35 mg/kg intravenously for 8 weeks), and (iii) diabetic rats treated with MNCs (1×10^6 cells by intracavernosal injection). The HUCB-MNCs isolated by the Ficoll-Hypaque technique were obtained from eight healthy donors and administered to diabetic rats after 4 weeks.

Main Outcome Measures: The ratio of intracavernosal pressure to mean arterial pressure ratio; the protein expression of endothelial and neuronal markers, such as von Willebrand factor, neuronal nitric oxide synthase, hypoxia-inducible factor-1α, and vascular endothelium growth factor; and the relative area of smooth muscle to collagen using western blotting and Masson trichrome staining were determined.

Results: Diabetic rats demonstrated a significantly decreased ratio of intracavernosal pressure to mean arterial pressure (0.26 ± 0.04; $P < .01$) and treatment with MNCs restored erectile function in diabetic rats (0.67 ± 0.05) compared with control rats (0.56 ± 0.02). In bath studies, neurogenic relaxant and contractile responses were significantly decreased in diabetic cavernosal tissues, which were restored by treatment. The ratio of smooth muscle to collagen was partly recovered by treatment, whereas von Willebrand factor levels were not altered in any group. Neuronal nitric oxide synthase and vascular endothelium growth factor levels were decreased, which were not restored by treatment. Increased hypoxia-inducible factor-1α protein expression in the diabetic group was completely normalized in MNC-treated diabetic samples.

Conclusion: These results suggest that HUCB-MNC treatment can enhance the recovery of erectile function and promote numerous activities such the contribution of the hypoxia-inducible factor-1α and von Willebrand factor pathway to the neurogenic erectile response of diabetic rats. HUCB-MNCs in the healing process could involve an adaptive regenerative response and appear to be a potential candidate for cell-based therapy in ED of men with diabetes. It is evident that HUCB could provide a realistic therapeutic modality for the treatment of diabetic ED.

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Key Words: Human Umbilical Cord Blood Mononuclear Cells; Erectile Dysfunction; Diabetes; von Willebrand Factor; Hypoxia Inducible Factor; Intracavernosal Pressure

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INTRODUCTION

Erectile dysfunction (ED) is a common complication of diabetes, which greatly affects quality of life. In men with diabetes, the risk of developing ED is threefold higher than in healthy men.[1–4] Compared with the other complications of diabetes, the development of ED begins at a younger age and the severity and incidence of dysfunction increase with the duration of the disease. Although the etiology of diabetic ED is multifactorial, endothelial dysfunction is recognized as a mainstay in the

pathophysiology of the disease. Furthermore, the efficacy of common ED therapies is low for diabetes-associated ED. For instance, patients with diabetes often exhibit a poor response to first-line oral phosphodiesterase type 5 inhibitors.[5]

Therapies based on stem cells have recently received increasing attention for their potential to restore erectile function impaired by different chronic and acute conditions.[6] Treatment with mesenchymal stem cells from bone marrow and adipose tissue has produced positive effects on erectile function in various animal models of ED.[7] Preclinical studies have shown that stem cell transplantation can correct impaired erectile function in diabetic animals.[8] Depending on the cell type, recent research has demonstrated that transplanting the stem cells can exert a paracrine effect on surrounding penile tissues and differentiate into smooth muscle, endothelium, and neurons.[9] The paracrine effect of human urine-derived stem cells improved erectile function in type 2 diabetic rats by increasing the endothelial expression and contents of smooth muscle.[10]

Umbilical cord blood (UCB) is one of the most important and promising sources of hematopoietic stem cells (mononuclear cells [MNCs]) and contains high levels of primitive, multipotent stem, and progenitor cells.[11–14] MNCs obtained from UCB are composed of lymphocytes, monocytes, and progenitor cells and a limited number of stem cells such as hematopoietic stem cells and mesenchymal stem cells.[15,16] Human UCB (HUCB) cells, which have easy availability, safety, low immunogenicity, and tumorigenicity, are the most widely accepted source of stem cells and have not been tested in erectile tissue regeneration.[17,18] The aim of this study was to examine the effectiveness of the potential capacity of intracavernosal HUCB-MNC treatment to recover erectile function in diabetic rats.

METHODS

Animal Model

Thirty male Sprague-Dawley rats weighing 300 to 350 g were randomly divided into control, diabetic, and HUCB-treated diabetic groups. Diabetic rats received a single intravenous injection of streptozotocin (35 mg/kg), which was dissolved in citrate buffer (pH = 5.5). Seventy-two hours after streptozotocin injection, blood glucose levels were measured using a blood glucose meter (Accu-Chek, Roche, Mannheim, Germany). All animal experiments were performed in compliance with the laws and institutional guidelines approved by the institutional animal care and use committee of Ankara University (Ankara, Turkey; approval number 2014-23-154).

Isolation and Injection of HUCB-MNCs

HUCB samples were collected from healthy adult volunteers (n = 8). The protocol to use HUCB and to obtain informed consent was approved by the Ankara University health sciences institutional review board (20-854-14). UCB samples were obtained by puncture of the umbilical vein of the umbilical cords of neonates during normal or cesarean delivery and collected in tubes containing ethylenediaminetetraacetic acid. UCB samples were diluted equally with phosphate buffered saline (PBS; pH = 7.4) and transferred to centrifuge tubes containing Ficoll-Paque solution. The cells were separated by the standard density gradient technique using a Biocoll separation solution (isotone density = 1.077 g/mL; Biochrom AG, Berlin, Germany). Two parts of blood were layered over one part of Histopaque before centrifugation at 1,200 rpm for 20 minutes at room temperature to isolate low-density HUCB-MNCs. The cloudy interface layer (buffy coat) of the MNCs was carefully removed by pipetting and transferring it to a new tube and washing twice with PBS through centrifugation at 300 rpm for 10 minutes. Subsequently, the obtained cell population was rapidly frozen in freezing medium. Before cell transplantation in the corpus cavernosum (CC) of rats, the UCB cells were thawed and evaluated for viability using trypan blue and diluted with PBS at a concentration of 1×10^6 MNCs per 100 μL.

Four weeks after streptozotocin injection, the rats were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally). The penis was exposed, and rats in each group received a corresponding injection of PBS 100 μL or 1×10^6 MNCs in PBS 100 μL into the CC at the mid-penile level with a 25-gauge needle.[19,20] After the injection of MNCs, pressure was applied for 1 minute to the injection site to prevent backflow of the treatment suspension.[21,22]

In Vivo Evaluation of Erectile Function

For the in vivo evaluation of erectile function, intracavernosal pressure (ICP; mm Hg) was measured. The rats were anaesthetized with sodium pentobarbital (50 mg/kg intraperitoneally), the trachea was exposed and cannulated with polyethylene-240 tubing to maintain the airway, and the carotid artery was cannulated with polyethylene-50 tubing to measure the main arterial pressure (MAP; mm Hg) with a transducer (Statham, Oxnard, CA, USA) attached to a data acquisition system (BIOPAC MP 100 System, BIOPAC Systems, Santa Barbara, CA, USA). A 25-gauge needle filled with heparin 250 U/mL and connected to polyethylene-50 tubing was inserted into the right crura of the penis and connected to a pressure transducer to measure ICP continuously. The right major pelvic ganglion and cavernosal nerve were identified and a stainless-steel bipolar-hook stimulating electrode was placed around the cavernosal nerve. The cavernosal nerve was stimulated (2.5, 5, and 7.5 V, 15 Hz, 30-ms pulse width) with a square pulse stimulator (Grass Instruments, Quincy, MA, USA). At the end of the study, the rats were killed, and the penises were removed, immediately frozen in liquid nitrogen, and stored at −80°C until further processing.

Isometric Tension Measurements

The penis was placed in a Petri dish containing Krebs bicarbonate solution (NaCl 118.1 mmol/L, KCl 4.7 mmol/L, KH_2PO_4 1.0 mmol/L, $MgSO_4$ 1.0 mmol/L, $NaHCO_3$ 25.0