THE JOURNAL OF
SEXUAL MEDICINE

ORIGINAL RESEARCH

BASIC SCIENCE

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

Tugba Cengiz, MS,[1] Ecem Kaya, PhD,[2] Didem Yilmaz Oral, PhD,[1] Isil Ozakca, PhD,[1] Nur Bayatli, PhD,[1] Arzu Zeynep Karabay, PhD,[2] Tugba Altun Ensari, MD,[3] Tuna Karahan, MD,[4] Enis Yilmaz, MS,[1] and Serap Gur, PhD[1]

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.

J Sex Med 2017;14:50–58. Copyright © 2016, International Society for Sexual Medicine. Published by Elsevier Inc. All rights reserved.

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

Received August 17, 2016. Accepted November 22, 2016.

[1]Department of Pharmacology Faculty of Pharmacy, Ankara University, Ankara, Turkey;

[2]Department of Biochemistry, Faculty of Pharmacy, Ankara University, Ankara, Turkey;

[3]Etlik Zubeyde Hanim Women's Health Education and Research Hospital, Ankara, Turkey;

[4]Department of Anatomy, School of Medicine, Ankara University, Ankara, Turkey

Copyright © 2016, International Society for Sexual Medicine. Published by Elsevier Inc. All rights reserved.

http://dx.doi.org/10.1016/j.jsxm.2016.11.314

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

mmol/L, $CaCl_2$ 2.5 mmol/L, glucose 11.1 mmol/L) and oxygenated with a mixture of 95% O_2 and 5% CO_2. The cavernosal tissue (1 × 1 × 8 mm) was dissected and mounted under 1*g* of resting tension in a 20-mL organ bath with one end attached to an electrode holder and the other to a wire connected to a force transducer and allowed to equilibrate for 60 minutes at 37°C. Electrical field stimulation (EFS) of the autonomic nerves (duration = 15 seconds; amplitude = 20 V; frequency = 1−20 Hz; pulse width = 0.5 millisecond) was accomplished by the use of platinum electrodes (Grass Instruments). Preparations were preincubated for 30 minutes with guanethidine (5 μmol/L) and atropine (1 μmol/L) to block noradrenergic neurotransmission and muscarinic responses, respectively. EFSs (1−20 Hz; n = 8) were evoked after pre-contraction of CC strips with phenylephrine (Phe; 10^{-5} M). Cumulative concentration-response curves of relaxation responses induced by acetylcholine (ACh; 10^{-8} to 10^{-3} M; n = 8) and sodium nitroprusside (10^{-8} to 10^{-3} M; n = 8) were obtained. Cumulative concentration-response curves of contractile responses induced by Phe (10^{-8} to 10^{-3} M; n = 8) were evaluated. The direct sympathetic stimulation-related contraction was induced with EFS (1−40 Hz; n = 8) of the tissue strips. The contractile response was elicited only by the adrenergic nerve component of the EFS, and tissues were treated with the non-selective nitric oxide synthase (NOS) inhibitor L-N(G)-nitroarginine methyl ester (100 μmol/L) and the muscarinic receptor antagonist atropine (1 μmol/L) for 30 minutes to eliminate nitrergic and cholinergic influences.[23] All tissue responses were normalized to grams of tissue weight.[24]

Masson Trichrome Staining

Cross-sections of penile tissue samples (n = 6 from each group) were fixed in 10% formalin, embedded in paraffin, and cut into 8- to 10-μm thick sections. These sections were stained with Masson trichrome (Sigma Chemical Co, St Louis, MO, USA) and photographed using light microscopy (DM4000B and DFC280 color digital camera system, Leica Microsystems, Wetzlar, Germany). The images were analyzed for two distinct populations of collagen and smooth muscle fibers with computerized imaging software (Image J, National Institutes of Health, Bethesda, MD, USA).[25] The red-staining area (smooth muscle) was divided by the blue-staining area (collagen) in the mid-shaft cross-section of the penis.

Western Blotting

Rat penile tissue was homogenized in RIPA lysis buffer containing a protease inhibitor cocktail (Cell Signaling Technology, Beverly, MA, USA). Protein extracts were quantified and 20 μg of protein was loaded per well onto a 7.5% sodium dodecyl sulphate-polyacrylamide gel electrophoresis gel, fractionated, and transferred to a nitrocellulose membrane by semi-dry electrotransfer for 3 hours at 100 V. After soaking in blocking buffer, membranes were incubated overnight at 4°C with primary antibodies (neuronal NOS [nNOS] 1:1,000, BD Transduction Laboratories, San Diego, CA, USA; vascular endothelium growth factor [VEGF] 1:100, Santa Cruz Biotechnology, Dallas, TX, USA; hypoxia-inducible factor-1α [HIF-1α] 1:500, Novus Biologicals, Littleton, CO, USA; von Willebrand factor [vWF] 1:500, Lifespan Biosciences, Seattle, WA, USA). Membranes were incubated with a horseradish peroxidase−linked secondary antibody and visualized with a chemiluminescent detection system (LumiGLO, KPL, Gaithersburg, MD, USA). Band intensity was quantified by densitometry (Quantity One 4.6.1, Bio-Rad, Hercules, CA, USA). The values were normalized to those of glyceraldehyde 3-phosphate dehydrogenase, and these results were expressed as fold changes.[26]

Statistical Analysis

All results are expressed as mean ± standard error of the mean. Statistical differences were determined by analysis of variance followed by Bonferroni complementary analysis, with a *P* value less than 0.05 considered statistically significant.

Drugs

All drugs were purchased from Sigma Chemical Co.

RESULTS

Characteristics of Animals

Body weight was significantly lower in diabetic rats than in control and MNC-treated diabetic rats, which was normalized by treatment (Table 1 and Figure 1A). Fasting blood glucose levels were significantly higher ($P < .001$) in diabetic rats than in controls (Figure 1B). No significant differences in blood glucose levels were found between the diabetic group and the treated group.

Effect of HUCB on Erectile Function

Figure 1C presents the ICP/MAP ratios for all groups. The diabetic group had the lowest ICP/MAP ratio at 54% for 7.5 V ($P < .01$; Figure 1C and Table 1). In addition, the ICP/MAP ratio of the HUCB-treated diabetic group was significantly higher than that of the diabetic group ($P < .001$).

Total ICP values by stimulation of the cavernosal nerve were decreased in diabetic rats compared with control rats, which was reversed at all voltage levels by HUCB treatment (Figure 1D).

In Vitro Relaxant and Contractile Responses

As shown in Figure 2A, the relaxation response to EFS in the diabetic group was significantly decreased compared with the control group. The HUCB-MNC−treated diabetic group showed a significantly increased EFS-induced relaxation response compared with the diabetic group ($P < .001$; Table 1 and Figure 2A). Also, nitrergic relaxation induced by EFS in

Table 1. Characteristic parameters and functional responses in groups*

	Control	Diabetic	MNC
Body weight (g)	406.6 ± 15.0	328.0 ± 7.4‡	383.0 ± 18.0
Blood glucose (mg/dL)	70.8 ± 0.3	436.3 ± 59.0§	477.0 ± 58.0§
ICP/MAP (7.5 V)	0.56 ± 0.02	0.26 ± 0.04‡	0.67 ± 0.05
Total ICP (mm Hg;7.5 V)	4833.5 ± 419.0	1381.6 ± 27.5‡	3871.0 ± 424.0
EFS (20 Hz)	57.3 ± 1.1	15.0 ± 6.0§	82.7 ± 5.1†
ACh (1 mmol/L)	82.33 ± 1.76	20.0 ± 2.3§	8.9 ± 4.7§
Phe (1 mmol/L)	73.1 ± 5.0	25.5 ± 1.6§	57.4 ± 4.7
EFS (40 Hz)	55.1 ± 2.5	19.9 ± 5.3‡	48.0 ± 4.5

ACh = acetylcholine; EFS = electrical field stimulation; ICP = intracavernosal pressure; MAP = mean arterial pressure; MNC = mononuclear cell; Phe = phenylephrine.
*Data are presented as mean (standard error of the mean) for 8 to 10 rats.
†$P < .05$; ‡$P < .01$; §$P < .001$ vs control values by analysis of variance and Bonferroni post hoc test.

HUCB-treated rats at 15 and 20 Hz was significantly potentiated compared with control rats.

The ACh-mediated relaxation in diabetic rats was significantly lower than in control rats (Figure 2B). MNC treatment had no effect on the ACh-induced relaxant response in diabetic rats compared with control rats (Figure 2B). No difference in the endothelium-independent NO-related relaxation response to sodium nitroprusside was observed among groups (data not shown).

The contractile response to Phe in diabetic rats was significantly lower than in control and HUCB-treated diabetic rats ($P < .001$; Table 1 and Figure 2C). MNC treatment improved the Phe-induced contractile response in diabetic rats.

EFS-induced contractile response in the diabetic group was lower than in the control group ($P < .01$; Table 1 and Figure 2D). The MNC-treated diabetic group showed significantly increased contractile response to EFS compared with the diabetic group ($P < .01$; Figure 2D).

Western Blot Analysis

The protein expression of nNOS was significantly decreased in diabetic rats compared with control rats ($P < .001$; Figure 3A). Treatment with MNCs did not alter nNOS protein levels in the diabetic group ($P < .001$; Figure 3A). No difference in the level of vWF protein expression was observed among groups (Figure 3B). The protein expression of VEGF in the CC from

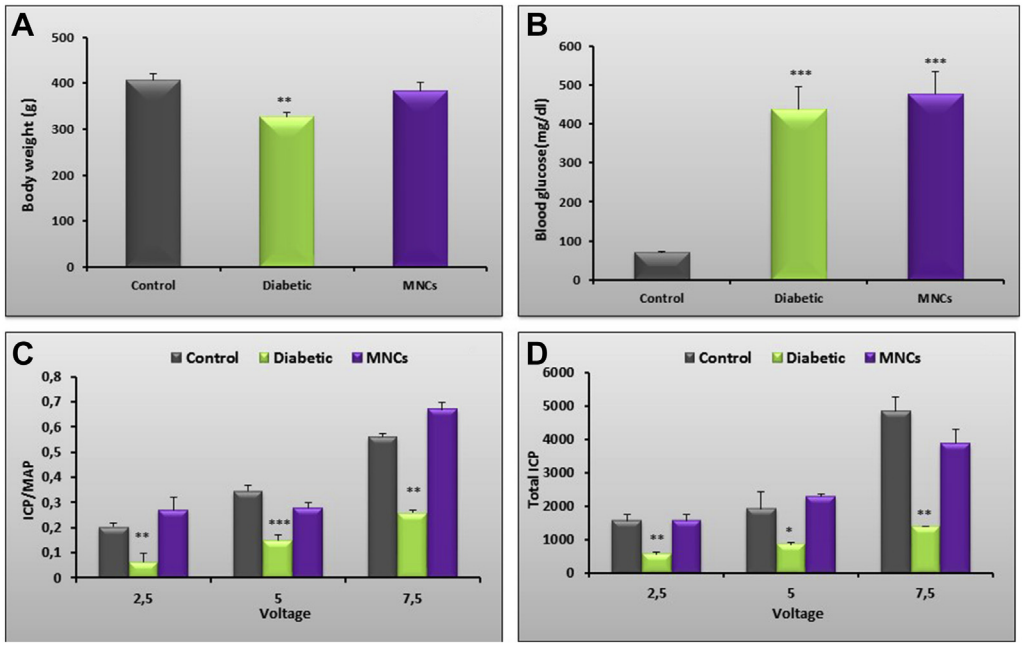


Figure 1. Panel A displays body weights in all groups. Panel B displays blood glucose levels in all groups. Panel C displays the ratio of intracavernosal pressure (ICP) to main arterial pressure (MAP) in all groups. Panel D displays total ICP values in all groups. Data are presented as mean (standard error of the mean) for 8 to 10 rats. *$P < .05$; **$P < .01$; ***$P < .001$ vs control group (analysis of variance and Bonferroni post hoc test). MNCs = mononuclear cells. Figure 1 is available in color at www.jsm.jsexmed.org.

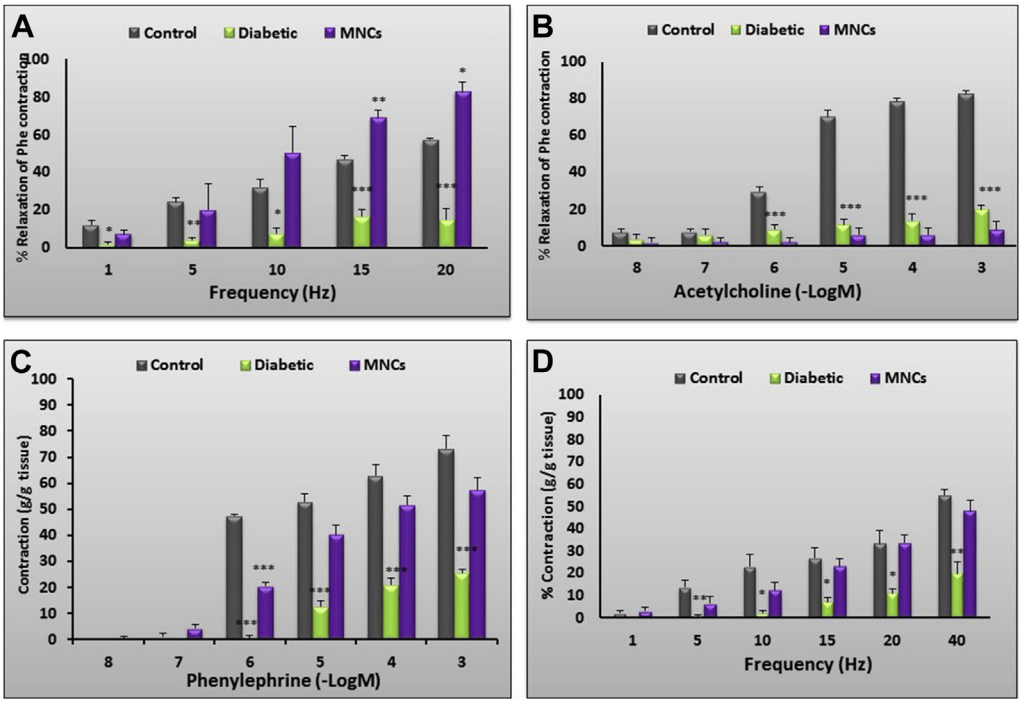


Figure 2. Panel A displays dose-response relaxation curves of corpus cavernosum strips to electrical field stimulation in all groups. Panel B displays dose-response relaxation curves of corpus cavernosum strips to acetylcholine in all groups. Panel C displays phenylephrine-induced contractile responses in all groups. Panel D displays electrical field stimulation—induced contractile responses in all groups. Data are presented as mean (standard error of the mean) for 8 to 10 rats. $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$ vs control group (analysis of variance and Bonferroni post hoc test). MNCs = mononuclear cells. Figure 2 is available in color at www.jsm.jsexmed.org.

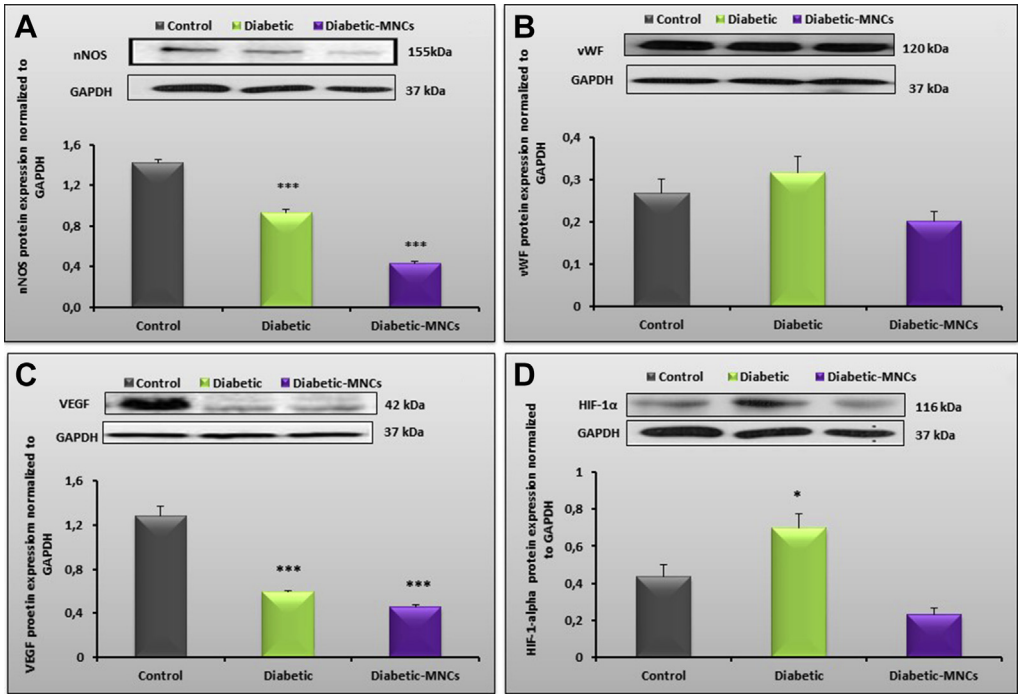


Figure 3. Panel A displays western blot analysis of rat penises showing protein expression of neuronal nitric oxide synthase (nNOS) in all groups. Panel B displays western blot analysis of rat penises showing protein expression of von Willebrand factor (vWF) in all groups. Panel C displays western blot analysis of rat penises showing protein expression of vascular endothelium growth factor (VEGF) in all groups. Panel D displays western blot analysis of rat penises showing protein expression of hypoxia-inducible factor-1α (HIF-1α) in all groups. Data are presented as mean (standard error of the mean) for four to six rats. $^{*}P < .05$; $^{***}P < .001$ vs control group (analysis of variance and Bonferroni post hoc test). GAPDH = glyceraldehyde 3-phosphate dehydrogenase; MNCs = mononuclear cells. Figure 3 is available in color at www.jsm.jsexmed.org.

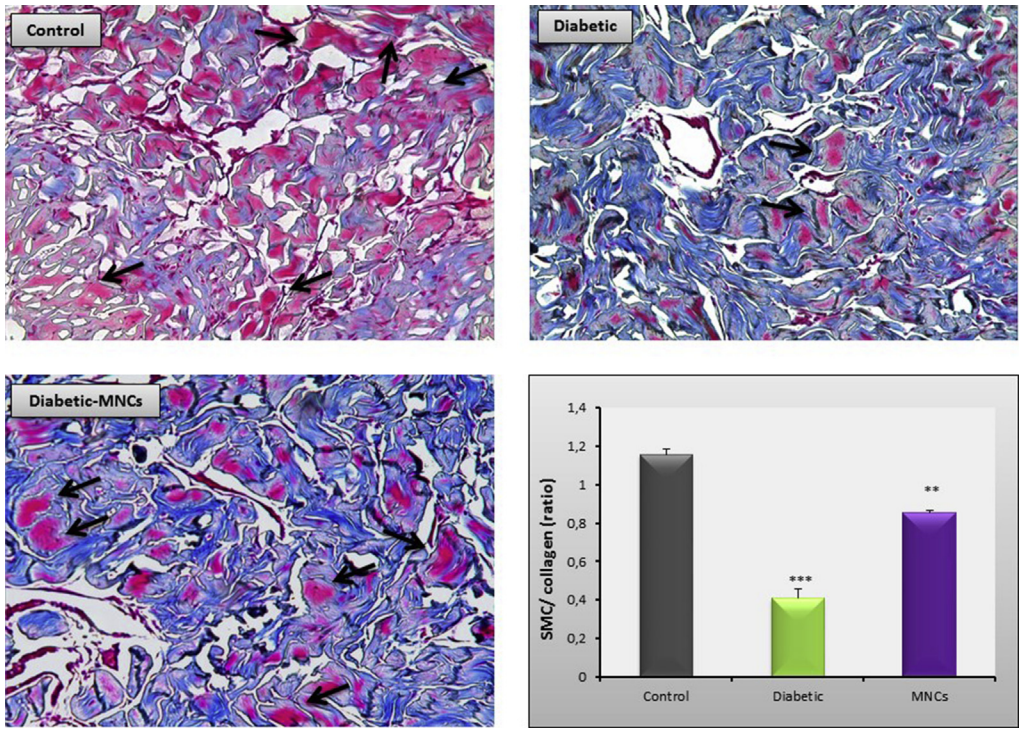


Figure 4. Panels display the ratio of smooth muscle cells (SMCs) to collagen in all groups. Tissue samples from diabetic rats show a decreased SMC/collagen ratio compared with the control group, which was reversed by treatment with human umbilical cord blood. Data are presented as mean (standard error of the mean) for four to six rats. $^{**}P < .01$; $^{***}P < .001$ vs control group (analysis of variance and Bonferroni post hoc test). MNCs = mononuclear cells. Figure 4 is available in color at www.jsm.jsexmed.org.

diabetic rats and MNC-treated rats was lower than in control rats ($P < .001$; Figure 3C). HIF-1α protein expression showed a significant increase in the CC from diabetic rats ($P < .05$; Figure 3D). Protein levels of HIF-1α were improved in the HUCB-treated group ($P < .05$; Figure 3D).

Ratio of Smooth Muscle Cells to Collagen

Masson trichrome staining was used to determine the relative area of collagen to muscle fibers in the cavernosal tissue (Figure 4). The staining showed that the ratio of smooth muscle cells to collagen in diabetic rats was markedly decreased (Figure 4) compared with HUCB-treated diabetic rats (Figure 4) and control rats ($P < .001$; Figure 4). The HUCB-treated group showed partial improvement compared with the control group ($P < .01$; Figure 4).

DISCUSSION

In this study, we showed that HUCB-MNC treatment reversed impaired erectile activity by stimulation of the cavernosal nerve in diabetic rats, EFS and ACh-induced relaxation responses in diabetic CC were significantly decreased, and HUCB treatment completely reversed decreased EFS- but not ACh-induced relaxations. However, sympathetic neurogenic and α_1-receptor—mediated contractions were decreased, which were reversed by the treatment; the ratio of smooth muscle cells to collagen decreased in diabetic samples, which was partly reversed by the treatment; and western blot analysis showed an increase in the protein expression of HIF-1α in the CC from diabetic rats that was completely reversed by the treatment.

This study found decreased body weight and increased blood glucose levels in the diabetic group compared with the control group. In our study, body weight (but not blood glucose levels) in diabetic rats was improved by the MNC treatment. Similarly, a previous study reported that decreased body weight but not increased blood glucose level was partly reversed after a single intracavernous injection of mouse clonal bone marrow—derived stem cells.[8] Furthermore, Naruse et al[27] reported that injection of endothelial progenitor cells (1×10^6 cells per rat) from HUCB-MNCs did not lower blood glucose levels in diabetic rats.

In our data, diabetes decreased in vivo the erectile response to cavernosal nerve stimulation and the in vitro relaxant response of the CC to EFS. HUCB treatment successfully normalized responses to nitrergic nerve stimulation in vivo and in vitro in the diabetic group. In this study, decreased nNOS protein levels were not corrected in HUCB-treated diabetic rats. The reason for the difference between in vivo erectile response and nNOS levels is not clear. Non-protection in nNOS values in the treated group could depend on the given number of cells to diabetic rats. In a previous study, injection of 2×10^6 HUCB mesenchymal stem cells into the CC of rats with cavernous nerve crush injury improved erectile function by increasing nNOS-positive nerve fibers in the dorsal penile nerve and the ratio of smooth muscle to collagen in the CC.[28] It is likely that even one more higher

dose of HUCB-MNCs could be effective in improving nNOS protein levels and to fully recover the ratio of smooth muscle to collagen (fibrosis), which was partly restored in HUCB-MNC—treated diabetic rats. In this study, we also found that HIF-1α protein expression increased in the diabetic group, which was reversed by the MNC treatment. Thus, HIF-1α and nNOS might have different thresholds for cellular responses. Although nNOS protein levels were decreased in the HUCB-MNC—treated group, its activity might be protected under the normoxia that is provided by lower HIF-1α protein levels.

Isolated CC strips from diabetic rats showed a weaker response to ACh, suggesting endothelial dysfunction, which was not corrected by the HUCB-MNC treatment. Previous studies have reported decreased endothelial relaxation to ACh in the diabetic group.[29–32] In this study, we found HUCB cells did not improve the decrease in VEGF protein expression; therefore, the impaired VEGF signaling pathway in the CC could be another key contributing factor to diabetic endothelial dysfunction. vWF is used as a specific marker of endothelium integrity in the vasculature.[27] In the present study, vWF protein levels of penile tissue did not differ among groups. In addition, there was no significant difference in vWF mRNA content between the endothelial cells of diabetic and non-diabetic rats.[33,34]

In the present study, sympathetic neurogenic contractile responses to EFS and the contractile response to Phe in the CC strips were decreased in diabetic rats compared with control rats, which was recovered by MNC treatment. There have been conflicting results on the contractile responses of diabetic erectile tissue.[35,36] The contractile response has been reported to be lower[37,38] or unchanged[39,40] in diabetic rats. The reason for this difference could be the methodology used to measure the contractile response to Phe and EFS.

We found that intracavernous injection of HUCB cells partly increased the content of smooth muscle in the CC of diabetic rats. A previous study showed that the content of smooth muscle in the CC of rats after transplantation of bone marrow—derived MSCs was significantly increased compared with diabetic controls.[41]

In conclusion, HUCB stem cells induced complete prevention of the impairment caused by diabetes on erectile responses in vivo and in vitro EFS-induced relaxation. HUCB-MNC intracavernous injection treatment could improve erectile function by inhibiting CC fibrosis and exerting neuro-regenerative effects in a diabetic rat model. Taken together, these findings provide a greater understanding of the mechanisms responsible for ED and could aid in the development of novel cell-based therapeutic strategies with future applications in regenerative medicine, particularly in the management of ED associated with diabetes.

Corresponding Author: Serap Gur, Department of Pharmacology, Faculty of Pharmacy, Ankara University, 06100, Tandogan, Ankara, Turkey. Tel: +90-312-203-3137; fax: +90-312-213-1081; E-mail: serapgur@ankara.edu.tr

Conflicts of Interest: The authors report no conflicts of interest.

Funding: None.

STATEMENT OF AUTHORSHIP

Category 1

(a) Conception and Design
Tugba Cengiz; Ecem Kaya; Didem Yilmaz Oral; Serap Gur

(b) Acquisition of Data
Tugba Cengiz; Ecem Kaya; Didem Yilmaz Oral; Isil Ozakca; Nur Bayatli; Serap Gur

(c) Analysis and Interpretation of Data
Tugba Cengiz; Ecem Kaya; Didem Yilmaz Oral; Arzu Zeynep Karabay; Tugba Altun Ensari; Tuna Karahan; Enis Yilmaz; Serap Gur

Category 2

(a) Drafting the Article
Tugba Cengiz; Ecem Kaya; Didem Yilmaz Oral; Isil Ozakca; Nur Bayatli; Arzu Zeynep Karabay; Tugba Altun Ensari; Tuna Karahan; Enis Yilmaz; Serap Gur

(b) Revising It for Intellectual Content
Tugba Cengiz; Ecem Kaya; Didem Yilmaz Oral; Isil Ozakca; Nur Bayatli; Arzu Zeynep Karabay; Tugba Altun Ensari; Tuna Karahan; Enis Yilmaz; Serap Gur

Category 3

(a) Final Approval of the Completed Article
Tugba Cengiz; Ecem Kaya; Didem Yilmaz Oral; Isil Ozakca; Nur Bayatli; Arzu Zeynep Karabay; Tugba Altun Ensari; Tuna Karahan; Enis Yilmaz; Serap Gur

REFERENCES

1. Corona G, Giorda CB, Cucinotta D, et al; Gruppo di studio S-D. Sexual dysfunction at the onset of type 2 diabetes: the interplay of depression, hormonal and cardiovascular factors. J Sex Med 2014;11:2065-2073.
2. Corona G, Giorda CB, Cucinotta D, et al; Group S-DS. Sexual dysfunction in type 2 diabetes at diagnosis: progression over time and drug and non-drug correlated factors. PLoS One 2016;11:e0157915.
3. Mazzilli R, Elia J, Delfino M, et al. Prevalence of diabetes mellitus (DM) in a population of men affected by erectile dysfunction (ED). Clin Ter 2015;166:e317-e320.
4. Rastrelli G, Corona G, Mannucci E, et al. Vascular and chronological age in subjects with erectile dysfunction: a cross-sectional study. J Sex Med 2015;12:2303-2312.
5. Ruan Y, Li M, Wang T, et al. Taurine supplementation improves erectile function in rats with streptozotocin-induced type 1 diabetes via amelioration of penile fibrosis and endothelial dysfunction. J Sex Med 2016;13:778-785.
6. Gur S, Kadowitz PJ, Hellstrom WJ. A review of current progress in gene and stem cell therapy for erectile dysfunction. Expert Opin Biol Ther 2008;8:1521-1538.
7. Soebadi MA, Moris L, Castiglione F, et al. Advances in stem cell research for the treatment of male sexual dysfunctions. Curr Opin Urol 2016;26:129-139.

8. Ryu JK, Kim DH, Song KM, et al. Intracavernous delivery of clonal mesenchymal stem cells rescues erectile function in the streptozotocin-induced diabetic mouse. **Andrology 2016; 4:172-184.**
9. Gokce A, Peak TC, Abdel-Mageed AB, et al. Adipose tissue-derived stem cells for the treatment of erectile dysfunction. **Curr Urol Rep 2016;17:14.**
10. Ouyang B, Sun X, Han D, et al. Human urine-derived stem cells alone or genetically-modified with FGF2 improve type 2 diabetic erectile dysfunction in a rat model. **PLoS One 2014; 9:e92825.**
11. Prasad VK, Kurtzberg J. Umbilical cord blood transplantation for non-malignant diseases. **Bone Marrow Transplant 2009; 44:643-651.**
12. Aridas JD, McDonald CA, Paton MC, et al. Cord blood mononuclear cells prevent neuronal apoptosis in response to perinatal asphyxia in the newborn lamb. **J Physiol 2016; 594:1421-1435.**
13. Mayani H, Alvarado-Moreno JA, Flores-Guzman P. Biology of human hematopoietic stem and progenitor cells present in circulation. **Arch Med Res 2003;34:476-488.**
14. Golchin N, Kheirandish M, Sharifi Z, et al. Quantification of viral genome in cord blood donors by real time PCR to investigate human herpesvirus type 8 active infection. **Transfus Apher Sci 2015;53:378-380.**
15. Lee OK, Kuo TK, Chen WM, et al. Isolation of multipotent mesenchymal stem cells from umbilical cord blood. **Blood 2004;103:1669-1675.**
16. Bieback K, Kern S, Kluter H, et al. Critical parameters for the isolation of mesenchymal stem cells from umbilical cord blood. **Stem Cells 2004;22:625-634.**
17. Bennet L, Tan S, Van den Heuij L, et al. Cell therapy for neonatal hypoxia-ischemia and cerebral palsy. **Ann Neurol 2012;71:589-600.**
18. Li J, McDonald CA, Fahey MC, et al. Could cord blood cell therapy reduce preterm brain injury? **Front Neurol 2014; 5:200.**
19. Liu G, Sun X, Bian J, et al. Correction of diabetic erectile dysfunction with adipose derived stem cells modified with the vascular endothelial growth factor gene in a rodent diabetic model. **PLoS One 2013;8:e72790.**
20. Lin G, Qiu X, Fandel T, et al. Tracking intracavernously injected adipose-derived stem cells to bone marrow. **Int J Impot Res 2011;23:268-275.**
21. Albersen M, Fandel TM, Lin G, et al. Injections of adipose tissue-derived stem cells and stem cell lysate improve recovery of erectile function in a rat model of cavernous nerve injury. **J Sex Med 2010;7:3331-3340.**
22. Xu Y, Guan R, Lei H, et al. Therapeutic potential of adipose-derived stem cells-based micro-tissues in a rat model of postprostatectomy erectile dysfunction. **J Sex Med 2014; 11:2439-2448.**
23. Carneiro FS, Giachini FR, Lima VV, et al. Adenosine actions are preserved in corpus cavernosum from obese and type II diabetic db/db mouse. **J Sex Med 2008;5:1156-1166.**
24. Gur S, Kadowitz PJ, Sikka SC, et al. Inhibition of sympathetic neuroeffector transmission in human corpus cavernosum. **BJU Int 2012;110:856-862.**
25. Hotta Y, Ohno R, Kataoka T, et al. Effects of chronic vardenafil treatment persist after end of treatment in rats with acute arteriogenic erectile dysfunction. **J Sex Med 2012;9:1782-1788.**
26. Lei H, Xin H, Guan R, et al. Low-intensity pulsed ultrasound improves erectile function in streptozotocin-induced type I diabetic rats. **Urology 2015;86:1241.e11-1241.e18.**
27. Naruse K, Hamada Y, Nakashima E, et al. Therapeutic neovascularization using cord blood-derived endothelial progenitor cells for diabetic neuropathy. **Diabetes 2005;54:1823-1828.**
28. Zhu JQ, Lu HK, Cui ZQ, et al. Therapeutic potential of human umbilical cord blood mesenchymal stem cells on erectile function in rats with cavernous nerve injury. **Biotechnol Lett 2015;37:1515-1525.**
29. Ryu JK, Tumurbaatar M, Jin HR, et al. Intracavernous delivery of freshly isolated stromal vascular fraction rescues erectile function by enhancing endothelial regeneration in the streptozotocin-induced diabetic mouse. **J Sex Med 2012; 9:3051-3065.**
30. Tang L, Yi R, Yang B, Li H, et al. Valsartan inhibited HIF-1alpha pathway and attenuated renal interstitial fibrosis in streptozotocin-diabetic rats. **Diabetes Res Clin Pract 2012; 97:125-131.**
31. Toque HA, Nunes KP, Yao L, et al. Activated Rho kinase mediates diabetes-induced elevation of vascular arginase activation and contributes to impaired corpora cavernosa relaxation: possible involvement of p38 MAPK activation. **J Sex Med 2013;10:1502-1515.**
32. Ohmasa F, Saito M, Tsounapi P, et al. Edaravone ameliorates diabetes-induced dysfunction of NO-induced relaxation in corpus cavernosum smooth muscle in the rat. **J Sex Med 2011;8:1638-1649.**
33. Ruggeri ZM, Ware J. The structure and function of von Willebrand factor. **Thromb Haemost 1992;67:594-599.**
34. Ribau JC, Hatton MW, Richardson M. Changes in the aortic endothelium and plasma von Willebrand factor levels during the onset and progression of insulin-dependent diabetes in BB rats. **Atherosclerosis 1998;139:291-299.**
35. Toque HA, Tostes RC, Yao L, et al. Arginase II deletion increases corpora cavernosa relaxation in diabetic mice. **J Sex Med 2011;8:722-733.**
36. Hafez G, Gonulalan U, Kosan M, et al. Acetylsalicylic acid protects erectile function in diabetic rats. **Andrologia 2014; 46:997-1003.**
37. Elcioglu HK, Kabasakal L, Ozkan N, et al. A study comparing the effects of rosiglitazone and/or insulin treatments on streptozotocin induced diabetic (type I diabetes) rat aorta and cavernous tissues. **Eur J Pharmacol 2011; 660:476-484.**
38. Zhang X, Kanika ND, Melman A, et al. Smooth muscle myosin expression, isoform composition, and functional activities in rat corpus cavernosum altered by the streptozotocin-induced

type 1 diabetes. **Am J Physiol Endocrinol Metab 2012; 302:E32-E42.**

39. Nunes KP, Toque HA, Caldwell RB, et al. Extracellular signal-regulated kinase (ERK) inhibition decreases arginase activity and improves corpora cavernosal relaxation in streptozotocin (STZ)-induced diabetic mice. **J Sex Med 2011;8:3335-3344.**
40. Lau LC, Adaikan PG. Possibility of inhibition of calcium-activated chloride channel rescuing erectile failures in diabetes. **Int J Impot Res 2014;26:151-155.**
41. Qiu X, Lin H, Wang Y, et al. Intracavernous transplantation of bone marrow-derived mesenchymal stem cells restores erectile function of streptozocin-induced diabetic rats. **J Sex Med 2011;8:427-436.**