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Adipose-Derived Stem Cell-Derived Exosomes Ameliorate Erectile Dysfunction in a Rat Model of Type 2 Diabetes

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ABSTRACT

Background: The efficacy of adipose-derived stem cells (ADSCs) in alleviating erectile dysfunction (ED) of diabetic rats has been demonstrated mainly through a paracrine effect. However, exosomes (EXOs), which are important bioactive substance vectors secreted by ADSCs, have never been associated with ED.

Aim: To investigate the effect of ADSC-derived EXOs on erectile function in a type 2 diabetic ED rat model.

Methods: EXOs were isolated from the supernatants of cultured ADSCs by ultracentrifugation. We constructed a type 2 diabetic rat model using a high-fat diet and low-dose streptozotocin administered by intraperitoneal injection. In total, 24 diabetic rats were randomly assigned to three groups and were treated with an intracavernous injection of ADSC-derived EXOs, ADSCs, or phosphate buffered saline. Another eight age-matched rats underwent sham operation and composed the normal control group.

Outcomes: Intracavernous pressure and mean arterial pressure testing and histologic and western blot analyses were performed 4 weeks after the intracavernous injection.

Results: ADSC-derived EXOs and ADSCs administered by intracavernous injection led to an increase in the ratio of intracavernous pressure to mean arterial pressure compared with that for phosphate buffered saline treatment. Histologic and western blot analyses demonstrated an increased ratio of smooth muscle to collagen, increased expression of an endothelial marker (CD31), a smooth muscle marker (α-smooth muscle actin), and antiapoptotic protein Bcl-2 and decreased the expression of the apoptotic protein cleaved caspase-3 and apoptosis of endothelial and smooth muscle cells in the corpus cavernosum tissue after EXO or ADSC injection compared with values for the phosphate buffered saline treatment.

Clinical Translation: The present results are expected to provide a scientific foundation for clinical application in the near future.

Strengths and Limitations: Although the results demonstrated that intracavernous injection of ADSC-derived EXOs could ameliorate ED of diabetic rats, the optimum dose and times of injection remain for further study.

Conclusions: ADSC-derived EXOs, similarly to ADSCs, were capable of rescuing corpus cavernosum endothelial and smooth muscle cells by inhibiting apoptosis and thus promoting the recovery of erectile function in type 2 diabetic rats. **Chen F, Zhang H, Wang Z, et al. Adipose-Derived Stem Cell-Derived Exosomes Ameliorate Erectile Dysfunction in a Rat Model of Type 2 Diabetes. J Sex Med 2017;XX:XXX–XXX.**

Key Words: Adipose-Derived Stem Cells; Exosomes; Diabetes; Erectile Dysfunction

INTRODUCTION

Diabetes mellitus (DM) is an independent risk factor for erectile dysfunction (ED). Men with DM are three times more likely to develop ED than men without DM. The incidence of DM-related ED has reached 32% to 90%.[1] Many studies have shown that ED in type 2 DM is associated with apoptosis and dysfunction of the corporal endothelium and smooth muscle, which leads to cavernosal systolic and diastolic dysfunction.[2,3] Considering the lower responsiveness to conventional therapy

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with phosphodiesterase type 5 inhibitors[1,4–6] and the complicated pathogenesis of DM-related ED, there is a great need to develop more effective novel modalities aimed at decreasing apoptosis of the corpus cavernosum endothelium and smooth muscle to treat DM-related ED.

Stem cell transplantation for the treatment of ED has been proved as a promising new therapeutic approach for ED because of the multipotent differentiation ability and paracrine actions. Adipose-derived stem cells (ADSCs) isolated and cultured from adipose tissue are a type of mesenchymal stem cell (MSC). ADSCs closely resemble other MSCs in immune phenotype, cell morphology, differentiation, and therapeutic potential.[7] Although their exact mechanism is not clear, ADSCs have been used to treat ED and to significantly improve the erectile function of experimental animals.[8,9] It is commonly believed that ADSCs exert their biological effects of restoring erectile function in rats with DM-related ED not by directly differentiating into endothelial cells, smooth muscle cells, or neuron-like cells to replace damaged ones but by secreting abundant bioactive substances (ie, paracrine effects) to repair damaged penile tissues.[10] In addition to many trophic factors, cytokines, and signal molecules, ADSCs could actively secrete abundant particles called exosomes (EXOs),[11] which might not only decrease tissue injury but also enhance tissue repair.

Recently, increasing attention has been paid to EXOs, which are produced by almost all cell types, including MSCs[12]; EXOs are defined as phospholipid bilayer vesicles with a diameter of 40 to 100 nm. EXO formation starts in endosomes as budding of the limiting endosomal membrane away from the cytosol, thus generating vesicle-laden endosomes, which are referred to as multivesicular endosomes and multivesicular bodies. Fusion of these late endosomes with the plasma membrane releases the intraluminal vesicles as EXOs,[13,14] which contain numerous proteins and lipids and nucleic acid material. EXOs can deliver their cargo to adjacent or distant cells and locations and can control critical processes. EXOs are important in the physiologic function of ADSCs. A wealth of findings has shown a role for ADSC-derived EXOs in revascularization and vascular protection,[15,16] indicating a possible therapeutic potential of these vesicles in cardiovascular disease.

Currently, the role of EXOs has been examined in several diseases models, including myocardial ischemia-reperfusion injury,[17] acute kidney injury,[18] pulmonary hypertension,[19] and stroke.[20] Pascucci et al[21] found that ADSC-derived EXOs stimulate vascular endothelial cell proliferation and migration and promote angiogenesis in vitro. To the best of our knowledge, no studies exist on the effects of EXOs on improving erectile function of rats with DM-related ED.

The present study resulted from the hypothesis that the EXOs released from ADSCs might exert their beneficial effects on alleviating ED through decreasing the apoptosis of corpus cavernosum cells. In our study, the present findings showed for the first time that EXO administration can rescue endothelial and smooth muscle cells of the corpus cavernosum and ameliorate ED in a type 2 DM rat model generated by a high-fat diet combined with multiple injections of low-dose streptozotocin (STZ). Therefore, EXOs could represent a novel therapeutic strategy for DM-associated ED and replace ADSCs, thus avoiding the adverse consequences of cell therapy.

METHODS

Animals

Six-week-old male Sprague-Dawley rats were obtained from the Laboratory Animal Center of Southern Medical University (Guangzhou, China). Four animals were placed in a rat cage. The animals were maintained on a 12-hour light-dark cycle and had access to water ad libitum at the Center of Experiment Animal of Nanfang Hospital. The care and treatment were approved by the ethics committee of Nanfang Hospital.

Isolation and Characterization of ADSC-Derived EXOs

ADSCs were isolated from the inguinal adipose tissue using a modified version of a previously published protocol.[22] Briefly, the adipose tissue was isolated, minced into small pieces, and incubated in a solution containing 0.15% collagenase type I (Sigma, St Louis, MO, USA). After centrifugation at 1,500 rpm for 10 minutes, the supernatant was removed, and the resulting pellet was exposed to erythrocyte lysis buffer to remove red blood cells. The remaining cells were suspended in Dulbecco's Minimal Essential Medium and F12 supplemented with 10% fetal bovine serum, filtered through a 100-μm mesh strainer, and cultured. ADSCs at the third passage were used for this study.

As described in the previously published protocol,[23] conventional culture medium was replaced with serum-free culture medium when the cells reached 80% confluence, and the ADSCs were cultured for an additional 48 hours. Then, the medium was collected, and EXOs were isolated through multistep centrifugation. After centrifugation at 300g for 10 minutes, 2,000g for 20 minutes, and 10,000g for 20 minutes to eliminate dead cells and debris, the supernatant was transferred to a 100-kDa MWCO ultrafiltration centrifuge tube (Amicon-Ultra, Millipore, Bedford, MA, USA) and centrifuged for 30 minutes at 1,000g. Subsequently, the condensed supernatant was filtered through a 0.22-μm filter to eliminate impurities; this step was followed by ultracentrifugation at 110,000g for 70 minutes to pellet the EXOs. The recovered EXOs were washed with phosphate buffered saline (PBS) and centrifuged again at 110,000g for 70 minutes. The pellets were resuspended in PBS. The total protein concentration in the EXOs was quantitated using a Micro Bicinchoninic Acid Protein Assay Kit (Pierce, Rockford, IL, USA), according to the manufacturer's recommended protocol. Protein levels of CD63, CD81, and calnexin were determined using western blot. The morphology and ultrastructure of EXOs were analyzed using transmission electron microscopy.