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Implanted Muscle-Derived Stem Cells Ameliorate Erectile Dysfunction in a Rat Model of Type 2 Diabetes, but Their Repair Capacity Is Impaired by Their Prior Exposure to the Diabetic Milieu

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ABSTRACT

Introduction: Muscle-derived stem cells (MDSCs) and other SCs implanted into the penile corpora cavernosa ameliorate erectile dysfunction in type 1 diabetic rat models by replenishing lost corporal smooth muscle cells (SMCs) and decreasing fibrosis. However, there are no conclusive data from models of type 2 diabetes (T2D) and obesity.

Aim: To determine whether MDSCs from obese Zucker (OZ) rats with T2D at an early stage of diabetes (early diabetic SCs isolated and cultured in low-glucose medium [ED-SCs]) counteract corporal veno-occlusive dysfunction and corporal SMC loss or lipo-fibrosis when implanted in OZ rats at a late stage of diabetes and whether MDSCs from these OZ rats with late diabetes (late diabetic SCs isolated and cultured in high-glucose medium [LD-SC]) differ from ED-SCs in gene transcriptional phenotype and repair capacity.

Methods: ED-SCs and LD-SCs were compared by DNA microarray assays, and ED-SCs were incubated in vitro under high-glucose conditions (ED-HG-SC). These three MDSC types were injected into the corpora cavernosa of OZ rats with late diabetes (OZ/ED, OZ/LD, and OZ/ED-HG rats, respectively). Untreated OZ and non-diabetic lean Zucker rats functioned as controls. Two months later, rats were subjected to cavernosometry and the penile shaft and corporal tissues were subjected to histopathology and DNA microarray assays.

Main Outcome Measures: In vivo erectile dysfunction assessment by Dynamic Infusion Cavernosometry followed by histopathology marker analysis of the penile tissues.

Results: Implanted ED-SCs and ED-HG-SCs improved corporal veno-occlusive dysfunction, counteracted corporal decreases in the ratio of SMCs to collagen and fat infiltration in rats with long-term T2D, and upregulated neuronal and endothelial nitric oxide. LD-SCs acquired an inflammatory, pro-fibrotic, oxidative, and dyslipidemic transcriptional phenotype and failed to repair the corporal tissue.

Conclusion: MDSCs from pre-diabetic rats injected into the corpora cavernosa of rats with long-term T2D improve corporal veno-occlusive dysfunction and the underlying histopathology. In contrast, MDSCs from rats with long-term uncontrolled T2D are imprinted by the hyperglycemic and dyslipidemic milieu with a noxious phenotype associated with an impaired tissue repair capacity. SCs affected by diabetes could lack tissue repair efficacy as autografts and should be reprogrammed in vitro or substituted by SCs from allogenic non-diabetic sources.

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Key Words: Corpora Cavernosa; Corporal Veno-Occlusive Dysfunction; Diabetes; Dyslipidemia; Fibrosis; Hyperglycemia; Myostatin; Obesity; Smooth Muscle; Stem Cell Damage

INTRODUCTION

Erectile dysfunction (ED) is a major complication of type 2 diabetes (T2D) affecting more than 60% to 75% of men with diabetes.[1,2] It impairs the quality of life of patients and their partners and leads to considerable health costs.[3,4] Erectile dysfunction also is prevalent in obese patients and those with metabolic syndrome.[5–7] The risk factors and histopathology that underlie ED are very similar to those affecting the media smooth

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muscle in the arterial tree in arteriosclerosis and hypertension.[8,9] More than 40% of patients with T2D and ED are refractory to palliative treatment with oral phosphodiesterase type 5 inhibitors to induce an erection,[10,11] and there is no convincing evidence that improving glycemic control or lifestyle alone reverses ED.[12–14] Therefore, new therapies for long-term correction of the underlying histopathology of the penile corpora cavernosa are needed to ameliorate ED.

The prevalent form of ED in T2D is vasculogenic, presenting mostly as corporal veno-occlusive dysfunction (CVOD),[15,16] the loss of compliance of the corporal smooth muscle to relax and compress the veins against the rigid tunica albuginea to retain blood during an erection. CVOD is a corporal smooth muscle cell (SMC) dysfunction caused by SMC loss or damage and compounded by endothelial damage and fibrosis.[17]

A novel therapy to repair penile corporal damage and CVOD induced by T2D is based on the corporal implantation of adult stem cells (SCs) that can differentiate into SMCs and other cell lineages impaired by the diabetic milieu. Most experimental studies of ED in diabetes have been conducted in streptozotocin-induced type 1 diabetes in rats and mice,[18] but they do not reflect the impact of obesity and dyslipidemia occurring in T2D and streptozotocin is a toxic agent that damages the pancreas and induces hypoinsulinemia. The reports show amelioration of ED with adipose-derived stromal vascular, bone marrow mesenchymal, and endothelial progenitor SCs that are native or modified by gene transfer.[19]

There is a paucity of studies on rodent models of T2D, metabolic syndrome, or obesity, such as the obese Zucker (OZ) rat, that at approximately 4 months of age is considered initially a model of metabolic syndrome, showing insulin resistance, borderline hyperglycemia, and mild dyslipidemia and overweight, but that at approximately 6 to 7 months of age evolves, similar to humans, into a model of frank T2D with moderate hyperglycemia, hyperinsulinemia, high hemoglobin A_{1C}, morbid obesity, and severe dyslipidemia accompanied by nephropathy and arterial media fibrosis.[19–23] SC therapy of ED in T2D is limited to a study on rat adipose-derived SCs that was performed in a rat model of streptozotocin-induced diabetes and the other study is questionable because of the origin of corporal damage corrected by SCs (see Discussion).[24,25] In humans, there is a report of transient return of morning erections and response to phosphodiesterase type 5 inhibitors in seven patients with diabetes who were awaiting penile prostheses that reverted to unresponsive ED within 1 year.[26]

Muscle-derived SCs (MDSCs) are promising because of easy access to biopsy specimens and their capacity to differentiate into angiogenic, neural, and other lineages[27–30] under paracrine and juxtacrine cues from the host tissue. Our group applied them as therapy for ED[31,32] and for other conditions,[33–35] but they have not been tested in T2D. A major obstacle for their clinical translation and for SCs in general is that if they are used as autologous implants from the same patient, then they would be subjected in the muscle to the same T2D milieu that in the corpora cavernosa damages the SMCs and other differentiated cells. Although allergenic implants of SCs from non-diabetic subjects might overcome this problem, immuno-rejection risks have to be controlled.

The purpose of this study was to investigate the hypotheses that (i) intracorporal implantation of MDSCs counteracts corporal loss of SMCs, lipo-fibrosis, and CVOD in the OZ rat, (ii) the tissue repair capacity of MDSCs isolated from donors with long-term T2D is impaired by their exposure to the diabetic milieu, and (iii) this is associated with alterations of their global transcriptional gene expression signature in markers of inflammation, fibrosis, dyslipidemia, and other noxious changes that clinically might decrease the effectiveness of autografts in patients with T2D.

METHODS

Animals and MDSC Isolation

Animals were used and treated according to the NIH's Principles of Laboratory Animal Care and a protocol approved by the institutional animal care and use committee. Male obese Zucker *fa/fa* (OZ) rats were used at 3 months of age, when they are only mildly hyperglycemic and slightly overweight, compared with their lean non-diabetic counterparts (LZ).[20–23] Allergenic MDSCs were isolated and named "early diabetic SCs" because they were not exposed long term to the considerable hyperglycemic and dyslipidemic milieu of frankly diabetic aged OZ rats. Another set of MDSCs was isolated from these older OZ rats (7 months old), already severely obese and dyslipidemic but with only moderately higher glycemia (see Results). These MDSCs, in contrast to the early diabetic SCs, were exposed for approximately 4 additional months to the more intense and prolonged T2D milieu and named "late diabetic SCs" (LD-SCs).

MDSCs were isolated from hind-limb muscles from the two OZ groups ($n = 2$ per group) using the pre-plating procedure, a validated method for MDSC isolation, as in our previous reports for mice and Fisher 344 rats.[31–35] Pre-plate fraction 6 is the cell population containing MDSCs that was subjected to Sca 1 selection and flow cytometry as described previously. Low (5.6 mmol/L) and high (22.2 mmol/L) concentrations of glucose were used for early diabetic SC and LD-SC maintenance, respectively. To determine the effect of high glucose on early diabetic SCs, they were incubated for 1 week in the 22.2-mmol/L medium and named early diabetic high-glucose SCs (ED-HG-SCs).

Animal Procedures

OZ rats at 7 months of age were divided in four groups ($n = 8$ per group): OZ/UT, which received only intracorporal saline injection (untreated); OZ/early diabetic, which received intracorporal injection of early diabetic SCs (10^6 cells); OZ/early diabetic HG, which received intracorporal injection of early diabetic HG-SCs; and OZ/LD, which received intracorporal injection of LD-SCs. An additional group, LZ/UT, was

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