Adipose Derived Stem Cells Modified by Vascular Endothelial Growth Factor Gene for Correction of Diabetic Erectile Dysfunction

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Introduction:
The prevalence of diabetes mellitus is increasing. Erectile dysfunction (ED) was three times more common and usually appeared ten years earlier in diabetic men than in nondiabetic men. Current treatments include oral therapy with a phosphodiesterase type 5 inhibitor (i.e. sildenafil), self-injection therapies, testosterone therapy and vacuum penile pump devices. However, each of these treatments has its complications or side effects. The core of pathogenesis in diabetes mellitus-related ED (DMED) is endothelial dysfunction. Our previous study demonstrated that vascular endothelial growth factor (VEGF) levels were abnormal in the penile corpus cavernosum tissue of DMED rats. Recently, we have successfully induced adipose stem cells (ASCs) from normal rats to differentiate into endothelial cells. The aim of this study was to determine whether ASCs expressing VEGF as a therapeutic approach can repair injured endothelial cells in the penile corpus cavernosum of DMED rats.

Materials and Methods
ASCs were isolated from adipose tissue at the groin area in normal Sprague-Dawley rats, cultured and expanded in vitro. A VEGF transcript (NM_001025368) was mutated from a clone (EHS1001-68950485313912) by PCR. VEGF-lentivirus was enveloped with plasmid pGC-FU-GFP, pHelper 1.0 and pHelper 2.0 in 293T cells. The vectors transduced efficiency of VEGF was assessed (multiplicities of infection=20). Stable expression of VEGF in ASC was confirmed by immunofluorescence staining, ELISA and Western blot analysis. Diabetes was induced in rats by intraperitoneal injection of streptozotocin 40mg/kg and utilized the apomorphine 100μg/kg skin-pop to screen the DMED models. Five groups were used (n=12 rats per group): 1) Lentivirus-VEGF intracavernous injection (40μl); 2) ASCs implantation at 5x10^5 cells into corpora cavernosum (40μl, 12,500 cells/μl); 3) ASC expressing VEGF gene at 5x10^5 cells; 4) PBS injection (40μl) as a negative control. The first 4 groups used DMED rats; a fifth group consisted of normal rats as controls. Erectile function including intracavernosal pressure (ICP) and mean systemic arterial pressure (MAP) was measured at days 7 and 21 after injection. The penile tissues were harvested and VEGF system function, including VEGF, fetal liver kinase 1(Flk-1), and endothelial nitric oxide synthase (eNOS) were assessed with immunohistochemistry and Western blot analysis.

Results:
ELISA and the Western blot analyses confirmed 95% ASCs expressing VEGF. The VEGF level in the culture medium reached peak at 12 days after infection. Histological analysis demonstrated that ASCs can survive better on day 7 in corporal tissue and erectile function improved on day 21 after intracavernous injection of cells expressing VEGF. Improved erectile function was associated with increased expression of VEGF, Flk-1 and eNOS protein confirmed by Western blot analysis. ASC implantation or lentivirus-VEGF injection alone each increased erectile function of DMED rats, but the degree of increase was lower than in the ASCs-expressing VEGF group. This change was also accompanied by upregulation of penile VEGF, Flk-1 and eNOS protein expression.

Discussion and Conclusions:
In the present study, ASCs were successfully transfected with lentivirus VEGF-GFP genes and secreted VEGF in vitro. ASCs expressing VEGF produced a therapeutic effect to restore erectile function and enhance VEGF systemic functions in DMED rats. This study highlights the potential clinical use of adult stem cell-based therapy for the treatment of ED.

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Disclosures
The authors have nothing to disclose.