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Effectiveness of Intracavernous Delivery of Recombinant Human Hepatocyte Growth Factor on Erectile Function in the Streptozotocin-Induced Diabetic Mouse

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ABSTRACT

Introduction: Diabetic erectile dysfunction is a disease mostly of vascular origin and men with diabetic erectile dysfunction respond poorly to oral phosphodiesterase-5 inhibitors. Hepatocyte growth factor (HGF) is a pleiotropic factor that plays an essential role in the regulation of cell proliferation, survival, and angiogenesis.

Aim: To determine the effectiveness of recombinant human (rh)-HGF in restoring erectile function in diabetic mice.

Methods: Four groups of mice were used: control non-diabetic mice and streptozotocin-induced diabetic mice receiving two successive intracavernous injections of phosphate buffered saline (days −3 and 0), a single intracavernous injection of rh-HGF (day 0), or two successive intracavernous injections of rh-HGF (days −3 and 0). We also examined the effect of rh-HGF in primary cultured mouse cavernous endothelial cells and in major pelvic ganglion culture in vitro, which was incubated under a normal-glucose (5 mmol/L) or a high-glucose (30 mmol/L) condition.

Main Outcome Measures: Two weeks after treatment, we measured erectile function by electrical stimulation of the cavernous nerve and the penis was harvested for histologic studies.

Results: Repeated intracavernous injections of rh-HGF protein induced significant restoration of erectile function in diabetic mice (89−100% of control values), whereas a single intracavernous injection of rh-HGF protein elicited modest improvement. Rh-HGF significantly induced phosphorylation of its receptor c-Met, increased the content of endothelial cells and smooth muscle cells, and decreased the generation of reactive oxygen species (superoxide anion and peroxynitrite) and extravasation of oxidized low-density lipoprotein in diabetic mice. Under the high-glucose condition, rh-HGF protein also promoted tube formation in mouse cavernous endothelial cells and enhanced neurite sprouting in major pelvic ganglion culture in vitro.

Conclusion: The dual angiogenic and neurotrophic effects of HGF could open a new avenue through which diabetic erectile dysfunction can be treated.

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Key Words: Erectile Dysfunction; Diabetes Mellitus; Hepatocyte Growth Factor; Angiogenesis; Neural Regeneration

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INTRODUCTION

Erectile dysfunction (ED) is predominantly a disease of vascular origin and affects up to 75% of all men with diabetes.[1,2] Oral phosphodiesterase-5 inhibitors are most commonly used for ED, but these medications have less efficacy in men with diabetes because of insufficient endogenous nitric oxide (NO) bioavailability from severe endothelial dysfunction and neuropathy.[3,4] Therefore, a new therapeutic strategy is required to regenerate damaged endothelial cells and neuronal cells, which are the major sources of endogenous NO and are prerequisite for physiologic penile erection.

Functional and structural derangements of vascular endothelial cells in the corpus cavernosum play a critical role in the pathophysiology of ED from various causes, such as diabetes and cavernous nerve injury. We and other investigators have reported that increased production of reactive oxygen species (ROS) in the corpus cavernosum tissue plays an important role in diabetes-induced functional and structural impairments in the cavernous endothelium, such as the decrease in endothelial NO synthase enzyme activity, endothelial cell apoptosis, and subsequent loss of endothelial cell content.[3,5,6] Therefore, the regeneration of cavernous endothelial cells and the restoration of their function is a top priority to cure ED.

Hepatocyte growth factor (HGF) is a pleiotropic factor that plays an essential role in the regulation of cell proliferation, survival, angiogenesis, and differentiation in a variety of organs.[7] As a potent angiogenic growth factor, HGF is reported to stimulate the growth of endothelial cells.[8] Importantly, the mitogenic activity of HGF is stronger than that of vascular endothelial growth factor in human aortic endothelial cells.[9,10] HGF is secreted mainly from fibroblast-like cells, whereas its receptor c-Met, a tyrosine kinase, is expressed in epithelial cells and in endothelial and smooth muscle cells of the blood vessels.[11]

We and other investigators have reported that regeneration of damaged endothelial cells using angiogenic factors, such as angiopoietin-1 and vascular endothelial growth factor, is a useful tool to restore erectile function in the diabetic ED model.[5,12] Therefore, we reasoned that HGF might be a potential target for therapeutic angiogenesis by regenerating functional cavernous endothelial cells and smooth muscle cells, which ultimately leads to the restoration of physiologic penile erection.

In the present study, we examined the effectiveness of recombinant human HGF (rh-HGF) protein in restoring erectile function in a diabetic ED model. We also assessed the efficacy of rh-HGF protein in primary cultured mouse cavernous endothelial cells (MCECs) and in primary cultured major pelvic ganglia (MPG) in vitro, which was exposed to a high-glucose (HG) condition to mimic in vivo diabetic ED.

METHODS

Animals and Treatment

Animal experiments performed were approved by the institutional animal care and use subcommittee of our university. Diabetes was induced in 8-week-old C57BL/6J mice by intraperitoneal injections of streptozotocin (STZ; 50 mg/kg) for 5 days consecutively.[5,12] Eight weeks after diabetes was induced, the animals were anesthetized with ketamine (100 mg/kg) and xylazine (5 mg/kg) intramuscularly and randomly distributed into four groups: control non-diabetic mice and STZ-induced diabetic mice receiving two successive intracavernous injections of phosphate buffered saline (PBS; 20 μL, days -3 and 0), a single intracavernous injection of rh-HGF (4.2 μg in PBS 20 μL, day 0; Sino Biological Inc, Beijing, China), or two successive intracavernous injections of rh-HGF (days -3 and 0) into the midportion of the corpus cavernosum (n = 6 per group). Fasting and postprandial blood glucose levels were determined with an Accu-Check blood glucose meter (Roche Diagnostics, Mannheim, Germany) before the mice were sacrificed. Blood samples were obtained from the tail vein.

Measurement of Erectile Function

Two weeks after treatment, erectile function was measured as previously described.[3] Systemic blood pressure was measured using a non-invasive tail-cuff system (Visitech Systems, Apex, NC, USA) just before the measurement of intracavernous pressure (ICP), because vibration during electrical stimulation of the cavernous nerve did not allow an accurate assessment of blood pressure. The ratios of maximal ICP and total ICP to mean systolic blood pressure were calculated to normalize for variations in systemic blood pressure.

Histologic Examinations

For histologic examinations, the penile tissue was fixed in 4% paraformaldehyde for 24 hours at 4°C, and frozen tissue sections (7-μm thick) were incubated with antibodies to platelet endothelial cell adhesion molecule-1 (PECAM-1; an endothelial cell marker; 1:50; Chemicon, Temecula, CA, USA), fluorescein isothiocyanate (FITC)-conjugated antibody to smooth muscle α-actin (a smooth muscle cell marker; 1:200; Sigma-Aldrich, St Louis, MO, USA), c-Met (1:50; Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA), oxidized low-density lipoprotein (LDL; 1:400; Abcam, Cambridge, MA, USA), cleaved caspase-3 (1:500; Cell Signaling, Beverly, MA, USA), nitrotyrosine (1:50; Upstate Biotechnology, Waltham, MA, USA), 5′-bromo-2′-deoxyuridine (BrdU; 1:50; Sigma-Aldrich), βIII-tubulin (1:200; Abcam), and tetramethylrhodamine- (Zymed Laboratories, South San Francisco, CA, USA) or FITC- (Molecular Probes Inc., Eugene, OR, USA) conjugated secondary antibodies. Signals were visualized and digital images were obtained with a confocal microscope (FV1000; Olympus, Tokyo, Japan). Quantitative analysis of histologic examinations was done with an image analyzer system (Image J 1.34; National Institutes of Health, http://rsbweb.nih.gov/ij/).

In Situ Detection of Superoxide Anion

An oxidative fluorescent dye, hydroethidine (Molecular Probes Inc), was used to evaluate levels of superoxide anion in situ as previously described.[13] The numbers of ethidium bromide fluorescence-positive cells in cavernous endothelium were counted at a screen magnification of $\times$400 in six different regions. Values were expressed per high-power field.

Cell Culture and In Vitro Tube Formation Assay

The MCECs were prepared and maintained as previously described.[13] Cells at passages 2 to 3 were used for the