

·Original Article·

Improvement in erectile dysfunction after insulin-like growth factor-1 gene therapy in diabetic rats

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Abstract

Aim: To determine whether adenoviral gene transfer of insulin like growth factor-1 (IGF-1) to the penis of streptozotocin (STZ)-induced diabetic rats could improve erectile capacity. **Methods:** The STZ diabetic rats were transfected with AdCMV- β gal or AdCMV-IGF-1. These rats underwent cavernous nerve stimulation to assess erectile function and their responses were compared with those of age-matched control rats 1 to 2 days after transfection. In control and transfected STZ diabetic rats, IGF-1 expression were examined by reverse transcription polymerase chain reaction (RT-PCR), Western blot and histology. The penis β -galactosidase activity and localization of the STZ diabetic rats were also determined. **Results:** One to two days after transfection, the β -galactosidase was found in the smooth muscle cells of the diabetic rat penis transfected with AdCMV- β gal. One to 2 days after administration of AdCMV-IGF-1, the cavernosal pressure, as determined by the ratio of maximal intracavernous pressure-to-mean arterial pressure (ICP/MAP) and total intracavernous pressure (ICP), was increased in response to cavernous nerve stimulation. Transgene expression was confirmed by RT-PCR, Western blot and histology. **Conclusion:** Gene transfer of IGF-1 significantly increased erectile function in the STZ diabetic rats. These results suggest that *in vivo* gene transfer of IGF-1 might be a new therapeutic intervention for the treatment of erectile dysfunction (ED) in the STZ diabetic rats. (Asian J Androl 2007 Jan; 9: 83–91)

Keywords: erectile dysfunction; gene therapy; cavernosometry; insulin like growth factor-1

1 Introduction

Erectile dysfunction (ED) is very common among diabetic patients [1]. Men with diabetes develop ED at

an earlier age than other men, with significantly high prevalence, ranging from 20% to 85% [2]. The etiology of ED in diabetes is multifactorial. There is a greater incidence of peripheral neuropathy, microangiopathy and arterial insufficiency in individuals with diabetes and ED compared with those with normal function [3]. The changes in endocrine function and the central nervous system control of sexual arousal might have an important role in the pathogenesis of ED associated with diabetes [3].

The association of ED and diabetes is also supported by evidence from experimental models using rats. The

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streptozotocin (STZ)-induced diabetic rat has been used as a model system to study sexual dysfunction in human diabetes [4]. Several possible explanations exist for diabetes-induced decreased erectile function in the STZ diabetic rat, including autonomic nerve dysfunction, decreased nerve conduction properties, alteration in the release or postsynaptic action of neurotransmitters, such as nitric oxide (NO), altered smooth muscle and vascular function, inhibition of insulin-like growth factor (IGF) binding protein and derangement of central nervous system control of behavioral drive or autonomic outflow [5, 6]. However, the underlying molecular causes of ED associated with diabetes need to be explored deeply.

IGF-1 is a single-chain polypeptide with structural homology to proinsulin. Some recent studies show that IGF-1 plays a key role in the regeneration of nitric oxide synthase (NOS)-containing nerve fibers in the dorsal and intracavernosal nerves [7], and administration of IGF-1 can facilitate the regeneration of NOS-containing nerve fibers in penile tissue and enhance the recovery of erectile function after bilateral cavernous nerve cryoablation [8]. Impairment of erection in chronic renal failure in rats is attributable to a disturbance in *NOS* gene expression with concomitant changes in IGF-1 [9]. In diabetic rats with ED downregulation of IGF-1 protein expression in penile cavernosum is found [10].

Gene transfer to the penis has successfully improved erectile capacity [11]. Low expression of IGF-1 has been reported in the penile cavernosum of diabetic rats [10]. We hypothesized that gene transfer of IGF-1 might restore erectile function in diabetic rats. Therefore, we investigated the effects of gene transfer of IGF-1 to the penis of STZ diabetic rats to determine if IGF-1 overexpression can improve erectile function.

2 Materials and methods

2.1 Experimental animals

Wuhan University Animal Care and Use Committee approved all procedures in the current study. A total of 40 adult male Sprague–Dawley rats (Wuhan University, Wuhan, China) were randomly divided into four groups. Group 1 includes 10 age-matched control rats that received i.p. injections of citrate buffer (100 mmol/L citric acid and 200 mmol/L disodium phosphate, pH 7.0). The other thirty rats were all received i.p. injection of STZ at a dose of 60 mg/kg. Total body weight and blood glucose levels in serum samples obtained from the tail vein of all rats were determined before and after i.p. injection of citrate buffer or STZ with a Super GlucoCard II blood glucose meter (Arkray, Kyoto, Japan). The rats were considered diabetic if blood glucose was greater than 200 mg/dL. After 2 months, 10 rats of STZ-treated rats were treated with the vehicle (3% sucrose in phosphate-buffered saline [PBS]) (group 2), 10 were transfected with AdCMV- β gal (group 3) and 10 were transfected with AdCMV-IGF-1 in the penis (group 4). Blood glucose levels and total body weight of control, STZ diabetic rats administrated with the vehicle (3% sucrose in phosphate-buffered saline (PBS)), and rats transfected with AdCMV- β gal and AdCMV-IGF-1 were recorded (Table 1).

2.2 Adenovirus vectors

AdCMV- β gal and AdCMV-IGF-1, replication-deficient recombinant adenovirus carrying β -galactosidase reporter gene and *IGF-1* gene under the control of cytomegalovirus (CMV) promoter, were generated using standard methods [12]. The AdCMV-IGF-1 virus has a

Table 1. Weight and blood glucose in 40 control and streptozotocin (STZ) diabetic rats. Data were expressed as mean \pm SD. ^b*P* < 0.05, compared with the initial level.

	Control	Diabetic		
		Vehicle	AdCMV- β gal	AdCMV-IGF-1
Weight (g)				
Initial	321 \pm 12	313 \pm 8	324 \pm 5	329 \pm 11
Final	521 \pm 30 ^b	279 \pm 9 ^b	265 \pm 11 ^b	269 \pm 7 ^b
Blood glucose (mg/dL)				
Initial	97 \pm 6	101 \pm 8	98 \pm 5	101 \pm 6
Final	105 \pm 2	384 \pm 11 ^b	392 \pm 17 ^b	389 \pm 13 ^b

concentration of 1×10^8 viral particles (vp)/mL and a titer of 3×10^{10} plaque-forming units (pfu)/mL. The AdCMV- β gal virus also has a concentration of 1×10^8 vp/mL and a titer of 1×10^{10} pfu/mL. Virus was suspended in PBS with 3% sucrose and maintained at -70°C until use.

2.3 Intracavernosal injection of virus into the STZ diabetic rats' penis

Male STZ diabetic Sprague–Dawley rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and placed in a supine position on a surgical table. Using a 30-gauge needle attached to a microliter syringe, 2 mL of vehicle (3% sucrose in PBS), AdCMV- β gal (1×10^8 vp/mL), or AdCMV-IGF-1 (1×10^8 vp/mL) was injected into the corpus cavernosum in different sides. Immediately before instillation, blood drainage through the dorsal veins was halted by circumferential compression of the penis at the base with an elastic band. Compression was released 5 min after injection of 2 mL of the vehicle or virus.

2.4 Measurement of erectile responses

Erectile function was assessed by measuring intracavernous pressure (ICP) following electrostimulation of the cavernous nerves, as described by Christ *et al.* [13]. Rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and placed on a surgical table 1–2 days after vehicle or virus administration. A carotid artery was cannulated (PE 50 tubing) for the measurement of systemic arterial pressure. Systemic arterial pressure was measured continuously with a data acquisition system (RM-6200 multichannel electrophysiology; Shenzhen Electronics, Shenzhen, China). The left jugular vein was cannulated (PE 50 tubing) for the administration of fluids and supplemental anesthesia. The bladder and prostate were exposed through a midline abdominal incision. The cavernosal nerve was identified posterolateral to the prostate on one side, and TENS stimulator (Emidue, Rome, Italy) with a stainless steel bipolar hook was placed around the cavernosal nerve. The skin overlying the penis was incised, and the right crura was exposed by removing part of the overlying ischiocavernosus muscle. A 25-gauge needle filled with 250 units/mL heparin and connected to PE 50 tubing was inserted into the right crura. Systemic arterial and intracavernosal blood pressure was measured by the multichannel electrophysiology. The cavernosal nerve was stimulated by the electronic stimulator at a frequency of 15 Hz and pulse width of 30 s in each rat.

Cavernous nerve stimulation at 2.5, 5 and 7.5 V was used in the current protocol to achieve significant erectile responses. The duration of stimulation was 1 min with a rest period of 2–3 min between subsequent stimulations. The ratio of maximal ICP-to-mean arterial pressure (ICP/MAP) and the total ICP determined by the area under the curve in mmHg per second were recorded in every rat. After measurement of the erectile response, rats were killed with an i.p. overdose of pentobarbital (80 mg/kg) and the penile shaft was removed for reverse transcription polymerase chain reaction (RT-PCR), Western blot and immunohistochemical analysis.

2.5 X-gal histochemistry for β -galactosidase activity

The activity of β -galactosidase was evaluated using the β -galactosidase assay system (Blossom Biotechnologies, Beijing, China) and X-gal (X-gal 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside) staining. The transfected rats were killed as described above, and the penile tissues were cut into 2-mm sagittal sections, incubated in X-gal assay system for 2 h at 24°C , rinsed in PBS, and postfixed in 7% (v/v) buffered formalin for 6 h. The sections then were placed in 20% (v/v) sucrose for 12 h, overlaid with OCT compound (Brayotime Biotechnology, Beijing, China) and frozen in liquid nitrogen. Cryostat (7 mm) sections were mounted on poly-L-lysine-coated slides and counterstained with eosin Y. Protein concentrations of the samples were determined using the Bradford Protein Assay (Brayotime Biotechnology, Beijing, China). Normalized β -galactosidase activity was expressed as relative light units of β -galactosidase per milligram of protein.

2.6 RT-PCR analysis of IGF-1 gene expression

After the functional study was completed, a cavernous tissue was obtained and maintained at -80°C until processing for mRNA expression analysis. Total RNA was extracted from the rat cavernous tissue of the four groups. In 20 μL reaction buffer, 1 μg total RNA was resuspended in dihexadecylphosphatidylcholine (DHPC)-treated water. The reverse transcription (RT) reaction was done using an RT system procedure (Promega, Wisconsin, USA). Briefly, 5 mmol/L MgCl_2 , $1 \times$ RT buffer (10 mmol/L tris-HCl, 50 mmol/L KCl and 0.1% Triton X-100), 1 mmol/L deoxynucleoside triphosphate [dNTP] mixture (equal amounts of deoxyadenosine triphosphate [dATP], deoxycytidine triphosphate [dCTP], deoxyguanosine triphosphate [dGTP] and deoxythymidine triphosphate

[dTTP]) (Sigma-Aldrich, Missouri, USA), 1 µg/µL recombinant RNasin (Jingmei Biotech, Shenzhen, China) ribonuclease inhibitor, 15 U avian myeloblastosis virus RT (high concentration) and 0.5 µg oligodeoxy-thymidine(oligo-dT) 15 primers were added to the RNA mixture. The reaction was done at 37°C for 5 min and 42°C for 60 min, followed by 10 min of heating at 95°C to destroy the enzyme and RNA. RT reaction (1 µL) was amplified in 20 µL reaction buffer containing 1× polymerase chain reaction (PCR) buffer, 0.5 Taq (Promega, Wisconsin, USA) DNA polymerase, 0.25 mmol/L dNTP mixture (equal amounts of dATP, dCTP, dGTP, dTTP), 0.2 mmol/L sense and antisense primers, and 0.25 µL dimethyl sulfoxide (DMSO). Cycle parameters consisted of the denaturing step at 94°C for 1 min, 1 min annealing step at 55°C for 1 min and extension step at 72°C for 1 min with 40 cycles per amplification. PCR products were electrophoresed on 1% agarose gel (Jingmei Biotech, Shenzhen, China), stained with 0.5 µg/mL ethidium, and visualized and photographed on an ultraviolet transilluminator bromide (SIM International, California, USA). The gene expression of *IGF-1* relative to *β-actin* was quantified by densitometry.

2.7 Western blot analysis

The STZ diabetic rats were killed and cavernous tissues were homogenized using a Polytron (Brinkmann Instruments, NY, USA) in ice-cold protease inhibitor buffer (50 mmol/L Tris-HCl, pH 7.4, 1 mmol/L EDTA, 1 mmol/L egtazic acid) (Jingmei Biotech, Shenzhen, China). Following centrifugation at 48 000 × *g* for 1 h at 4°C the cytosolic supernatant liquid was removed. The particulate fraction was re-suspended and rehomogenized in protease inhibitor buffer containing 1 mol/L KCl and then centrifuged at 48 000 × *g* for 1 h at 4°C. The supernatant liquid was discarded. The particulate was suspended and homogenized in protease inhibitor buffer, again. Then 10% (v/v) 200 mmol/L CHAPS solution was added to the homogenate and mixed for 30 min at room temperature, and centrifuged at 48 000 × *g* again. The sample protein concentrations were evaluated using the Pierce Protein Assay. The membrane fraction supernatant for IGF-1 were mixed with an equal volume of 2% sodium dodecyl sulfate (SDS)/1% β-mercaptoethanol and fractionated using 8% SDS/polyacrylamide gel electrophoresis (SDS-PAGE) (70 µg per lane). Proteins were then transferred to a Hybond-ECL (Amersham Biosciences, Beijing, China) nitrocellulose membrane, blocked for 1 h with blotto-Tween (5% nonfat dry milk

and 0.1% Tween-20). The primary polyclonal rabbit anti-IGF-1 IgG (1:200) (Boster, Wuhan, China) were added and incubated at 4°C overnight. The labeled goat antirabbit IgG secondary antibody conjugated to horseradish peroxidase were added and incubated for 2 h. The bands were visualized using enhanced chemiluminescence.

2.8 Immunohistochemical analysis

For histochemical staining of IGF-1, the cavernous tissue were frozen in OCT compound, and serial 5 mm cross-sections were cut. After immersion fixation in acetone (4°C), the sections were incubated in 0.1% sodium azide/0.3% hydrogen peroxide and then incubated with 5% normal goat serum/PBS-Tween 20 to block the nonspecific protein binding sites. A monoclonal antibody for IGF-1 (1:100) (Boster, Wuhan, China) was applied for 60 min at room temperature, followed by incubations with goat antirabbit IgG secondary antibody conjugated to horseradish peroxidase (1:200, 20 min). After immersion in 0.1 mol/L sodium acetate buffer (pH 5.2) for 30 s, IGF-1 immunoreactivity was visualized with 3-amino-9-ethylcarbazole and hematoxylin counterstaining. The expression of IGF-1 protein was visualized by densitometry using the YPS 2000 Pathology Picture Analysis System (Shanghai Huanyan, Shanghai, China).

2.9 Statistical analysis

Data were analyzed using analysis of variance with repeated measures and the Mann-Whitney *U*-test for multiple group comparisons using Statview 4.5 (SAS Institute, Cary, NC, USA) software. Values were considered significant at *P* < 0.05. Data were expressed as mean ± SD.

3 Results

3.1 Evaluation of the erectile function

The cavernous nerve-induced erectile response was measured 1–2 days after transfection with AdCMV-βgal or AdCMV-IGF-1. The increases in ICP/MAP and total ICP (the area under the curve) in response to cavernosal nerve stimulation (2.5, 5 and 7.5 V) in the AdCMV-IGF-1-treated rats were significantly greater than those in the AdCMV-βgal-treated rats or vehicle-treated rats (*P* < 0.05). The significant decreases in ICP/MAP and total ICP were observed in vehicle-treated rats and AdCMV-βgal-transfected rats when compared with control rats (*P* < 0.05, Figure 1). The representative ICP tracing in response to

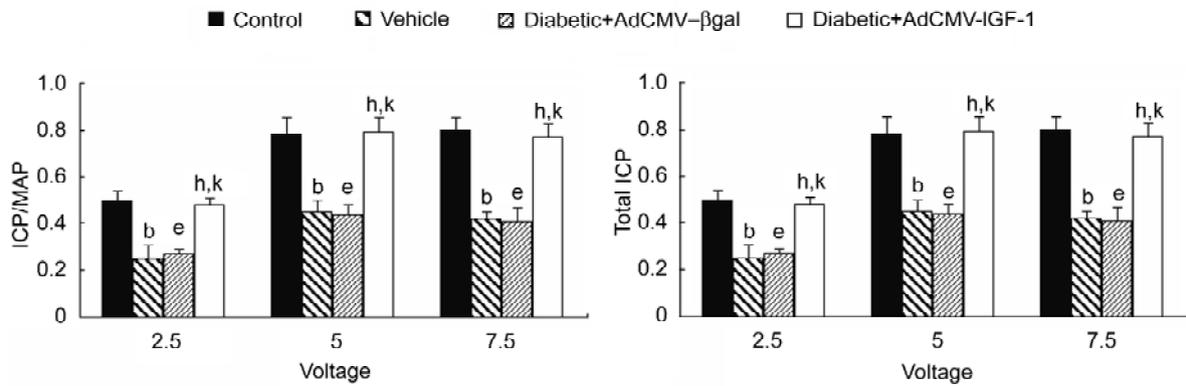


Figure 1. The effect of insulin like growth factor-1 (*IGF-1*) gene transfer on erectile function in streptozotocin (STZ) diabetic rats, as measured by ratio of intracavernous pressure-to-mean arterial pressure (ICP/MAP) and total ICP (the area under curve) in the control, vehicle-treated and transfected with AdCMV-βgal or AdCMV-IGF-1 STZ diabetic rats. ^{b,e}*P* < 0.05, compared with the control; ^{h,k}*P* < 0.05, compared with the STZ diabetic rats treated with vehicle; ^{h,k}*P* < 0.05, compared with the STZ diabetic rats transfected with AdCMV-βgal.

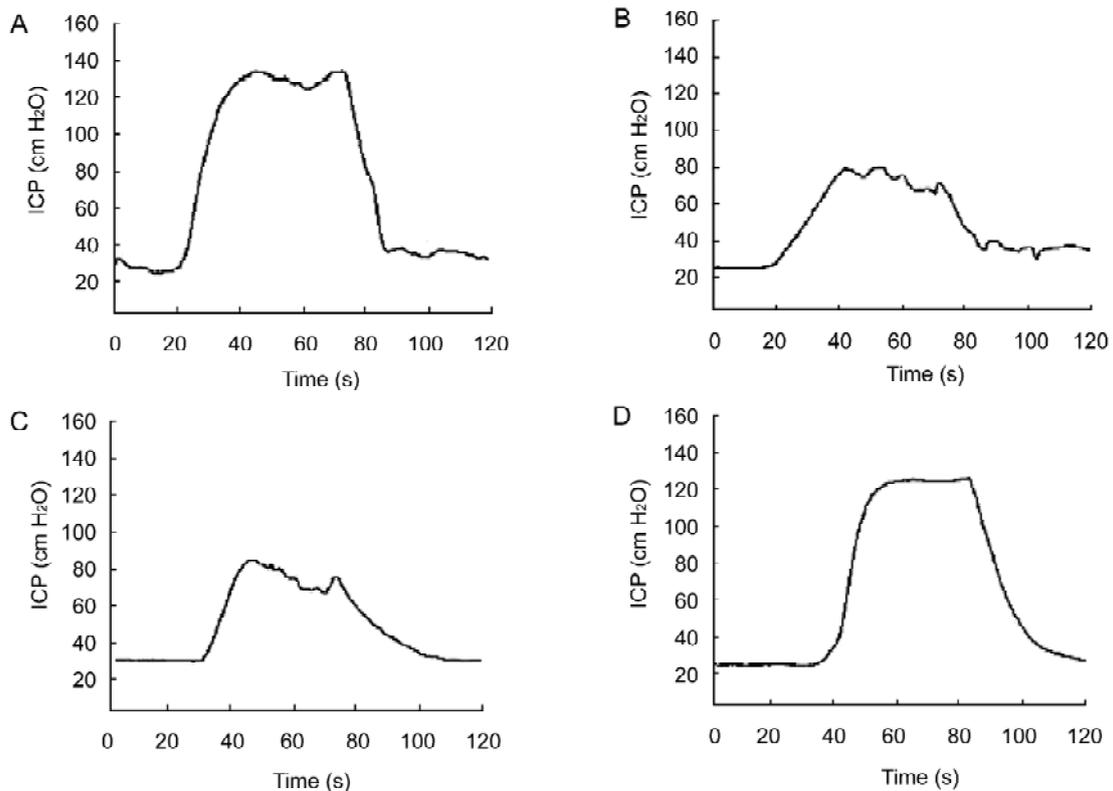


Figure 2. Representative intracavernous pressure (ICP) in response to electrostimulation of cavernous nerve. Vehicle-treated (B) and AdCMV-βgal-transfected streptozotocin (STZ) diabetic rats (C) showed decreased ICP compared with the control (A). The AdCMV-IGF-1 STZ diabetic rats (D) showed increased ICP compared with vehicle treated or AdCMV-βgal-transfected STZ diabetic rats.

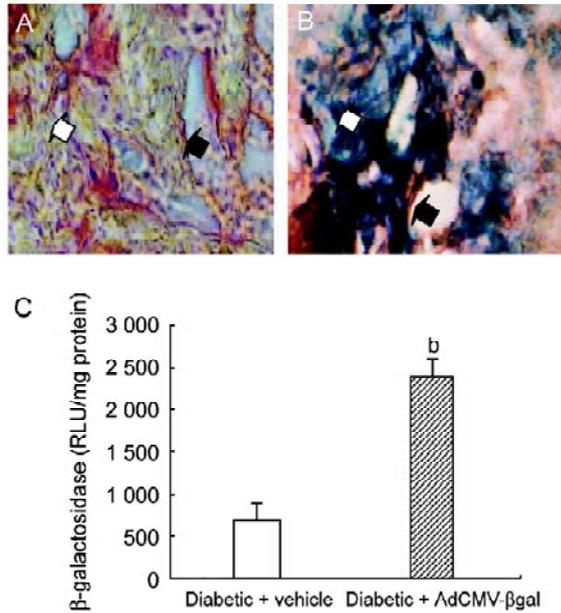


Figure 3. β -galactosidase expression in the corpus cavernosum of streptozotocin (STZ) diabetic rats. (A): Rats treated with vehicle showed no staining for β -galactosidase. (B): AdCMV- β gal-transfected rat shows localization of β -galactosidase in the corpus cavernosum. White arrowheads show the smooth muscle cells and the black arrowheads show the sinusoids. (C): Magnitude of β -galactosidase activity in corpus cavernosum after intracavernous administration of vehicle or AdCMV- β gal. ^b $P < 0.05$, compared with the vehicle-treated rats.

electrostimulation of cavernous nerve is indicated in Figure 2.

3.2 Evaluation of β -galactosidase activity

Histochemical analysis of β -galactosidase was assessed in the penile tissue of rats 1–2 days after transfection with AdCMV- β gal or vehicle: a typical 2 mm section of penis is shown in Figure 3A. β -galactosidase protein was expressed diffusely throughout the corpus cavernosum and localized in the smooth muscle of corpus cavernosum of rats treated with AdCMV- β gal. β -galactosidase staining was not observed in the corpus cavernosum of rats treated with the vehicle. β -galactosidase activity was quantified in the penis using chemiluminescence. Penile tissue from rats treated with the vehicle showed very lower β -galactosidase activity than those treated with AdCMV- β gal ($P < 0.05$, Figure 3B).

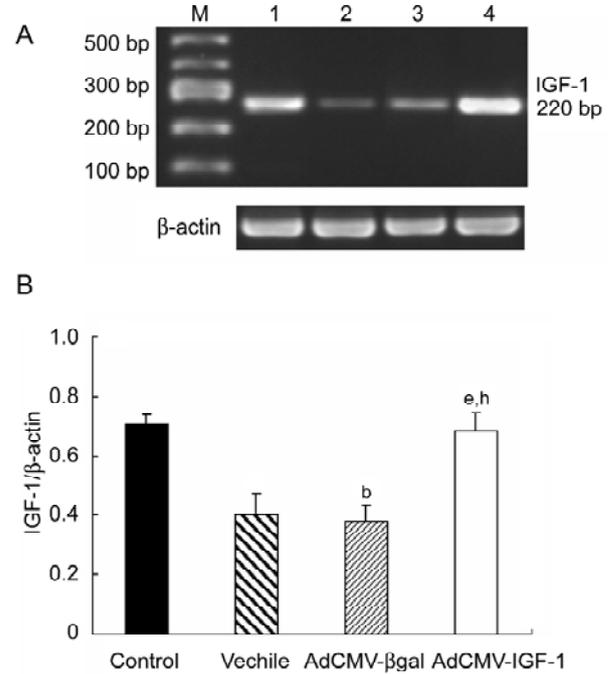


Figure 4. (A): Insulin like growth factor-1 (IGF-1) mRNA expression in penile cavernous tissue according to reverse transcription polymerase chain reaction (RT-PCR). IGF-1 mRNA transcripts were present in rat cavernous tissue of controls (lane 1) and in streptozotocin (STZ) diabetic rat cavernous tissue 1–2 days after the vehicle treatment (lane 2) and gene transfer of AdCMV- β gal (lane 3) or AdCMV-IGF-1 (lane 4). M, marker. (B): Mean quantitative gene expression \pm SE of IGF-1 was analyzed using densitometry. Data were calculated as ratio of IGF-1 to β -actin expression. ^b $P < 0.05$, compared with the control; ^{c,h} $P < 0.05$, compared with the vehicle-treated rats or AdCMV- β gal-transfected rats.

3.3 IGF-1 mRNA expression

Figure 4 shows IGF-1 mRNA transcripts in the penile cavernous tissue of age-matched control rats and 1–2 days after intracavernous administration of vehicle, AdCMV- β gal or AdCMV-IGF-1 in STZ diabetic rats on RT-PCR. We used β -actin mRNA sequence as housekeeping gene. IGF-1 mRNA transcripts were present in the rat cavernous tissue of all groups. Cavernous IGF-1 mRNA levels were significantly lower in STZ diabetic rats transfected with AdCMV- β gal than those in the control rats ($P < 0.05$, Figure 4B). One to two days after transfection with AdCMV-IGF-1, IGF-1 mRNA levels in cavernous tissue were significantly higher in STZ diabetic rats transfected with AdCMV-IGF-1 than those in STZ diabetic rats transfected with AdCMV- β gal or treated with the vehicle

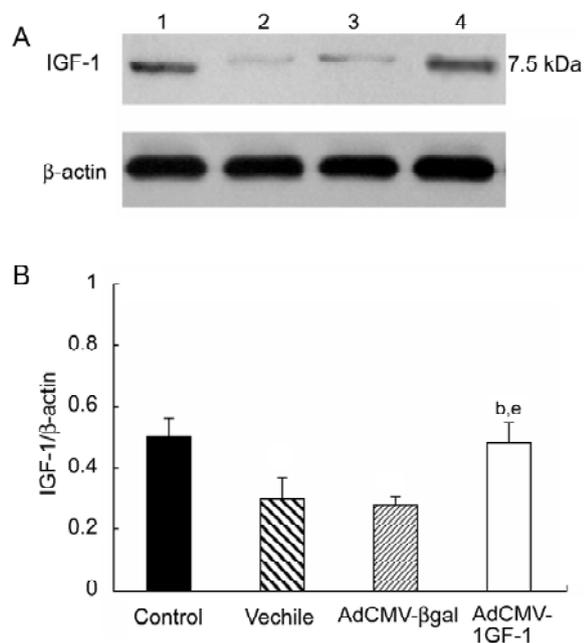


Figure 5. (A): Western blot analysis reveals expression of insulin like growth factor-1 (IGF-1) protein in cavernous tissue of controls (lane 1), and in streptozotocin (STZ) diabetic rat cavernous tissue 1–2 days after vehicle treatment (lane 2) and gene transfer of AdCMV-βgal (lane 3) or AdCMV-IGF-1 (lane 4). (B): Mean quantitative protein expression ± SE of IGF-1 was analyzed using densitometry. Data were calculated as ratio of IGF-1 to β-actin expression. ^{b,e} $P < 0.05$, compared with the vehicle-treated rats or AdCMV-βgal-transfected rats.

($P < 0.05$, Figure 4B).

3.4 Western blot analyses of IGF-1 protein expression

The 7.5-kDa IGF-1 protein band can be detected in cavernous tissue of age-matched control rats and 1–2 days after intracavernous administration of AdCMV-βgal or AdCMV-IGF-1 in STZ diabetic rats (Figure 5). Cavernous IGF-1 protein levels were significantly higher in the control and STZ diabetic rats transfected with AdCMV-IGF-1 than those in rats transfected with AdCMV-βgal or treated with the vehicle ($P < 0.05$, Figure 5B).

3.5 Immunohistochemical analyses of IGF-1 localization

IGF-1 protein was also determined by immunohistochemistry in STZ rats 1–2 days after transfection with AdCMV-IGF-1 and in the control rats. Figure 6 shows that IGF-1 protein expression was positive in the sinusoidal spaces and cavernous smooth muscle cells. However,

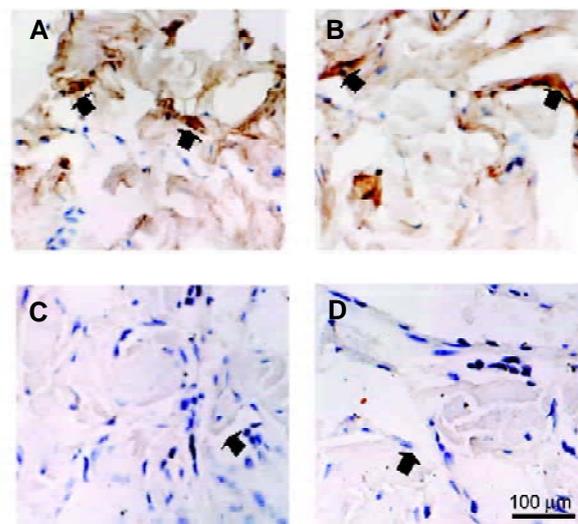


Figure 6. Immunohistochemical localization of insulin like growth factor-1 (IGF-1) expression in the control (A, ×400), AdCMV-IGF-1-transfected (B, ×400), AdCMV-βgal-transfected rats (C, ×400) and vehicle-treated rats (D, ×400). Brown staining and arrowheads denote presence of IGF-1-positive cells. The IGF-1 protein expression was higher in AdCMV-IGF-1-transfected rats and control than that in AdCMV-βgal-transfected rats or vehicle-treated rats.

cavernous IGF-1 protein expression was higher in STZ diabetic rats transfected with AdCMV-IGF-1 and the control than that in those transfected with AdCMV-βgal or treated with the vehicle (Figure 6).

4 Discussion

Although the role of IGF-1 in diabetic associated ED is not completely clear, some studies suggested that it might be a vital growth factor gene in ED [6–10]. In the current study we investigated the role of IGF-1 in impaired corpus cavernosum in STZ-induced diabetes. Our results indicate a decrease in IGF-1 protein expression in the STZ diabetic rats. Adenoviral gene transfer of IGF-1 was associated with increased cavernous expression of IGF-1 mRNA and protein as well as the reversal of diabetic related ED. Moreover, to our knowledge we report for the first time that direct injection of adenovirus encoding the *IGF-1* gene to the corpora cavernosa of STZ diabetic rats can improve impaired erectile function.

IGF-1 is a member of the growth factor family. Growth factors represent a system of signals that organize and coordinate cellular proliferation [12]. They are

mediators of physiologic and pathologic cellular growth and repair, including embryogenesis, wound healing and carcinogenesis. IGF-1 are single-chain polypeptides with structural homology to proinsulin. They regulate proliferation and differentiation of a multitude of cell types and are capable of exerting insulin-like metabolic effects. Unlike insulin, they are produced by most of body tissues and are abundant in the circulation. Part of the allure of IGF-I as a therapeutic agent is its wide range of biologic effects and its actions on many different tissues. IGF-I mediates many, if not most, of the anabolic effects of circulating growth hormones. It stimulates bone formation, protein synthesis, glucose uptake in muscle and myelin synthesis. Growth hormones are known to stimulate hepatic production of IGF-1, which is the mediator of most growth hormone functions.

Recently, IGF-1 has been found to enhance regeneration of NOS-containing penile nerves after cavernous neurotomy in rats [7, 14]. Administration of IGF-1 can facilitate the regeneration of NOS-containing nerve fibers in penile tissue and enhance the recovery of erectile function after bilateral cavernous nerve cryoablation [8]. Abdel-Gawad *et al.* [9] found a significant decrease in the amount of *IGF-1* gene expression in the major pelvic ganglia of rats with renal failure and concluded that impairment of erection in chronic renal failure in the rat is attributable to a disturbance in *NOS* gene expression with concomitant changes in IGF-I system. El-Sakka *et al.* [10] also observed the downregulation of IGF-1 protein expression in penile cavernosum of diabetic rats with ED. Our data further indicate that a decreased abundance of IGF-1 is involved in diabetic corpus cavernosum.

The STZ-induced diabetic rat has been used as a model for type I diabetes by several investigators in various scientific fields. ICP responses to neurostimulation are significantly attenuated in STZ diabetic rats [15]. Adenoviral vectors are a useful method for introducing genetic material into vascular tissue to alter vascular function [11, 15]. This approach has been used to improve erectile function in corpus cavernosum with functional impairments. In a study by Christ *et al.* [13], a gene therapy approach with the K^+ channel *hSlo* gene was successful in restoring erectile function in STZ-induced diabetic rats, and dose dependence and duration were evaluated. Some other genes, such as vascular endothelial growth factor (VEGF) [16], neurotrophin-3 [17], vasoactive intestinal polypeptide [18] and NOS isoforms [11], have also been used to improve erectile response in

the diabetic rat.

Accordingly, in the current study we observed a significant decrease in IGF-1 protein expression in the STZ diabetic penis 2 months after the induction of diabetes. These biochemical changes were observed at a time when erectile function was significantly attenuated, suggesting that decreases in IGF-1 expression contribute in part to ED associated with diabetes. More importantly, adenoviral mediated over-expression of IGF-1 in the corpus cavernosum of STZ diabetic rats increased IGF-1 protein expression, producing functional changes and reversing impaired erectile function in STZ diabetic rats. Over-expression of the IGF-1 supports the concept of future gene therapy trials in patients with diabetes to improve reduced erectile function. To our knowledge, this is the first study reporting the use of adenoviral mediated transfer of IGF-1 to treat ED in diabetic rats. However, there are many problems that still need to be explored, such as evaluation of the effects of the dose and duration of gene transfer.

The results show that adenoviral mediated gene transfer of IGF-1 to the corpus cavernosum restores erectile capacity to cavernous nerve stimulation in STZ diabetic rats. To our knowledge, this is the first study of the use of adenoviral gene transfer of IGF-1 to improve erectile function in the diabetic rat. In addition, the decrease in IGF-1 protein might play an essential role in the pathophysiology of diabetic related ED and IGF-1 contributes significantly to the physiology of erection. Adenoviral mediated transfer of IGF-1 could be an exciting new form of therapy for ED associated with diabetes.

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