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Gene therapy as future treatment of erectile dysfunction

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Abstract

Importance of the field—Erectile dysfunction (ED) is a major men's health problem. Although the high success rate of treating ED by phosphodiesterase 5 (PDE5) inhibitors has been reported, there are a significant number of ED patients who do not respond to currently available treatment modalities.

Areas covered in this review—To understand the current status of gene therapy application for ED, gene therapy approaches for ED treatment are reviewed.

What the reader will gain—Gene therapy strategies that can enhance nitric oxide (NO) production or NO-mediated signaling pathways, growth factor-mediated nerve regeneration or K^+ channel activity in the smooth muscle could be promising approaches for the treatment of ED. Although the majority of gene therapy studies are still in the preclinical phase, the first clinical trial using non-viral gene transfer of Ca^{2+}-activated, large-conductance K^+ channels into the corpus cavernosum of ED patients showed positive results.

Take home message—Gene therapy represents an exciting future treatment option for ED, especially for people with severe ED unresponsive to current first-line therapies such as PDE5 inhibitors although the long-term safety of both viral and non-viral gene therapies should be established.

Keywords

gene therapy; erectile dysfunction; nitric oxide; growth factor; K^+ channel

1. Introduction

It has been projected that approximately 150 million men in the world suffer from erectile dysfunction (ED), which is defined as the inability to achieve or maintain an erection of sufficient rigidity for vaginal penetration and completion of the sexual act [1 2]. The etiology of ED can be classified into two major categories: organic and psychological[3]. Major causes for organic ED include aging, particularly in men older than 50 years[4–6], cardiovascular diseases such as atherosclerosis and hypertension [7, 8] diabetes [9], and radical prostatectomy[10–12]. These causes lead to two forms of organic ED, vasculogenic and neurogenic, which often seen together in an ED patient [13].

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Although oral phosphodiesterase type 5 (PDE5) inhibitors (sildenafil, tadalafil, and vardenafil) are effective for the treatment of ED, it is not always efficacious in all patients. For example, in the clinical trials using sildenafil, the failure of treatment was often seen in men with diabetes, non-nerve sparing radical prostatectomy, and high disease severity [3, 12]. For the patients with organic causes of ED, the overall response rate to sildenafil was 68%, and lower response rates were observed in diabetic patients (for type I, 59%; for type II, 64%) and in patients who had undergone prostatectomy for prostate cancer (43%) [14, 15]. The response rate to sildenafil is also known to decreases as the patient age increases [16]. Therefore, improved therapies based on a better understanding of the fundamental mechanisms inducing ED are needed especially when the PDE5 inhibitor treatment fails.

Gene-based therapy has been proposed as one of potential new therapies for PDE5-resistant ED, and preclinical studies have been performed to examine the feasibility of gene therapy for ED induced by various causes such as aging, diabetes and cavernous nerve injury. Since the nitrergic pathway principally contributes to cavernous smooth muscle relaxation, resulting in penile erection, genes involved in NO synthesis such as NO synthase (NOS) have been tested for potential gene therapies of ED. For the neurogenic type of ED induced by diabetes or cavernous nerve injury, genes encoding different types of neurotrophic factors, which can enhance nerve regeneration, have been proposed for ED gene therapy. In addition, K^+ channel genes, which functionally enhance relaxation of cavernous smooth muscle, have also been tested as another category of genes for ED gene therapy. The current status of gene therapies for ED has been summarized in a recent thorough review by Harraz et al. [17]. Thus, in this article, we will review and update the information on gene therapies for the management of ED, especially focusing on three major categories of gene therapies, respectively, targeting the NO system, growth factors and K^+ ion channel mechanisms, including our recent studies using HSV vector-based neurotrophic factor gene therapies in rat models of ED (Fig. 1).

2. Physiology of penile erection

Penile erection is induced by dilation of penile arteries and relaxation of smooth muscle of the corpus cavernosum that increase penile blood inflow, and venous occlusion that decreases penile blood outflow. Although the venous occlusion is achieved passively by mechanical compression of the emissary veins, smooth muscle relaxation of penile arterial and cavernous tissues is principally induced by nitric oxide (NO), which is a potent vasodilator that is generated by metabolism of arginine via neuronal NO synthase (nNOS) in the nonadrenergic, noncholinergic (NANC) nerves and endothelial NOS (eNOS) in the endothelium of penile arteries and cavernosal sinusoids [18–21]. A third isoform (inducible NOS, iNOS), which is Ca^{2+}-insensitive, also plays a role in penile erection [22]. NANC nerves that release NO are parasympathetic carried through the cavernous nerves, and activated by parasympathetic nerve stimulation. Sympathetic nerve stimulation in the cavernous nerves release norepinephrine and is responsible for cavernous smooth-muscle contraction and detumescence after orgasm [23]. NO produced in nerves or endothelial cells then diffuses to smooth muscle cells and activates soluble guanylate cyclase, resulting in increased cyclic guanosine monophosphate (cGMP) formation, which mediates smooth muscle relaxation [20]. Other molecules such as vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), substance P, and pituitary adenylate cyclase-activating polypeptide (PACAP) also play important roles in penile erection [24].

3. Modulation of the nitrergic system

3.1. NOS

It has been well documented that reduced NO production due to a significant decline in NOS expression is often associated with ED induced by aging [25, 26] or diabetes [27, 28]. Thus, restoration of endogenous NO synthesis and/or enhancement of the NO-related cascade in the penis have been regarded as potential treatments for ED[17].

Adenoviral vector-mediated overexpression of eNOS has been examined in aged or diabetic rats [29]. When recombinant adenovirus containing the eNOS gene was injected into the corpus cavernosum of aged rats, eNOS transgene expression as well as cGMP level in cavernous tissues were increased. The increase in intracavernosal pressure in response to cavernous nerve stimulation was also enhanced in eNOS-treated rats. These effects of eNOS gene therapy were detected at 1 day and 5 days after administration in aged rats injected with adenoviruses driven by the Cytomegarovirus (CMV) virus and Rous sarcoma virus (RSV) promoters, respectively [29, 30]. In diabetic rats, eNOS gene transfer using adenoviral vectors is associated with an increase in eNOS protein expression and constitutive NOS activity as well as an increase in nitric oxide biosynthesis as shown by increased levels of cavernous nitrate and nitrite formation at 1–2 days after treatment. Furthermore, in eNOS-treated rats, intracavernosal pressure (ICP) after cavernosal nerve stimulation was similar to that observed in normal animals [31]. In addition, the combination of adenoviral-mediated eNOS gene therapy and intravenous sildenafil in diabetic rats also resulted in a synergistic erectile response that is greater than either of these therapies [32]. The efficacy of eNOS gene therapy was also tested using mesenchymal stem cells (MSC) transduced with adenovirus containing the eNOS gene. When eNOS-modified MSC were injected into the penis of aged rats, the erectile response was improved in association with increased eNOS protein levels, NOS activity, and cGMP levels in corporal tissues at 7 and 21 days after injection [33].

In addition to eNOS, gene delivery of other NOS subtypes such as nNOS and iNOS into the penis also improve erectile function in rats. Magee et al. reported that the adenovirus-mediated gene transfer of an nNOS variant responsible for erection, penile nNOS was effective in stimulating the erection of aged rats at 18 days when given by electroporation, without inducing the expression of cytotoxic genes[34]. In terms of iNOS gene therapy, plasmid mediated gene transfer of iNOS has been shown to increase ICP during cavernosal nerve stimulation and enhances NOS activity in aged rats at 10 days after treatment [35]. Another study using transfection of the iNOS gene via plasmid vectors, adenoviral vectors, or adenovirus-transduced myoblast cells in healthy rats has shown that myoblast mediated transfer produced superior erectile response to cavernous nerve stimulation with enhanced iNOS activity in the cavernous tissue compared with animals treated with adenoviral or plasmid vectors alone [36].

3.2. Other gene therapies targeting the nitrergic mechanisms

3.2.1. Protein Inhibitor of NOS (PIN)—In the rat, the protein inhibitor of NOS (PIN), which binds to nNOS to suppress its activity, is expressed in the pelvic ganglion as well as the cavernosal and dorsal nerve of the penis [37]. When plasmid constructs for short hairpin RNA (shRNA) targeting PIN were injected into the penile corpus cavernosum of aged rats, an increase in intracavernosal pressure following electrical field stimulation of the cavernous nerve was raised above the value of young rats 1 month after the injection[38]. In addition, PIN mRNA and protein expression in penile tissues was decreased by >70% by the shRNA. Thus the antisense PIN gene therapy, which can enhance the nitrergic mechanism, could ameliorate ED in the aged rat[38].

3.2.2. Anti-arginase—It has been reported that NO production can be controlled not only by activities of NOS isozymes but also by activities of arginase isozymes and that inhibition of arginase activity can enhance NO-dependent relaxation of smooth muscle tissue in human penile corpus cavernosum[39]. It is also known that arginase is upregulated in the cavernous tissues of diabetic humans and animals[40, 41]. Gene transfer of anti-sense arginase via an adenoviral vector reportedly decreases arginase protein and mRNA in the penile tissues of aged mice, in association with improved cGMP production and erectile function in response to cavernous nerve stimulation [41].

3.2.3. cGMP-dependent protein kinase G1 (PKG1)—The release of NO induces elevation of cGMP levels via stimulation of guanylate cyclase activity, which leads to relaxation of smooth muscle tissue in penile corpus cavernosum, resulting in penile erection. One of the effector proteins for cGMP is cGMP-dependent protein kinase-1 (PKG-1), and PKG-1 knockout mice exhibit ED [42]. It has also been shown that PKG1 is reduced in the corpus cavernosum smooth muscle of diabetic rabbits and rats [43, 44]. Adenoviral-mediated transfer of the PKG-1α gene into the penile corpus cavernosum of diabetic rats restores PKG activity to the levels observed in control rats and improved erectile function during cavernous nerve electrostimulation [44].

4. Growth factors

4.1 Neurotrophin-3 (NT3)

Previous studies suggest autonomic neuropathy as the primary etiology for ED in men with diabetes [45, 46]. A significant decrease in NOS containing nerve fibers in the dorsal and intracavernous nerves is found in streptozotocin (STZ)-induced diabetic rats [47]. Neurotrophin-3 (NT-3) is a member of the neurotrophin gene family, and Lin et al. reported that male rat major pelvic ganglia (MPG) cultures treated with NT3 induced the most fiber outgrowth[48].

The study by Bennett et al. investigated whether herpes simplex virus (HSV) vector-mediated NT-3 delivery is applicable for the treatment of ED induced by diabetes in rats[49]. After 4 weeks, replication defective HSV vector expressing the β-galactosidase gene (HSV-LacZ) or NT-3 (HSV-NT-3) were injected directly into the cavernous nerve sheath. Four weeks later the animals underwent measurement of intracavernous pressure (ICP) during cavernous nerve electrostimulation. Staining for lacZ and NOS in the major pelvic ganglia was also performed. β-Galactosidase staining revealed lacZ positive neurons in the MPG.. Maximal ICP of the HSV-NT3 treated group was significantly higher than that of the HSV-LacZ treated group. The mean number of NOS positive neurons per section in the NT3 group was significantly higher than that in the lacZ control group. These data suggest that HSV vectors encoding neurotrophic factors such as NT3 might facilitate cavernous nerve regeneration/repair/survival and increased nNOS expression in pelvic ganglion neurons to restore erectile function in diabetic rats[49].

4.2. Glial cell derived neurotrophic factor (GDNF) and neurturin (NTN)

ED resulting from cavernous nerve injury is a major complication following extirpative surgery of pelvic organs such as the prostate, bladder and rectum. ED is an exceedingly common side effect in men receiving radical prostatectomy[50]. Surgical trauma associated with removing the prostate where neurovascular bundles run alongside can produce neuropraxia, local inflammation and other immune responses that can ultimately lead to ED. Even with nerve-sparing surgery, it takes many months to recover erectile function[11, 12].

Glial cell line-derived neurotrophic factor (GDNF) family ligand such as GDNF and neurturin (NTN) is known to be important for survival and regeneration of cavernous nerves[51, 52]. GDNF is a member of the transforming growth factor-b (TGF-β) superfamily and is well known for its activities in promoting the survival and extension of sympathetic and parasympathetic axons [53]. GDNF is also known to be expressed in the penis of adult rats and retrogradely transported in penile parasympathetic and sensory nerves[51, 52]. Recent reports also demonstrate that GDNF family receptors are expressed in penis-projecting neurons and they are involved in the survival and regeneration of these neurons[51, 54]. Neurturin (NTN), another member of GDNF family, has also been known as a target-derived survival and/or neuritogenic factor for postganglionic neurons innervating the penis [51, 54–56]. Laurikainen et al. showed that NTN mRNA was expressed in smooth muscle of penile blood vessels and the corpus cavernosum in adult rats, whereas glial cell line-derived neurotrophic factor receptor α 2 (GFRα2) and Ret (common GDNF family receptor) mRNAs were expressed in all cell bodies of the penile postganglionic neurons [55]. Furthermore, mice lacking the GFRα2 receptor have significantly less NOS-containing nerve fibers, which are responsible for penile erection, in the dorsal penile and cavernous nerves [55]. Other studies also demonstrated the role of the NTN-GFRα2 pathway in survival and regeneration for sacral parasympathetic neurons, especially for those innervating the penis, by examining their signaling pathways in these neurons[51, 54, 56]. In addition, GFRα2 and nNOS expression both decreased with age, suggesting the possible involvement of a NTN-GFRα2 pathway in the age-related alteration of nNOS expression[57]. A recent report also showed that treatment with neurturin protein at the site of cavernous nerve crush injury facilitated recovery of erectile function[58].

Based on these data, recent studies have shown the feasibility of HSV vector-mediated neurotrophic factor delivery for the treatment of ED induced by cavernous nerve injury in rats [59, 60]. GDNF and NTN were used as penile neurotrophic factors in these studies, and erectile function by measurement of ICP along with arterial pressure and nerve regeneration by a retrograde tracing study using Fluorogold (FG) following the nerve injury and the HSV vector administration was investigated.

In male rats with bilateral cavernous nerve injury induced by using a clamp and dry ice without transection, the effecte of HSV-GDNF and HSV-NTN (1.0×10^5, 10^7 and 10^9 pfu) administered around the damaged nerve after the injury were evaluated. HSV vector expressing green fluorescent protein (GFP) and LacZ (HSV-LacZ) were used as a control vector. ICP during pelvic nerve stimulation was measured at 2 and 4 weeks after the nerve injury. FG was injected into the penile crus 7 days before histological experiments. Approximately 60% of MPG cells were GFP-positive after the viral administration, indicating that the inserted genes were well transported to MPG. ICP of the groups which received the high titer HSV-GDNF and HSV-NTN exhibited significant recovery of ICP compared with the HSV-LacZ group or HSV-untreated group at 4 weeks after the nerve injury (Fig. 2). However, since the post-treatment ICP value did not reach the control level, further studies may be needed to evaluate whether the increased ICP level after GDNF or NTN gene transfer would be sufficient for intercourse. The high titer HSV-GDNF and HSV-NTN groups had more FG-positive cells in MPG than the HSV-LacZ group, indicating that delivery of these vectors is capable of rescuing the nerve damage [59, 60]. These data suggest that administration of HSV vector-mediated neurotrophic factors such as GDNF or NTN around injured cavernous nerves could hasten the process of nerve regeneration and promote the recovery of erectile function after cavernous nerve injury.

4.3. Brain derived neurotrophic factor (BDNF)

Brain derived neurotrophic factor (BDNF) is known to enhance nerve growth. In rats with cavernous nerve injury induced by freezing, adenoviral vector-mediated BDNF gene transfer

into the corpus cavernosum exhibited significantly higher ICP during cavernous nerve stimulation and had greater nNOS staining in major pelvic ganglion neurons compared with the control gene (LacZ)-treated group [61]. It is also shown that adenoviral BDNF gene transfer is effective to restore erectile function in response to cavernous nerve stimulation and reduce neural and vascular damages of cavernous tissues in a rat model of hypercholesterolemia induced by high fat diet [62].

4.4. Vascular Endothelial derived Growth Factor (VEGF)

Vascular endothelial growth factor (VEGF) is a multifunctional protein that stimulates angiogenesis and increases vascular permeability. Previous studies showed that VEGF treatment could restore erectile function in animal models of ED such as hypercholesterolemic rabbits and diabetic rats[62–65]. In diabetic rats, transfection with DNA encoding VEGF into the corpus cavernosum showed increased smooth muscle content as well as improved ICP during cavernous nerve electrostimulation compared to controls [66]. In another study using diabetic rats, adenovirus vector mediated gene transfer VEGF and another angiogenic factor, angiopoietin-1, into the corpus cavernosum has been demonstrated to completely restore erectile function during electrical stimulation of the cavernous nerve in association with increased angiogenesis, eNOS phosphorylation, and cGMP expression in cavernous tissues 2 and 8 weeks after treatment while intracavernous injection of either angiopoietin or VEGF adenoviral vector alone elicited partial improvement [67]. Intracorporal treatment with adenoviral VEGF has also been shown to prevent castration-associated ED in rats[68].

4.5. Insulin-like growth factor-1 (IGF-1)

It has been demonstrated that IGF-I can enhance regeneration of NOS-containing nerves after cavernous nerve injury in rats [69, 70]. In addition, expression of IGF-1 has been shown to be reduced in cavernous tissues of diabetic rats[71]. Adenoviral vector-mediated gene transfer of IGF-1 into the corpus cavernosum in diabetic rats reportedly improves erectile function during cavernous nerve stimulation in association with increases expression of IGF-1 mRNA and protein in cavernous tissues [72].

5. Modulation of K^+ channels

K^+ ion channels are important to stabilize the membrane potential and reduce the excitability of nerves and muscle cells including penile smooth muscle cells [73, 74]. Thus the feasibility of gene transfer of K^+ channels for the treatment of ED has been investigated in animals as well as humans.

5.1. Large-conductance Ca^{2+}-activated potassium channel (BK or Maxi-K)

Ca^{2+} activated K^+ channels are one of major groups of six/seven transmembrane potassium-selective channels. These channels are divided into several groups according to the genetic, electrical and structural properties of each channel[75]. Large-conductance, Ca^{2+} activated K^+ (BK or Maxi-K) channels, which are activated by membrane potential depolarization, increases in cytosolic Ca^{2+} concentration and/or NO/cGMP mediated mechanisms, contribute to hyperpolarization of smooth muscle cell membranes, resulting in a decrease in calcium influx and penile corporeal muscle relaxation[75–77]. Several previous studies by the Christ and Melman's group investigated the efficacy of intracavernous gene transfer of naked hSlo cDNA that encodes the human BK channel α-subunit for the treatment of ED. The hSlo cDNA was inserted into a mammalian plasmid, where expression is driven by the cytomegalovirus promoter. The plasmid cannot replicate itself, but is designed to efficiently replicate the inserted DNA sequence in the presence of the appropriate enzymes and substrates in the host nucleus. The plasmid containing the desired DNA sequence (i.e., hSlo

DNA) enters the nucleus of the host cell, and transcribe the desired mRNA, leading to production of a functional BK channel protein [78]. In aged or diabetic rats, intracavernous injection of the plasmid containing hSlo DNA induced significant elevation in ICP in response to cavernous nerve stimulation in association with the overexpression of hSlo in cavernous tissues for up to 4 months after the treatment[79–81]. More recently, the same group has reported that intracavernous injection of a smooth-muscle-specific gene transfer vector (pSMAA-hSlo) encoding the pore-forming α-subunit of the human large-conductance, Ca^{2+}-sensitive K^+ (BK) channel also induces increased ICP response to cavernous nerve stimulation in aged rats[82] or an improvement of sexual behavior and papaverine-induced erectile responses in male cynomolgus monkeys with ED secondary to diet-induced atherosclerosis[83].

To date, a phase 1 safety clinical trial using the plasmid containing hSlo DNA has been completed, and this is the first and only clinical trial of gene therapy for humans with ED [84, 85]. The trial was an open-label, sequential, single-dose, intracavernous instillation of seven doses of the hSlo plasmid in 20 men with moderate-to-severe erectile dysfunction as defined by the international index of erectile function (IIEF) score. The recent review by Melman et al. [86] summarized the encouraging results of the trial as follows: (1) of greatest importance in a phase 1 trial, that is, the primary endpoint, safety, showed no serious short or long-term (2 years) transfer-related adverse events, (2) there was no plasmid evident in the semen of the participants, (3) there were no clinically relevant changes in any of the physical or chemical parameters measured including ECG. and (4) in two of the men who responded with improved erections after transfer, the response lasted for 6 months as in the preclinical trials in aged or diabetic male rats.

5.2. ATP-sensitive K^+ channels (K_{ATP})

ATP-sensitive K^+ channel (K_{ATP}) is also involved in the control of smooth muscle tone. A previous study has shown using isolated penile corporal tissue strips from diabetic patients have shown a significant decrease in the relaxation responses to $\boldsymbol{K_{ATP}}$ channel modulators such as pinacidil and levcromakalim [87]. Thus a recent study examined the efficacy of intracavernous injection with naked cDNA of the $\boldsymbol{K_{ATP}}$ channel gene (Kir6.1 + SUR2B or Kir6.2 + SUR2B) for erectile function in aged rats. The $\boldsymbol{K_{ATP}}$ channel gene transfer enhanced ICP responses to cavernous nerve stimulation compared to age-matched control rats, which were associated with an increase in cavernosal levels of K $\boldsymbol{K_{ATP}}$ channel mRNA (Kir 6.1 and 6.2)[88].

6. Viral vs. non-viral gene therapy

Both non-viral and viral delivery vehicles have been employed to deliver genes to the penis and other tissues. Each delivery system has its own advantages and disadvantages for delivery to different target cells and tissues. Overall, a major advantage of the non-viral delivery systems has been the low immunogenicity of this approach compared to viral vectors which all show some level host response to the vector itself which may only amplify any host response to the therapeutic gene. In addition, the non-viral systems generally exhibit the short-term nature of vector-delivered transgene expression due to the limited maintenance of the delivery vehicle in the transduced cells. On the other hand, viral vectors are more efficient at delivering their genetic payload to the target cell compared to non-viral systems because over millennia viruses have acquired efficient methods to deliver their own genetic material to cells in order to replicate their genomes and further propagate themselves. Another advantage is that most viral vectors can be readily produced and purified for in vivo gene transfer while the non-viral methods are limited by production of sufficient quantities of DNA for transduction [89]. However there are potential risks with viral

vectors that have limited the use of viral vectors in humans, such as endogenous viral recombination, cancer development and immunological reactions[90].

In addition, HSV vectors have several significant advantages over other viral vectors for the treatment of peripheral nervous system disorders. Replication-defective recombinant vectors, which lack multiple essential gene functions and are non-toxic in vivo[91–94] have been generated to increase the overall safety for clinical therapeutic applications. These vectors can be prepared to high titer and purity without contamination from wild-type [95–97]. Thus, delivery of the replication defective HSV vector to neurons innervating the site of vector delivery at the time of surgery could reduce the complications associated with systemic delivery of trophic factors, as the vector would express the neurotrophin solely within the targeted neurons [59, 60].

7. Expert opinion

The high success rate of treating ED has been reported because of the recent advances of new oral therapies including PDE5 inhibitors. However, there are still a significant number of ED patients who do not respond to currently available treatment modalities, especially severe ED cases with diabetes, aging or cavernous nerve injury, for whom new treatment alternatives are of great demand. Thus the possibility of gene therapy application for ED has been explored in the last decade (Fig. 1). However, there are significant safety concerns that delay the clinical translation of gene therapy modalities to human patients. The recent clinical study using non-viral gene therapy of the Ca^{2+} activated BK (Max-K) channel for ED patients shows great promise for future development of gene-based therapy of ED. In addition, the phase I safety studies using non-replicating HSV vectors encoding human enkephalin, an opiate gene, are underway in patients with cancer pain to elucidate the safety of locally applied viral gene therapy[98, 99]. Thus it is hoped that the long-term safety of both viral and non-viral gene therapy can be established in near future before large scale human studies are considered. Overall, gene therapy for ED represents an exciting future treatment option, especially for people with severe ED unresponsive to current first-line therapies such as PDE5 inhibitors.

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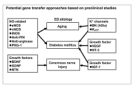

Figure 1. Summary of potential targets of ED gene therapy

Potential gene transfer approaches targeting the nitric oxide (NO) system, growth factors, K^+ channels that are discussed in this review are summarized. Based on the results of preclinical studies, the candidate genes for different ED etiologies such as aging, diabetes mellitus or cavernous nerve injury are connected by arrows.

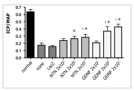

Figure 2. Intracavernous pressure (ICP) responses at 4 weeks after the bilateral cavernous nerve injury

Rats treated with higher titers (2×10^5 and 10^7 pfu) of HSV-GDNF or NTN exhibited significant recovery of ICP along with mean arterial pressure (ICP/MAP) compared with the HSV-LacZ control vector group or HSV-untreated nerve-injured group at 4 weeks after the nerve injury. Asterisk indicates $P<0.05$ versus "none" (virus-untreated group) by student's t-test and ANOVA. Cross mark indicates $P<0.05$ versus "LacZ" (HSV-LacZ group) by student's t-test and ANOVA.