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·*Review*·

Gene therapy and erectile dysfunction: the current status

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Abstract

Current available treatment options for erectile dysfunction (ED) are effective but not without failure and/or side effects. Although the development of phosphodiesterase type 5 (PDE5) inhibitors (i.e. sildenafil, tadalafil and vardenafil) has revolutionized the treatment of ED, these oral medications require on-demand access and are not as effective in treating ED related to diabetic, post-prostatectomy and severe veno-occlusive disease states. Improvement in the treatment of ED is dependent on understanding the regulation of human corporal smooth muscle tone and on the identification of relevant molecular targets. Future ED therapies might consider the application of molecular technologies such as gene therapy. As a potential therapeutic tool, gene therapy might provide an effective and specific means for altering intracavernous pressure "on demand" without affecting resting penile function. However, the safety of gene therapy remains a major hurdle to overcome before being accepted as a mainstream treatment for ED. Gene therapy aims to cure the underlying conditions in ED, including fibrosis. Furthermore, gene therapy might help prolong the efficacy of the PDE5 inhibitors by improving penile nitric oxide bioactivity. It is feasible to apply gene therapy to the penis because of its location and accessibility, low penile circulatory flow in the flaccid state and the presence of endothelial lined (lacunar) spaces. This review provides a brief insight of the current role of gene therapy in the management of ED. *(Asian J Androl 2007 Jan; 9: 8–15)*

Keywords: gene therapy; nitric oxide synthase; erectile dysfunction; calcium-sensitive potassium channel; vascular endothelial growth factor; calcitonin gene-related peptide

1 Introduction

Erectile dysfunction (ED) has been broadly defined as the inability to achieve or maintain an erection sufficiently rigid for satisfactory sexual intercourse [1]. ED is estimated to affect more than 100 million men worldwide [1]. ED is associated with multiple risk factors including smoking, hypertension, hyperlipidemia, vascular disease and diabetes (some of these features are part of the metabolic syndrome) [1–4]. The development of phosphodiesterase type 5 (PDE5) inhibitors (i.e. sildenafil, tadalafil and vardenafil) has revolutionized the treatment of ED [1]. Orally administered medications are effective

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in the majority of ED cases and are less invasive compared with other modes of treatment, such as intracavernosal injection/intraurethral administration of alprostadil. Furthermore, they confer more spontaneity than vacuum devices [1]. However, these oral medications are not without side effects, which is one of several causes of discontinuation [5–7]. The drugs require on-demand access and have limitations in their effectiveness in treating ED related to diabetic, post-prostatectomy and severe veno-occlusive disease states with only 50% to 60% of these cases benefiting from PDE5 inhibitor therapy [8]. PDE5 inhibitor failure can cause considerable distress in relationships if the drug is perceived to be the only effective and/or acceptable treatment for ED [9]. This has prompted the development of new approaches, including gene-based therapy for ED, which might also maintain the long-term efficacy of PDE5 inhibitors.

Gene therapy has gained acceptance as a possible treatment modality in diseases such as cancer and inborn errors of metabolism, and has been evaluated in several clinical trials, and preclinical studies in animal models [10–13]. As a therapeutic tool in treating ED, it might provide a safe, effective and specific means for altering intracavernous pressure (ICP) "on demand" without affecting resting penile function. It might also "cure" underlying conditions in ED, including fibrosis [14]. It is feasible to apply to the penis because of its location and accessibility, low penile circulatory flow in the flaccid state and the presence of endothelial lined (lacunar) spaces [8]. Figure 1 summarizes the sequence of events associated with use of gene therapy for the treatment of ED.

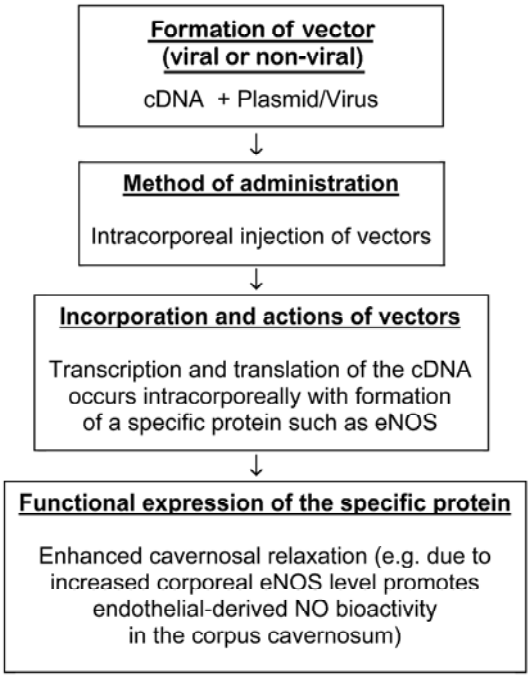

Figure 1. Schematic diagram summarizing use of gene therapy to treat erectile dysfunction (ED). NO, nitric oxide; NOS, nitric oxide synthase; eNOS, endothelial NOS.

2 Vectors of gene transfer: viral and nonviral

The concept of gene therapy entails transferring genetic material to the target cell or tissues using viral and nonviral vectors [8]. Viral vectors include retrovirus and adenovirus, whereas nonviral vectors include naked DNA and cationic liposomes. Viruses are generally very efficient gene-transfer vehicles, unlike nonviral vectors [8]. However, viral vectors are limited in usage because of potential induction of mutagenesis and carcinogenesis [14]. Also, reduction/disappearance of transgene expression occurs with repeated administration of a viral vector as a result of induced immune response [8]. Second generation (helper-dependent) adenovirus vectors have been used to reduce cellular toxicity and immune response as well as to increase efficiency [14]. Nonviral vectors were developed to overcome problems associated with viral gene delivery. They confer low immunogenicity, unlike viral vectors, but lack organ or cell specificity, which limit their application in gene therapy [15]. Recently, a water-soluble lipopolymer was evaluated as a potential gene carrier to the corpus cavernosum [16]. The advantages and disadvantages of viral and nonviral vectors are summarized in Table 1. In addition, *ex vivo* gene therapy, combining gene transfer with stem cell implants and using transformed endothelial cells injected intracorporally [17] as well as directly implanting muscle cells into the penis have been attempted [18]. These new approaches require extensive research prior to clinical application.

Studies involving urogenital organs, including the penis, have used different genes [19–21]. However, nitric oxide synthase (NOS) is the main focus for gene therapy to treat ED (see section 3).

This review briefly considers the latest advances in ED gene therapy.

3 Gene therapy and NOS

Table 1. The advantages and disadvantages of viral and nonviral vectors.

	Viral vectors (e.g. retrovirus, adenovirus)	Nonviral vectors (e.g. naked DNA, cationic liposomes)
Advantages	1) Very efficient gene-transfer vehicles	1) Low immunogenicitys
	2) Can accommodate large transgenic sequence (e.g. with Human Simplex Virus)	2) non-pathogenicity
		3) Locally restricted treatment for a local problem
		4) No detectable local or systemic side effects
		5) Relatively long-lasting (e.g. with "naked" DNA; up to 4 months)
		6) Low oncogenic potential
Disadvantages	1) Mutagenesis	1) Not as efficient as gene-transfer vehicles (low integration rate) compared to viral vectors
	2) Carcinogenesis	
	3) Induction of immune response	2) Lack organ or cell specificity

The modulation of the synthesis of nitric oxide (NO), the main mediator of penile erection, is an attractive target for gene therapy. The synthesis of NO is catalyzed by NOS. NO activates soluble guanylate cyclase in the cytoplasm of the corpus cavernosum, which leads to elevation of intracellular cyclic guanosine monophosphate (cGMP) concentrations. The elevated cGMP levels activate protein kinase G. This causes a reduction in intracellular calcium levels, which inhibits cavernosal contraction by preventing the calcium-dependent activation of myosin light chain kinase [21]. Experiments using animal models show that the content and/or enzyme activity of penile NOS is significantly reduced in diabetes and aging [22–25]. Therefore, increasing penile NOS content, which can be achieved by gene therapy with NOS constructs, might be a viable therapy for ED.

There are three NOS isoforms: endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) [14]. All three have been investigated as a potential gene therapy to modulate the erectile response.

The penis is essentially a modified vascular tissue. Therefore, it is not surprising that initial promising gene therapy studies investigating NOS in non-penile vascular tissues have crossed over to studies on the penis in ED. In the case of eNOS, one of the early studies was the application of a construct of the bovine eNOS cDNA into the rat carotid artery submitted to balloon injury of the endothelium [26]. In the study, inhibition of the neointimal vascular lesion was demonstrated. Similar findings were confirmed in other studies [27, 28] in rats and pigs. Furthermore, Chen *et al.* [29] found hyperexpression of eNOS in the vascular adventitia and endothelium of canine basilar artery incubated with adenoviral construct of eNOS. This was associated with inhibition of the contractile response of the artery *in vitro*. This beneficial effect of eNOS gene transfer is similarly shown in the corpus cavernosum, where it partially restores NO synthesis and erectile function in streptozotocin (STZ)-induced diabetic rats [30]. In the study, the peak and total ICP to cavernous nerve stimulation were significantly increased in the diabetic rats to a value similar to that in control rats. This physiological improvement in erection through eNOS gene transfer is not only confined to diabetes but has also been demonstrated in penile organs of age-related ED in the rat [31]. Bivalacqua *et al.* [31] showed an enhanced expression of eNOS by transfection using an adenoviral vector in the aged rat. There was a significant increase in the erectile response to cavernosal nerve stimulation, similar to the response observed in younger rats. Interestingly, the overexpression of eNOS following gene transfer together with sildenafil enhanced cavernosal responses to cavernosal nerve stimulation in the STZ-diabetic rat, which was similar to the response observed in the controls. More importantly, the total erectile response was greater in diabetic rats receiving eNOS gene therapy plus a PDE5 inhibitor than in the rats receiving sildenafil or eNOS gene therapy alone [32]. Similar findings were demonstrated in aged rats with concomitant gene therapy with eNOS transfection and a PDE5 inhibitor [33]. All these findings suggest that eNOS contributes significantly to the physiology of penile erection and indicate that *in vivo* adenoviral gene transfer of eNOS can be beneficial to treat diabetic and age-related ED. In addition, gene therapy

with eNOS constructs can be an option to treat ED when monotherapy fails and might offer a solution to delay PDE5 inhibitor failure and to maintain the efficacy of these drugs.

Adenovirus-mediated iNOS gene transfer has been shown to cause significant NO synthesis in vascular smooth muscle [34] and inhibition of apoptosis in hepatic tissue [35]. Ding *et al.* [36] showed that antisense knockdown of iNOS inhibits induction of experimental autoimmune encephalomyelitis in SJL/J mice. However, Garban *et al.* [37] were among the first to demonstrate that gene therapy using iNOS is feasible to treat ED. Their aim was to determine if gene therapy with rat penile iNOS construct could restore the age-related decline in the ICP response observed in 20-month-old rats, when compared to 5-month-old rats. Rat penile iNOS cDNA (i.e. "naked" DNA) was injected intracorporally. A significant enhancement in the cavernous nerve-stimulated ICP was noted for up to 10 days post-injection of the iNOS construct. The plasmid iNOS cDNA was detected in the rat penile DNA preparation by polymerase chain reaction (PCR), and iNOS hyper-expression was shown by reverse transcription PCR and Western blot. These results suggest that the low basal expression of iNOS in the penis of old rats might be increased by gene therapy with iNOS constructs. Birder *et al.* [38] found that NO was liberated into the culture medium following transfection of cultured myoblasts with iNOS, which could be measured directly by a porphyrinic microsensor. Tirney *et al.* [39] and Chancellor *et al.* [18] showed that myoblast-mediated gene therapy was more successful for delivering iNOS into the corpus cavernosum than direct adenovirus injection or plasmid transfection using a rat model. In their studies, iNOS gene expression in the rat penis was time-dependent, being maximal at day 4 following injection. Furthermore, the maximal ICP response to nerve-stimulation was elevated 2-fold, but the basal, or resting, ICP was also 10-fold greater in the rats with the iNOS transgene. These studies indicate that physiologically relevant amounts of iNOS can be delivered through penile injection either directly packaged with adenovirus, or indirectly through a shuttle vector/cell type (i.e. the myoblast cells). In addition, iNOS cDNA can be use as a potential antifibrotic agent to reverse fibrotic changes that impair cavernosal function because gene transfer of iNOS cDNA regressed the fibrotic plaque in a rat model of Peyronie's disease [40]. However, iNOS is not widely considered a gene target because, unlike nNOS and eNOS, it is not known to participate in the physiological control of penile erection [14].

A variant of the nNOS isoform, named PnNOS (for penile nNOS), is present in the rat and human penis [41, 42]. This nNOS variant is different from the one expressed in the CNS. In most rat models, ED was accompanied by a decrease of the activity of NOS [43–45]. However, only under chronic conditions [45–47] was this decrease in activity accompanied by a similar reduction in the content of nNOS. This is in contrast to eNOS levels, which remain constant [48]. Studies suggest that PnNOS (exists in alpha and beta forms) is probably the NOS isoform responsible for erectile neurotransmission. Its beta form survives in the nNOS knockout mouse [49], because PnNOS has been detected in the corpus cavernosum of this animal at the same level when compared with the wild type mouse [42]. Therefore, nNOS is a good candidate for gene therapy of ED and, in particular, PnNOS [41 ,42] because of the tissue-specific control of its enzyme activity. This was confirmed by Magee *et al.* [50] who showed that intracavernosal gene therapy with PnNOS construct in a helper-dependent adenovirus rectified the aging-related ED for at least 18 days following treatment. Combination with tissue-specific promoters and adenoviral or adeno-associated virus constructs of the cDNA for the beta form might be more efficient than the alpha form of PnNOS for gene therapy for ED as the former might be more insensitive to endogenous inhibition by the "Protein Inhibitor of nNOS" (PIN) [14]. Interfering with the binding and subsequent NOS inhibition of PIN on the alpha form of PnNOS by gene therapy provides a promising target for therapeutic stimulation of NO synthesis in the penis. Already, evidence shows that counteracting PIN by gene therapy is effective in treating ED in the aging rat model that exhibits both neurogenic ED and corporal veno-occlusive dysfunction [14].

4 Gene therapy and the human calcium-sensitive potassium channel subtype

Potassium (K) channels are modulators of human corporal smooth muscle tone, by their ability to modulate corporal smooth muscle membrane potential, transmembrane calcium flux, and, therefore, the free intracellular calcium concentration [51–53]. The calcium-sensitive, maxi-K channel [51–53] is one of the most prominent K currents present in human corporal smooth

muscle cells. The K channel is encoded by the *hSlo* cDNA gene.

Christ *et al.* [54] were able to prevent the age-related decline in erectile capacity in rats following intracavernous injection of naked *hSlo* cDNA. Essentially, a piece of DNA, encoding the alpha-subunit of the maxi-K channel is inserted in a mammalian plasmid. A plasmid is a circular, double stranded piece of DNA that contains all of the essential genetic machinery to ensure the replication of the inserted sequence. 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 is the "vector" for transporting the inserted DNA into the host cell. Eventually, the plasmid containing the desired DNA sequence is able to get into the nucleus of the host cell, and use the available nuclear genetic and enzymatic machinery to transcribe the desired messenger RNA (mRNA), which eventually results in production of a functional maxi-K channel protein. Certainly, similar strategies would apply for the incorporation and expression of any gene of interest, as illustrated in Figure 1.

In the study by Christ *et al.* [54], the *hSlo* cDNA/pcDNA transfection was sustained for at least 2 months and was also measurable with an increased ICP response to electrical field stimulation. Christ *et al.* [55] were also able to show similar effects in diabetic rats following intracorporal injection of hSlo cDNA. The gene transfer restored erectile capacity in the diabetic rats *in vivo*. The overexpression of *hSlo* was associated with increased cavernous nerve-stimulated ICP responses compared with responses in corresponding control animals. These studies clearly document that maxi-K channel therapy works and can restore both age-related and diabetic-induced decline in erectile capacity observed in rats [54, 55]. Recently, gene transfer with hMaxi-K was safely administered to men with ED without adverse events in the first human trial for gene transfer (intracavernous) therapy with the maxi-K channel for the treatment of ED [56]. This is a phase 1 trial and we await data on clinical efficacy before we can propose any role of maxi-K channel as a gene target to treat ED.

5 Gene therapy and vascular endothelial growth factor (VEGF) and other molecular targets

The causes of ED are most often associated with alterations in blood flow to or from the penis. Gene therapy with vasculogenic/angiogenic agents is proposed to treat ED where vascular insufficiency is so severe as to produce a problem with vascular perfusion to the erectile tissue of the penis because VEGF therapy in laboratory animals or humans with peripheral arterial disease produces increases in tissue vascularity [57]. Four previously described VEGF isoforms have been detected in both rat and human corporal tissue [58]. The identification of the relevant human VEGF isoforms enables genetic manipulation of VEGF in the penis as treatment for ED. Gholami *et al.* [57] showed that ED as a result of neurological and vascular changes related to hyperlipidaemia seems to be alleviated by VEGF as well as adeno-associated virus mediated brain derived neurotrophic factor given intracavernously in rats. In addition, intracavernosal VEGF injection and adeno-associated virus-mediated VEGF gene therapy were also found to prevent and reverse venogenic ED in rats [58]. These studies indicate that intracavernous injection of either VEGF protein or VEGF gene might be a preferred therapy to preserve erectile function in hyperlipidaemic as well as venogenic-related ED patients in whom testosterone therapy is contraindicated.

Nerve growth factor and neurotrophin-3 (NT3) are neurotrophic factors that might protect nerves from mechanical and metabolic damage. Benett *et al.* [59] investigates the effects of herpes simplex virus (HSV)-mediated delivery of NT3 for the treatment of diabetic ED using a rat model. ED improved after NT3 gene therapy in diabetic rats (STZ-induced) with a significant increase in maximal ICP induced by electrical stimulation compared with controls. They conclude that gene therapy for the treatment of diabetic ED is feasible with HSV vectors and that NT3 gene therapy might be applicable for the treatment of ED associated with diabetes.

Vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) are both peptide neurotransmitters localized in the corpora cavernosa. Studies indicate possible involvement of both neurotransmitters in modulating cavernosal relaxation in erection [60, 61]. They are downregulated in diabetes and the aging rat penis, respectively [62, 63]. Two separate studies demonstrate that intracavernous injection with VIP cDNA and adenoviral-mediated gene transfer of prepro-CGRP (AdRSV-CGRP) improves erectile response in the diabetic and aged rat, respectively. VIP cDNA is easily incorporated into the corpus cavernosum, and the expression is sustained for more than 2 weeks in the dia-

betic rat penis (*in vivo*). Therefore, VIP and CGRP could be potential targets for gene therapy to treat diabetic and age-related ED, respectively.

PDE5 catalyses the degradation of cGMP, which promotes erectile response to sexual stimulation. Therefore, inhibiting the enzyme enhances cGMP action and, hence, promotes erection. This enzyme provides an attractive target for gene therapy to treat ED. In an *in vitro* study, PDE5 gene antisense oligodeoxynucleotide (ASON) was transfected into human corpus cavernosum smooth muscle cells [64]. Following transfection, the level of cGMP in smooth muscle cells was significantly higher than that in controls [64]. In addition, Lue *et al.* [65] demonstrate that a specific small interfering RNA (siRNA) could downregulate PDE5, resulting in prolonged cGMP accumulation and improved erection in rats. These promising findings provide future experimental groundwork for the gene therapy of ED using the PDE5 gene ASON or by silencing PDE5 using the siRNA.

eNOS suppressed by RhoA/Rho-kinase and erectile response to cavernosal nerve stimulation is impaired in the diabetic corpus cavernosum [66]. Bivalacqua *et al.* [66] demonstrated that inhibition of RhoA/Rho-kinase by transfection of the STZ-diabetic rat penis with an adenoassociated virus encoding the dominant-negative RhoA mutant (AAVTCMV19NRhoA) restored cavernosal eNOS protein, constitutive NOS activity, and cGMP levels to those found in control rats. Also, the AAVT19NRhoA gene transfer improved erectile responses in the STZ-diabetic rat to values similar to control. Therefore, erectile function in diabetes can be restored by gene therapy targeting RhoA/Rho-kinase.

6 Conclusion

Gene therapy for ED is still in its infancy. However, most gene-based strategies for the treatment of ED show apparent preclinical success. The fact that intracavernous injection and cellular incorporation of naked DNA leads to the subsequent expression of functional protein [54, 67] is an important discovery. This obviates the necessity for using an adenoviral or retroviral vector for the treatment of ED. Furthermore, the use of "naked" DNA would have the additional benefit of minimizing the possibility of insertional mutagenesis when using more aggressive vector-based gene therapy treatments [68]. Like any new therapy, a bottleneck might hamper further progression or development. Technical obstacles exist in the identification of specific strategies in finding the best safety profile, the greatest specificity for altering ICP "on demand" and the longest half-life of the protein targets in the gene therapy of ED. Furthermore, restrictions in the clinical development of gene therapy lie in the optimization of the safety, specificity and longevity of relevant protein targets used. Gene therapy would represent a major advance in the treatment of ED if successful. Given the encouraging findings of preclinical studies reviewed here the future of gene therapy for ED is promising.

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