

REVIEW

Gene transfer for the therapy of erectile dysfunction: progress in the 21st century

A Melman¹

Department of Urology, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, USA

Gene transfer represents the next potential era of advancement in medicine for the prevention of the effects of aging or for treatment of genetic or acquired disease. For gene transfer to be a practical successor to today's oral and minimally invasive therapies, the product must have a high safety profile and a long duration of effectiveness to correct the need for on-demand administration. Several types of vectors have been used in preclinical studies, but because of widely publicized adverse events, progress using viral vectors in humans has been limited. There is a current phase I human trial using naked DNA as the vector with the maxi-K gene to modify cellular contractility. Preliminary results in the safety trial thus far have shown no treatment-related adverse events, no transfer to the semen, and the possibility of efficacy in one participant.

International Journal of Impotence Research advance online publication, 10 November 2005; doi:10.1038/sj.ijir.3901412

Keywords: gene therapy; gene transfer; ED; naked DNA; hMaxi-K; potassium channels; smooth muscle

Introduction

Gene transfer represents the next potential era of advancement in medicine for the prevention of the effects of aging or for treatment of genetic or acquired disease. The concept of gene therapy is that the intermittent or single addition of a selective gene causes the body to manufacture natural proteins using the patient's own cells as minibioreactors.¹ These naturally produced proteins can correct the abnormalities of cellular function and can correct abnormal physiological function. This presentation summarizes past attempts at gene transfer and introduces an exciting new gene transfer program that is currently in human trial.

The current era of medical therapy for the treatment of erectile dysfunction (ED) began in 1982 with the discovery that the intracavernous

injection of the nonspecific phosphodiesterase inhibitor (PDE) papaverine could induce erection.² Intraurethral, intracavernous, and oral therapy with vasodilating agents were developed and were adopted rapidly into the clinical armamentarium.³⁻¹² The problem with each of these treatments is that they must be used on demand, thereby reducing the spontaneity of the sexual act. Furthermore, the most frequently used drugs, the oral PDEs, are effective in only approximately 60% of men, have significant side effects, and are contraindicated in the presence of certain diseases or concomitant medications.^{13,14} The recent report of nonarteritic anterior ischemic optic neuropathy in a small number of men with diabetes who were using the PDEs has created a potential for reduced demand in the use of that family of drugs in men who need them the most.¹⁵

Given the limitations of the current therapies, there is a great potential for advancement in using gene transfer for therapy of ED and other potential genitourinary smooth muscle diseases. The safe and efficient use of vectors that allow delivery of the gene into the cells of interest (e.g., viruses and plasmids), allowing the new gene to bypass the body's natural defense mechanisms, are the challenge to the field. Serious adverse events, including death, caused by the host response to viral vectors used to gain entry of the recombinant DNA into the cells of interest thus far have limited

Correspondence: Dr A Melman, Department of Urology, Montefiore Medical Center/Albert Einstein College of Medicine, 3400 Bainbridge Avenue, Fifth Floor, Bronx, NY 10467-2490, USA.

E-mail: amelman@montefiore.org

¹The author is a directing member of Ion Channel Innovations, LLC the sponsor of the clinical trial of hMaxi-K

Received 24 August 2005; revised 31 August 2005; accepted 1 September 2005

the widespread interest and progress in the field. In particular, for non-life-threatening applications in urologic diseases such as ED, pharmaceutical industrial interest has been quiescent. Therefore, progress in the field has been left to the urological laboratories in several medical centers with long-standing interest in ED.^{16–24} To date, the reports are from studies in the preclinical, early stage that use a variety of vectors and genes. The response to the transfer for the most part has been measured within days to a few weeks after transfer. A representative summary of these studies is shown in Table 1.

For gene transfer to be a practical successor to today's oral and minimally invasive therapies, the product must have a high safety profile and a long duration of effectiveness to correct the need for on-demand administration. The penis is an organ uniquely suitable for gene transfer because of its

anatomic features, its ultrastructural features, and the presence of potassium channels within it.

Suitability of the penis for gene transfer

Anatomic features

The most obvious reason why the penis is ideally suited for gene transfer is that it is an external organ that is readily accessible and whose anatomy permits a tourniquet to be placed at its base. The tourniquet limits any potential biodistribution of gene to other organs, thus diminishing the potential objection to theoretical problems related to direct intravenous injection of a gene product. Second, the penile portion of the paired corpora cavernosa is in direct communication with itself through the fenestrated midline septae. Therefore, an injection

Table 1 Comparison of gene therapy studies for ED

<i>Authors, year, and reference no.</i>	<i>Vector</i>	<i>Gene</i>	<i>Animal model</i>	<i>Duration</i>
Rogers <i>et al</i> (2003) ¹⁶	1. Recombinant VEGF protein 2. AAV 3. AAV- <i>LacZ</i>	1. VEGF 2. <i>LacZ</i>	Male Sprague–Dawley rats 3–6 months of age (350–450 g)	1 mo after administration
Chancellor <i>et al</i> (2003) ¹⁷	1. Plasmid 2. Adenovirus 3. Adenovirus-transduced myoblast cells	1. iNOS	Adult male Sprague–Dawley rats (250–400 g); no disease	2, 4, or 7 d after administration
Champion <i>et al</i> (1999) ¹⁸	1. Replication-deficient recombinant adenovirus	1. eNOS gene (AdCMVeNOS) 2. Stereotype 5-encoding nuclear-targeted β -galactosidase (AdCMV β -gal)	Male 40-week-old Sprague–Dawley rats (350–400 g); no disease	1 d after adenovirus administration
Garban <i>et al</i> (1997) ¹⁹	1. Plasmid (exogenous iNOS cDNA contracts)	1. iNOS inducers	5-, 20-, and 30-mo-old male Fischer 344 rats	3–21 d after administration
Magee <i>et al</i> (2002) ²⁰	1. Plasmid cDNA construct (pCMV-PnNOS) 2. Helper-dependent adenovirus (Adv-CMV-PnNOS)	1. β -galactosidase 2. PnNOS	Male Fischer 344 rats: young rats (5 mos old), aged rats (24 mos old), and retired breeders (9–11 mos old) were used	1. Assessment of β -galactosidase expression was estimated 11 d after administration 2. After 18 days for PnNOS administration
Bivalacqua <i>et al</i> (2001) ²¹	1. Adenoviral-mediated transfer (AdRSVCGRP) 2. Adenovirus (AdRSV β gal)	1. Prepro-CGRP 2. Nuclear-targeted β -galactosidase	12-wk (225–300 g) and 60-wk-old (450–550 g) Brown Norway rats	5 d after administration
Bivalacqua <i>et al</i> (2003) ²³ and Shen <i>et al</i> (2005) ²⁴	1. Adenovirus (AdCMV β -gal) 2. Adenovirus (AdCMVeNOS)	1. β -galactosidase 2. eNOS	Total of 44 adult male CD rats divided into four groups	1–2 d after administration
Shen <i>et al</i> (2005) ²⁴	pcDNA	VIP	61 STZ-induced diabetic rats	3, 7, and 14 d after administration

mo = months; d = day/s; AAV = adeno-associated virus; iNOS = inducible nitric oxide synthase; STZ = streptozotocin; VEGF = vascular endothelial growth factor; VIP = vasoactive intestinal polypeptide.

of gene product on one side of the penis traverses the midline and is available to the other side of the penis. Third, the blood flow of the corpora at rest is very low at approximately 5 ml/min, ensuring a long dwell time in the cavernous bodies even in the absence of a tourniquet. Finally, the sinusoidal spaces of the corpora allow for several milliliters of the gene transfer product to be administered into to a vascular space lined by endothelial cells. Preclinical studies have shown that naked DNA is readily transported across these endothelial cells into the smooth muscle of the trabeculae.²⁵

Ultrastructural features

Gap junctions are membrane channels consisting of connexin 43 protein molecules that form pores between cells that allow rapid passage of intracellular ions and second messengers.²⁶⁻²⁸ Regulation of the open state of the channels is accomplished by phosphorylation of the channel proteins and the

electrical state of the cell. The presence and natural redundant function of the channels allows the penile corpora to function as a syncytium, so that even if neurotransmission signals are diminished because of disease or trauma, cell-to-cell communication is possible. The effectiveness of the gap junction channels allows use of a safe but inefficient vector, such as naked DNA, with uptake rates of approximately 10% used for effective, physiologically relevant gene transfer in the smooth muscle of the genitourinary system (Figure 1).

Potassium channels

Potassium channels are ubiquitous in smooth muscle, and their action controls the moment-to-moment membrane potential of the cell. That control translates into whether the smooth muscle cell is contracted or relaxed, and thereby controls the organ function.²⁹ Previous reports have shown that there are at least four potassium channels present in the plasma membranes of the cells of

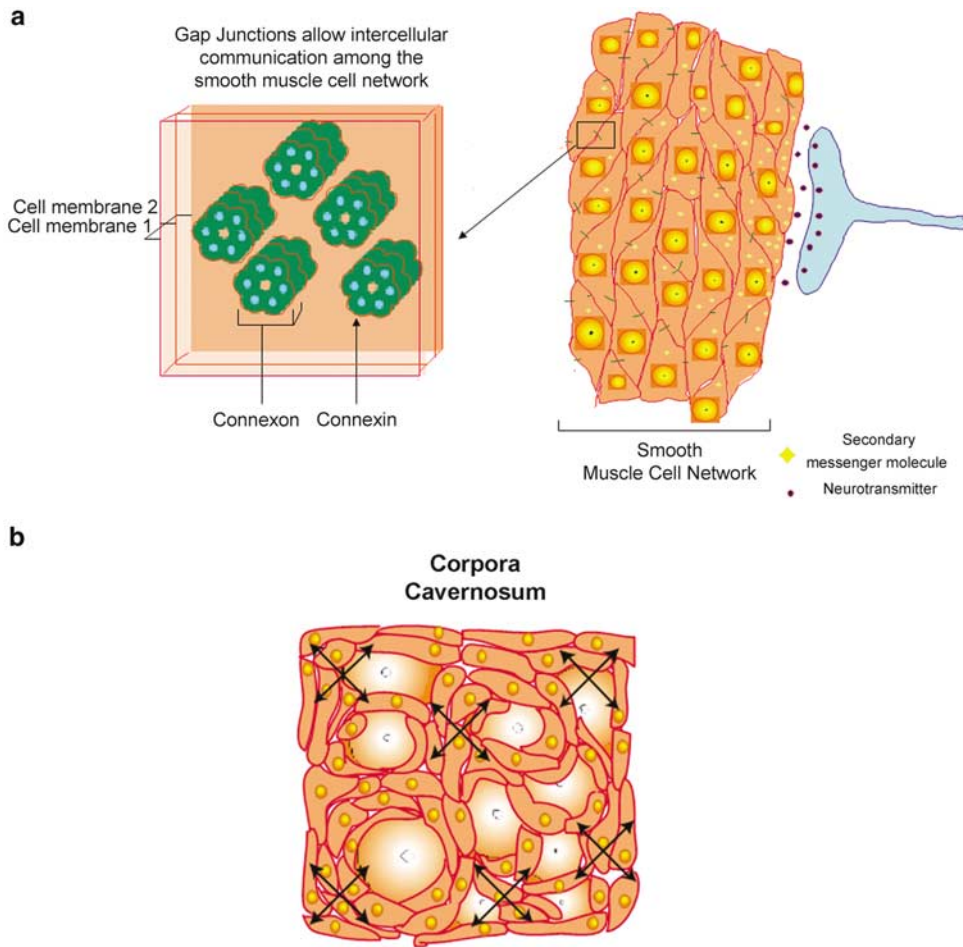


Figure 1 (a) Diagram showing the structure of the intercellular hexamer connexin 43 channels that allow rapid transfer of ions and second messengers between smooth cells of the penis. The modulation of the relatively sparse neural network of the corpora is directed across the corporal cells via the gap junction network. (b) Diagram showing the structure of the cells and possible directions of ion transfer around the endothelial-lined sinuses.

the human corpora.^{28–32} These channels are the Ca^{2+} -activated K^+ (Maxi-K), the voltage-dependent K^+ (K_V), the ATP-sensitive K^+ (K_{ATP}), and the inward rectifier K^+ (K_{IR}). The role of the potassium channels in smooth muscle regulation is to respond to physiological intracellular events by opening and allowing K^+ flow down its electrochemical gradient out of the cell, thereby lowering of the membrane potential of the cell to a more negative state. That action – hyperpolarization of the cell – limits the entry of calcium into the smooth muscle cell through voltage-dependent calcium channels. The physiological relevance is that gene transfer offers the ability to overexpress a potassium channel gene in tissue that has a reduction in neuronal input or a ‘K channelopathy’ each as a result of disease or aging, but will not cause a permanent state of relaxation of the organ, for example, priapism, after gene transfer to the penis (Figure 2).

Safe and effective vector

The next step in the gene transfer program was to choose a safe vector that would allow the transfer to occur, yet pass the scrutiny of the Food and Drug

Administration (FDA) for potential therapy of a nonfatal disease for which other therapies were already available. The vector that meets each of these requirements is naked DNA. Most important is that naked DNA does not cause an allergic response, as do the viral vectors, and, as shown in Figure 3, it is not integrated into the host chromosome, as also occurs with viral vectors.

Although it has been reported that naked DNA would not be useful for therapy of disease because of a lack of efficacy and duration, the preclinical data we have collected belie that suggestion. Previous publications have reported both the efficacy and long duration in two rat models of human disease (aging and diabetes mellitus).^{33,35} The presence of the aforementioned connexin 43 gap junction and the presence of potassium ion channels that control the moment-to-moment inward fluxes of the contractile Ca^{2+} ions have allowed the development of a gene transfer product we have named *hMaxi-K*. *hMaxi-K* is composed of a safe, nonallergenic plasmid vector designed to treat ED in humans (see Figure 4).

The physiological effect of gene transfer with *hMaxi-K* has been verified over several years in hundreds of animals using models of aging and diabetes.^{25,33–35} In these animal models, the effect of

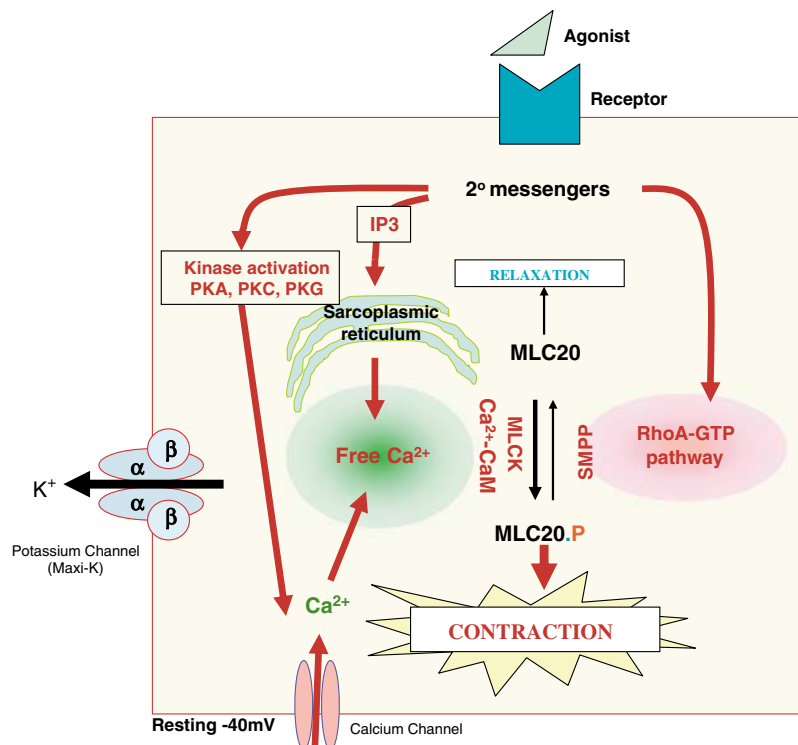


Figure 2 Diagram showing the relationship to the potassium ion channels, intracellular calcium ion, procontractile and relaxant second messengers, and contractile proteins of the smooth muscle cell. As smooth muscle contractility is primarily regulated by intracellular Ca^{2+} , the moment-to-moment control of calcium entry by potassium channel activity regulates the tone of the smooth muscle cell. Furthermore, the change to the open state of the channel is in response to an event. Most of the time, the K^+ channels are in the closed state. With the opening of the channel, the membrane potential of the cell becomes more negative (i.e., hyperpolarized) and the voltage-sensitive calcium channel closes and prevents influx of calcium ion into the cell.

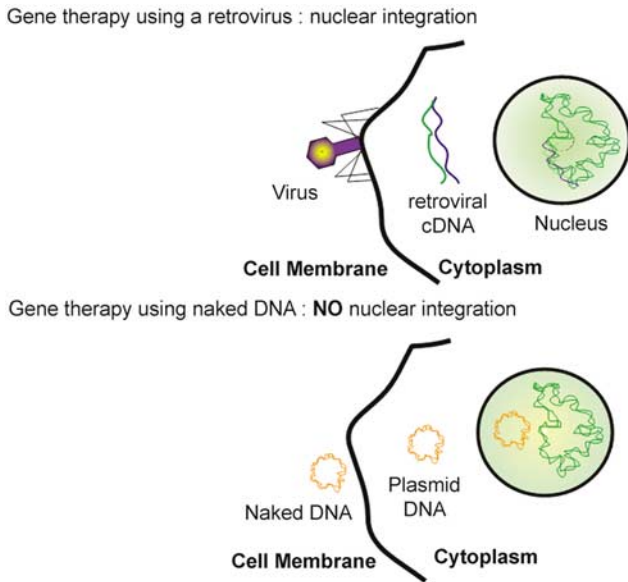


Figure 3 Diagram showing the difference between the use of viral and naked DNA to effect gene transfer. In the upper panel, in which a retrovirus is used as the vector, the DNA passes across the cell and nuclear membrane into the nucleoplasm, where it is integrated into the chromosomal apparatus. When naked DNA is used to effect the transfer, the DNA passes across both membranes but is not incorporated into the nuclear apparatus.

transfer of Maxi-K in doses ranging from 10 to 1000 μg has been tested on erectile function (see Figure 5). Although a response was observed at all levels of transfer, a sustained dose-dependent response was observed for at least 4 months in an streptozotocin (STZ)-induced diabetes model and for 6 months in a model of aging.^{33,35}

Preliminary results of a phase I safety trial

As of this writing, there is one FDA-approved human trial studying the effects of gene transfer for the potential therapy of ED. An Investigational New Drug (IND) application to the FDA was approved in August 2003 under the aegis of Ion Channel Innovations, LLC. The latter was created to form an independent funding base to develop the project. A phase I sequential dosing trial was initiated early in 2004. The sites are the Departments of Urology of the Mt Sinai School of Medicine (Natan Bar-Chama, MD, principal investigator) and New York University School of Medicine (Andrew McCullough, MD, principal investigator). Enrollment into the trial proceeded slowly because of the potential of unknown risks of gene transfer. The FDA required that the participant men and women who were potentially fertile use condoms during sex to

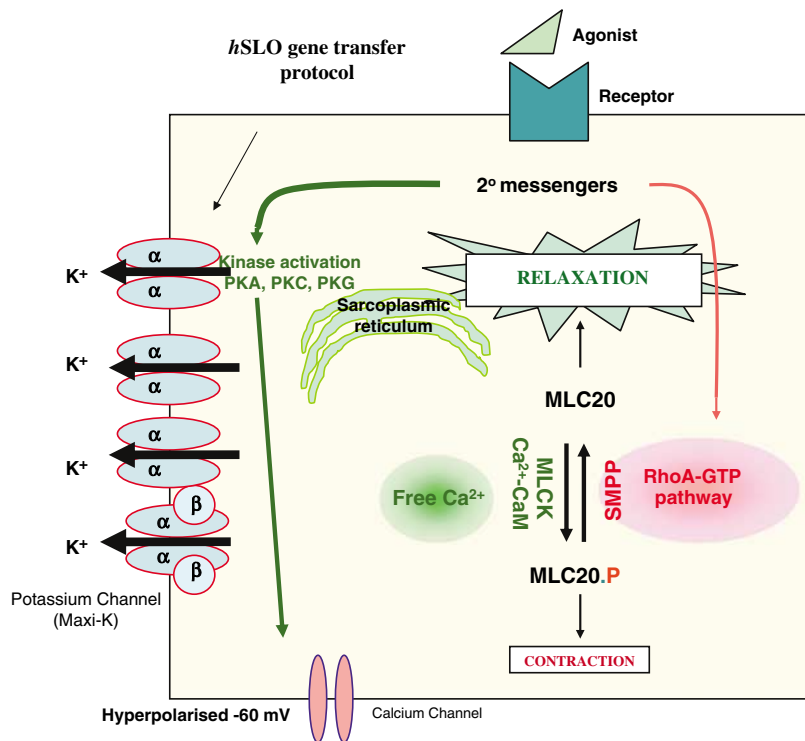


Figure 4 Diagram of a representative cell into which the α -subunit of the Maxi-K channel has been transferred. Three additional Maxi-K channels are shown in the cell membrane. As it is not known if the additional channels expressed by the *hMaxi-K* possess the β -subunit, that unit was not included. In the presence of an appropriate neural or ionic signal, the potassium channels open, hyperpolarize the cell to approximately -60mV , and inhibit the influx of calcium ion, thus causing the cell to relax.

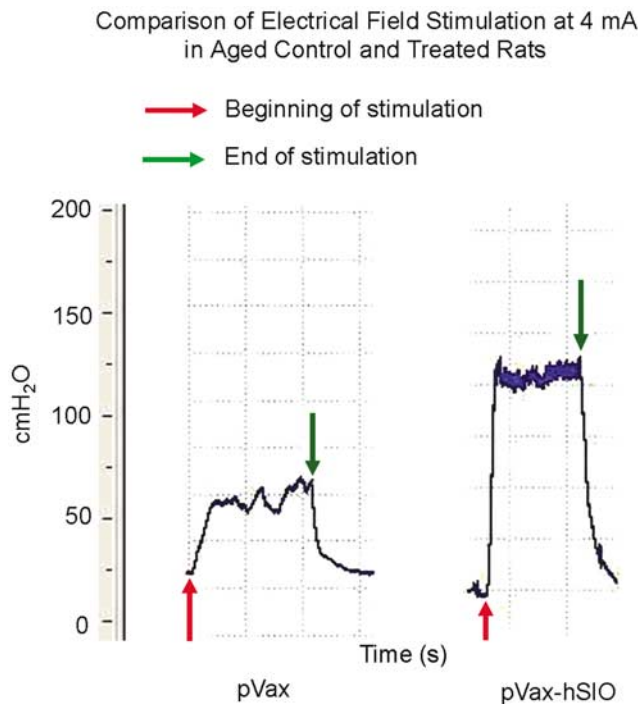


Figure 5 Diagram showing the effect of 1 min of electrical stimulation of the cavernous nerve of two aged male rats. The control animal received transfer of the vector only (pVax), and the experimental animal received 100 μ g of pVax-hSlo (*hMaxi-K*). Note that in the animal in which gene transfer was carried out 1 week earlier with *hSlo*, the maximal intracavernous pressure was 125 cm H₂O compared with a maximal pressure of approximately 70 cm H₂O in which vector only was transferred. When the electrical signal was stopped, the cavernous pressure elevation dropped immediately in both animals.

eliminate the possibility of transfer of the gene to a fetus. Gene transfer trials also require 15 years of follow-up after completion of the study. A preliminary result after transfer in six men at two doses was reported recently.³⁶

At this time, three dose levels, 500, 1000, and 5000 μ g, have been instilled sequentially into three groups of three men each. Results of the trial from the first six participants who received the 500- and 1000- μ g doses recently were published.³⁶ The 5000- μ g dose corresponds to an instillation of 46 μ g in the rat, well below the 1000- μ g instillation used in the preclinical studies. Preliminary data have shown no gene transfer-related serious adverse events in any of the trial participants. There have been no abnormal alterations of serum electrolyte, hormonal, or inflammatory factors related to the transfer. Transfer of the gene to spermatozoa is a particular concern of the FDA. Participants have had to submit post-transfer semen specimens for analysis. To date, there has been no evidence of transfer of *hMaxi-K* to the semen, as measured with polymerase chain reaction, in any specimen, in any participant, at any time up to 6 months after transfer. In the first two

groups, 500 and 1000 μ g, there has been no evidence of efficacy as determined by IIEF and Rigiscan. However, in the third group, one participant has reported a significant improvement in the hardness and duration of erection as estimated by questions 3 and 4 of the IIEF, verified by his sexual partner, in the second and third month after transfer. That finding if replicated in additional men may indicate the clinical utility of the *hMaxi-K* gene transfer.

Conclusions

In summary, the current state of gene transfer for the potential therapy of ED has been reviewed. The adverse atmosphere for the use of viral vectors for therapy of nonfatal disease has promulgated the use of a safe but less efficacious vector, naked DNA. Despite the prevailing opinion in the literature that naked DNA is of short-acting duration, our pre-clinical results suggest the opposite to be true after transfer into the rat penis. The rationale has been presented for the use of *Maxi-K*, and we report the early results of the first human clinical trial of gene transfer for the treatment of ED. To date, after transfer of three of the chosen doses in this single dose-escalation phase I trial in which gene transfer of the *hMaxi-K* gene was administered to nine participants, there have been no drug-related adverse events and no transfer of the plasmid to the participant's semen. Yet to be determined is the efficacy of gene transfer in a dose-dependent manner in humans that is equivalent to 100–1000 μ g in the rat. At present, sequential instillation of two higher doses equivalent to 69 and 92 μ g in the rat is planned. The results of these higher doses will be reported subsequently.

Acknowledgments

I thank Kelvin Davies, PhD, for creating the illustrations used in this manuscript.

References

- 1 Rolland A. Gene medicines: the end of the beginning? *Adv Drug Deliv Rev* 2005; **57**: 669–673.
- 2 Virag R. Intracavernous injection of papaverine for erectile failure. *Lancet* 1982; **2**: 938.
- 3 The European Alprostadil Study Group. The long-term safety of alprostadil (prostaglandin-E1) in patients with erectile dysfunction. *Br J Urol* 1998; **82**: 538–543.
- 4 Goldstein I, Lue TF, Padma-Nathan H, Rosen RC, Steers WD, Wicker PA. Oral sildenafil in the treatment of erectile dysfunction. Sildenafil Study Group. *N Engl J Med* 1998; **338**: 1397–1404.
- 5 Hedlund H, Hedlund P. Pharmacotherapy in erectile dysfunction agents for self-injection programs and alternative application models. *Scand J Urol Nephrol Suppl* 1996; **179**: 129–138.

- 6 Ishii N, Watanabe H, Irisawa C, Kikuchi Y, Kubota Y, Kawamura S *et al.* Intracavernous injection of prostaglandin E1 for the treatment of erectile impotence. *J Urol* 1989; **141**: 323–325.
- 7 Kunelius P, Lukkarinen O. Intracavernous self-injection of prostaglandin E1 in the treatment of erectile dysfunction. *Int J Impot Res* 1999; **11**: 21–24.
- 8 Lea AP, Bryson HM, Balfour JA. Intracavernous alprostadil. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic potential in erectile dysfunction. *Drugs Aging* 1996; **8**: 56–74.
- 9 Padma-Nathan H, Steers WD, Wicker PA. Efficacy and safety of oral sildenafil in the treatment of erectile dysfunction: a double-blind, placebo-controlled study of 329 patients. Sildenafil Study Group. *Int J Clin Pract* 1998; **52**: 375–379.
- 10 Porst H. Erectile dysfunction. New drugs with special consideration of the PDE 5 inhibitors. *Urol A* 2004; **43**: 820–828.
- 11 Stief CG. Phosphodiesterase inhibitors in the treatment of erectile dysfunction. *Drugs Today (Barcelona)* 2000; **36**: 93–99.
- 12 Linet O. Intracavernous prostaglandin E1 in erectile dysfunction. *Clin Invest* 1994; **72**: 139–149.
- 13 Lue TF. Erectile dysfunction. *N Engl J Med* 2000; **342**: 1802–1813.
- 14 Gresser U, Gleiter CH. Erectile dysfunction: comparison of efficacy and side effects of the PDE-5 inhibitors sildenafil, vardenafil and tadalafil – review of the literature. *Eur J Med Res* 2002; **7**: 435–446.
- 15 Pomeranz HD, Bhavsar AR. Nonarteritic ischemic optic neuropathy developing soon after use of sildenafil (Viagra): a report of seven new cases. *J Neuroophthalmol* 2005; **25**: 9–13.
- 16 Rogers RS, Graziottin TM, Lin CS, Kan YW, Lue TF. Intracavernosal vascular endothelial growth factor (VEGF) injection and adeno-associated virus-mediated VEGF gene therapy prevent and reverse venogenic erectile dysfunction in rats. *Int J Impot Res* 2003; **15**: 26–37.
- 17 Chancellor MB, Tirney S, Mattes CE, Tzeng E, Birdler LA, Kanai A *et al.* Nitric oxide synthase gene transfer for erectile dysfunction in a rat model. *BJU Int* 2003; **91**: 691–696.
- 18 Champion HC, Bivalacqua TJ, Hyman AL, Ignarro L, Hellstrom WJ, Kadowitz PJ. Gene transfer of endothelial nitric oxide synthase to the penis augments erectile responses in the aged rat. *Proc Natl Acad Sci USA* 1999; **96**: 11648–11652.
- 19 Garban H, Marquez D, Magee T, Moody J, Rajavashisth T, Rodriguez JA *et al.* Cloning of rat and human inducible penile nitric oxide synthase. Application for gene therapy of erectile dysfunction. *Biol Reprod* 1997; **56**: 954–963.
- 20 Magee TR, Ferrini M, Garban HJ, Vernet D, Mitani K, Rajfer J *et al.* Gene therapy of erectile dysfunction in the rat with penile neuronal nitric oxide synthase. *Biol Reprod* 2002; **67**: 20–28.
- 21 Bivalacqua TJ, Champion HC, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ. Gene transfer of prepro-calcitonin gene-related peptide restores erectile function in the aged rat. *Biol Reprod* 2001; **65**: 1371–1377.
- 22 Bivalacqua TJ, Usta MF, Champion HC, Adams D, Namara DB, Abdel-Mageed AB *et al.* Gene transfer of endothelial nitric oxide synthase partially restores nitric oxide synthesis and erectile function in streptozotocin diabetic rats. *J Urol* 2003; **169**: 1911–1917.
- 23 Bivalacqua TJ, Armstrong JS, Biggerstaff J, Abdel-Mageed AB, Kadowitz PJ, Hellstrom WJ *et al.* Gene transfer of extracellular SOD to the penis reduces O₂-* and improves erectile function in aged rats. *Am J Physiol Heart Circ Physiol* 2003; **284**: H1408–H1421.
- 24 Shen Z-J, Wang H, Lu Y-L, Zhou X-L, Chen S-W, Chen Z-D. Gene transfer of vasoactive intestinal polypeptide into the penis improves erectile response in the diabetic rat. *BJU Int* 2005; **95**: 890–894.
- 25 Christ GJ, Rehman J, Day N, Salkoff L, Valcic M, Melman A *et al.* Intracorporal injection of hSlo cDNA in rats produces physiologically relevant alterations in penile function. *Am J Physiol* 1998; **275**: H600–H608.
- 26 Christ GJ, Wang HZ, Venateswarlu K, Zhao W, Day NS. Ion channels and gap junctions: their role in erectile physiology, dysfunction, and future therapy. *Mol Urol* 1999; **3**: 61–73.
- 27 Moreno AP, Campos de Carvalho AC, Christ G, Melman A, Spray DC. Gap junctions between human corpus cavernosum smooth muscle cells: gating properties and unitary conductance. *Am J Physiol* 1993; **264**: C80–C92.
- 28 Melman A, Christ GJ. Integrative erectile biology. The effects of age and disease on gap junctions and ion channels and their potential value to the treatment of erectile dysfunction. *Urol Clin N Am* 2001; **28**: 217–231.
- 29 Nelson MT, Quayle JM. Physiological roles and properties of potassium channels in arterial smooth muscle. *Am J Physiol* 1995; **268**: c799–c822.
- 30 Fan SF, Brink PR, Melman A, Christ GJ. An analysis of the Maxi-K⁺ (KCa) channel in cultured human corporal smooth muscle cells. *J Urol* 1995; **153**: 818–825.
- 31 Christ GJ, Spray DC, Brink PR. Characterization of K currents in cultured human corporal smooth muscle cells. *J Androl* 1993; **14**: 319–328.
- 32 Karicheti V, Christ GJ. Physiological roles for K⁺ channels and gap junctions in urogenital smooth muscle: implications for improved understanding of urogenital function, disease and therapy. *Curr Drug Targets* 2001; **2**: 1–12.
- 33 Melman A, Zhao W, Davies KP, Bakal R, Christ GJ. The successful long-term treatment of age related erectile dysfunction with hSlo cDNA in rats *in vivo*. *J Urol* 2003; **170**: 285–290.
- 34 Christ GJ. Gene therapy treatments for erectile and bladder dysfunction. *Curr Urol Rep* 2004; **5**: 52–60.
- 35 Christ GJ, Day N, Santizo C, Sato Y, Zhao W, Sciafani T *et al.* Intracorporal injection of hSlo cDNA restores erectile capacity in STZ-diabetic F-344 rats *in vivo*. *Am J Physiol Heart Circ Physiol* 2004; **287**: H1544–H1553.
- 36 Melman A, Bar-Chama N, McCullough A, Davies K, Christ G. The first human trial for gene transfer therapy for the treatment of erectile dysfunction: preliminary results. *Eur Urol* 2005; **48**: 314–318.