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Erectile Dysfunction—Andrology

The First Human Trial for Gene Transfer Therapy for the Treatment of Erectile Dysfunction: Preliminary Results

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Abstract

Objective: To test the safety of a single intracavernous injection of a plasmid vector (*h*Maxi-K) that expresses the *hSlo* gene, that encodes the α-subunit of the Maxi-K channel, for the treatment of erectile dysfunction (ED).

Methods: Six men, thus far have fulfilled the entry criteria of the protocol and had gene transfer with *h*Maxi-K. Three received a dose of 500 μg and three received a dose 1000 μg of the gene product, injected intracavernously as naked DNA. Dosing at 5000 μg and higher is planned.

Results: The primary end point of the phase I trial is safety. No drug-related adverse events or significant laboratory changes have occurred after the gene transfer. Moreover, there is no evidence of the gene in semen at the one copy per μg total DNA in any of the participants.

Conclusion: Preliminary results indicate that, in a single dose escalation study, ion channel gene transfer with *h*Maxi-K can be administered safely to men with ED without adverse events.

Keywords: Erectile dysfunction; Smooth muscle; Gene transfer; Naked DNA; Ion channel therapy; Potassium channels; *h*Maxi-K; Maxi-K channel

1. Introduction

Despite the success and popularity of the oral PDE-5 inhibitors for the treatment of erectile dysfunction (ED) there are still several unmet needs in men suffering with the problem. All of the extant oral therapies are on-demand, i.e., a pill must be first taken with a temporal relationship to the sexual act, so that planning is required. The effect of PDE-5 inhibitors is short-lived, with a duration lasting from hours to a few days so that the medication must be taken repeatedly. The side-effect profile, such as nasal stuffiness, and headache although short-lived, is significant. Furthermore, the effectiveness of PDE-5 inhibitors and other medical therapies is limited in men with diabetes, and there are contraindications to the administration of PDE-5 inhibitors to all men taking nitrate compounds. In that light, a recently published European study has shown that diabetes mellitus, aging, and hypertension are the most important risk factors for the development of ED [1].

In this scenario, we have endeavored to develop an improved, durable treatment for ED based on more than a decade of mechanistic insight into the physiology, pharmacology and electrical excitability of corporal smooth muscle cell tone. The goal of this report is to briefly outline the background, rationale and extensive preclinical testing that led to the development of ion channel gene transfer with *h*Maxi-K, review the

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governmental regulatory pathway for gene transfer and preliminary clinical trial results with gene therapy.

2. Methods

*h*Maxi-K was developed in and patented by members of the Department of Urology research laboratory of the Albert Einstein College of Medicine. Extensive animal trials to develop the concept were done using the male rat model of erectile dysfunction resulting from diabetes mellitus and aging [2,3].

2.1. The regulatory pathway

In the United States, human gene transfer trials require submission to the Recombinant DNA Advisory Committee (RAC) of the Office of Biotechnology Activity of the National Institutes of Health. That committee is charged with advising the FDA concerning initiating a gene transfer study. A public presentation was made to the RAC at a meeting on June 20–21, 2002 (see http://www4.od.nih.gov/oba/RAC/meeting.html for details). Subsequently, an Investigational New Drug application was submitted to and approved by the Center for Biologics Evaluation and Research (CBER) of the FDA on August 1, 2003. Thereafter, the study protocol and participant and partner informed consent documents were submitted to a central institutional review board and institutional biohazard committee (Biomedical Research Alliance of New York (BRANY). Approval was granted so that specific sites could conduct the gene transfer trial at their respective institutions.

2.2. The therapeutic product

The *hSlo* cDNA (the ''active'' component of ion channel therapy) is a linear piece of human DNA that encodes the α, or pore-forming, subunit of the human smooth muscle Maxi-K channel (*hSlo*). In the plasmid construct used to generate the therapeutic product, *hSlo* was subcloned into a commercially available, non-viral, closed- loop (circular), double-stranded piece of DNA (pVAX; Invitrogen, Carlsbad, CA) to create a ''naked'' DNA plasmid (see Fig. 1). This plasmid contains both the gene encoding the human smooth muscle Maxi-K channel as well as the genetic signals to allow eukaryotic transcription of the *hSlo* gene. This naked DNA construct is referred to as *h*Maxi-K. *h*Maxi-K in phosphate-buffered saline was produced in an FDA-approved facility under GMP (good manufacturing practices) conditions for the injection into volunteer participants in a phase I trial.

2.3. Study initiation and design

The trial was begun in March 2004. The study was designed to evaluate the safety and tolerability of *h*Maxi-K administered intracorporally. The open label, sequential three-arm study was designed to evaluate a single administration of three escalating doses of *h*Maxi-K injected into the corpus cavernosum of the penis. The trial is six months long with an annual 15 year follow-up as required by the FDA.

Because the phase I trial is a safety study, the doses chosen, 500, 1000, and 5000 μg, were based upon lowest range of *h*Maxi-K used in the pre-clinical studies. Rapid breakdown of the product in blood ($t^{1/2}$ of ≈30) minutes further reduces the opportunity of unwanted biodistribution. In this trial, a tourniquet was placed on the base of the penis for 30 minutes, prior injection of the gene product thereby insuring that the vector effect is largely limited to the penis.

The study population is men with erectile dysfunction attributable to an underlying, stable medical condition but who are otherwise in good health. The primary outcome is the safety and tolerability of *h*Maxi-K as measured by effect of study drug administration upon clinical and laboratory assessments. That assessment including EKG, general blood electrolyte and liver chemistries, hematologic parameters, sex hormone, cortisol, and thyroid profiles, urine and semen analysis. The DNA of semen was evaluated for the presence of pVAX-hSlo plasmid with PCR. A general and specific genitourinary physical examination was done in men before and after the gene transfer. The effect of *h*Maxi-K on ED was evaluated in all participants. Specifically, participants were assessed for change from baseline using the International Index of Erectile Function (IIEF-5) questionnaire and Rigiscan™ [4–6]. The potential participants and their sexual partners were first given the respective informed consents. If they agreed to participate in the trial they underwent a screening physical and laboratory examination. Those results were sent to an independent Data Safety Monitoring Board (DSMB) composed of four physician- scientists conversant with gene transfer trials. If given permission for the trial by the DSMB, the participant had repeat blood tests as a baseline and was given the gene transfer on that day. He was then seen at one week and at monthly intervals for six months. Subsequently annual visits for 15 years are planned. The schedule of visits and tests is shown in Table 1.

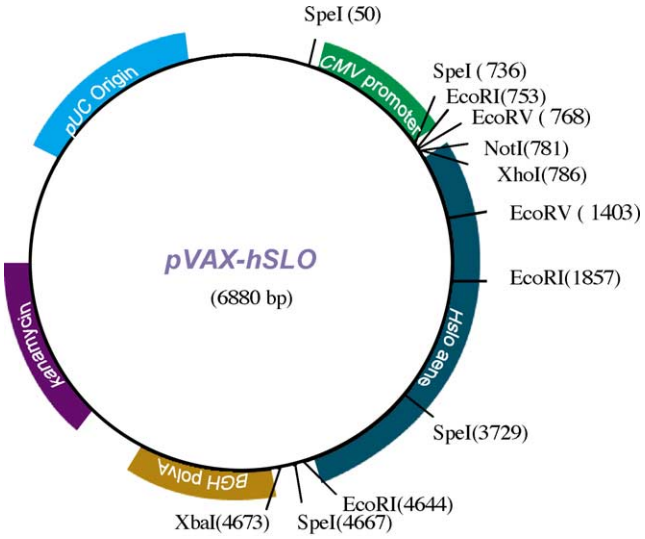

Fig. 1. Plasmid construct (*h*Maxi-K, 6880 bp). CMV promoter (137–724) viral. *hSlo* gene (888–4428) human. BGH polyadenylation signal: (4710–4940) bovine. kanamycin gene (5106–5901) bacterial. PUC origin (6200–6874) bacterial. Plasmid Description: *h*Maxi-K is a double stranded naked plasmid DNA molecule carrying the human cDNA encoding the alpha, or pore forming subunit of the human smooth muscle maxi-K channel *hSlo*. *hSlo* is under control of the CMV promoter positioned upstream of the transgene and the construct also contains the Bovine Growth Hormone poly A site, kanamycin resistance gene and pUC origin of replication.

3. Results

This is the first human trial for gene transfer in men with ED. The primary end point of this dose-escalation study was to test the safety of a single intracavernous injection of *h*Maxi-K. At present, 15 men have been screened for the trial. Six of the men, each of whom had

Table 1
Schedule of events

Test or Procedure	Visit 1 (−2 Week)	Visit 2 (Week 0)	Visit 3 (Week 1 ± 2 days)	Visit 4 (Week 2 ± 2 days)	Visit 5 (Week 4 ± 3 days)	Visit 6 (Week 8 ± 3 days)	Visit 7 (Week 12 ± 3 days)	Visit 8 (Week 24 ± 3 days)
Informed consent process	X							
Medical history	X	X[e]	X	X	X	X	X	X
Physical examination	X	X[e]	X	X	X	X	X	X
Physical examination of penis[a]	X	X[e]	X	X	X	X	X	X
Blood tests	X[d]	X[e,g]	X[d]	X[g]	X[d]	X[g]	X[g]	X[d]
Urine analysis[c]	X	X	X	X	X	X	X	X
Semen collection for *hSlo* cDNA		X[e]	X	X	X	X	X[h]	X[h]
ECG	X	X[f]	X	X	X	X	X	X
Rigiscan™	X		X	X	X	X	X	X
IIEF[b]		X[e]	X	X	X	X	X	X
Study drug administration		X						

[a] PE of penis includes: inspection, palpation.
[b] IIEF completed by both the patient and his partner at all time points.
[c] Urine analysis includes: microscopic RBC and WBC, protein, glucose, and specific gravity.
[d] Blood tests include: Hematology (CBC with differential, platelet count, PTT, PT, sedimentation rate, CRP), Chemistry (BUN, Cr, Na^+, K^+, Mg^{2+}, Ca^{2+}, CO_2, Cl, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein), Endocrine (testosterone, random cortisol, TSH, T4).
[e] Test or procedure will be done prior to administration of study drug.
[f] ECG will be done prior to administration of study drug and at 1 and 3 hours following administration of study drug.
[g] Blood tests include: Hematology (CBC with differential, CRP), Chemistry (BUN, Cr, Na^+, K^+, Mg^{2+}, Ca^{2+}, CO_2, Cl, albumin, alkaline phosphatase, ALT, AST, total bilirubin, total protein).
[h] Semen specimen collected only if *hSlo* DNA was detectible in either of two preceding semen specimens. If semen still positive at week 24 we will ask participant to return monthly until two successive semen specimens are negative for *hSlo* DNA.

used other available therapies that were either unsuccessful or unpalatable, fulfilled the entry criteria and received *h*Maxi-K. *h*Maxi-K, was injected into the cavernous body of men with moderate to severe ED. The demographics of the patient population are summarized in Table 2.

Three men received the lowest planned dose (500 μg) and three received an intermediate dose (1000 μg) of *h*Maxi-K. Three men will now be entered to receive the highest planned dose of 5000 μg. Of primary importance is that there have been no drug-related adverse events after administration of *h*Maxi-K. As noted above, transfer with naked DNA as a vector is noted for the lack of immunogenicity seen with viral vectors. Because germline transmission is a concern, careful analysis of the testis in preclinical studies has not shown evidence of the gene in the testicular tissue at any time after transfer (data not shown). In this trial to date, there has been no detectable evidence of *h*Maxi-K in semen measured down to the 1 copy/μg of total DNA level in any participant at any time after transfer for the six months of the trial. Thus, the primary end point of the trial, safety, has been tested and thus far has shown no drug-related adversity. These preliminary results indicate that, at the doses chosen, ion channel gene transfer with *h*Maxi-K can be administered to men with ED without adverse events. At the low dose schedule used to this date efficacy has not been observed.

Table 2
Patient characteristics and preliminary results

Patient no.	Age (yrs)	Drug-related adverse event	Cause of erectile dysfunction	IIEF-5 at baseline
(500 μg)				
1	56	None	Vascular	0
2	79	None	Hypertension	4
3	60	None	Diabetes	9
(1000 μg)				
4	46	None	Diabetes	0
5	65	None	Vascular	7
6	65	None	Vascular	5

4. Discussion

Gene transfer with ion channel therapy specific to the smooth muscle organ of interest offers a potential new treatment for ED with duration of action that may last for months without advanced planning. The preclinical study data have not indicated contraindications to its use in man. Furthermore, published results of preclinical studies suggest that the therapy may be effective in ameliorating the ED associated with diabetes and aging [3,7].

4.1. Development of the gene transfer concept for ED

The mechanism of action of this novel therapy uses a final common pathway, alteration of smooth muscle cell excitability, for its effect. The preclinical evidence showing safety, effectiveness and long duration of action has been highly significant, and thus, we proposed that the *h*Maxi-K vector also be injected intracavernously as naked DNA in the clinical trial [2,7,8]. Naked DNA is devoid of the significant side- effects seen with viral vectors [9,10]. Nonetheless, because ED is a nonfatal disease, stringent requirements needed to be met before the Food and Drug Administration (FDA) granted permission for the use of naked DNA in a human trial. The reasons that naked DNA is not chosen routinely as a vector in gene transfer protocols to treat cancer and genetic illness are the reported lack of efficiency in transfer into the cells of interest, as well as the reportedly short duration of effect for those indications [10]. However, our preclinical studies in a rodent model of aging suggested that the therapeutic effect produced by this plasmid may remain for up to 6 months after a single injection [3]. Similarly in a rat model of diabetes, the same effect was found to last for at least 4 months [7]. This activity of *h*Maxi-K may represent a distinct advantage over current drug therapy for ED, where there is a need for on-demand treatment that provides only a temporary effect.

4.2. Why might low efficiency gene transfer with hMaxi-K be effective for the treatment of ED?

Unlike gene transfer potential therapy for therapy of cancer where virtually 100% of the cells must be affected, only a small percentage of the cells of the corpora need to incorporate the gene of interest. The reason is that in the penis intracellular concentrations of ions and second messengers in smooth muscle cells (e.g., K^+, Ca^{2+}, cAMP, cGMP, etc.) are affected by release of neurotransmitters, and in turn, the changes can be propagated rapidly from cell-to-cell throughout the penile corporal smooth muscle network via the gap junctions [11–13]. Gap junctions are specific intercellular protein pores located in the membranes of apposing smooth muscle cells of the penis, that permit the relatively nonselective exchange of the numerous physiologically relevant intracellular second messengers and ions. Thus, the importance of gap junctions in coordinating corporal smooth muscle function, and thus, erectile capacity, cannot be overstated. For example, even after severe nerve loss, such as can occur with diabetes or radical prostatectomy, there may remain a sufficient level of intercellular coupling to allow the corporal tissue to function as a syncytium following appropriate sexual stimulation (Fig. 1).

In essence then, gap junctions serve as the anatomic substrate for the signal amplification necessary for smooth muscle contraction and relaxation during neural stimulation. In the absence of gap junctions, more aggressive gene incorporation strategies (e.g., adenoviral or retroviral vectors) would need to be considered, thus increasing the risk of side effects. Such side effects include adverse immune responses and tissue inflammation caused by the viral vector [9,10]. Inflammatory responses to the viral vector actually may inhibit the activity of the virus-based therapy and may prohibit repeated administrations. Because *h*Maxi-K is a naked DNA, there is no viral vector involved. Therefore, in the presence of gap junctions, nonviral gene therapy may offer the best opportunity to achieve the appropriate therapeutic response while minimizing adverse effects.

4.3. Rationale for the use of the large conductance, calcium-sensitive Maxi-K Channel in gene transfer

Contraction and relaxation of smooth muscle is critical to the storage and conduit functions of hollow organs such as the bladder, gut, blood vessels, and penis. K channels play an important role in this process by virtue of their ability to alter the membrane potential and excitability of smooth muscle cells [14]. Their primary effect is to modulate Ca^{2+} influx through Ca channels (i.e., L-type, voltage-dependent). The amount of Ca^{2+} that enters the cell through these channels is a major determinant of the free intracellular calcium levels inside the smooth muscle cell, which in turn determine the degree of smooth muscle cell contraction.

Prominent among the K-channel subtypes found in smooth muscle is the large- conductance, calcium-sensitive K channel, referred to as the Maxi-K channel [15]. Increased Maxi-K channel activity is associated with corporal smooth muscle cell relaxation and penile erection. Moreover, alterations in K-channel physiology and function increasingly are being recognized as major contributing factors to the development of the vascular pathologic characteristics associated with diabetes, including ED [16–18]. The use of ion channel therapy for the treatment of ED is based on the principle that rapid, robust and syncytial corporal smooth muscle relaxation is needed to obtain and maintain erection [19–21]. Using this principle, *h*Maxi-K was developed to treat ED, and potentially other smooth muscle diseases. Intracorporal administration of *h*Maxi-K presumably increases expression of the Maxi-K channel in smooth muscle cells, which, with appropriate stimuli, will generate increased efflux of K^+ across the cell membrane, resulting in enhanced cellular hyperpolarization, and thus, decreased entry of Ca^{2+} ions. Importantly, when the sexual/neural stimulation is terminated,

the K^+ efflux also stops, so that priapism has not been observed [3]. Thus the enhanced effect on outward K^+ currents across the smooth muscle cells allows the smooth muscle of the corpora to relax, and in turn, the corporal sinusoids to become engorged with blood, and the penis to become rigid.

5. Conclusions

In summary, we report the early results of the first human clinical trial of gene transfer for the treatment of ED. To date, after transfer of two of the chosen doses in this single dose-escalation phase I trial in which gene transfer of the *h*Maxi-K gene was administered to six participants, there were no drug-related adverse events and no transfer of the plasmid to the participant's semen The next planned transfer dose is 5000 μg, a dose is equivalent to 46 μg in the rat, well below the highest 1000 μg dose given in the preclinical studies. in which there were no adverse histopathological or blood chemistry results. It is not now known how the efficacy of gene transfer in the rat that was effective in a dose-dependant manner from 100 to 1000 μg will be comparable to the dosing schedule in this human trial. The results of the higher chosen doses will be reported at the completion of the trial.

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