Plasmid-based Gene Transfer for Treatment of Erectile Dysfunction and Overactive Bladder: Results of a Phase I Trial*

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Abstract

Background: Ion Channel Innovations has developed a gene transfer product, *h*Maxi-K, and has begun clinical trials to investigate the effect of increased expression of Maxi-K channels in the smooth muscle of the penis or bladder in patients with erectile dysfunction and those with overactive bladder. The primary function of K channels is to modulate Ca^{++} influx through Ca-channels (i.e., L-type, voltage-dependent). The amount of Ca^{++} 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 determines the degree of smooth muscle cell contraction. Increased Maxi-K channel activity is associated with smooth muscle cell relaxation, resulting in, for example, penile erection and detrussor muscle relaxation. A phase I clinical trial that used *h*Maxi-K has been completed and a similar trial to assess safety of the transfer for overactive bladder is about to begin.

Objectives: To assess the safety and tolerability of escalating *h*Maxi-K doses by clinical evaluations and laboratory tests, and to measure efficacy objectives by means of the International Index of Erectile Function scale.

Methods: In the erectile dysfunction trial 11 patients with moderate to severe erectile dysfunction were given a single-dose corpus cavernosum injection of *h*Maxi-K, a "naked" DNA plasmid carrying the human cDNA encoding for the gene for the α, or pore-forming, subunit of the human smooth muscle Maxi-K channel, hSlo. Three patients each were given 500, 1000, and 5000 µg and two patients were given 7500 µg doses of *h*Maxi-K and followed for 24 weeks. Patient responses were validated by partner responses.

Results: There were no serious adverse events and no dose-related adverse events attributed to gene transfer for any patient at any dose or study visit. No clinically significant changes from baseline were seen in physical evaluations (general and genitourinary), hematology, chemistry and hormone analyses, or in cardiac events evaluated by repeated electrocardiograms. Importantly, no plasmid was detected in the semen of patients at any time after the injections. Patients given the two highest doses of *h*Maxi-K had apparent sustained improvements in erectile function as indicated by improved IIEF-EF domain scores over the length of the study. One patient given 5000 µg and one given 7500 µg reported EF category improvements that were highly clinically significant and were also maintained through the 24 weeks of study.

Conclusions: Efficacy conclusions cannot be drawn from results of a phase 1 trial with no control group. However, the promising primary safety outcomes of the study and preliminary indications of effectiveness provide evidence that *h*Maxi-K gene transfer is a viable approach to the treatment of erectile dysfunction and other smooth muscle diseases with targeted access.

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Erectile dysfunction and overactive bladder are both smooth muscle-related urogenital disorders in which altered potassium channel physiology may play a central role [1]. Potassium channels modulate smooth muscle cell excitability, thereby affecting the degree of smooth muscle cell contraction and relaxation, which ultimately translates into control of hollow organ function [2]. At least four types of potassium channels are present in the plasma membranes of human smooth muscle cells [2–6]. With respect to penile erection, these ion channels respond to endogenous intracellular events by opening and allowing K^+ to flow down its electrochemical gradient out of the smooth muscle cell. The resulting hyperpolarization, in turn, limits calcium entry and promotes relaxation of the corporal and arterial smooth muscle cells. Similarly, detrusor overactivity is related, at least in part, to enhanced bladder detrusor smooth muscle cell contraction (i.e., myogenic DO). Because detrusor smooth muscle contraction is dependent on transmembrane influx of calcium ions, any therapy that diminishes this process will inhibit some aspects of the aberrant detrusor contractility associated with DO. Thus, a gene transfer approach that provides the ability to locally overexpress a potassium channel gene in a target tissue could theoretically overcome the age- or disease-related changes in end-organ contractility that contribute to both overactive bladder and erectile dysfunction. In this regard, Ion Channel Innovations has thus far focused on recombinant *hSlo*, which encodes the α, or pore-forming subunit of the human Ca^{2+}-activated K^+ potassium channel (Maxi-K).

As noted above, the preclinical work leading to the ICI Erectile Dysfunction clinical trial targeted the key role played by endogenous potassium channels [7–10]. In fact, the penis is an organ uniquely suitable for gene transfer because of its anatomic and ultrastructural features. Gap junctions, key ultrastructural features of cell-to-cell communication, allow the penile corporal smooth muscle to function as a synctium, and thereby enable the use of inefficient transfer vectors,

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IIEF = International Index of Erectile Function
EF = erectile function
DO = detrusor overactivity

such as naked DNA. Despite a likely *in vivo* efficiency of cellular incorporation of ≤ 10%, naked DNA can still be used for effective, physiologically relevant gene transfer in the smooth muscle of the genitourinary system [9,11]. For the studies summarized herein, the *h*Slo gene was subcloned into a commercially available expression vector (pVAX; Invitrogen, CA, USA) to construct *h*Maxi-K, the naked DNA construct. Preliminary reports of the use of *h*M*axi*-K in the first human gene transfer trials for the treatment of erectile dysfunction were reported [9,12] and complete trial results were recently published [13]. The first human trial of *h*Maxi-K for OAB will be underway shortly.

Patients and Methods

This study was conducted in compliance with federal and international regulations for human clinical trials.

Gene transfer product – *h*Maxi-K

The construction of *h*Maxi-K and final testing prior to release for clinical use has been described in detail [7,13]. The *h*Maxi-K plasmid construct was diluted in phosphate-buffered saline–20% sucrose buffer immediately prior to patient administration.

Study design and patient

This open-label, sequential four-arm, phase 1 study evaluated a single administration of four escalating doses of *h*Maxi-K injected into the corpus cavernosum of the penis. Of the 15 men screened. 11 qualified for entry. Three men each were treated with 500, 1000, or 5000 µg doses and two were given 7500 µg. The dose level selection was based on the lowest range of *h*Maxi-K used in preclinical studies in rodents [14]. *h*Maxi-K was injected into the corpus cavernosum of patients after placing a tourniquet (Actis® venous flow controller; Vivus, Inc., Menlo Park, CA) at the base of the penis. The tourniquet remained in place for 30 minutes to ensure that the vector was largely limited to the penis.

The men participating in the study were 18 years or older. They had erectile dysfunction attributable to underlying, stable medical conditions and Rigiscan™ results diagnostic for the condition [15]. They were otherwise in good health, with normal blood pressure and normal general and genitourinary physical examination at screening. Patients and their sexual partners signed the respective informed consents and the approved patients returned for the baseline visit 2 weeks later when gene transfer was given. They were seen after 1 week and then monthly for 6 months.

The primary objective of this study was to assess the safety and tolerability of a single injection of *h*Maxi-K at four escalating dose levels. This was measured by assessment of changes in clinical evaluations and laboratory tests that included general and genitourinary physical examinations, blood pressure and heart rates, ECG, general blood electrolyte and liver chemistries, hematologic parameters, endocrine tests, thyroid profiles, and urine and semen analysis. Adverse events were assessed and recorded at each visit. The DNA of semen was tested for the presence of pVAX-*h*Slo plasmid using reverse transcriptase-polymerase chain reaction with primers specific to the plasmid [7]. The key secondary study objective was assessment of the effect of *h*Maxi-K on erectile dysfunction using the erectile function domain category of the International Index of Erectile Function scale [16,17]. The erectile function domain, questions 1–5, and 15 of the IIEF has been validated to assess erectile changes only [17]. Additional IIEF sub-domain scores were recorded to confirm the IIEF-ED, including the mean intercourse satisfaction score, questions 6, 7 and 8, as an indicator of overall sexual satisfaction.

Results

The study was conducted from May 2004 to May 2006. Fifteen men were screened for the study and 11 men fulfilled the criteria for enrollment and were given injections of *h*Maxi-K. The mean age of the study population was 59.0 ± 10.6 years (range 42–80 years); six subjects were Caucasian, four were African-American, and one was Hispanic. The duration of erectile dysfunction ranged from 1 to 20 years and the mean baseline IIEF-EF score was 6.8 ± 4.05. Nine subjects were categorized with severe erectile dysfunction and two subjects with moderate dysfunction according to standard classifications [17].

Primary study endpoint – Safety

Table 1 lists the adverse events reported by patients during the study. All three patients given 500 µg, one of three patients given 1000 µg, and one of three patients given 5000 µg had adverse experiences. All reported that the events occurred at least 30 days after the gene transfer and none were considered related to the gene product transfer by the investigators. No patients reported any discomfort from the injection and no local physical events related to the injections were observed.

No clinically significant changes were seen in the general or genitourinary physical examinations during the study. No emergent transfer-related cardiac events were noted or reported during

Table 1. Adverse event summary by dose

	Dose (µg)				
	500 (n=3)	1000 (n=3)	5000 (n=3)	7500 (n=2)	Total
Patients reporting at least one adverse event	3 (100%)*	2 (67%)**	1 (33%)***	0 (0%)	6 (54.5%)
Patients with adverse events related to study treatment	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Patients with serious adverse events	2 (67%)	0 (0%)	0 (0%)	0 (0%)	2 (18.2%)
Patients with adverse events leading to early withdrawal	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

* One patient had knee arthroscopy, one had atrial flutter with ablation reported as severe, and one had kidney stone removal by lithotripsy, also reported as severe. The atrial flutter and lithotripsy were also classified as serious adverse events.

** One patient reported acid reflux, sciatic pain and an upper respiratory infection and one patient had a parasitic intestinal infection and foot edema.

*** One patient had a bladder stone removed.

the study, and no significant changes in ECGs as determined by shift analysis (no normal to abnormal occurrences) were observed with the exception of atrial flutter considered unrelated to treatment in one patient.

No clinically significant changes were seen in the mean blood chemistry or endocrine test values at the end of the study or at any of the interim study visits. In addition, no clinically significant changes from normal to abnormal in any blood chemistry, endocrine, hematology or urinalysis values were seen at any visit for any patient. Mean systolic and diastolic blood pressures and heart rates did not show notable changes over time in each dose group. However, individual subject values varied from visit to visit, but no clinically significant pattern of changes was evident. No adjunctive therapies or changes in therapy were required.

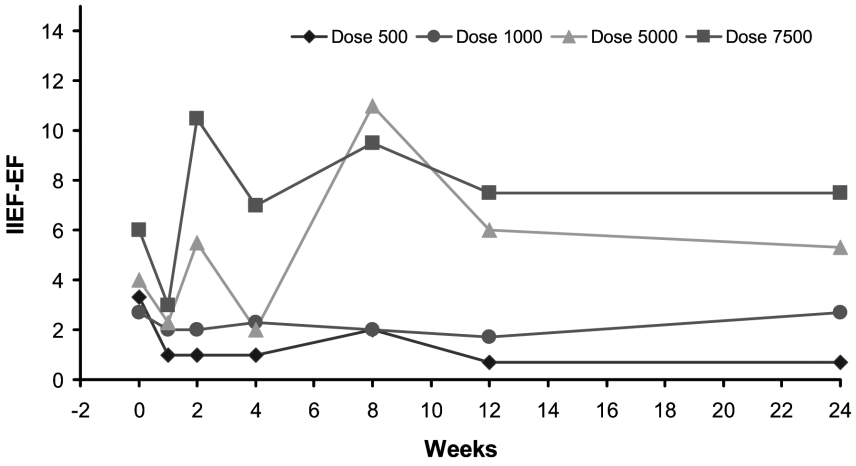

Figure 1. *h*Maxi-K: change in patient IIEF-mean intercourse satisfaction (Items 6, 7, 8): scores over time by dose for each patient

There was no detectable evidence of *h*Maxi-K in semen down to the 1 copy/µg of the total DNA level in any participant at any of the visits.

Secondary study endpoints – Efficacy

Decreased IIEF-EF domain scores at each dose were observed 1 week after injection. Mean scores for the two lower dose groups (500 and 1000 µg) fluctuated around the baseline values throughout the study. However, improvements in the mean IIEF-EF scores were observed for the two higher dose groups (5000 and 7500 µg) beginning 2 weeks after transfer. Improvements were maintained in both groups through the 24 weeks of study. The positive changes from baseline for most patients were small and did not indicate improvement by IIEF scoring. However, two patients, one given 5000 µg and the other 7500 µg, showed notable improvement in IIEF-EF beginning 2 weeks after transfer and continuing improvement (from severe to mild or to no erectile dysfunction) at 4 weeks. The improvement was maintained through the 24 week study.

Figure 1 displays the IIEF mean intercourse satisfaction score for each patient at each visit. The results showed that for those men who responded to the transfers at the two higher doses there was a clinically significant increase in sexual satisfaction

Discussion

The results of this first human trial of gene transfer therapy for erectile dysfunction suggest that gene transfer focused on ion channel therapy in the smooth muscle of organs such as the penis and bladder offers a promising new treatment strategy. This novel therapeutic approach may address limitations of current therapies for disorders like erectile dysfunction and overactive bladder.

The primary objective of this study was to determine if single escalating doses of *h*Maxi-K given to men with erectile dysfunction would be tolerated and safe. The most important finding of the study is that single injections of *h*Maxi-K at doses of 500, 1000, 5000, and 7500 µg were well tolerated and safe, and furthermore, that no safety issues emerged during the 6 months of follow-up. No significant drug-related changes from baseline were seen in physical evaluations (general and genitourinary), hematology, chemistry and hormone analyses, or in cardiac events evaluated by repeated ECGs (one patient with preexisting atrial arrhythmia had a recurrence approximately 1 month after dosing). Importantly, no plasmid was detected in the semen of patients at any time after the injections.

Certainly, conclusions about efficacy cannot be drawn from results of phase 1 trials without randomized controlled groups. Nonetheless, efficacy measurements, made at each study visit, may provide insight into potential clinical activity. The IIEF is the standard instrument accepted as the best measure of efficacy in erectile dysfunction clinical trials, and patients given the two highest *h*Maxi-K doses had apparent sustained improvements in erectile function indicated by improved scores of the IIEF-EF domain over the length of the study. Specifically, one patient in the 5000 µg group and one in the 7500 µg group reported relatively equivalent EF improvements that approached the no erectile dysfunction IIEF-EF score and they maintained the improvement for 24 weeks. Sexual satisfaction scores confirmed patient improvement.

Since the participants in this trial were not blinded to their treatments the improvement in IIEF score may have been a consequence of their belief in the effectiveness associated with the treatment. However, the preliminary results are consistent with the supposition that gene transfer with *h*Max-K has significant potential as a therapy for patients with erectile dysfunction. Overall, the results indicate that further studies in a larger group of patients, with the addition of a placebo control and multiple doses, should be conducted to confirm the safety and efficacy of *h*Maxi-K in patients with erectile dysfunction. The positive results of this first *h*Maxi-K gene transfer therapy in patients provide promise of a platform technology, that is, a similarly novel approach to other smooth muscle-related diseases such

as overactive bladder (i.e., detrusor overactivity), irritable bowel syndrome or asthma.

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Capsule

Stromal cells in tumors

Solid tumors may be seen as a malignant mass of epithelial cells, but in fact they contain normal cells such as fibroblasts and the endothelial and smooth muscle cells that comprise tumor blood vessels. The molecular conversations between malignant cells and these stromal cells can influence tumor growth; thus, stromal cells have become possible targets for cancer therapy. In contrast to tumor cells, stromal cells are widely believed to be genetically stable and hence would not be expected to develop resistance to therapy. Pelham et al. have investigated the possibility that tumor-associated stromal cells, like their malignant neighbors, acquire genetic alterations during tumor progression. They used high resolution DNA copy-number analysis to study human breast and colorectal tumors that had grown in mice for 30 to 150 days, an experimental design that allowed the stromal components to be readily identified by virtue of their mouse origin. Surprisingly, the stromal cells had undergone amplification or deletion of several genes, some of which can plausibly be linked to tumorigenesis. The magnitude of the genetic changes suggests that clones of mutant host cells had been selected during tumor establishment or progression. Whether these changes reflect a selective pressure placed on stromal cells by the tumor in order to invoke a favorable microenvironment or, conversely, a host-initiated selection of mutant stromal cells designed to suppress tumor progression, is not yet clear.

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