Future Strategies for Treating Erectile Dysfunction

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Erectile dysfunction affects over half of all men between 50 and 70 years of age, and by the age of 40, about 40% of men may suffer from some form of erectile dysfunction. Many disease states, such as diabetes, hypertension, depression, and vascular disease, are associated with the condition, which may occur many years prior to the onset of these disorders. The phenomenal success of sildenafil in improving erections in men with erectile dysfunction is due to the fact that the drug, as a phosphodiesterase inhibitor, improves the relaxation of smooth muscle cells, which become dysfunctional with the aging process. However, not everyone responds to this medication, mainly because the efficacy of the drug is directly dependent on the release of nitric oxide from the nerve terminals of the cavernosal nerve, and this may become defective with aging/certain disease states. The goal of gene therapy for organic impotence is to allow the patient to sustain physiologically elicited erections without resorting to pharmacological treatment immediately prior to the sexual act. Experimental efforts in gene therapy for erectile dysfunction are likely to continue intensively in a series of directions, some specific to the nature of the selected gene to be manipulated or the physiology of the corpora cavernosa itself, and others extrapolatable from the advancement of gene therapy in general. [Rev Urol. *2002;4(suppl 3):S48–S53*]

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Erectile dysfunction (ED) is defined as the inability of a man to attain or maintain an erection long enough to complete the sexual act,[1] and it affects over half of all men between 50 and 70 years of age.[2] Recent data suggest that by the age of 40, about 40% of men may suffer from some form of ED, and this increases to about 70% by the end of the sixth decade of life.[3] This high prevalence underscores the fact that from a biological point of view, ED is in reality a part of the aging process. The determinants of whether and at what age any man becomes dysfunctional are probably related to a number of genetic and environmental factors. For example, many disease states (eg, diabetes, hypertension, depression,

and vascular disease) are associated with ED, and the development of ED in these men is extremely variable, occurring at times many years prior to the onset of these disorders. This review will focus on methods of enhancing smooth muscle relaxation, in particular the upregulation of nitric oxide (NO) production through gene therapy.

Background

The penis should be considered as a specialized vascular organ and an extension of the vascular system. Anatomically, it consists of sinusoidal blood vessels surrounded by a syncytium of vascular smooth muscle cells[4,5] that begin with aging to become dysfunctional, resulting in an inability of these smooth muscle cells to relax normally following sexual stimulation; this is what primarily leads to the development of erectile dysfunction.

The phenomenal success of sildenafil in improving erections in men with ED[6] is due to the fact that the drug, as a phosphodiesterase (PDE) inhibitor, improves the relaxation of these same smooth muscle cells that become dysfunctional with the aging process. However, not every person with ED responds to this medication, mainly because the efficacy of the drug is directly dependent on the release of NO from the nerve terminals of the cavernosal nerve, which may become diminished with aging and certain disease states. In addition, in many men the diminished[7] amount of vascular smooth muscle cells present with aging and certain disease states is insufficient to allow the drug to have a clinical effect. Although overall response rates in the broad ED population are high, in some organic forms of ED as many as 35%–50% of men with ED may not respond to this medication[8,9] and usually need more invasive treatment to effect a clinical response, eg, the use of intracorporeal injections into the penis with drugs (phentolamine, papaverine and prostaglandin E1) that also promote smooth muscle relaxation.[10] However, it is well known that many men discontinue the use of these on-demand erectogenic injections[11] and are reluctant to advance to more invasive therapies that may require surgery.[12]

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The high discontinuation rate of intracorporeal injections by men is surprising, because the success rate of such therapy in improving erections is close to 90%. Such high success rates demonstrate that delivery of a drug directly to the penis, essentially bypassing the peripheral vascular system, provides a pharmacological level of the drug within the cavernosal bodies that cannot be achieved with any oral drugs. Because the oral drugs and the intracorporeal drugs are, in actuality, the same medication, the observation that the intracorporeal injections have almost twice the efficacy rate of the oral drugs suggests that this discrepancy between the results of the two delivery systems is strictly a concentration-dependent issue.

The endogenous biochemical events within the penis that affect an erection make up the nitric oxide–cyclic guanosine monophosphate (NO-cGMP) system.[13] The cGMP ultimately stimulates certain kinases to uncouple calcium intracellularily within the smooth muscle cells of the corpora, resulting in smooth muscle relaxation. If smooth muscle relaxation is impaired due to a myopathy, an inability of the neurons to release adequate amounts of NO, or the development of products within the penile tissues that counteract the erectogenic effects of the NO-cGMP pathway, then ED may ensue.

The classical complaint in this clinical setting of inadequate smooth muscle relaxation is "inability to achieve a full erection" or "loss of erection" prior to completion of the sexual act or at the time of penetration. When nonresponders to sildenafil treatment are investigated as to the etiology of their disorder, venous leakage or failure to store, which is the most common cause of ED, now takes a back seat to the other etiologies.[14] This is probably due to the fact that those patients who are going to respond to the PDE therapies are those with "failure to store," because the PDE inhibitors work by enhancing the relaxation of the corporal smooth muscle, and most of the successes will be those patients with this myopathy.

Relaxation of the corporal smooth muscle is the key to the development of an erection.

Because relaxation of the corporal smooth muscle is the key to the development of an erection, any future strategies that deal with the treatment of ED will probably focus on this target. The enhancement of smooth muscle relaxation may occur either by upregulating the production or activity of NO within the corporal tissues, by developing a way to increase the quantity of corporal smooth muscle cells, or both. Recent

data from Chitaley and colleagues suggest that there may be an NO-dependent vasoconstrictive pathway, called Rho kinase, that stimulates the smooth muscle cells to constrict.[15] These investigators theorize that when NO is released by the cavernosal nerves at the beginning of the erectile process, it has a dual effect. One is an inhibitory effect on the Rho kinase system, resulting in inhibition of the vasoconstriction of the smooth muscle cells, and the other is the well-known vasorelaxing effects on the smooth muscle cells of the formation of cGMP, as elucidated above. The discovery of the Rho kinase system is still too recent to conclude whether it may have a future therapeutic role in the treatment of ED, and we still await the availability of genetically grown smooth muscle cells that may be transplanted into the corporal bodies.

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Gene Therapy for Erectile Dysfunction

The penis is the ideal organ for gene therapy. It hangs off the body as an appendage and although it communicates with the peripheral vascular system, experience with the use of intracorporeal injections for the treatment of ED has taught us that drugs may be injected directly into the corporal bodies without fear of the product(s) escaping—at least in large and significant quantities—into the systemic circulation. Furthermore, if one could inject into the penis a product that would have an effect for between 6 months and a year or even longer, even the sildenafil nonresponders might also be candidates for such treatment, because the patient would not have to time the ingestion of the medication nor worry about its side effects on the peripheral vascular system.

The goal of gene therapy for organic impotence is to allow the patient to sustain physiologically elicited erections without resorting to pharmacological treatment immediately prior to the sexual act, as is currently done today with either intracorporeal injections or oral administration. This implies a stable biological correction of some facets of the impaired erectile mechanism, which could be defined as a medium or long-term cure rather than a palliative intervention. Therefore, even if the complementary DNA (cDNA) construct may be given by injection to the penis, the treatment is sporadic because it is expected that the effects will last for weeks, months, or even years, according to the vector, promoter, and delivery procedure used.

In the specific case of NO, it is assumed that erectile dysfunction results from a reduction in the synthesis of this mediator in the penile nerve terminals and/or an impaired mechanical compliance of the target cavernosal smooth muscle to the relaxation induced by NO, possibly combined with a putative increase in the response to or the levels of contractile factors. Therefore the modulation of endogenous penile NO synthesis through gene therapy of the corpora cavernosa with NO synthase (NOS) cDNA, may achieve a more stable and biologically controlled effect than that caused by vasoactive drugs injected into the corpora cavernosa, provided the enhanced NOS levels are activated only upon physiological stimulation in the penis. This is a likely scenario based on what is known about the neural control of penile erection and the mechanism of NOS activation. The neural dependence for eliciting the response would resemble the effects of oral PDE inhibitors like sildenafil,[16] except that the correction would be long-term or even permanent and not necessarily dependent on any type of drug prior to intercourse.

The initial demonstration that gene therapy for ED is feasible and that the modulation of NOS expression is a valid target was published in 1997.[17] This study reported that treatment of rats with a small amount (5 μg) of a construct containing the rat penile inducible NOS (iNOS) enzyme coding region in a plasmid under the control of the cytomegalic virus (CMV) promoter in a lipofectamine preparation, injected directly into the corpora cavernosa, improved aging-related erectile dysfunction. At 10 days after injection, the intracavernosal pressure elicited by neural stimulation in 20-month-old "aged" rats treated with the recombinant iNOS DNA was significantly increased (46%) over the 5-month-old young adult control animals. The intracorporeal pressure in the aged rats even surpassed the 5-month-old controls, without any NO-induced side effects. The recombinant iNOS protein appears to be activated only when the cavernosal relaxation is initiated by a physiological stimulus, because no priapism

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or hypotension was observed. This justifies the hypothesis that the therapeutic increase of penile NOS levels may ameliorate a deficient or insufficient NO synthesis responsible for erectile dysfunction. An important point to consider is that the syncytial nature of the corpora cavernosal smooth muscle, derived from the cell-to-cell communication through gap junctions, may compensate for the restricted site of delivery of iNOS cDNA (or any other gene) into the tissue.[18]

Very recently, endothelial NOS (eNOS) has been shown to improve erectile dysfunction in the aged rat through the administration into the corpora cavernosa of an adenoviral (AdV) eNOS construct where the expression of the recombinant protein was driven, as above, by the CMV promoter.[19] As expected, eNOS gene transfer increased the expression of eNOS and the calcium Ca2+-dependent NOS activity in penile tissue. The results in terms of cavernosal pressure were comparable to those seen in the earlier study with iNOS.

Neuronal nitric oxide synthase (nNOS) is certainly a strong candidate for gene therapy of erectile dysfunction because of the location of variants of this isoform in the nerve terminals of the penis. The recent cloning of the penile-specific nNOS variant (PnNOS)[20] allows for a strategy that is particularly attractive because of the putative tissue-specific control of enzyme activity that may be conferred by its 34 amino acid insert. This insert may provide some type of tissue specificity to NOS activation during the erectile response through a differential response of PnNOS as compared to the brain-type nNOS.

Although NOS is an obvious candidate for potential manipulation by gene therapy, many other genes expressed in the penis control critical processes in the complex process of erection and are therefore amenable to this approach. One good example is the hSlo cDNA, which encodes for the α subunit of the human smooth muscle maxi-K+ channel.[21] A study by Christ and colleagues[21] demonstrated first that expression of reporter β-galactosidase triggered by a plasmid under a CMV promoter persisted for up to 75 days in the corpora cavernosa when it was transfected into the rat penis as naked DNA. Intracorporeal injection of a similar naked vector encoding for the hSlo cDNA (100 μg) increased intracavernosal pressure response to electric field stimulation (EFS) in aged rats over their respective controls for at least 2 months. Although the equivalent amount in men would be rather excessive (close to 3 mg plasmid DNA), this study shows that overexpression of the maxi-K+ channel in the cavernosal smooth muscle may be an effective way to modulate intracellular Ca2+ levels and transmembrane Ca2+ flux in this tissue and therefore improve erectile dysfunction. Interestingly, a single intracorporeal injection of the same naked plasmid construct as above improved the erectile response in 9-month-old rats one week after the injection.[22]

Another approach[23] has been based on the possibility of increasing by gene transfer the expression in the penis of growth factors that may be essential for correcting tissue damage involved in neurogenic and vasculogenic erectile dysfunction, namely in nerve regeneration and angiogenesis, respectively. In both cases, the selected cDNAs were cloned in adeno-associated virus (AAV) vectors, a strategy that may end up being superior at least to the early generation AdV vectors because it reduces immunogenicity and prolongs expression. In addition, AAV are neurotrophic, and this may be particularly useful for nerve regeneration interventions. In one of the studies[24] it was shown histochemically that intracavernosous injection of the construct for brain-derived neurotrophic factor (AAV-BDNF) can prevent in rats, after bilateral cavernous nerve freezing, the degeneration of nNOS-containing neurons in the pelvic ganglion and can stimulate nerve regeneration. As a consequence, the erectile response to EFS in the animals with neurogenic impotence that were treated with AAV-BDNF was notably increased after 4 weeks and particularly after 8 weeks. This is promising for the recovery of erectile function after bilateral cavernous injury, such as after radical prostatectomy.

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Future Directions

Experimental efforts in making gene therapy for erectile dysfunction a viable therapeutic alternative are likely to continue intensively in a series of directions, some specific to the nature of the selected gene to be manipulated or the physiology of the corpora cavernosa itself, and others extrapolatable from the advancement of gene therapy in general. In the first category, in the case of NOS gene therapy, it may be envisaged that the selection of the NOS isoform cDNA will initially be based on studies in the rat using strong promoters such as CMV without tissue specificity, and

comparing the efficiency of the later-generation AdV or AAV cassettes with special liposome formulations or other methods of delivery of plasmid constructs.

The isoform selected will in turn dictate the choice of the most adequate gene promoter to favor expression of the protein in the respective target tissue. For PnNOS, specific promoters for neural tissue such as the neuronal specific enolase (NSE)[25] should restrict expression to nerves and ganglions. Even if the levels of PnNOS protein are elevated throughout the central and peripheral nervous system, the actual stimulation of catalytic activity is the only factor determining the increase in NO synthesis, and this should occur in the penis only upon appropriate sexual, pharmacological, or electrical stimulation, according to the animal models. In addition, the knowledge of several endogenous factors that control PnNOS activity, such as the protein inhibitor of NOS (PIN) or CAPON, may spur the design of gene transfer approaches based on inhibiting the expression of these proteins with ribozyme or antisense approaches[26] or competing with their binding to PnNOS. The same considerations can be applied to the other genes discussed above regarding selection of promoters, vectors, or cofactors. Novel vectors and transgenic mice are available where the recombinant cDNA is placed under a promoter regulatable by very low nonhazardous doses of a drug, such as doxycycline, ecdysone, or RU486,[27,28] and active only in a given tissue.

If a regulatable promoter is combined with a vector assuring long-term expression, the production of the recombinant protein may remain silent everywhere in the organism after the actual transfection or infection and be activated only in a specific tissue when the drug is given. Suspension of the drug stops further expression, and the cycle may be repeated. If two recombinant genes (eg, NOS and vascular endothelial growth factor) are placed under control of a different regulatable promoter, it would be possible to activate their expression separately or together according to the drug used. It is hypothetically possible to combine temporal expression with oral treatments with cofactors or regulators of the respective enzyme activity so that the basal frame of the gene product is enhanced at will, and then the protein is activated in a more conventional way by direct modulation of a temporarily hyperexpressed product. Although at first sight gene therapy for erectile dysfunction in men may appear rather remote at this moment, the advances in recombinant DNA technology and delivery procedures in the last few years may drastically change this perception in the near future. ■

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Main Points

- The penis consists of sinusoidal blood vessels surrounded by a syncytium of vascular smooth muscle cells that begin with aging to become dysfunctional, resulting in an inability of these smooth muscle cells to relax normally following sexual stimulation.
- As a phosphodiesterase inhibitor, sildenafil improves the relaxation of the smooth muscle cells.
- Of those men with organic erectile dysfunction (ED), up to 35%–50% may not respond to sildenafil, because the efficacy of the drug is directly dependent on the release of nitric oxide from the nerve terminals of the cavernosal nerve, which may become diminished with aging and certain disease states.
- The success rate of intracorporeal injections in improving erections is close to 90% demonstrating that bypassing the peripheral vascular system provides a pharmacological level within cavernosal bodies that cannot be achieved with oral drugs.
- Endogenous biochemical events within the penis that affect an erection make up the nitric oxide–cyclic guanosine monophosphate (NO-cGMP) system.
- The cGMP ultimately stimulates certain kinases to uncouple calcium intracellularly within the smooth muscle cells of the corpora, resulting in smooth muscle relaxation.
- Enhancement of smooth muscle relaxation may occur either by upregulating the production or activity of NO within the corporal tissues, by developing a way to increase the quantity of corporal smooth muscle cells, or both.
- The penis is the ideal organ for gene therapy because, although it communicates with the peripheral vascular system, a drug may be injected directly into the corporal bodies without fear of the product escaping in significant quantities into the systemic circulation.

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Summary of Discussion Following Dr. Rajfer's Presentation

Dr. Rajfer articulated the main point of his presentation as this: with ED, the future treatment for ED is probably going to be non-oral drugs. Gene therapy is possible, he believes, and if all the kinks are worked out, it may be applicable not only to oral drug responders, but to a larger proportion of ED patients.

Dr. McCullough asked Dr. Rajfer to make some prediction as to what ED therapy might look like in 10 years, but Rajfer preferred not to guess, emphasizing that research in gene therapy for ED is in its embryonic stages.

Dr. Carson asked of Rajfer, "What do you think the electroporation is actually doing? Why is that such a huge advantage?" Rajfer responded that, as yet, nobody knows, but that this fascinating issue is one that is being investigated in his laboratory. "The good thing about [electroporation]," he said, "is you don't need to use as much product, to get the same transfection. It is believed that it alters the cell membrane potential, and opens up the pores. That allows the gene vector to get into the system."

Dr. Carson then asked the question, "Do you think that the use of the maxi-K+ gene, in gene therapy, is better than the nitric oxide synthase approach? Worse? The same? Should you combine them?" Rajfer responded that, again, this isn't known, and that more data is needed to show the mechanism of action of that product. So far, the human maxi-K+ gene has been used in the rat model, and has shown efficacy. It is highly unusual, he added, that a human protein will work in a rat model. The question is, will it work in humans?

Dr. Rajfer emphasized that what he'd mainly attempted to show in his presentation is the most common cause of ED: aging tissue. We are just starting to understand, he said, what the molecular events are that occur with aging in the penis. The challenge is to find a way to target the aging process.

"This is so different from the current dogma with ED," Dr. McCullough observed, "because now we have age as the major issue here. It's not just peripheral vascular disease, the predictor of coronary disease. It's simply the age as the major issue and it's inescapable and not preventable"

Although Dr. Rajfer pointed out the NOS pathway as a potential major culprit in the aging effect on erection, Dr. Steers reminded him of the powerful Rho-kinase system that might also be driving a more "contracted state" that would not respond to the relaxation message. "You have two enzymes that are conflicting."