

Potential Application of Gene Therapy for the Treatment of Erectile Dysfunction

Review

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Erectile dysfunction (ED) is a condition defined by the inability to attain or maintain penile erection sufficient for satisfactory sexual intercourse (NIH Consensus Conference, 1993). In 1995, it was estimated that approximately 152 million men worldwide suffered from ED, with projections for 2025 growing to a prevalence of 322 million affected men (Aytac et al, 1999). In the past, ED was believed to be primarily caused by nonspecific psychological causes; however, in the past 2 decades, the majority of cases have been attributed to an organic etiology. Although ED patients can have a number of medical conditions, organic ED is usually associated with vascular risk factors such as arteriosclerosis, hypertension, diabetes mellitus, Peyronie disease, and renal disease (Benet and Melman, 1995; Laumann et al, 1999; Hellstrom and Bivalacqua, 2000). In addition, pelvic trauma and pelvic surgery (radical prostatectomy or radical cystectomy) can cause ED by either vascular or nerve damage.

Since the early 1980s, our understanding about the pharmacology of the erectile mechanism has advanced significantly. Basic research in corporal cavernosal smooth muscle (CCSM) physiology and identification of the central mediators involved in the erectile process has contributed to the development of pharmacological agents that can effectively treat ED patients. At present, the diagnosis and treatment of ED has evolved to the point where virtually every patient suffering from ED can be successfully treated. Vacuum erection devices, intracavernosal injection therapy, intraurethral suppositories, oral medications, penile vascular procedures, and surgical implantation of prosthetic devices offer most men a viable option to correct their ED. However, despite the overall success and efficacy of the aforementioned therapies, there are implicit side effects, complications, and contraindications. Therefore, the development of future therapeutic options for the treatment of ED should focus on

those strategies with fewer adverse effects and an absence of contraindications. Gene therapy for the treatment of ED may become a viable and relatively noninvasive therapeutic option. The purpose of this review is to examine the possible role of gene therapy for the treatment of ED and, hopefully, to foster interest and future investigation.

Physiology of Penile Erection

In order to understand the potential role of gene therapy for ED treatment, it is essential to understand the neural and vascular pathways that function during penile erection. The process of penile erection is dependent on an intact central and peripheral nervous system and stable hormonal status. Normal erectile function involves 3 synergistic and simultaneous processes: 1) neurologically mediated increase in penile arterial inflow, 2) relaxation of cavernosal smooth muscle, and 3) restriction of venous outflow from the penis (Andersson and Wagner, 1995). ED occurs as a result of the failure of any one or all of these processes.

The penis is composed of 3 bodies of tissue separated by connective tissue septa. The singular corpus spongiosum supports and protects the urethra along the ventral surface of the penis. The paired corpora cavernosa, which lie dorsally and adjacent to each other, function as blood-filling reservoirs and provide structure to the penis in the erect state. The cavernosal bodies are composed of a network of vascular sinuses supplied by the helicine arteries (terminal branches of the cavernosal arteries). In the flaccid state, the smooth muscle trabeculae, which support the vascular sinuses, are tonically contracted and permit only a small amount of arterial inflow. The release of neurotransmitters from cavernous nerve terminals and smooth muscle endothelium in response to sexual stimulation results in cavernosal smooth muscle relaxation and, ultimately, penile erection.

The penis is innervated by both autonomic and somatic nerve fibers (Stief et al, 1997; Lue, 2000). Cholinergic nerves, nonadrenergic/noncholinergic nerves (nitric oxide), and other factors such as vasoactive intestinal peptide and calcitonin gene-related peptide mediate cavernosal smooth muscle relaxation (Andersson and Wagner, 1995). In addition, shear stress and muscarinic receptors on trabecular endothelium stimulate the production of nitric oxide. Nitric oxide originating from nonadrenergic/noncholinergic nerves and the trabecular endothelium diffuses into smooth muscle cells where it directly interacts

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with the soluble form of guanylate cyclase to increase intracellular levels of cyclic guanosine monophosphate (cGMP) (Burnett et al, 1992; Rajfer et al, 1992). The increase in cGMP then influences a number of cellular processes that result in smooth muscle relaxation, primarily through activation of the cGMP-dependent protein kinase and ion channels (Moreland et al, 1999). This mechanism reduces intracellular Ca^{2+} via Ca^{2+} sequestration, extrusion, or both, and opens potassium channels causing hyperpolarization of CCSM (Christ, 1997). Similar to the more familiar cyclic adenosine monophosphate (cAMP) pathway, cGMP activity is terminated by hydrolysis of the 3'5' bond by the type 5 phosphodiesterase. Vasoactive intestinal peptide, calcitonin gene-related peptide, and prostaglandin-mediated pathways, which produce an increase in intracellular cAMP, also likely contribute to smooth muscle relaxation in the penis (Bivalacqua et al, 1999). The increased corporal arterial inflow results in an increase in intracavernosal pressure and volume. As the penis expands in length and girth, the intracavernosal pressure eventually exceeds that of the subtunical venular plexus, thereby compressing it between the tunica albuginea and the peripheral sinusoids. This process of veno-occlusion traps blood in the corporal bodies, causing penile tumescence and rigidity.

The balance between contractile systems (α -adrenergic, endothelin, angiotensin, thromboxane A_2) and vasodilatory second-messenger systems (adenylate cyclase-cAMP and guanylate cyclase-cGMP) determines the tone of corpora cavernosa smooth muscle of the penis. One important factor that should be considered in the erectile process that contributes to the overall vascular tone of the penis is the presence of intercellular channels known as gap junctions in the membranes of the cavernosal smooth muscle cells (Christ et al, 1996; Christ, 1997). Communication among smooth muscle cells of the corpora cavernosa allows the passage and movement of physiologically relevant ions (K^+ , Ca^{2+}) and second messengers (cGMP, cAMP) that govern syncytial relaxation and contraction of the corpora cavernosal smooth musculature necessary for unified penile erection and detumescence (Christ et al, 1996; Christ, 1997; Christ and Melman, 1998).

What Is Gene Therapy?

Over a century ago, Gregor Mendel attempted to explain the inheritance of biological characteristics. He proposed that each inheritable property of an organism is controlled by a factor, now called a gene, that is present in all cells. The birth of genetics aimed to understand what genes are and exactly how they function to determine physiological processes in an organism. In the 1940s, a number of future Nobel prize-winning researchers proposed that genes were made up of DNA, and this DNA governed the bi-

ological and physiological characteristics of man. Over the past 20 years, scientists have developed molecular biological techniques (Southern, Western, and Northern blotting; polymerase chain reaction; and DNA sequencing) to help study genes and their functions in life processes. This technology has evolved logarithmically to the point that scientists are now performing gene cloning in animals and mapping the entire human genome. From a medical standpoint, the future of gene therapy will be to use this knowledge to treat human diseases. Therefore, the goal of gene therapy for an acquired disease or genetic abnormality is to introduce novel genetic material into an appropriate cell in an attempt to restore normal cellular function.

Somatic gene therapy can be defined as the ability to introduce genetic material (RNA or DNA) into an appropriate cell type in vitro or in vivo, thus altering gene expression of that cell in order to produce a therapeutic effect (Nabel et al, 1994). Gene therapy involves a number of finite sequences: the administration of a desired gene into the body, delivery of the gene to a targeted cell that is subsequently transported into the nucleus, and then expression of the therapeutic product (Figure 1). In the past 10 years, there has been a substantial amount of basic and clinical research in the field of gene therapy that can be attributed to a solid foundation of scientific advances in eukaryotic gene expression, viral genetics, and, more importantly, the cloning of human disease-related genes (Mulligan, 1993). In the past, gene-therapy approaches have been used to correct or treat disorders that had an underlying genetic component. However, gene therapy has evolved to the point where treatment of any disease process can be theoretically accomplished as long as there exists a therapeutic gene that can either effectively restore or supplement defective functions or antagonize the expression of a mutant gene.

In order for this to occur, a number of important criteria must be met. First, any successful gene-therapy approach to human disease requires the existence of a therapeutic gene. Second, an appropriate vector must be chosen that will safely and effectively deliver the desired gene into the target tissue or cell. Third, a method for delivering this vector to a localized cell that allows maximal incorporation of the desired gene must be determined. Each of these criteria must be tailored to the specific disease being treated. Accordingly, the following review of erectile dysfunction gene therapy will examine the different vector systems currently available to deliver various genes to specific tissues or cells, explain why these technologies may be useful in the treatment of erectile dysfunction, and justify which specific therapeutic genes are likely to be used for erectile dysfunction gene therapy in the future.

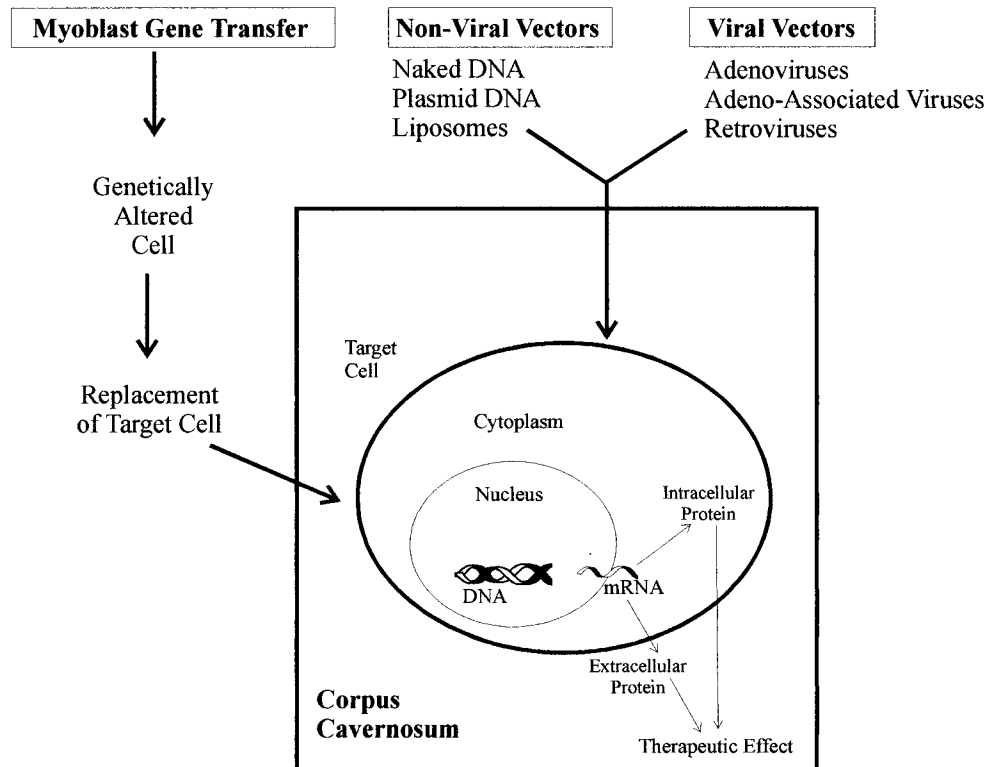


Figure 1. General schematic of gene-therapy approaches to replace a targeted cell or overexpression of a therapeutic gene for the treatment of erectile dysfunction. This figure demonstrates the administration of a desired gene or cell into the corpus cavernosum and subsequent transportation into the nucleus and alteration of cellular function. More specific delineation of the mechanisms for vector incorporation of a specific gene into a targeted cell and the alteration in cellular function can be found in the reference list.

Methods of Gene Transfer

In order to transfer genetic material to a variety of cells, DNA or RNA must be delivered in a vehicle or vector that allows efficient gene transfer. Some examples of vectors used to date for gene transfer include nonviral vectors (naked DNA, plasmid DNA, and liposomes), adenoviruses, adeno-associated viruses (AAVs), and retroviruses (Mulligan, 1993; Ledley, 1995; Costantini et al, 2000). Each of these gene transfer vehicles offers different gene transduction efficiencies and possesses distinct advantages and disadvantages. The ideal vector would be one that would allow efficient transduction and long-term stable transgene expression while demonstrating little or no adverse side effects, such as risk of infection, immunogenicity, or host-cell mutagenesis. However, despite numerous experimental attempts, the ideal vector has not yet been identified. Therefore, a number of vectors have been developed, each with their own unique properties that can be applied to certain cell types and diseases. This section summarizes some of the gene-therapy vectors that have been used to date.

Nonviral Vectors—Nonviral gene transfer is dependent on the normal cellular mechanisms of mammalian cells for the uptake and intracellular transport of macromolecules (Ledley, 1995). Gene transfer via naked DNA has

been shown to produce low but detectable transgene expression, particularly in skeletal and cardiac muscle (Lin et al, 1990; Wolff et al, 1990). The major drawback of using naked DNA is the relatively low efficiency rate (usually less than 1% of target cells) and transient expression (Table). Plasmid DNA is easy to construct and can be produced in large quantities. Plasmid vectors are generally not associated with immune responses either to the DNA or transgene product and do not exhibit an inflammatory response. However, the use of plasmid DNA vectors has been limited to low efficiencies of transduction in vivo (Bally et al, 1999). Most authorities hypothesize that this low transduction efficiency may be due to a lack of receptor-mediated endocytosis of plasmid DNA and its poor nuclear translocation after incorporation into the lysosomal compartment following cellular uptake (Rhodes et al, 1998; Bally et al, 1999). In order to increase the transgene expression, DNA has been incorporated into lipid vesicles called liposomes. Liposomes facilitate increased stability of the desired DNA and increase cellular entry by promoting fusion with the plasma membrane. Although liposomes have been shown to increase transgene expression in vitro, they have been less successful in vivo (Nabel et al, 1990; Takeshita et al, 1994). Another possible approach to genetically alter a

Advantages and disadvantages of vectors for gene therapies

Vector	Advantage	Disadvantage
Naked DNA	Extremely safe; insertion of large DNA segments	Low (<1%) gene transfer efficiency
Liposomes	Increased efficiencies when compared to naked DNA	Low gene transfer efficiency; transient expression
Retrovirus	Stable long-term expression; high-efficiency gene transfer	Requires dividing cells; integration into host DNA (insertion mutagenesis)
Adenovirus	Highest efficiency of gene transfer; infects both dividing and nondividing cells	Transient expression; immunogenic
Adeno-associated virus	High efficiency gene transfer	Immunogenic; insertion of small DNA segments; transient expression

particular cell or tissue is performed by myoblast-mediated gene therapy. This approach involves the use of cells, skeletal muscle or endothelial cells, to introduce genes encoding therapeutic proteins into the body (Stockdale et al, 1990; Smythe et al, 2000). This is performed by removing cells from the body and genetically engineering these cells in culture and then reintroducing these cells into a particular tissue, where they become integrated into a pre-existing tissue. Myoblast-mediated gene transfer is safe and offers stable delivery of a particular genetically altered cell into a tissue. In summary, nonviral vector systems generally have relatively low transgene expression in vivo but do not cause host immune and inflammatory responses (Table).

Retroviral Vectors—Retroviral gene therapy has been used to deliver genes to both experimental animals and humans (Tolstoshev, 1992; Jejurikar et al, 1997). A major advantage of using retroviral vectors is the ability to remove all viral genes and replace them with the therapeutic gene. However, there are major drawbacks with retroviral vectors that may limit their therapeutic applicability (Table). First, it is very difficult to produce high titer stocks of these viruses (unlike adenoviruses and AAVs), which makes it difficult to use in vivo. Second, successful gene therapy with retroviruses requires cell proliferation (Miller et al, 1990). This is a major problem for ED gene therapy because the smooth muscle and endothelial cells of the corpora cavernosa do not actively proliferate. Third, retroviruses integrate into the DNA of dividing cells; thus, the risk of insertional mutagenesis is present.

Adenovirus Vectors—Adenoviral gene therapy offers a significant number of advantages over the aforementioned vector systems (Table). Adenoviral vectors are frequently used for gene transfer because of their high cellular transduction efficiency in a wide variety of cell types and tissues in vivo (Rosenfeld et al, 1991; Stratford-Perricaudet et al, 1992). Adenovirus vectors can be produced at very high titers, thereby allowing efficient gene transfer to a specific tissue with small volumes of the virus. Furthermore, adenoviruses transfect both dividing and nondividing cell types and do not insert into the cell's genome (Csete et al, 1995). The biggest disadvantage of first-generation adenoviral vectors is the expression of viral pro-

teins in infected cells, which can trigger a host immune and inflammatory response that precludes repeated administration of this vector as well as long-term and transient expression of the desired gene. However, there has been great interest in the development of second-generation or helper-dependent (gutless) adenovirus vectors in which all viral genes are deleted (Schiedner et al, 1998; Morral et al, 1999). This is important because it minimizes the possibility of host immune response, increases the cloning capacity, and, more importantly, allows repeated administration over time. This factor is an important consideration in the treatment of ED via gene-therapy techniques because of the necessity to repeatedly administer a therapeutic gene over time into the penis. The helper-dependent or gutless virus has been used to efficiently increase gene expression in vivo while decreasing cellular toxicity and immune response (Schiedner et al, 1998). Therefore, the future application of this gutless virus variation may represent an important new vector that offers long-term transduction efficiency with little or no host immune responses for both dividing and nondividing cells.

Adeno-Associated Viral Vectors—AAV, a nonpathogenic human parvovirus, is thought to be an attractive vehicle for use in human gene therapy. One important feature of recombinant AAV vectors is their ability to be stably maintained in host cells as integrated proviruses. AAVs have several characteristics that make them attractive as gene transfer vectors: 1) no known pathogenicity, 2) high efficiency and the ability to remain latent, 3) a minimal number of antigens ensuring minimal immunogenicity, 4) the ability to transduce nondividing cells, albeit at a lower frequency than dividing cells, and 5) the ability to infect a broad range of cell types (Robbins et al, 1998; Carter and Samulski, 2000) (Table). However, as with retroviruses and adenoviruses, there are a number of significant problems that must be examined before AAVs can be used in humans. Although AAV vectors seem to transfect a number of cell types in vitro, it is still unclear as to which cell types can be stably transfected in vivo (Romano et al, 1998). Another potential problem is the fact that AAVs are often co-infected with adenoviruses, making it difficult to prepare large quantities of

pure AAV vectors. AAV vectors do not produce large titers because deletion of internal sequences appears to reduce the titers from 10- to 10000-fold. In fact, when there is a deletion of the viral genes, this process does not allow the vectors to be integrated in a site-specific manner, thus promoting the possibility of insertional mutagenesis (Rabinowitz et al, 1999).

Gene Therapy and Erectile Dysfunction

The question that must be answered is: "Why is gene therapy for the treatment of erectile dysfunction a viable therapeutic option in humans?" This section will discuss the rationale behind gene therapy's role in the future management strategies of ED.

Gene therapy has been proposed as a viable treatment option for cardiovascular diseases (arteriosclerosis, congestive heart failure, pulmonary hypertension) because of the vascular origin of these disorders (Nabel et al, 1994; Heistad and Faraci, 1996; Champion et al, 1999a). This, by biological extension, suggests that gene therapy may be employed to treat vascular diseases of the penis; in most cases, ED can be considered a manifestation of vascular disease. One problem that arises in cardiovascular gene therapy is that genes can be transferred or incorporated into the wrong peripheral vascular bed or organ. However, this is not a problem for ED gene therapy because the penis has an easily accessible external location (Christ and Melman, 1998; Lue, 1999). Hence, a tourniquet can be placed around the base of the penis and the desired gene can be administered directly into the corpora cavernosa, allowing less entry into the systemic circulation.

Recently, at the University of Pennsylvania, there was a death of a patient as a result of adenoviral gene therapy for an inherited liver disorder. This young man's death was caused by a pathological increase in inflammatory cells that attacked the patient's lungs, liver, and brain, resulting in organ failure. This is a potential risk of gene therapy when a gene-therapy vector is administered into the systemic circulation. However, this potential adverse effect is minimized when gene-therapy vectors are used for treatment of ED because the penis has its own external circulation, allowing a gene to be transferred and localized in 1 organ, lessening the risk of systemic spillover.

Another problem that other gene-therapy approaches have encountered is the determination of the number of cells that must be transfected in order to produce a therapeutic effect that would change the physiology and function of the organ or vasculature positively. It is often difficult to transfect a large number of cells, and if an inadequate number are not transfected, there may not be a physiologically relevant change in function. However, in the penis, only a small number of cells need to be transfected because the CCSM are interconnected by gap junc-

tions (Christ, 1997; Christ et al, 1999). Moreover, the vascular smooth muscle cells of the penis have a relatively low turnover rate, thus allowing a desired gene to be expressed for long periods of time. Christ and Melman (1998) have concluded that these gap junctions permit the intercellular exchange of ions and second messengers that allow CCSM cells that are not directly activated with a given neuronal signal to be effected via this intercellular communication. Because naked DNA does not have a high transduction efficiency, this principle allows for lower transfection efficiencies to cause physiologically relevant changes in erectile function. It also means that lesser amounts of viral vectors need to be administered, which would in turn cause fewer immunogenic reactions.

The principle mechanism of penile erection involves a delicate balance between the relaxation and contraction of arterial and trabecular smooth muscle in the corpora cavernosa of the penis. Any alteration, no matter how small, that may occur in this mechanism may have dramatic effects on erectile function. This is an important concept, because if ED gene therapy can restore the normal balance between the relaxatory and contractile mechanisms, then erectile physiology can be restored to normal functioning. For example, if ED gene therapy can restore the depletion of nitric oxide synthesis in the penis that is observed in diabetes, then erectile function can potentially be restored to normal in diabetic patients suffering from ED. In theory, a variety of strategies can be used to restore normal potency in disease processes that cause ED in humans. These theories serve as the motivation for exploring gene-therapy techniques to restore erectile function in humans.

Which Genes to Use for ED Gene Therapy?

Smooth muscle relaxation is the necessary step needed to achieve a normal erection. Therefore, molecules and enzymes that influence the signal-transduction pathway of corporal smooth muscle relaxation represent potential targets for ED gene therapy. Figure 2 describes one approach to cavernosal smooth muscle relaxation using the endothelial nitric oxide synthase (NOS) gene by various nonviral and viral vectors or replacement of an endothelial cell. Several researchers have envisioned the use of gene therapy for the treatment of ED. Garban and associates first demonstrated that gene therapy can be performed in the penis by use of naked complementary DNA (cDNA) encoding the penile-inducible NOS gene, and they recorded an improvement in erectile function in aged rats after injection with the rat penile-inducible NOS (Garban et al, 1997; Gonzalez-Cadavid et al, 1999). Rehman et al (1997) found that intracavernous injection of cDNA encoding for neuronal NOS could significantly increase intracavernosal pressure above that of age-matched controls. Christ and colleagues demonstrated that injec-

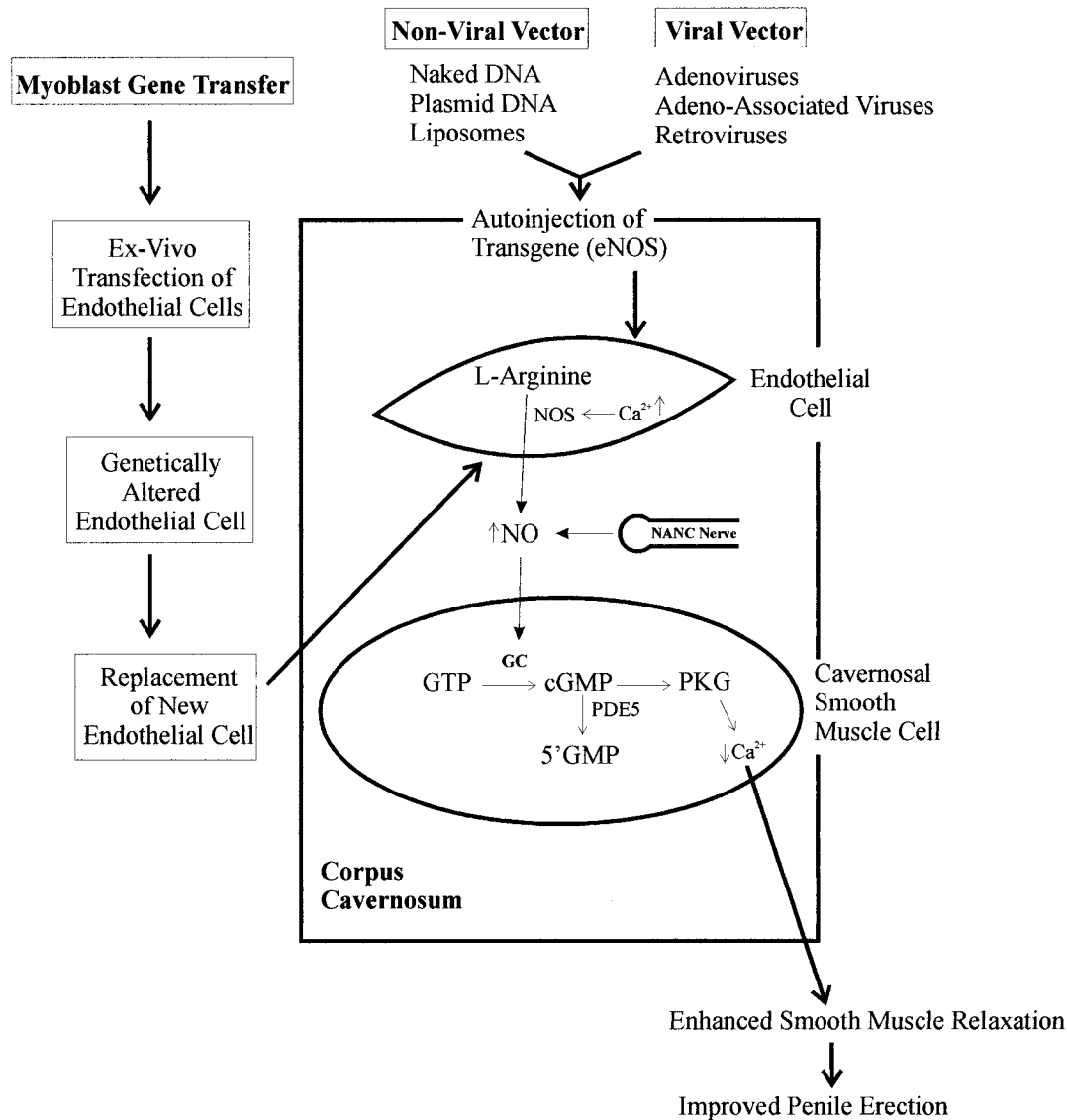


Figure 2. General schematic of one gene-therapy approach to alter vascular tone of the penis. This mechanism involves replacement of endothelial cells or overexpression of the endothelial nitric oxide synthase (NOS) gene. Nitric oxide (NO), synthesized by nonadrenergic/noncholinergic (NANC) nerves and endothelial cells, diffuses into the smooth muscle cell, a process that activates guanylate cyclase (GC) and increases intracellular cyclic guanosine monophosphate (cGMP) synthesis. This results in cavernosal smooth muscle relaxation and penile erection. In aging and diabetes, there is a decrease in NOS expression that is associated with erectile dysfunction. This gene-therapy process causes overexpression of the endothelial NOS gene, increased NO synthesis, enhancement of smooth muscle relaxation, and improvement in erectile function. PKG indicates protein kinase G; GTP, guanosine triphosphate; PDE, phosphodiesterase; and GMP, guanosine monophosphate.

tion of hSlo cDNA, which encodes for the large-conductance calcium-sensitive maxi-K channel, into the rat corpora cavernosa can increase gap-junction formation and enhance erectile responses to nerve stimulation in aged and diabetic rats (Christ et al, 1998; Christ et al, 1999). They also found that hSlo cDNA was expressed for at least 2 months in the penis (Christ et al, 1998).

More recently, our laboratory reported that adenoviral gene transfer of endothelial NOS could reverse age-related erectile dysfunction in rats (Champion et al, 1999b; Bivalacqua et al, 2000a; Bivalacqua et al, 2000b). In our

studies, we utilized the cytomegalovirus and Rous sarcoma adenoviruses and noted that expression of endothelial NOS was sustained for 1 month in the corpora cavernosa of the rat penis. We also documented that 5 days after transfection with the adenoviruses encoding for endothelial NOS (AdCMVeNOS or AdRSVeNOS), aged rats had significant increases in erectile function as determined by cavernosal nerve stimulation and pharmacological injection with the endothelium-dependent vasodilator, acetylcholine, and the type 5 phosphodiesterase inhibitors, zaprinast and sildenafil (Champion et al,

1999b; Bivalacqua et al, 2000a; Bivalacqua et al, 2000b). The intracavernous response to cavernosal nerve stimulation was studied 1 month after transfection with the AdRSVeNOS virus, and erectile function was still significantly higher than those of age-matched controls, suggesting that the adenovirus encoding for endothelial NOS was sufficient to reverse age-related ED in rats for up to 1 month. Similar results with adenoviruses encoding for the inducible NOS (iNOS) gene have been found in aged rats in another laboratory (Huard et al, 1998). We believe that adenoviral gene transfer offers significant advantages over other vehicles because this vector will incorporate a particular gene into both dividing and nondividing cells, and it has higher efficiencies of transduction when compared to naked DNA constructs. However, second- and third-generation adenoviruses must be utilized to reduce the host immunological responses that have been seen with first-generation adenoviruses. Recently, Wessells and Williams (1999) demonstrated that transplanted endothelial cells, which may be genetically modified, when injected into the rat corpora cavernosa, persist in the corporal sinusoids for up to 2 weeks, suggesting that this may be a more efficient and safer method of gene transfer.

These innovative studies provide evidence that in vivo gene transfer can have beneficial physiological effects on penile erection. If genes encoding for any of the NOS isoforms, the maxi-K channel, or other genes that influence the vascular tone of the penis (ie, calcitonin gene-related peptide, chloride channels, or vascular endothelial growth factor) can be incorporated into the human corpora cavernosa and influence the vascular tone of the penis for several months, then this may represent an exciting new treatment option for many men suffering from organic ED. For example, diabetic men with depressed nitric oxide synthesis due to neuropathy will more likely benefit from NOS gene-transfer therapy compared to men with vascular organic ED, who likely have multiple etiologies contributing to their ED. Theoretically, patients on ED gene therapy would not require other treatment modalities during this time period. Gene therapy is an exciting and ever-growing new field in urology; however, clinical use of gene transfer to restore erectile function must meet stringent regulatory approval before the first clinical trials can be performed.

Conclusion

The application of gene therapy for the treatment of ED represents an exciting new field that still requires more basic research before in vivo gene-therapy techniques can be applied to humans. Furthermore, new viral and non-viral vector systems that offer longer gene-transfer efficiency, higher levels of expression of the transduced gene, and little or no immunogenic reactions represent important therapeutic parameters to be manipulated in devel-

oping gene therapy for safe application in the future treatment of ED.

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