Neuroregenerative Strategies After Radical Prostatectomy

Robert C. Dean, MD, Tom F. Lue, MD

Department of Urology, University of California San Francisco, San Francisco, CA

Patients with erectile dysfunction (ED) following radical prostatectomy (RP) continue to present to practicing urologists. Although nerve-sparing RP has decreased the rates of ED significantly, new therapies for cavernosal nerve protection and recovery are now being developed. This report discusses the many agents available in neuroregeneration and neuroprotection to aid in the recovery of erectile function. Multiple agents and strategies have been used for neuroprotection and neuroregeneration of the cavernosal nerve following RP and in nerve injury models. Many of these agents display promise for the treatment of impotence. Early treatment for patients recovering from RP is becoming the standard of care. Natural recovery of erections may take as long as 18 to 24 months post RP; however, treatment plans may reduce the time to erectile recovery.

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With the development of the anatomic radical prostatectomy (RP) described by Walsh and Donker,[1] impotence rates following RP have decreased significantly. Patients' recovery of erectile function following RP, however, continues to be a challenge for the practicing urologist. With impotence rates remaining quite varied among these patients, even with nerve-sparing RP, there continues to be a strong interest in the discovery of the pathophysiology of erectile dysfunction (ED) in this patient population.

Theories abound on the cause of ED after nerve-sparing RP. Tissue cell death within the penis, neurapraxia, vasculogenic injury, and loss of the veno-occlusive mechanism have all been cited as possible causes of ED following RP. The evidence for each of these mechanisms remains limited; therefore, some of these theories are controversial. However, even though the causes of injury are debatable, preventive strategies have been investigated.

Neurapraxia

Neurapraxia is the "failure of conduction in a nerve in the absence of structural changes, due to blunt injury, compression, or ischemia; return of function normally ensues."[2] Some urologists believe that although the cavernosal nerves are spared during a nerve-sparing RP, the nerves are usually injured by direct trauma or by stretch during the operation.[3] This injury would account for the immediate delay in erectile function following RP but its subsequent return months later. Few men recover erectile function within the first 6 months following RP.[4] Some studies have found that a period of up to 18 months is required before maximum recovery is seen.[5]

Also supporting the neurapraxia theory is the delay in the response to phosphodiesterase-5 (PDE-5) inhibitor therapy in RP patients. Recent studies have demonstrated a considerable decrease in the enzyme nitric oxide synthase (NOS) in animals with bilateral cavernosal nerve resection.[6] If, after RP, the cavernosal nerves are not functioning because of neurapraxia but their function recovers in 8-18 months, one can understand how the benefits of PDE-5 inhibitors are not seen until this recovery phase begins. Response rates for sildenafil in the treatment of ED are poor for the first 9 months following RP but improve steadily, even up to 2 years.[7] These findings also support the theory of neurapraxia.

Although the neurapraxia theory has gained some acceptance, there is evidence against this theory. In a study by Zippe and colleagues,[8] successful sildenafil therapy following RP was related to the presence of neurovascular bundles and to the dose of sildenafil, but not to the time interval between surgery and drug therapy. In this study, there was no difference in response rate among men who initiated therapy 0 to 6 months after RP, those who started 6 to 12 months after RP, and those who began more than 12 months after RP. If neurapraxia did occur in this cohort, an increased response rate would be expected in the group beginning therapy furthest from surgery.

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Many factors may be involved in the nerve injury mechanism in RP patients. Nerve stretch, direct injury, increased pelvic fibrosis, and nerve entrapment may all play a role in the nerve-sparing RP patient experiencing impotence. Whether the cause of ED is true nerve injury or neurapraxia remains difficult to ascertain in this patient population.

Smooth Muscle Apoptosis

Increased programmed cell death, or apoptosis, has been demonstrated in the rat penis after cavernosal denervation.[9] Specifically, increased cell death is seen in the smooth muscles of the rat penis after cavernosal nerve injury. Although programmed cell death is essential for a multicellular organism's growth and development, it is an accelerated or generalized cell death that may lead to tissue volume loss and fibrosis. After RP, both clinicians and patients have perceived a volume loss of the penis, which may be a consequence of apoptosis of the smooth muscle of the corpora cavernosa.

Klein and associates[9] demonstrated apoptosis of the erectile tissue of the rat penis following denervation. Increased DNA fragmentation and elevation of sulfated glycoprotein-2 (SGP-2), both markers of apoptosis, were noted in the rat corpora following denervation. In a recent investigation, User and coworkers[10] demonstrated an apoptotic smooth muscle cell population in the subtunical location in denervated rat penis. Again, this implies that the smooth muscle cells of the corpora depend on neural input for stability. The loss of function of the subtunical smooth muscle cells may contribute to the loss of the veno-occlusive mechanism, thus causing ED. In this study, rats underwent bilateral or unilateral cavernosal neurotomy, or a sham operation. At different time intervals, the rats were killed and the penile wet weight, DNA content, and protein amount were measured. In addition, immunohistochemical analysis was performed on the penile tissue. Both penile wet weight and DNA content were significantly decreased in the bilateral cavernosal neurotomy group versus the unilateral denervation group and the controls. Also, apoptotic staining of the group penis was highest in the subtunical smooth muscle cells and highest on postoperative day 2. Other muscle cell lines, such as skeletal and cardiac, have been shown to rely on innervations through neurotrophic mediators for their survival.[11,12] It is a reasonable assumption that

cavernosal smooth muscle may also be reliant on neurotrophic mediators.[13] However, in our bilateral nerve injury rat model, we did not see a significant difference in smooth muscle content between the sham and nerve-injury groups at 3 months (author data on file). In addition, because the subtunical cells are not typical smooth muscle cells, the significance of a short-term increase in the rate of apoptosis of these cells remains to be elucidated.

sufficient for satisfactory sexual intercourse, compared with 3 patients (20%) in group 2. The authors therefore recommend early postoperative administration of alprostadil injections to rehabilitate the penis after RP. Intracavernous injection of alprostadil seems a reasonable approach; however, our preference is to give a papaverine and phentolamine combination, because of the high rate of painful erections associated with alprostadil in post-prostatectomy patients.

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Therapeutic Strategies

Current Strategy

Although many articles have reported various response rates to pharmacologic therapies for ED after RP, few have addressed strategies for the recovery of spontaneous erections. In 1997, Montorsi and colleagues[14] reported the first study using intracavernous injection of alprostadil to enhance the recovery of spontaneous erections after RP. The study consisted of 30 potent patients with clinically localized prostate cancer. The subjects underwent nerve-sparing radical retropubic prostatectomy and were then randomized to alprostadil injections 3 times per week for 12 weeks (group 1, 15 patients) or observation with no erectogenic treatment (group 2, 15 patients). Patients were assessed at the 6-month follow-up by sexual history, physical examination, color Doppler sonography of the cavernous arteries, and polysomnographic recording of nocturnal erections. The authors reported that in group 1, 12 patients (80%) completed the entire treatment schedule and were evaluated at the long-term follow-up. Eight patients in this group (67%) reported the recovery of spontaneous erection

The introduction of sildenafil ushered in a simple, effective oral treatment for ED and high hopes for improving erectile function. However, so far, there has been only one report on sildenafil and the recovery of spontaneous erections in patients after RP. In an industry-sponsored study, 76 potent men with normal International Index of Erectile Function (IIEF) score and normal nocturnal penile erection underwent nerve-sparing RP. At week 4, group 1 (51 men) was instructed to take sildenafil 50-100 mg nightly, and group 2 (25 men) was given a placebo for 36 weeks. At week 48 (2 months after discontinuing the drug/placebo), a follow-up study was performed. In group 1, 14 of the 51 men (27%) recovered spontaneous "normal" erections, whereas in group 2, only 1 of 25 men (4%) recovered normal erections. The authors postulate that this effect may be the result of improved endothelial function combined with neuronal regeneration and neuroprotection.[15]

After a thorough review of recent literature, Gontero and Kirby[16] concluded that current scientific evidence in support of an early postoperative use of erectile aids is based mainly on indirect proof of cavernosal damage that may follow the temporary postoperative "erectile silence." Intracavernous injections or a vacuum device may be the best first-line treatment option for the first few months after the procedure, as their mechanism of action does not require intact neural tissue for erection. Thereafter, oral PDE-5 inhibitor therapy may be a reasonable choice for patients who can achieve at least a partial erection. A PDE-5 inhibitor may not be effective when spontaneous erections are absent. Because the rehabilitation of sexual function aims to prevent cavernosal tissue damage by providing oxygenation to the erectile tissue, it is possible that the choice of a potentially ineffective treatment may jeopardize the results of a reasonable nerve-sparing procedure.

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Future Strategies

Neurotrophic and neuroprotective factors. At the forefront of post-prostatectomy research is the use of neurotrophic and neuroprotective agents for the improvement of erectile function. The application of multiple agents to rats after injury to the cavernosal nerve has demonstrated earlier

Table 1
Current and Future Strategies for Cavernosal Nerve Recovery

Current Treatments	Future Therapies
Alprostadil (intracavernous injection)	Nitric oxide synthase (iNOS, eNOS, nNOS)
Oral phosphodiesterase inhibitor (sildenafil, vardenafil, tadalafil)	Immunophilin ligands (FK506, GPI-1046)
	Neurotrophic factors (BDNF, VEGF, bFGF, neurturin)

iNOs, inducible nitric oxide synthase; eNOS, endothelial nitric oxide synthase; nNOS, neuronal nitric oxide synthase; BDNF, brain-derived neurotrophic factor; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor.

recovery of cavernosal nerve function (Table 1).

Agents studied have included growth hormone,[17] neurturin,[18] immunophilins,[19] and prostaglandin E1.[20] Also, vascular endothelial growth factor (VEGF)[21] and Sonic hedgehog protein[22] have demonstrated neurite outgrowth properties. Basic fibroblast growth factor (bFGF) is one of the most abundant neurotrophic factors noted in the developing rat penis.[13] In addition to their biochemical effects on neural regeneration, some factors have demonstrated physiologic importance as well. Brain-derived neurotrophic factor (BDNF),[23] nonimmunosuppressant immunophilin ligands,[24] and insulin-like growth factor-1[25] have been shown to improve erections in rats following cavernosal nerve injury.

A great deal of enthusiasm has developed over the use of immunophilins for neural regeneration following nerve injury. After receptor proteins FKBP and cyclophilin, the immunophilins that bind to FK506 and cyclosporine, were reported in extraordinarily high levels in the brain,[26] it was discovered that immunophilins modulate the release of numerous neurotransmitters and influence nitric oxide (NO) formation.[27] Next, FK506 was studied in rat nerve-injury subjects. In vivo, FK506 promoted nerve regeneration and function in the crush-injured sciatic nerves.[28,29] Also, neuroprotective properties were seen in rats following cavernosal nerve injury when FK506 was administered. Preserved penile erections were seen as early as 1 day following treatment with FK506.[19] In combination, bFGF and FK506 enhanced nerve healing by accelerating early nerve regrowth following sciatic nerve injury in rats.[30] Also, GPI-1046 (a nonimmunosuppressive immunophilin ligand) stimulated regeneration of injured sciatic nerve axons and myelin in rats.[31] GPI-1046 is an FK506 derivative that binds to FK506-binding protein 12. In the central nervous system, GPI-1046 promoted sprouting of serotonin-containing fibers following injury, and GPI-1046 induced regenerative sprouting from spared nigrostriatal dopaminergic neurons following stereotaxic injury of the substantia nigra in rats. These neurotrophic actions of GPI-1046 suggest a therapeutic utility in neurodegenerative disease and injury.[31]

The extensive neural research in therapeutic neurogenesis is perhaps the next step in erectile preservation following prostatectomy. Human trials are needed and have begun. These therapies, if they continue to show functional promise, will be useful not only in RP patients but also in patients with pelvic injury and those receiving prostate radiation, in whom cavernosal nerve injury is known to occur.

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Gene therapy. The use of genetic material transfer with the aid of vectors has evolved from a molecular biology research tool to a possible therapeutic intervention for humans. This vast advance has pushed medical research into a new era. Gene therapy, which was primarily reserved for life-threatening diseases, is now being studied in many disease processes, including ED. In the late 1990s, studies began to emerge that used gene transfer technologies for ED in rat models.[32-34] Human trials employing FK506 and GPI-1046 as well as potassium channel gene therapy have just started.

George Christ, PhD, has classified gene therapy strategies for ED into 2 broad groups.[35] Borrowing from the discipline of economics, the 2 categories are based on either increasing the physiologic "supply" (strength) of the erectile stimulus or making the corporeal tissue more sensitive, thus

decreasing the physiologic "demand" for the erectile stimulus. Increasing the NO and NOS, increasing the actual nerve supply with BDNF, and increasing vascular supply with VEGF are examples of altering the supply. Decreasing the amount of stimulus needed for smooth muscle relaxation by altering the Ca^{2+} system is an example of manipulating the demand for erectile function.

The NO pathway has been a target of gene therapy, for obvious reasons. With an increase in the expression (increasing the supply) of this endogenous smooth muscle relaxant, the erectile response will improve. The NOS isoenzymes have been a target for this therapy. Transfection of the NOS via adenovirus has produced encouraging results. The intracavernosal pressure (ICP) in rats was significantly increased after the transfection of iNOS (inducible NOS isoform) to the rat corpora.[32,36] Transfection with eNOS (endothelial NOS)[37] and PnNOS (the penile-specific variant of neuronal NOS)[38] has also given elevated ICPs. In the rat model, this gene transfection therapy has produced a relatively short physiologic effect: 1 to 18 days. The long-term effects of the overexpression of NOS are not yet known.

Calcitonin gene-related protein (CGRP) is believed to improve erectile ability by producing a receptor-mediated increase in intracellular cyclic adenosine monophosphate levels and hyperpolarization by increasing K^+ channel activity. Bivalacqua and associates[39] produced an overexpression of CGRP in aged rats using an adenoviral-mediated delivery system. Also, an increase in nerve-stimulated ICP was seen at day 5 in this rat model. The resulting ICP was equivalent to that seen in younger rats. No detectable effects on blood pressure were observed; however, long-term side effects are not known.

Two gene therapies that fall in the demand category involve RhoA and K^+ channels. The RhoA/Rho kinase pathway, a calcium-sensitization mechanism, has recently been noted to be an important component in the tonic contraction of the corporeal smooth muscle (flaccidity).[40] By introducing a nonfunctional mutant form of RhoA via an adeno-associated virus, Bakircioglu and coworkers[41] demonstrated a decrease in calcium sensitization and an increase in the basal ICP. Cavernous nerve–stimulated ICP was also elevated. This group studied their rat model until day 7 post transfection. Again, long-term side effects of this therapy are not known at this time.

Potassium channels help regulate corporeal smooth muscle tone.[41-43] Gene therapy has been targeted to the pore-forming subunit calcium-sensitive K^+ channel or *hSlo*. An overexpression of *hSlo* may increase the responsiveness of the erectile apparatus to a low level of endogenous smooth muscle relaxants. The physiologic effects of a single intracorporeal injection of *hSlo*/pcDNA ("naked DNA") have lasted for up to

The physiologic effects of a single intracorporeal injection of hSlo*/pcDNA ("naked DNA") have lasted for up to 4 months.*

Main Points

- With impotence rates remaining quite varied among the patients of practicing urologists, even with nerve-sparing radical prostatectomy (RP), there continues to be a strong interest in the discovery of the pathophysiology of erectile dysfunction (ED) in this patient population.
- Some urologists believe that although the cavernosal nerves are spared during a nerve-sparing RP, the nerves are usually injured by direct trauma or by stretch during the operation. This injury would account for the immediate delay in erectile function following RP but its subsequent return months later.
- Both clinicians and patients have perceived a volume loss to the penis after RP, which may be a consequence of apoptosis of the smooth muscle of the corpora cavernosa.
- Intracavernous injections or a vacuum device may be the best first-line treatment option for the first few months after the RP procedure, as their mechanism of action does not require intact neural tissue for erection. Thereafter, oral phosphodiesterase-5 inhibitor therapy may be a reasonable choice for patients who can achieve at least a partial erection.
- At the forefront of postprostatectomy research is the use of neurotrophic and neuroprotective agents for the treatment of erectile dysfunction.
- Gene therapy, which was primarily reserved only for life-threatening diseases, is now being studied in many disease processes, including ED.

4 months.[33] *hSlo* has a minimal effect on baseline resting corporeal smooth muscle activity and therefore a low risk of causing priapism.

Two gene therapies that affect the physiologic supply are therapy with BDNF and therapy with VEGF. After injections with adeno-associated virus vector containing BDNF, rats with cavernosal nerve injury demonstrated improved ICP, and immunohistochemical studies showed an increase in the number of NOS-positive nerve fibers.[44] This gene therapy approach increases the number of nerves available to release NO, which would greatly benefit the nerve-injured patient, that is, the prostatectomy patient. Vast complexities exist with neuronal regrowth, and further studies are necessary.

To improve the vascular supply to the corporeal body, gene therapy with VEGF has been employed.[45-47] The strategy of increased angiogenesis may benefit patients with vascular injury or disease. In a rat model with a vascular injury (bilateral ligation of internal iliac arteries), there was recovery of erectile function by week 6 following injections of VEGF.[46,48]

Gene therapy has become an exciting new research modality with applications to human medical therapy. With any new science or therapy, caution is needed to create a safe treatment option for patients. Setbacks have occurred in this bold new approach to treating disease[49]; however, further studies continue. Excitement over these novel treatment ideas is warranted, but efficiency and safety are paramount in this relatively new science. ■

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