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Amelioration of Penile Fibrosis: Myth or Reality

Review

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ABSTRACT: Several changes have been reported to occur in the cavernosal tissue and tunica albuginea with aging. The atherosclerosis of the penis that occurs with aging causes a decrease in penile oxygen tension. A reduction in the number of smooth muscle cells (SMCs) has been demonstrated in relation to this change in oxygen tension. Changes in the ratio of penile collagen have also been observed and could explain the decrease in penile elasticity and compliance with aging. Chronic ischemia is therefore associated with fibrosis but also with nitric oxide–cGMP reduction. The sensitivity of the α-adrenoceptors on the SMCs increases with aging. Furthermore, androgen deprivation produces penile tissue atrophy, alterations in dorsal nerve structure, alterations in endothelial morphology, reductions in trabecular SM content, increases in deposition of extracellular matrix, and increases in accumulation of adipocytes in the subtunical region of the corpus cavernosum. All of these modifications can explain the prevalence of erectile dysfunction with aging. The aim of this review is to address the underlying etiology of corporal fibrosis, especially aging, cavernosal nerve damage, androgen deprivation, and tunical fibrosis. Finally, we will address the proposed amelioration and reversal of fibrosis in terms of correcting, at least partially, the relative SMC loss that occurs with aging, diabetes, or cavernosal nerve damage and its impact on prevention of erectile dysfunction–associated cavernosal fibrosis.

Key words: Corpora cavernosa, tunica albuginea, aging.

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Erectile dysfunction (ED) is associated with loss of smooth muscle cells (SMCs), and an increase in fibrosis has been repeatedly reported in corporal tissue of patients with ED (Yaman et al, 2003; Iacono et al, 2005). The relationship of oxygen tension and cavernosal fibrosis has been clearly demonstrated in previous studies (Moreland et al, 1995; Moreland, 1998). Transforming growth factor β1 (TGF-β1) increases collagen synthesis in human corpus cavernosal SMCs in culture and is induced by hypoxia (Moreland et al, 1995). Furthermore, hypoxia can induce TGF-β1 expression and inhibit prostaglandin E (PGE) synthesis (Moreland, 1998). In a cell culture study, one application of PGE1 was sufficient to significantly suppress TGF-β1–induced collagen synthesis (Moreland et al, 1995). In humans, a correlation between oxygen tension in the penis has been demonstrated with the percentage of SM fibers. The number of muscular fibers is therefore dependent on good oxygenation of the penis (Sattar et al, 1995).

Ultimately, cavernosal fibrosis will result in corporal veno-occlusive dysfunction (CVOD), which is mainly due to failure of the corporal SM mass to achieve sufficient relaxation. The relaxation is necessary for passive veno-occlusion of the subtunical veins to occur. Therefore, any process that decreases the content or function of the corporal SM, or ultimately corporal fibrosis, will predispose to the development of CVOD. This process specifically includes cavernosal nerve injury following radical prostatectomy and diabetes (Mulhall et al, 2002).

In Peyronie disease (PD), the fibrosis is characterized by an increase in collagen over the intracellular compartment and is associated with the production of profibrotic factors, such as TGF-β1, plasminogen activator inhibitor 1, and reactive oxygen species during oxidative stress (El-Sakka et al, 1997b; Bivalacqua et al, 2000; Gonzalez-Cadavid and Rajfer, 2005). This fibrosis is accompanied by the induction of inducible nitric oxide synthase (NOS2A), which acts as an endogenous antifibrotic mechanism in response to the profibrotic processes (Bivalacqua et al, 2000; Davila et al, 2004). The expression of NOS2A accompanying fibrosis and oxidative stress also has been seen in rat models for aging of arterial vessels, cavernosal nerve damage, types 1 and 2 diabetes, and chronic smoking (Ferrini et al, 2004; Ferrini et al, 2006a; Kovanecz et al, 2006). This agrees with studies in the NOS2A knockout mouse in which NOS2A depletion intensified experimental fibrosis not only in the urogenital and vascular systems but also in kidney and liver (Hochberg et al, 2000; Chen et al, 2005).

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The aim of the current review is to highlight the underlying etiology of corporal fibrosis and eventually tackle the interesting idea of "proposed amelioration and reversal of penile fibrosis."

Architecture of Healthy Corpora Cavernosa and Tunica Albuginea

Corpora Cavernosa—The penis is composed of 2 corpora cavernosa and a single ventral corpus spongiosum. Each corporal body consists of a loose trabecular meshwork of muscular and connective tissues. The corpora cavernosa share a common septum in the pendulous portion of the penis with many perforations that allow free passage of blood from one side to the other, allowing the 2 corpora to function essentially as a single unit. The corpora cavernosa in the young are composed of 40% to 52% SM, in the elderly with CVOD 19% to 36% SM, and in those with arterial impotence 10% to 25% SM (collagen is correspondingly increased; Wespes et al, 1997). Qualitative and quantitative differences in the collagenous architecture within the corpora of impotent patients have been reported (Goldstein and Padma-Nathan, 1990).

Tunica Albuginea—The tunica is composed of elastic fibers forming an irregular lattice network on which the collagen fibers rest. The tunica of the corpora cavernosa is a bilayered structure with multiple sublayers. The inner layer bundles contain the cavernous tissue and are oriented circularly. Radiating from this inner layer are intracavernosal pillars acting as struts, augmenting the septum that provides essential support to the erectile tissue (Brock et al, 1997). The outer layer bundles, oriented longitudinally, apparently determine to a large extent the variation in thickness and strength of the tunica. The bundles extend from the glans penis to the proximal crura and insert into the inferior pubic ramus but are absent in the 6 o'clock position. In contrast, the corpus spongiosum lacks an outer layer or intracorporeal struts, assuring low pressure during erection (Brock et al, 1997). Considering the anatomic and ultrastructural architecture, the 3-dimensional structure of the tunica affords great flexibility, rigidity, and tissue strength to the penis (Brock et al, 1997).

Collagen and elastic fibers of the tunica albuginea are the key structures of this compliant tissue and permit the increase in girth and length during tumescence while providing adequate resilience to return rapidly to the flaccid state with detumescence (Hsu et al, 1994). The tunica acts as a fibrous frame, with its columns penetrating into various depths within the corpus cavernosum; it prevents overstretching or compression of the vascular and nervous structures, which are under increasing intracavernosal pressure during erection. Collagen fibers are composed of aggregations of tropocollagen molecules and are normally arranged in an undulating pattern in the flaccid state. Elastic fibers, which are composed of elastin and microfibrils, can stretch to 150% of their normal length (Akkus et al, 1997).

Abnormal Fibrotic Reaction in the Tunica Albuginea—This minimal sexual trauma of partially rigid penis causes delamination of the septal fibers with extravasation of blood into the intralaminal spaces. These early lesions show a predominantly perivascular lymphatic and plasmacytic inflammatory cellular infiltrate in the areolar connective tissue sleeve below the tunica albuginea (Smith, 1966). We have found that surgical trauma of the tunica albuginea does not produce PD-like conditions in the rat penis (El-Sakka et al, 1998b). This finding supports the notion that entrapment of inflammatory cells and deposition of extracellular matrix (ECM) in the multilayered structure of the tunica is the key factor in induction of PD (Akkus et al, 1997).

The ultrastructural findings by transmission and scanning electron microscopy show that in normal tunica albuginea, elastic fibers form an irregular lattice network onto which the collagen fibrils lie. The multilayered nature of the tunica appears to be distinct, and the tunica is able to slide upon adjacent layers. In this way, flexibility is achieved. In PD plaques, collagen fibers are more densely packed, irregular, and premature, resulting in the noncompliant nature of the tunica in PD. The affected area of the tunica albuginea does not expand upon erection and therefore causes tethering and curvature of the penis (Brock et al, 1997; El-Sakka et al, 1998a).

Fibrotic Disease States

See the Table for a summary of the etiology and underlying mechanisms of disease states causing corporal fibrosis.

Aging and Corporal Fibrosis—The percentage of SMCs steadily decrease with aging. Corpora demonstrate excessive deposit of collagen fibers that results in corporal fibrosis, and these changes also occur in the media of the penile arteries (Ferrini et al, 2001, 2004). It has been postulated that these histologic changes in aged corpora are caused by increased oxidative stress and/or other profibrotic factors that stimulate SMC apoptosis and collagen deposition (Ferrini et al, 2004).

The essential factor that determines the ability to achieve normal penile corporal veno-occlusion is the percentage of corporal SM content, whereas the number of elastic fibers or endothelial cells does not seem to correlate with the occurrence of venous leakage (Wespes et al, 1997). Diminution of cavernosal SM content is

Table. *Underlying etiology and mechanisms of corporal and tunical fibrosis*

Etiology of Corporal Fibrosis	Underlying Mechanisms
Aging	• Loss of SMCs • Fibrosis in corpora cavernosa • CVOD • Excessive deposit of collagen fibers • Same changes occur in media of penile arteries because of increased oxidative stress and/or other profibrotic factors (that stimulate SMC apoptosis and collagen deposition)
Diabetes mellitus	• Excessive deposition of collagen and ECM accompanied by loss of functional cells that characterize tissue fibrosis • Appearance and accumulation of myofibroblasts or switch to synthetic phenotype producing ECM of original cell components, such as fibroblasts and/or SMCs in the penis • Diabetic model developed both abnormal corporal SMC relaxation and generalized fibrosis of arterial media; these processes seem to uniformly underlie CVOD • Exacerbation of fibrosis by iNOS deletion seen in iNOS ko diabetic mouse • Up-regulation of TGF-β1 expression and phosphoactivation of Smad pathway
Cavernosal nerve damage	• Penile biopsy after radical prostatectomy demonstrated replacement of corporal SM with collagen • CVOD develops in bilateral cavernosal nerve resection rats as result of early loss of corporal SMCs by neuropraxia-induced apoptosis, followed by fibrosis • Time course of iNOS induction supports antifibrotic role of iNOS
Androgen deprivation	• Penile tissue atrophy • Alterations in dorsal nerve structure • Alterations in endothelial morphology • Reduction in trabecular SM content • Increase in deposition of ECM • Accumulation of adipocytes in subtunical region of corpus cavernosum
Tunical fibrosis	• Characterized by increased collagen over intracellular compartment • Associated with production of profibrotic factors (eg, TGF-β1 and plasminogen activator inhibitor 1) • Myostatin or its cDNA construct increased myofibroblast number and collagen in tunica albuginea cells • Fibrin trapping • Collagen/elastin changes • Reactive oxygen species production • NO/NOS imbalance • Cellular transformation • Collagenase deficiency • Genetic predisposition/autoimmunity • Chromosomal/cytogenetic abnormalities • Cell cycle regulation aberration

Abbreviations: CVOD, corporal veno-occlusive dysfunction; ECM, extracellular matrix; iNOS, inducible nitric oxide synthase; SMC, smooth muscle cell; TGF, transforming growth factor.

significantly associated with CVOD, decreased values of erectile flow rates, and ultimately increased severity of ED (Wespes et al, 1997).

Changes in elastic fibers or collagen types can provoke mechanical alterations of the penis, which reduce its elasticity and compliance. The collagen in the corpus cavernosum tissue is predominantly types I, III, and IV. Type I collagen, which forms stiff bands of fibrils, has been shown to be less compliant than type III collagen, which is found predominantly in distensible elastic tissue and is essential for normal tensile strength. The endothelial cells are believed to be responsible for secretion of type IV collagen, which forms the basement membrane of blood vessels. In the penis, there is an equal abundance of types I and IV collagen with concomitant diminution of type III (Luangkhot et al, 1992).

The alterations in collagen configuration that relate to advanced glycosylation products in addition to the reduction of elastic fibers could be the mainstay in penile hemodynamic changes associated with aging (Jiaan et al, 1995; Akkus et al, 1997).

In addition to increased collagen deposition and reduction of elastic fibers, postmortem studies have revealed that aging is associated with increasing degrees of atherosclerotic vascular alteration in the arterial bed of the penis. The exact pathophysiologic mechanism of ischemia-induced fibrosis of the corpus cavernosum is not clearly understood; however, in vitro studies have suggested that it is likely to be caused by hypoxia-induced overexpression of TGF-β1 (Moreland, 1998).

TGF-β1 is a pleotrophic cytokine that has been shown to increase collagen synthesis in corpus cavernosum SMCs in vitro. Under ischemic conditions, TGF-β1

induces its own mRNA, leading to a further increase in TGF-β1 synthesis that reinforces the development of severe fibrosis (Moreland, 1998). Measuring the differential mRNA expression for various growth factors in young and aging rat penile tissues demonstrated that TGF-β1 is higher in older rats compared with young rats and seems to confirm the role of TGF-β1 in penile fibrosis (Dahiya et al, 1999). Furthermore, mRNA expression of nerve growth factor is reduced in older rat penile tissues. Therefore, age-related neuronal atrophy may be caused by the reduced synthesis or availability of target-derived neurotrophic factors.

The number of NOS fibers was reduced by half in old rats. These findings emphasize the role of NO in erectile physiology, and a reduction of NOS nerve fibers may be an important neurologic factor of age-related changes (Garbán et al, 1995; Carrier et al, 1997).

Diabetes and Penile Fibrosis—The excessive deposition of collagen and ECM, accompanied by the loss of functional cells that characterize tissue fibrosis, is due to the appearance and accumulation of myofibroblasts or to the switch to a synthetic phenotype producing ECM of the original cell components, such as fibroblasts and/or SMCs in the penis. The main factor in eliciting these cellular alterations is an insult to the tissue, be it: 1) acute and localized, in a specific site in the tunica albuginea in PD; 2) acute and diffuse throughout the corpora, such as in cavernosal nerve damage after radical prostatectomy; or 3) chronic and also diffuse throughout the corpora and penile artery walls, such as in aging, diabetes, and heavy smoking (Gonzalez-Cadavid, 2009). A similar exacerbation of fibrosis by inducible NOS (iNOS) deletion is seen in the iNOS ko mouse rendered diabetic by streptozotocin (STZ) injection. iNOS is also overexpressed in the aged arteries, and its blockade leads to an increase in fibrosis measured by SMC/collagen ratio. An identical loss of SMCs and increase in apoptosis occurs in the penile dorsal artery and aorta in the ZDF rat, a model for type 2 diabetes (Kovanecz et al, 2009).

Blockade of the Smad pathway, which is a common downstream signaling mechanism for both TGF-β1 and myostatin, is a potential antifibrotic strategy—up-regulation of TGF-β1 expression and phosphoactivation of the Smad pathway was shown to occur in the penis of the rat with STZ-induced diabetes (a model for type 1 diabetes; Zhang et al, 2008). Another promising approach is via the modulation of metalloproteinase expression by overexpression with the respective cDNA (Atkinson and Senior, 2003).

Cavernosal Nerve Damage and Corporal Fibrosis—An experimental study demonstrated that protein expression of collagens I and III was significantly higher in a neurotomy compared with a control group, which is consistent with increased expression of TGF-β1 (Diegelmann, 1997). On the other hand, another study showed that cavernous nerve neurectomy did not cause significant morphologic or functional changes in the penile erectile tissue of rats (Martinez-Pineiro et al, 1995). An interesting study investigated the effects of nerve injury alone; neurotomy was performed by electrical cauterization of the cavernous nerves to assure that all rat penises had been denervated for an identical period of time. The results of this study demonstrated protein expression and immunohistochemical staining of hypoxia-inducible factor 1α (HIF-1α).

HIF-1α expression was significantly higher in the neurotomy group, confirming the theory that hypoxia of rat penises was induced by the loss of nocturnal erections (Leungwattanakij et al, 2003). However, it is noteworthy that oxygen is brought to the penile tissues by the capillaries, not the sinusoids; evidence has yet to be confirmed that oxygen from the red blood cells in the sinusoids play a role in oxygenation of the corporal tissues.

Cavernosal nerve damage, such as that after radical prostatectomy, is associated with corporal fibrosis and loss of SMCs (Leungwattanakij et al, 2003). Penile biopsy after radical prostatectomy has demonstrated replacement of corporal SMCs with collagen (McCullough, 2008). Furthermore, CVOD developed in bilateral cavernosal nerve resection rats as a result of the early loss of corporal SMCs by neuropraxia-induced apoptosis, followed by fibrosis (Ferrini et al, 2009).

ED after radical prostatectomy may be attributed to vascular, neurogenic, and psychogenic etiologies. Because ED is significantly more common in men who undergo non–nerve-sparing prostatectomy than in men who undergo nerve-sparing prostatectomy, a neurogenic cause is recognized to be a main etiology of postprostatectomy ED (Gralnek et al, 2000). Moreover, the recovery rate of erectile function owing to neurapraxia from surgery is time related, and it may take 6 to 18 months after surgery for recovery to occur (Montorsi et al, 1997; Hong et al, 1999). Despite the introduction of nerve-sparing techniques, a significant number of men still develop ED after radical prostatectomy (Walsh and Donker, 1982). Overall maintenance of sexual potency after nerve-sparing radical prostatectomy has been reported to occur in 39% to 86% of men who have at least unilateral nerve preservation (Catalona and Bigg, 1990; Gralnek et al, 2000).

Androgen Deprivation and Corporal Fibrosis—Over recent years, the age-related decline of circulating testosterone in men has received increasing attention, not only in relation to sexual function but also in a wider context of male health (Figure). A decline in testicular function with a consequent decline in testosterone level

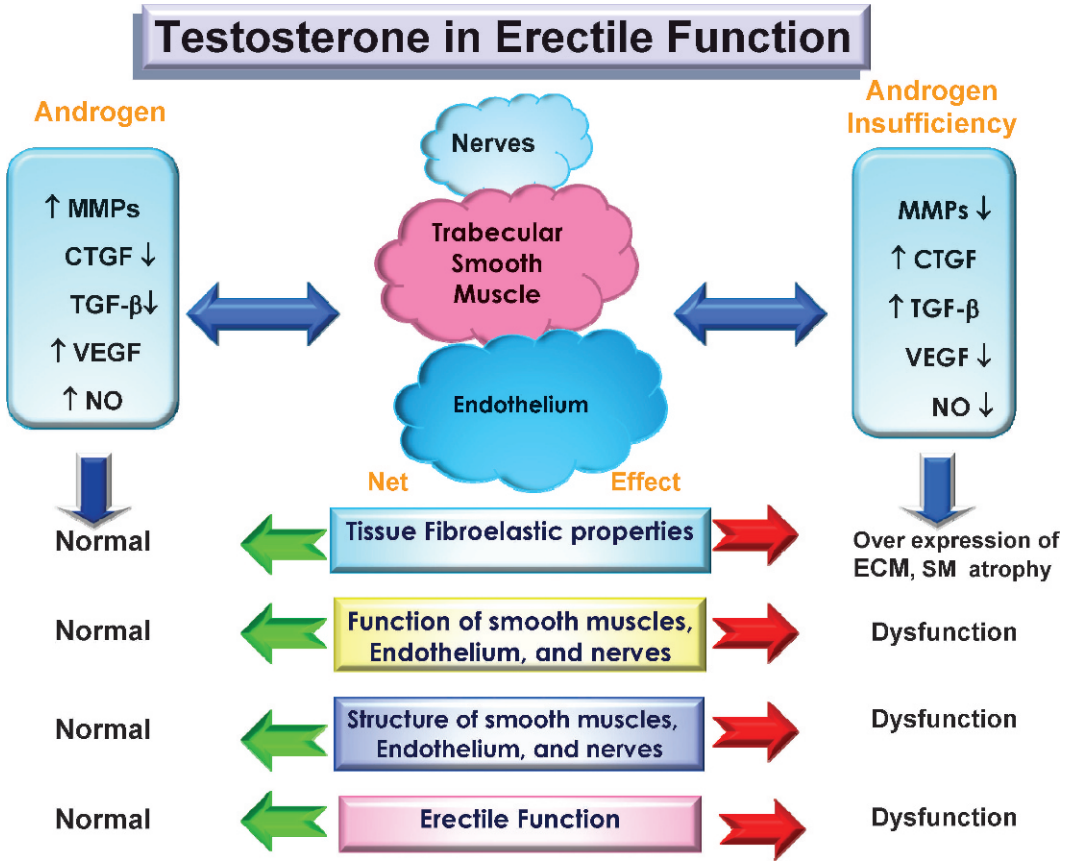

Figure. Effect of testosterone on erectile function. Color figure available online at www.andrologyjournal.org.

is a common occurrence in older men (Morales, 2003; El-Sakka and Hassoba, 2006). Moreover, experimental research has presented convincing evidence, so far mainly in laboratory animals, that testosterone has profound effects on tissues of the penis involved in the mechanism of erection and that testosterone deficiency impairs the anatomic and physiologic substrate of erectile capacity (Traish et al, 2007).

Androgen deprivation by surgical or medical castration results in a significant reduction in trabecular SM content and marked increase in connective tissue deposition (Traish et al, 2003). These structural alterations are also associated with loss of erectile function. Ultrastructural study has demonstrated that the cavernosal SM in castrated animals appears disorganized with a large number of cytoplasmic vacuoles, whereas in intact animals, SMCs exhibit normal morphology and are arranged in clusters (Rogers et al, 2003).

Several studies have provided demonstrations of the potential role of androgens in maintaining the structure and function of many pelvic ganglion neurons (Keast et al, 2002). Giuliano et al (1993) suggested that testosterone acting peripherally to the spinal cord enhances the erectile response of the cavernous nerve. Rogers et al (2003) demonstrated that castration altered the dorsal nerve ultrastructure in the rat concomitant with loss of erectile function.

In addition to the alterations in SM and connective tissue, fat-containing cells have been observed in the subtunical region of penile tissue sections from orchiectomized animals (Traish et al, 2005). This could lead to venous leakage, which was observed in a subset of hypogonadal patients with ED who improved upon testosterone administration (Yassin and Saad, 2006; Yassin et al, 2006). The alterations in cavernosal tissue composition and structure were accompanied by a reduced erectile response to pelvic nerve stimulation (Traish et al, 2005; Armagan et al, 2006).

There is renewed interest in understanding the mechanisms by which androgens regulate growth and differentiation of vascular SMCs. Singh et al (2006) hypothesized that androgens promote the commitment of pluripotent stem cells to a muscle lineage and inhibit their differentiation into an adipocyte lineage. The total number of circulating vascular progenitor cells may also be dependent on testosterone levels (Foresta et al, 2006).

Regulation of progenitor cell differentiation is a complex process, dependent on numerous hormones and growth factors and specific activation of a cascade of gene expression (Bélanger et al, 2002; Rosen et al, 2002).

Traish et al (2007) noted that there were marked structural changes in the cavernosal nerve from castrated animals compared with controls (sham-operated animals) or castrated animals treated with androgens. These structural alterations may be responsible in part for the marked reduction in the intracavernosal pressure (Armagan et al, 2006).

Reproducible results support the role for androgens in regulating the expression and activity of NOS isoforms in the corpus cavernosum in animal models (Zvara et al, 1995; Park et al, 1999). In castrated animals, testosterone or 5α-dihydrotestosterone administration restored the erectile response and NOS expression in the penis (Marin et al, 1999; Baba et al, 2000; Armagan et al, 2006).

In summary, animal investigations have revealed that androgen deprivation produces penile tissue atrophy, alterations in dorsal nerve structure, alterations in endothelial morphology, reduction in trabecular SM content, increase in deposition of ECM, and accumulation of adipocytes in the subtunical region of the corpus cavernosum.

Tunical Fibrosis—In PD, fibrosis of the tunica albuginea is characterized by increased collagen over the intracellular compartment. Several studies have demonstrated that fibrosis is associated with the production of profibrotic factors (eg, TGF-β1 and plasminogen activator inhibitor 1; El-Sakka et al, 1997a,b, 1998a; Davila et al, 2005). Regulation of collagen synthesis by many endogenous and exogenous factors, especially producers of oxygen free radicals such as ascorbic acid and other biologically active peptides such as epidermal growth factor and insulin-like growth factor, has been reported as playing a role in the pathogenesis of PD. TGF-β has gained considerable attention as a factor implicated in the cause of chronic fibrotic conditions. TGF-β is involved in numerous vital processes including inflammation, stimulation of intracellular matrix formation, production of fibroblasts, and normal healing (Sporn et al, 1987). Although growing evidence has implicated TGF-β as a cytokine, vital to tissue repair, its excessive action may be responsible for tissue damage caused by scarring in many serious diseases. The pathologic consequences of the action of TGF-β have been referred to as the "dark side" of tissue repair (Border and Ruoslahti, 1992). Inhibitors of TGF-β may be important future drugs in controlling this condition.

TGF-β has attracted much interest as a cytokine that affects the deposition of ECM and induces fibrosis in the tunica albuginea (El-Sakka et al, 1997a,b, 1998a). In another experiment that addressed the pathogenesis of PD, we demonstrated that a high frequency of microsatellite alterations and loss of heterozygosity were associated with PD, suggesting their role in the pathogenesis of this disease (Perinchery et al, 2000). Furthermore, exciting studies using this animal model have demonstrated activation of nuclear factor κB, which regulates the expression of several genes that encode adhesion molecules, after TGF-β injection and injury to the rat penis. NOS isoforms, particularly iNOS, have been revealed to modulate the onset and progression of fibroblast or wound healing. Inhibition of iNOS results in increased deposition of collagen around the TGF-β–induced lesions, suggesting that iNOS suppresses collagen production in PD. Bivalacqua et al (2000, 2001) demonstrated that iNOS was increased in PD probably because of inflammation. iNOS is the key control element for peroxynitrite formation, arginase II expression, and endothelial NOS (eNOS) downregulation in the induction of a PD-like condition in the rat (Bivalacqua et al, 2000, 2001). Recently, myostatin (a member of the TGF-β family) or its cDNA construct increased the myofibroblast number and amount of collagen in the tunica albuginea cells (Cantini et al, 2008).

Shen et al (2003) have demonstrated that the structure of the tunica albuginea in rats is also influenced by androgens. Four weeks after castration, the tunica was thinner with fewer elastic fibers, and the collagen appeared more disorganized. Depletion of elastic fibers and replacement fibrosis was also noted in intact rats treated with finasteride, although the thickness of the tunica did not differ from intact controls (Shen et al, 2003). PD has been reported to be associated with type 2 diabetes (El-Sakka and Tayeb, 2005) and with more impairment of vascular elements of erection in patients with diabetes (El-Sakka and Tayeb, 2009). The relationship between tunical fibrosis and androgen depletion or medical comorbidities is another interesting new dimension in understanding the pathogenesis of PD.

Proposed Amelioration and Reversal of Penile Fibrosis

The reversal of penile fibrosis is an interesting issue to all concerned researchers; however, it is still an unsettled issue. Promising recent results and future investigations might bring this dream to fruition. In this section, we will address some of the important available data.

Postradical Prostatectomy—Phosphodiesterase type 5 inhibitors. Animal studies using cavernous nerve injury models provide support for daily phosphodiesterase

type 5 inhibitor (PDE5-I) therapy in patients with postradical prostatectomy. Vignozzi et al (2006) reported their findings of protection against cavernous tissue protein and mRNA changes and preservation of PDE5 expression and tadalafil efficacy after a 3-month treatment course of daily tadalafil (2 mg/kg) following bilateral cavernous neurotomy in the rat. Using a similar rat model, Ferrini et al (2006a) demonstrated that long-term vardenafil may prevent CVOD after radical prostatectomy by preserving SM content and inhibiting corporal fibrosis. These mechanisms could be due to an effect on iNOS and could result in functional normalization of the dynamic infusion cavernosometry drop rate and SM/collagen ratio.

Sildenafil, a PDE5A-I, significantly raises the SMC/collagen ratio in the corpora cavernosa, mainly by stimulation of cell proliferation. Daily-use sildenafil has been shown to protect against structural changes secondary to cavernous nerve injury and to preserve erectile function in the rat (Mulhall et al, 2008).

An elegant study by Ferrini et al (2006a, 2007) demonstrated that CVOD in aged rats is associated with a significant reduction of the SMC/collagen ratio in the penile corpora cavernosa compared with young rats. Even more clinically relevant, it has been reported that long-term and continuous administration of sildenafil corrects aging-associated vasculogenic ED as measured by cavernosometry. Ultimately, in the aging rat model, a PDE5A-I corrected CVOD and ameliorated the underlying corporal fibrosis (Ferrini et al, 2007). Based on these results, one may assume that long-term sildenafil affects SM compliance by a mechanism additional to the elevation of the SMC/collagen ratio via the counteraction of oxidative stress or TGF-β1 expression or else by the alteration of collagen isoform composition (Ferrini et al, 2007).

PDE5A inhibitors partially preserve or restore the number of SMCs and reduce collagen deposition, but whether this is the main factor in the beneficial long-term effects of PDE5A-Is may vary according to the degree of corporal oxidative stress and hence of fibrosis (Ferrini et al, 2007). Another factor that affects corporal SM compliance and that may be influenced by long-term PDE5A-Is, as opposed to acute effects on SM relaxation, is the expression of contractile proteins, such as α-actin 2, smoothelin, and others, which are fundamental for the corporal SM relaxation/contraction process that operates in penile tumescence and detumescence. This expression is associated with the functional phenotype of the SMCs (ie, contractile vs synthetic; Moncada and Higgs, 2006). Also, levels of penile neuronal NOS and eNOS, which directly elicit an erection by releasing NO upon sexual stimulation, may be elevated by sustained high cGMP levels and thus improve compliance. Another important target that may be affected by PDE5-Is is the Rho kinase system; the mechanisms are alternative to protein tyrosine phosphatase, nonreceptor type 11 induction or VAV down-regulation. These mechanisms may be phosphorylation or direct inhibition of Rho kinase or the availability of a cGMP substrate for these processes (Chang et al, 2002; Mills et al, 2003).

The application of molecular technologies such as gene therapy could also have a future in amelioration or reversion of penile fibrosis. Gene therapy might also "cure" underlying conditions in ED, including fibrosis (Gonzalez-Cadavid and Rajfer, 2004). Furthermore, gene therapy might help prolong the efficacy of PDE5-Is by improving penile NO bioactivity (Lau et al, 2007). Therefore, increasing penile NOS content, which can be achieved by gene therapy with NOS constructs, might be a viable therapy for ED.

Bivalacqua et al (2004) demonstrated that inhibition of RhoA/Rho kinase by transfection of the STZ-diabetic rat penis with an adeno-associated virus encoding the dominant-negative RhoA mutant (AAVTCMV19NRhoA) restored cavernosal eNOS protein levels, constitutive NOS activity, and cGMP levels to those found in control rats. Also, the AAVT19NRhoA gene transfer improved erectile responses in the STZ-diabetic rat to values similar to control. Therefore, erectile function in diabetes can be restored by gene therapy targeting RhoA/Rho kinase (Bivalacqua et al, 2004).

Schwartz et al (2004) reported on 40 patients who had undergone radical prostatectomy and were treated with every-other-day sildenafil for 6 months; after the treatment period, cavernosal biopsy demonstrated SM preservation at 50 mg and decreased levels of fibrosis and substantially increased SM content at 100-mg doses. Sighinolfi et al (2006) observed an extended response to chronic PDE5-I treatment using sildenafil for up to 20 months; peak systolic velocity demonstrated a 10.5% improvement after treatment.

Padma-Nathan et al (2008) demonstrated in their multi-institutional randomized controlled trial on 76 men after nerve-sparing radical prostatectomy who used sildenafil 50–100 mg nightly for 36 weeks a 27% successful intercourse rate in sildenafil-treated men compared with 4% in placebo-treated men. Another nonrandomized prospective study of 132 men after radical prostatectomy reported improved functional outcomes for a rehabilitation protocol using PDE5-I. Men in the rehabilitation arm manifested improved erectile function parameters (22% of rehabilitation patients compared with 6% of nonrehabilitation patients; Mulhall et al, 2005). Although these preliminary results are encouraging, the possibility of selection bias and the heterogeneity of the treatments used for

rehabilitation necessitate further prospective studies to address the role of PDE5-I in penile rehabilitation.

PDE5-I on reversing fibrosis in other vascular beds. Preclinical data and preliminary clinical reports suggest that PDE5-Is may improve endothelial function and decrease arterial stiffness, introducing this class of compounds as potential drugs for patients with metabolic syndrome and diabetes mellitus (Gori et al, 2005; Hatzimouratidis and Hatzichristou, 2007).

Sildenafil is an approved treatment for pulmonary hypertension (Galiè et al, 2005). In addition to the management of pulmonary artery blood pressure, sildenafil may have other beneficial pulmonary effects. Sildenafil (25 mg/kg orally) has been shown to attenuate mucous production and markers of reactive airway disease (NO metabolites, neutrophil and macrophage count, and the proinflammatory cytokines tumor necrosis factor α and rat analog of human interleukin 8) when given prophylactically to rats exposed to the pulmonary toxin acrolein (which works by increasing oxidative stress) for 6-hour periods for 14 and 28 days (Wang et al, 2009).

Sildenafil improves endothelium-dependent, flow-mediated vasodilatation in patients with diabetes mellitus and chronic heart failure and in current smokers, and it provokes vasodilation of epicardial coronary arteries, improvement of endothelial dysfunction, and inhibition of platelet activation in patients with coronary artery disease (Katz et al, 2000). Tadalafil has also been shown to improve endothelial function in patients with increased cardiovascular risk (Rosano et al, 2005).

Of particular interest and relevance are the apparently significant effects of PDE5-Is on the prevention/attenuation of cardiac hypertrophy in the setting of heart failure, ischemia/reperfusion injury, and coronary vasospasm and preservation of the vascular endothelium. Furthermore, reversal of endothelial dysfunction may cause erectile and cardiovascular function improvements. The new data clearly suggest not only the interplay among those conditions but also raise a critical question for the development of a common prevention strategy. Can chronic use of PDE5-Is prevent or reverse endothelial dysfunction and possibly inhibit the atherosclerotic process? If the answer is yes, a new era is opened in the management of cardiovascular diseases.

Other agents that could affect penile fibrosis. Theoretically, fibrosis may be down-regulated by the application of vasoactive modalities such as intracavernous PGE1, which improves cavernosal blood flow and increases oxygenation to the corpus cavernosal SMCs (Daley et al, 1996). However, to date, there are no unequivocal data proving that opening up the cavernosal arteries with vasodilators increases oxygen tension to the cavernosal SMCs. Such intervention for prevention of cavernous fibrosis needs to be initiated early. Unfortunately, the precise time and frequency of these preventive treatments remain speculative. Future therapies may focus on noninvasive measures, such as topical PGE1 application, to increase patient compliance. Topical PGE1 is recognized to have a lower response rate in the induction of penile rigidity (McVary et al, 1999). However, hypoxic prevention measures do not necessarily require a rigid erection to increase oxygenation and benefit the cavernous SMCs under such postoperative circumstances. It is becoming more and more evident that corporal fibrosis is the main underlying etiology for ED in the majority of patients (Melman and Gingell, 1999). Regardless of age or etiology, 66% to 75% of cases of ED are caused by corporal fibrosis and ultimately CVOD. Therefore, if the cause of CVOD itself can be prevented, then the possibility remains that ED could possibly become a preventable condition.

PD—In the rat models of PD and/or cavernosal nerve damage, long-term overexpression of NOS2A and NO production via intratunical *NOS2A* cDNA gene transfer; long-term oral administration of the PDE5A-Is sildenafil and vardenafil, which elevate cGMP; or long-term treatment with the PDE4-I pentoxifylline, which increases cAMP, reduces penile fibrosis (Ferrini et al, 2006a,b). Vardenafil has been shown to slow and reverse the early stages of PD-like plaques in the rat, with amelioration of more advanced plaques. Once-daily (1 and 3 mg/kg) vardenafil treatment resulted in reduced collagen/SM and collagen III/I ratios and myofibroblasts and TGF-β1–positive cells and selectively increased the apoptotic index in the PD-like plaques (Ferrini et al, 2006b). In addition, it is conceivable that long-term treatment with PDE5A-Is could upregulate NOS2A expression via cGMP modulation and thus contribute to SMC protection (Kukreja et al, 2005; Rosanio et al, 2006). In the clinical setting, there have been occasional reports that long-term continuous oral administration of pentoxifylline or sildenafil may ameliorate ED (Korenman and Viosca, 1993; Montorsi and McCullough, 2005). Pentoxifylline, a nonspecific cAMP-PDE-I, has been shown to decrease the expression of collagen I and SM α-actin (Davila et al, 2004; Brant et al, 2006). Similar observations have been made with the application of sildenafil together with L-arginine. These findings can be explained by the observation that iNOS is expressed in human PD plaques and inhibition of iNOS leads to a significant exacerbation of tissue fibrosis. Furthermore, an antifibrotic regimen consisting of up-regulators of NO production (pentoxifylline and sildenafil) demonstrated amelioration of the corporal fibrosis associated with recalcitrant priapism (Rajfer et al, 2006). However, use

of pentoxifylline as a therapeutic agent for the treatment of PD and priapism is still investigational.

More recently, chronic treatment with tadalafil improved endothelial function and morning erections in patients with ED (Aversa et al, 2007), agreeing with the results of studies in rats in which long-term sildenafil improved endothelium-dependent cavernosal relaxations and the erectile response to cavernosal nerve stimulation in young animals (Behr-Roussel et al, 2005) and prolonged this response in old rats (Musicki et al, 2005).

In addition, the oxidative stress and TGF-β1 levels were not affected by sildenafil, thus differing from the effects of vardenafil in the rat models of cavernosal nerve damage or PD-like fibrotic plaques (Ferrini et al, 2006b). This is not surprising because cGMP is not a direct inhibitor of TGF-β1 expression but does interfere with TGF-β1 signaling both by blocking pSmad2 and pSmad3 nuclear translocation or SMAD-induced gene expression and by the conversion of latent TGF-β1 to its active form (Saura et al, 2005; Li et al, 2007). In fact, the inhibition of TGF-β1 expression by NO is not mediated by cGMP (Craven et al, 1997). In addition, in contrast to NO, cGMP is not a key modulator of oxidative stress, although it is possible that a sildenafil effect may be detected by markers of this process other than xanthine dehydrogenase. PDE5-Is did not affect the collagen III/I ratio, the increase or decrease of which is associated with tissue fibrosis in the penis (Ferrini et al, 2006 a,b).

Antagonizing TGF-β signaling through the use of neutralizing antibodies, soluble type II receptors (eg, TGF-β receptor II), and antisense oligonucleotides inhibits various types of TGF-β–mediated fibrosis (Hakenjos et al, 2000; Martin et al, 2000). The inhibitor of activin receptor-like kinase 5 (ALK5), a TGF-β type I receptor, was developed as a selective inhibitor of endogenous TGF-β signaling, and ALK5 inhibition has been reported to attenuate tissue fibrosis in kidney, lung, and liver (Bonniaud et al, 2005; de Gouville et al, 2005). PDE5-Is affect oxidative stress and TGF-β1 levels through interference with TGF-β1 signaling by blocking phospho-Smad2 and Smad3 nuclear translocation or Smad-induced gene expression and conversion of latent TGF-β1 to its active form (Li et al, 2007).

Prospective Directions to Ameliorate Penile Fibrosis—An emerging approach to treat corporal fibrosis is the replacement of the lost SMCs by implanted stem cells (Bivalacqua et al, 2007; Song et al, 2007). It was recently demonstrated that stem cells isolated from the skeletal muscle of mice can be implanted into the rat corpora cavernosa of old rats with ED and generate SMCs (Nolazco et al, 2008). By undergoing this conversion, the muscle-derived stem cells corrected the ED in the aged rats. The blockade of the Smad pathway, which is a common downstream signaling mechanism for both TGF-β1 and myostatin, is also a potential antifibrotic strategy because up-regulation of the expression of TGF-β1 and phosphoactivation of the Smad pathway have been shown to occur in the penis of the rat with STZ-induced diabetes (Zhang et al, 2008). Another promising approach is the modulation of metalloproteinase expression by overexpression with the respective cDNA (Atkinson and Senior, 2003). However, the pharmacologic modulation of endogenous stem cells in the penis to produce SMCs and to block myofibroblast generation could be the most promising approach. These endogenous stem cells may be good candidates for antifibrotic pharmacologic modulation, particularly with agents belonging to the NO/cGMP and TGF-β1 pathways. This approach could be more feasible than regular gene and stem cell therapies for ameliorating penile fibrosis and restoring the normal cellular pattern in penile tissue.

Conclusions

It may be concluded that correcting, at least partially, the relative SMC loss occurring with aging, diabetes, or cavernosal nerve damage should be a key therapeutic aim to prevent the ED associated with these conditions. Up-regulation of the NO/cGMP pathway may play a role in preventing and reversing fibrosis in the tunica albuginea and in the corpora cavernosa. Therefore, long-term and continuous treatment with sildenafil, and speculatively with the other available PDE5A-Is, may be pharmacologically effective for partially reversing the underlying alterations in the corpora that lead to ED, thus potentially curing this disorder, as opposed to the current discontinuous, on-demand, palliative administration of these compounds for eliciting an erection. Therapies aimed at blocking the TGF-β signaling pathway might be efficacious in amelioration or prevention of tunical fibrosis.

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