

Amelioration of Penile Fibrosis: Myth or Reality

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Abstract:

Several changes have been reported to occur in the cavernosal tissue as well as tunica albuginea with aging. The atherosclerosis of the penis that occurs with aging causes a decrease in penile oxygen tension. A reduction of smooth muscle cells (SMCs) has been demonstrated in relation with 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 (NO)-cyclic guanosine monophosphate reduction. The sensitivity of the α -adrenoceptors on the smooth muscle cells increases with aging. Furthermore, androgen deprivation produces penile tissue atrophy, alterations in dorsal nerve structure, alterations in endothelial morphology, reduction in trabecular smooth muscle content, increase in deposition of extracellular matrix (ECM) and accumulation of fat-containing cells (adipocytes) in subtunical region of corpus cavernosum. All those 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 in addition to tunical fibrosis. Finally we will address the proposed amelioration and reversion of fibrosis in terms of correcting, at least partially, the relative SMC loss occurring with: aging, diabetes, or cavernosal nerve damage and their impact on prevention of erectile dysfunction (ED) associated-cavernosal fibrosis.

Keywords: corpora cavernosa, tunica albuginea, fibrosis, aging,

Introduction:

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 ED patients (Yaman O 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- β 1 (TGF- β 1) increases collagen synthesis in human corpus cavernosal smooth muscle cells in culture, and is induced by hypoxia (Moreland et al, 1995). Furthermore, hypoxia can induce TGF- β 1 expression and inhibit prostaglandin E (PGE) synthesis (Moreland, 1998). In a cell culture study, one application of PGE1 was sufficient to significantly suppress TGF- β 1-induced collagen synthesis (Moreland et al, 1995) In human, a correlation between oxygen tension in the penis has been demonstrated with the percentage of smooth muscle 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 the failure of the corporal smooth muscle (SM) mass to achieve sufficient relaxation, which is necessary for the process of 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, specifically cavernosal nerve injury following radical prostatectomy and diabetes (Mulhall, 2002).

In Peyronie's disease (PD), where the fibrosis is characterized by an increase in collagen over the intracellular compartment, the fibrosis is associated with the production of profibrotic factors, such as TGF- β 1, plasminogen activator inhibitor-1, and reactive oxygen species (ROS) during oxidative stress (El-Sakka et al, 1997 a; Gonzalez-Cadavid and Rajfer, 2005; Bivalacqua et al, 2000). This is accompanied by the induction of the inducible nitric oxide synthase (NOS2A), which acts as an endogenous antifibrotic mechanism in response to the profibrotic processes (Davila et al 2004; Bivalacqua et al, 2000). The expression of NOS2A accompanying fibrosis and oxidative stress also has been seen in rat models for aging of the arterial vessels, cavernosal nerve damage, and types 1 and 2 diabetes, as well as in chronic smoking (Ferrini et al, 2006; 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).

The aim of the current review is to spot light on the underlying etiology of corporal fibrosis and eventually tackle the interesting idea "proposed amelioration and reversion of penile fibrosis"

The architecture of the healthy corpora cavernosa and tunica albuginea

Corpora cavernosa:

The penis is composed of two 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 two corpora to function essentially as a single unit. the corpora cavernosa in the young were composed of 40-52% smooth muscle, in the elderly with corporal veno-occlusive dysfunction 19-36% smooth muscle, and in those with arterial impotence 10-25% smooth muscle (collagen was correspondingly increased) (Wespes et al 1997). Qualitative and quantitative differences in the collagenous architecture within the corpora of impotent patients were 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. It

is composed of: The inner layer bundles which 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. It extends 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 anatomical and ultrastructural architecture, the three dimensional structure of the tunica affords great flexibility, rigidity and tissue strength to the penis (Brock et al., 1997).

Collagen and elastic fibers of 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 the overstretching or compression of the vascular as well as 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 injury causes delamination of the septal fibers with extravasation of blood into the intralaminar spaces. These early lesions showed a predominantly perivascular lymphatic and plasmacytic inflammatory cellular infiltrate in the areolar connective tissue sleeve below the tunica albuginea (Smith 1966). We found that surgical trauma of the tunica albuginea does not produce Peyronie's like condition in the rat penis (El-Sakka et al, 1998 b). This findings support the notion that entrapment of inflammatory cells and deposition of 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 is able to slide upon the adjacent layers. In this way flexibility is achieved. In Peyronie's plaques, collagen fibers are more densely packed, irregular, premature and 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, 1998 a).

Fibrotic Disease-States (Table1)

Aging and Corporal Fibrosis

The percentage of SMCs was steadily decreased with aging. Corpora demonstrates excessive deposit of collagen fibers that result in corporal fibrosis and these changes also occurs in the media of the penile arteries (Ferrini et al, 2001; Ferrini et al, 2004) . It has been postulated that these histologic changes in the aged corpora are caused by increased oxidative stress and/or other profibrotic factors that stimulate SMCs 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 smooth muscle 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 smooth muscle content was significantly associated with CVOD, decreased values of erectile flow rates and ultimately increased severity of ED (Wespes et al, 1997).

Changes of 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 the 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 which related 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 increase 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 pathophysiological mechanism of ischemia-induced fibrosis of the corpus cavernosum is not clearly understood however, in vitro study suggested that it is likely to be caused by hypoxia-induced overexpression of TGF- β 1 (Moreland 1998).

TGF- β 1 is a pleiotropic cytokine that has been shown to increase collagen synthesis in corpus cavernosum SMCs in vitro. Under ischemia conditions, TGF- β 1 induces its own mRNA, leading to a further increase in TGF- β 1 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- β 1 is higher in older rats compared with young rats and seems to confirm the role of TGF- β 1 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 neurological factor of age-related changes (Garban 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: (i) acute and localized, in a specific site in the tunica albuginea in PD; (ii) acute and diffuse throughout the corpora such as in cavernosal nerve damage after radical prostatectomy; or (iii) chronic and also diffuse throughout the corpora and the penile arteries wall such as in aging, diabetes, and heavy smoking (Gonzalez-Cadavid, 2009) In the wild type animal there is a progressive but mild fibrosis peaking at 20 months of age. A similar exacerbation of fibrosis by 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 SMCs/collagen ratio. An identical loss of SMCs and increase in apoptosis occurs in the penile dorsal artery and the aorta in the ZDF rat, a model for type 2 diabetes (Kovanecz et al, 2009).

The blockade of the Smad pathway, which is a common downstream signaling mechanism for both TGF β 1 or myostatin, is a potential antifibrotic strategy, as upregulation of the expression of TGF β 1 and phospho-activation 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:

Experimental study demonstrated, protein expression of collagens I and III was significantly higher in the neurotomy group, which is consistent with an increased expression of TGF- β 1 (Diegelmann, 1997). On the other hand, another study showed that cavernous nerve neurectomy did not cause significant morphological 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 in order to assure that all rat penes had been denervated for an identical period of time. The results of this study demonstrated that protein expression and immunohistochemical staining of (Hypoxia-inducible factor-1 α)

HIF-1 α were significantly higher in the neurotomy group, confirming the theory that hypoxia of rat penes 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 that oxygen from the RBCs in the sinusoids play a role in oxygenation of the corporal tissues is to be approved.

Cavernosal nerve damage such as, after radical prostatectomy is associated with corporal fibrosis and loss of SMCs (Leungwattanakij et al, 2003). Penile biopsy after radical prostatectomy demonstrated replacement of corporal SMCs with collagen (McCullough A 2008). Furthermore, CVOD develops in the bilateral cavernosal nerve resection rats as a result of the early loss of corporal SMCs by the 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 etiology is recognized to be a main etiology of post-prostatectomy ED (Gralnek et al, 2000). Moreover, the recovery rate of erectile function due to the neurapraxia from surgery is time-related, and it may take 6 to 18 months after surgery for it 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: (Figure1)

Over the recent years, the age-related decline of circulating testosterone in men has received increasing attention, not only in relation to sexual functioning but in a wider context of male health. A decline in testicular function with a consequent decline in testosterone level is recognized as 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 anatomical and physiological substrate of erectile capacity (Traish et al, 2007).

Androgen deprivation by surgical or medical castration results in a significant reduction in trabecular smooth muscle 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 demonstrated that, the cavernosal smooth muscle in castrated animals appears disorganized with a large number of cytoplasmic vacuoles, whereas, in the intact animals, the SMCs exhibit normal morphology and are arranged in clusters (Rogers et al, 2003).

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

In addition to the alterations in smooth muscle and connective tissue, fat-containing cells have been observed in the subtunical region of penile tissue sections from orchietomized animals (Traish et al 2005). This could lead to a venous leakage, which have been observed in a subset of hypogonadal patients with ED, who improved upon testosterone administration (Yassin et al 2006 a,b). 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 hypothesized that androgens promote the commitment

of pluripotent stem cells into a muscle lineage and inhibit their differentiation into an adipocyte lineage (Singh et al, 2006). 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, growth factors, and specific activation of a cascade of gene expression (Rosen et al, 2002; Belanger et al, 2002).

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

A reproducible result supports 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 5 α -dihydrotestosterone (DHT) 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 revealed that androgen deprivation produces penile tissue atrophy, alterations in dorsal nerve structure, alterations in endothelial morphology, reduction in trabecular smooth muscle content, increase in deposition of ECM and accumulation of fat-containing cells (adipocytes) in subtunical region of corpus cavernosum.

Tunica Fibrosis

In PD, fibrosis of tunica albuginea is characterized by increase collagen over intracellular compartment. Several studies had demonstrated that fibrosis is associated with production of profibrotic factors, (i.e. TGF- β 1 & plasminogen activator inhibitor-1) (El-Sakka et al, 1997 a; El-Sakka et al, 1997 b; El-Sakka et al, 1998 a; 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 (EGF), insulin like growth factor (IGF) have been reported as a factors 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. The role of 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). While growing evidence implicates 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 pathological 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 attained a lot of interest as a cytokine that affects the deposition of ECM and induces fibrosis in the tunica albuginea (El-Sakka et al, 1997 a; El-Sakka et al, 1997 b; El-Sakka et al, 1998 a). 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 demonstrated activation of nuclear factor kappa b (NF-KB), which regulates the expression of several genes and encode adhesion molecules, has been demonstrated 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 resulted in increased deposition of collagen around the TGF- β 1 induced lesions suggesting that iNOS suppressing collagen production in PD. Bivalacqua et al demonstrated that iNOS was increased in PD probably due to inflammation. iNOS is the key control element for peroxynitrite formation, arginase II expression, and eNOS down-regulation in the induction of a Peyronie's-like condition in the rat (Bivalacqua et al, 2000; Bivalacqua et al, 2001). Recently Myostatin (member of the TGF β family) or its cDNA construct increased the myofibroblast number and collagen in the tunica albuginea cells (Cantini et al, 2008).

Shen et al 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 was reported to be associated with type II diabetes (El-Sakka, and Tayeb, 2005) and with more impairment of vascular element of erection in diabetic patients (El-Sakka, and Tayeb, 2009). The relationship between tunical fibrosis and androgen depletion or medical co-morbidities is another interesting new dimension in understanding the pathogenesis of PD.

Proposed amelioration & reversion of penile fibrosis:

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

Post radical Prostatectomy:

Phosphodiesterase type 5 Inhibitors (PDE5-I):

Animal Studies Using cavernous Nerve Injury Models: Animal studies provide support for daily PDE5-I therapy in patients with post radical prostatectomy. Vignozzi et al reported their findings of protection against cavernous tissue protein and messenger RNA (mRNA) changes, and preservation of PDE5 expression and tadalafil efficacy after a 3-mo treatment course of daily tadalafil (2mg/kg) following bilateral cavernous neurotomy in the rat (Vignozzi et al, 2006). Using a similar rat model, Ferrini et al demonstrated that long-term vardenafil may prevent CVOD after radical prostatectomy by preserving smooth muscle content and inhibiting corporal fibrosis. These mechanisms could be due an effect on inducible NOS (iNOS) and result in functional normalization of the dynamic infusion cavernosometry drop rate and smooth muscle-to-collagen ratio. (Ferrini et al, 2006 a).

Sildenafil significantly raised the SMCs:collagen ratio in the corpora cavernosa, mainly by stimulation of cell proliferation. Daily-use sildenafil has demonstrated the ability to protect against structural changes secondary to cavernous nerve injury and to preserve erectile function in the rat (Donohue JF et al, 2006)

Elegant study by Ferrini et al demonstrated that CVOD in aged rats is associated with a significant reduction of the SMCs:collagen ratio in the penile corpora cavernosa compared with young rats, (Ferrini et al, 2006; Ferrini et al, 2007). Even more clinically relevant, it was reported that long-term and continuous administration of a PDE5A inhibitor, sildenafil, corrects aging-associated vasculogenic ED as measured by cavernosometry. Ultimately, in the aging rat model a PDE5A inhibitor corrects CVOD and ameliorates 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 SMCs:collagen ratio via the counteraction of oxidative stress or TGFβ1 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 inhibitors may vary according to the degree of corporal oxidative stress, and thus of fibrosis (Ferrini et al, 2007). Another factor that affects corporal SM compliance and that may be influenced by long-term PDE5A inhibitors, as opposed to acute effects on SM relaxation, is the expression of contractile proteins, such as ACTA2, 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 (e.g., contractile vs. synthetic) (Moncada , Higgs 2006). Also, penile neuronal NOS and endothelial NOS levels, that directly elicit an erection by releasing NO upon sexual stimulation, may be elevated by sustained high cGMP levels and thus improve compliance. Other important target that may be affected by PDE5 inhibitors is the Rho kinase system, by mechanisms

alternative to PTPN11 induction or VAV downregulation. These mechanisms may be phosphorylation or direct inhibition of Rho kinase, or the availability of cGMP substrate for these processes (Mills et al, 2003; Chan et al, 2002).

The application of molecular technologies such as gene therapy could also have a future prospect 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 the PDE5 inhibitors by improving penile nitric oxide 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.* 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, 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)

Clinical Trials of PDE5I after Prostatectomy: Schwartz et al reported on 40 volunteers who had undergone radical prostatectomy and were treated with every-other-day sildenafil for 6 months; after the treatment period, cavernosal biopsy demonstrated smooth muscle preservation at 50 mg, and decreased levels of fibrosis and substantially increased smooth muscle content at 100 mg doses (Schwartz et al, 2004). Sighinolfi et al observed an extended response to chronic PDE5-I treatment using sildenafil for up to 20 mo; peak systolic velocity demonstrated a 10.5% improvement after treatment (Sighinolfi et al, 2006).

Padma-Nathan et al 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 that 27% successful intercourse rate in sildenafil treated men compared with 4% in placebo treated men (Padma-Nathan et al. 2008). Another nonrandomized prospective study of 132 men after radical prostatectomy reported improved functional outcomes in men in a rehabilitation protocol utilizing PDE5I. Men in the rehabilitation arm manifested improved erectile function parameters (22% of rehab patients with compared with 6% of nonrehab patients), (Mulhall et al, 2005). While these preliminary results were encouraging, the possibility of selection bias and the heterogeneity of the treatments utilized for rehabilitation necessitate further prospective studies to address well the role of PDE5I in penile rehabilitation.

PDE5I on reversing fibrosis in other vascular beds:

Preclinical data and preliminary clinical reports suggest that PDE5 inhibitors 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).

Pulmonary: Sildenafil is an approved treatment for pulmonary hypertension (Galiè et al, 2005). In addition to the role in management of pulmonary artery blood pressure, sildenafil may have additional beneficial pulmonary effects. Sildenafil (25 mg/kg orally) has been shown to attenuate mucous production and markers of reactive airway disease (nitric oxide metabolites, neutrophil and macrophage count, and the proinflammatory cytokines TNF-alpha and the rat analogue of human IL-8) when given prophylactically in 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).

Cardiovascular: Sildenafil improves endothelium-dependent, flow-mediated vasodilatation in patients with diabetes mellitus and chronic heart failure and in current smokers, and provokes vasodilation of epicardial coronary arteries, improvement of endothelial dysfunction, and inhibition of

platelet activation in patients with CAD (Katz et al, 2000). Tadalafil has been also 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 PDE5I on the prevention/attenuation of cardiac hypertrophy in the setting of heart failure, ischemia/reperfusion injury, coronary vasospasm, and preservation of the vascular endothelium. Furthermore, reversal of endothelial dysfunction may cause erectile improvement as well as cardiovascular function. 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 inhibitors 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 prove 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 inducing 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, ED caused by corporal fibrosis and ultimately CVOD ranges between 66% and 75%. Therefore, if the cause of CVOD itself can be prevented, then the possibility remains that ED could possibly become a preventable condition.

Peyronie's disease:

In the rat models of PD and/or cavernosal nerve damage, long-term overexpression of NOS2A and nitric oxide production via intratunical NOS2a cDNA gene transfer, or long-term oral administration of the phosphodiesterase 5 (PDE5A) inhibitors sildenafil and vardenafil, which elevate cGMP, or long-term treatment with the phosphodiesterase 4 inhibitor pentoxifylline, which increases cAMP, reduces penile fibrosis (Ferrini et al, 2006 a, b). Vardenafil has been shown to slow and reverse the early stages of PD-like plaque in the rat, with amelioration of more advanced plaques. Once-daily (1 and 3 mg/kg) vardenafil treatment resulted in reduced collagen/smooth muscle and collagen III/I ratios, and myofibroblasts and tumour growth factor-beta1-positive cells, and selectively increased the apoptotic index in the PD like plaque (Ferrini et al, 2006 b). In addition, it is conceivable that long-term treatment with PDE5A inhibitors could upregulate NOS2A expression via cGMP modulation, and thus contribute to SMCs protection (Kukreja et al, 2005; Rosanio et al, 2006). In the clinical setting there have been occasional reports in men that long-term continuous oral administration of pentoxifylline or sildenafil may ameliorate ED (Korenman and Viosca, 1993; Montorsi, McCullough, 2005). Pentoxifylline a non-specific cAMP-phosphodiesterase inhibitor has been shown to decrease the expression of collagen I and α -smooth muscle actin (Davila et al, 2004 and Brant et al 2006). Similar findings have been observed with 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 upregulators 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, 2006), agreeing with studies in rats in which long-term

sildenafil both 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- β 1 levels were not affected by sildenafil, thus differing with the effects of vardenafil in the rat models of cavernosal nerve damage or Peyronie-like fibrotic plaque (Ferrini et al 2006 b). This is not surprising, since cGMP is not a direct inhibitor of TGF- β 1 expression but does interfere with TGF- β 1 signaling both by blocking pSmad 2 and 3 nuclear translocation or SMAD-induced gene expression and by the conversion of latent TGF β 1 to its active form (Li et al, 2007; Saura et al, 2005). In fact, the inhibition of TGF- β 1 expression by NO is not mediated by cGMP (Craven et al, 2005). 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 inhibitor did not affect the collagen III:I ratio, the alteration of which either as an increase or a decrease 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 (TGF β RII) and antisense oligonucleotides inhibit various types of TGF- β -mediated fibrosis (Hakenjos et al, 2000; Martin et al, 2000). Inhibitor of activin receptor-like kinase (ALK)5, a TGF- β type I receptor, was developed as a selective inhibitor of endogenous TGF- β signaling, and ALK5 inhibition was reported to attenuate tissue fibrosis in kidney, lung, and liver (de Gouville, et al, 2005; Bonniaud et al, 2005). PDE5I affects oxidative stress & TGF β 1 levels through interferes with TGF β 1 signaling by blocking phospho-Smad 2 & 3 nuclear translocation or Smad-induced gene expression and conversion of latent TGF β 1 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 (Song et al, 2007; Bivalacqua 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 (MDSC) corrected the ED in the aged rats. The blockade of the Smad pathway, which is a common downstream signaling mechanism for both TGF β 1 or myostatin, is also a potential antifibrotic strategy, as upregulation of the expression of TGF β 1 and phospho-activation of the Smad pathway was shown to occur in the penis of the rat with streptozotocin-induced 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). However, the pharmacological 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 pharmacological modulation, particularly with agents belonging to the NO/cGMP and TGF β 1 pathway. This approach could more feasible than regular gene and stem cell therapy 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 SMCs loss occurring with aging, diabetes, or cavernosal nerve damage should be a key therapeutic aim to prevent the ED associated with these conditions. Upregulation of 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 inhibitors, 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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Figure Legend

Figure 1: Effect of testosterone on erectile function.

Table 1: The underlying etiology and mechanisms of corporal and tunical fibrosis.

<i>Etiology of Corporal Fibrosis</i>	<i>Underlying Mechanisms</i>
<u>-Aging</u>	<ul style="list-style-type: none"> • loss of smooth muscle cells (SMCs). • fibrosis in the corpora cavernosa. • corporal veno-occlusive dysfunction (CVOD). • excessive deposit of collagen fibers. • same changes occur in media of penile arteries due to increased oxidative stress and/or other profibrotic factors (<i>that stimulate SMC apoptosis & collagen deposition</i>).
<p><u>Diabetes Mellitus</u></p> <p><u>-Cavernosal nerve damage</u></p> <p><u>-Androgen deprivation</u></p> <p><u>-Tunica Fibrosis</u></p>	<ul style="list-style-type: none"> • excessive deposition of collagen and ECM accompanied by loss of functional cells that characterize tissue fibrosis. • appearance and accumulation of myofibroblasts or the switch to a synthetic phenotype producing ECM of the original cell components, such as fibroblasts and/or SMC in the penis. • Diabetic model developed both abnormal corporal SMC relaxation and a generalized fibrosis of the arterial media. These processes seem to uniformly underlie CVOD • exacerbation of fibrosis by iNOS deletion is seen in the iNOS ko diabetic mouse. • upregulation of the expression of TGFβ1 and phospho-activation of the Smad pathway • penile biopsy after radical prostatectomy demonstrated replacement of corporal smooth muscle with collagen • CVOD develops in the bilateral cavernosal nerve resection rats as a result of the early loss of corporal SMC by the neuropraxia-induced apoptosis, followed by fibrosis. • the time course of iNOS induction supports the antifibrotic role of iNOS • penile tissue atrophy. • alterations in dorsal nerve structure. • alterations in endothelial morphology. • reduction in trabecular smooth muscle content. • increase in deposition of ECM. • accumulation of fat-containing cells (adipocytes) in subtunica region of corpus cavernosum. • fibrosis is characterized by increase collagen over intracellular compartment • fibrosis is associated with production of profibrotic factors, (i.e. TGFβ1 & plasminogen activator inhibitor-1) • Myostatin or its cDNA construct increased the myofibroblast number and collagen in tunica albuginea cells • Fibrin trapping • Collagen/elastin changes • ROS production • NO/NOS imbalance • Cellular transformation • Collagenase deficiency • Genetic Predisposition/Autoimmune • Chromosomal/Cytogenetic abnormalities • Cell cycle regulation aberration

Figure 1: Effect of testosterone on erectile function.

