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Decorin as a new treatment alternative in Peyronie's disease: preliminary results in the rat model

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Summary

The purpose of this study is to investigate the effect of decorin, a naturally occurring proteoglycan with anti-transforming growth factor beta (TGF-β) activity, on the rat model of Peyronie's disease (PD). Twenty-five adult male Sprague-Dawley rats were divided in three groups: I) TGF-β (0.5 μg) injected (*n*: 8); II) TGF-β injected and decorin treated (*n*: 8); and III) controls (*n*: 9). Decorin (0.5 μg per day) was given with intracavernous injection on the second, third, fourth and fifth day following TGF-β injection. All rats underwent electrical stimulation of the cavernous nerve after 6 weeks. Intracavernosal and arterial blood pressures were measured during this procedure. Cross-sections of the rat penises were examined using Mason trichrome and H&E stains. Statistical analyses were carried out using one-way ANOVA. Histopathological examinations confirmed the Peyronie's-like condition in TGF-β-injected rats, which exhibited a thickening of the tunica albuginea (TA), when compared to controls. Disorganisation of collagen on the TA was also prominent in TGF-β-injected rats, but not in decorin-treated and control rats. Decorin-treated rats showed significantly higher maximal intracavernosal pressure (MIP) responses to cavernous nerve stimulation, when compared to group 1 ($P < 0.05$). Our results indicate that decorin antagonises the effects of TGF-β in the rat model of PD and prevents diminished erectile response to cavernous nerve stimulation.

Introduction

Peyronie's disease (PD) is a connective tissue disorder that primarily affects the tunica albuginea (TA) of the penis and its adjacent erectile tissue. The disease is characterised by inelastic scar tissue formation that alters penile anatomy and may dramatically diminish erectile function (Ralph *et al.*, 2010). Patients with PD present either in their acute inflammatory phase, distinguished as fibrin deposition and lymphocytic infiltration that usually lasts about 12–18 months, or less commonly in the chronic phase when the deformity stabilises and pain disappears (Kadioglu *et al.*, 2006). Fibrosis, followed by calcification in some cases and even ossification, is the final consequence of all pathogenetic changes. Both clinical animal and clinical studies have demonstrated the key role of transforming growth factor beta (TGF-β) in the pathogenesis of PD (El-Sakka *et al.*, 1997a,b). Furthermore, recent evidence suggests that fibrosis, which often compromises the function of the tissue involved, is a result of significant changes in the structure of extracellular matrix, which is basically composed of structural proteins, such as collagen and elastin, embedded in proteoglycans and adhesive proteins.

Although alterations in collagen and elastin content have been demonstrated in several studies, changes in proteoglycan levels in extracellular matrix have not attracted attention in the pathogenesis of PD (Somers *et al.*, 1989). The role of proteoglycans, and especially that of decorin, is being questioned in a variety of diseases characterised by fibrosis. Experimental animal studies with decorin treatment have successfully prevented fibrosis in the kidney, lung and cerebral hemisphere in several disease models (Logan *et al.*, 1999; Fust *et al.*, 2005; Zhang *et al.*, 2010). This antifibrotic activity of decorin is briefly attributed to the fact that it directly interacts with TGF-β as well as collagen. The aim of this study is find out whether or not treatment with decorin

will have an antifibrotic effect in a TGF-β-induced rat model of PD.

Materials and methods

Animals and experimental design

Twenty-five 12-week-old male Sprague-Dawley rats (300–400 g) were divided in three groups: group 1 (*n*: 8) and group 2 (*n*: 8) received TGF-β (T 7039, 0.5 μg in a total volume of 0.25 ml; Sigma Chemical Co., St. Louis, MO, USA) injection into their TA. TGF-β was injected into the TA via a 28-G needle through a small pubic incision exposing the proximal half of the left side of the penis under sterile conditions. Group 3 animals (*n*: 9) served as control, and bovine serum albumin, which was used for TGF-β preparation, was injected into their TA. Beginning 2 days after TGF-β injection, rats in group 2 received intracavernosal decorin (D 8428, 0.5 μg in a total volume of 0.25 ml; Sigma) for four consecutive days; control rats in group 3 received intracavernosal saline. Decorin was administered directly into the corpus cavernosum of the rats using a 28-G needle. All animal experiments were approved by Experimental Research Institution, University of Istanbul, Istanbul, Turkey.

Penile haemodynamics

At the end of sixth week after TGF-β injection, erectile function was assessed. Anaesthesia was induced by intraperitoneal injection of (35 mg kg^{-1}) pentobarbital sodium and was maintained during the course of the experimental protocol by a subsequent intraperitoneal injection of pentobarbital sodium (5–10 mg kg^{-1}) as required. The animals were placed in the supine position, and the bladder and prostate were exposed through a midline abdominal incision. The major pelvic ganglia, pelvic nerves and the cavernous nerve were identified posterolaterally to the prostate on both sides. The penis was denuded of skin, and penile shaft was exposed. To monitor intracavernous pressure (ICP), a 23-gauge cannula was filled with 250 U ml^{-1} of heparin solution, connected to PE-50 tubing (Intramedic; Becton-Dickonson, Franklin Lake, NJ, USA) and inserted into the left corpus cavernosum. The carotid artery was also dissected and exposed, and systemic arterial blood pressure was monitored via a 25-gauge cannula placed in the carotid artery. Both pressure lines were connected to pressure transducers (Grass Model, PT 300; Grass Instrument Company, Quincy, MA, USA), which were, in turn, connected to a Grass polymetrograph (Grass Model 7400, Grass Instrument Division; Astro-Med Inc., West Warnick, RI, USA).

Direct electrostimulation of the cavernous nerve was performed with a delicate stainless steel bipolar hook electrode. Each probe was 0.2 mm in diameter; the two poles were separated by 1 mm. Monographic rectangular pulses were delivered by a signal generator (custom-made and with a built-in constant-current amplifier; Dr. Curtis Gleason, University of California, San Francisco). Stimulation parameters were frequency of 20 Hz, pulse width of 0.22 ms, voltage of 5 V and duration of 1 min. The application of 10 mA was used in the current protocol to achieve a significant and consistent erectile response. For each animal, electrical stimulations were repeated thrice on either side separately. The maximal amplitude of ICP during nerve electrostimulation was calculated from baseline value and included for statistical analysis.

Tissue procurement and histopathological examination

Following the electrical stimulation experiment in each rat, the penis was harvested and the animal was sacrificed. All penile tissue was immediately transferred to Holland's solution. After fixation, tissues were processed for paraffin embedding. Five-micron-thick tissue sections were applied to charged slides, deparaffinised and hydrated with distilled water. The sections were then stained with hematoxylin and eosin stains, as previously described. The thickness of the TA as well as the presence of fibrosis and the organisation of collagen fibres were assessed. Light microscope (BX50F; Olympus Optical Co., Tokyo, Japan) was used for histopathological examination.

Statistical analysis

The haemodynamic data were expressed as mean ± SD. One-way ANOVA was used to compare the results. A P value of less than 0.05 was used as the criterion for statistical significance. When statistical significant was present, ANOVA results were confirmed using the Tukey test.

Results

Histopathology

Peyronie's-like alterations, defined as massive thickening of the TA with fibrous-like nodules, were observed in 87.5% (*n*: 7) of the TGF-β-injected group 1 rats, while these changes were present in 37.5% (*n*: 3) of decorin-treated group 2 rats. TGF-β injection resulted in calcification within the TA in 2 (25%) rats. Histopathological examination of the TA in the control group did not reveal any significant changes, although haemorrhage in two rats and fibrosis in one rat were observed within corporeal bodies.

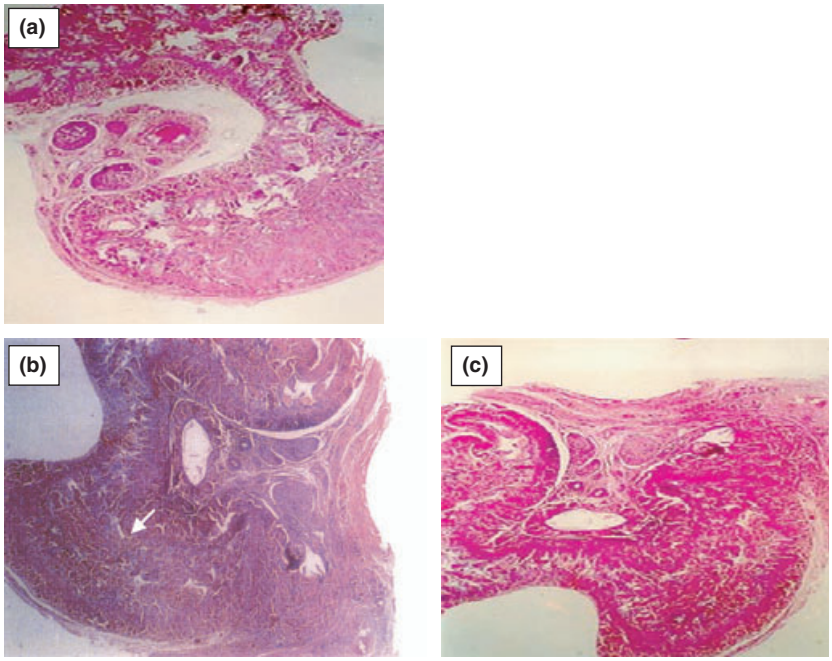

Fig. 1 H&E: In group 1, the thickness of the tunica is increased and there is fibrosis in the tunica albuginea. Also, note oedema around the neurovascular bundle (a); In group 2, normal thickness tunica albuginea, foci of haemorrhage within tunica albuginea (b); In group 3, a section of a control rat penis shows normal appearance, with regularly arranged collagen bundles in the tunica albuginea (c).

Alterations in the TA in TGF-β-injected rats were predominant on the same side of the penis that was injected with TGF-β. There were thickening in TA and inflammation around the neurovascular bundle; collagen bundles lost their normal wavy appearance within the TA in the majority of TGF-β-injected rats (Fig. 1).

Erectile response to electrical stimulation of the cavernous nerve

Baseline ICP, measured before direct electrical stimulation of the cavernous nerve, ranged between 8.8 and 20.6 mmHg, and the mean values were similar between groups. The mean basal arterial blood pressure levels did not reveal any significant difference between groups. Haemodynamic findings are given in Table 1. Following electrical stimulation of the cavernous nerve, an increase in ICP was noted to occur within 14–26 s, and the mean latency period of control group 3 was statistically shorter ($P < 0.001$). ICP increased about 5- to 7-fold in each group during erection.

The maximal intracavernosal pressure (MIP) was 110.1 ± 26.5 mmHg in the control group, 94.7 ± 19.9 mmHg in decorin-treated group 2 rats and 76.3 ± 13.7 mmHg in TGF-β-injected group 1 rats. Finally, *post hoc* statistical analysis demonstrates that there is difference between value of MIP of group 1 and 3. The mean duration of erection was 35.1 ± 7.3 s in TGF-β-injected group 1 rats and was found to be significantly shorter ($P < 0.001$) when compared to mean duration of erection observed in controls (57.1 ± 7.2 s) and decorin-treated rats (63.3 ± 16.4 s).

Table 1 Comparison of Haemodynamic findings (mean ± SD)

	Group 1 (TGF-β injected)	Group 2 (Decorin treated)	Group 3 (Control)	*P* value
Latent period (s)	20.3 ± 3.8	18.9 ± 6.8	12.1 ± 2.7	<0.001
MIP (mmHg)	76.3 ± 13.7	94.7 ± 19.9	110.1 ± 26.5	0.014
Maximum MAP(mmHg)	78.2 ± 17.0	78.0 ± 13.4	78.6 ± 15.0	0.99
Plato ICP (mmHg)	65.9 ± 11.4	82.1 ± 22.9	86.5 ± 20.7	0.09
MIP/max MAP	1.04 ± 0.4	1.21 ± 0.3	1.40 ± 0.3	0.15
Mean duration of erection (s)	35.1 ± 7.3	63.3 ± 16.4	57 ± 7.2	<0.001
Mean duration of plateau phase (s)	21.4 ± 3	33.7 ± 8.4	35 ± 8.8	<0.001

ICP, intracavernous pressure; BP, blood pressure.

Discussion

The pathophysiology of PD is not fully understood. However, PD is commonly perceived to be a disorder of inappropriate wound healing, and its development is presumably associated with an underlying genetic predisposition in addition to the presence of an inciting event. The most widely accepted theory correlates the

histological findings and symptoms to the impact of penile trauma during intercourse (Serefoglu & Hellstrom, 2011).

Several factors that induce fibrosis and also contribute to the pathogenesis of PD have been proposed. TGF-β-1 is found in human PD plaques and represents the main profibrotic factor in multiple tissues (El-Sakka *et al.*, 1997a,b). Histologic and ultrastructural changes such as chronic inflammatory infiltration, focal and diffuse elastosis, induration and disorganisation occurring in rat penises have been reported to be similar to those found in the TA of patients with PD (Gonzalez-Cadavid & Rajfer, 2009). Subsequent to TGF-β injection, a significant increase in myofibroblast counts and the levels of iNOS (inducible nitric oxide synthase) and a meaningful decrease in the levels of eNOS (endothelial nitric oxide synthase) relative to the control group were detected (Bivalacqua *et al.*, 2000, 2001). Numerous studies on animal models have suggested that TGFb-1 and myofibroblasts play an important role in the formation of PD plaques (Gonzalez-Cadavid, 2009).

The natural history of PD has not been clearly elucidated. Spontaneous resolution of deformity was reported to be less than 13% in a few studies (Gelbard *et al.*, 1990; Kadioglu *et al.*, 2002; Mulhall *et al.*, 2006). Lower rates of spontaneous resolution emphasises the need for treatment.

Although any robust confirmatory data demonstrating the indispensability of medical treatment is lacking, to achieve the best outcomes, medications are usually recommended during or immediately before the acute inflammatory phase of the disease. Aside from the most sophisticated medical therapy alternatives including oral agents, topical therapies and intralesional agents, a number of other therapeutic modalities have been used for PD (Bella *et al.*, 2007). A standard medical treatment modality for PD has yet to be defined.

There is evidence against the 0–50% beneficial effects of form of oral therapy, including vitamin E, potassium aminobenzoate, colchicine, tamoxifen and carnitine (Bella *et al.*, 2007; Gur *et al.*, 2011). Because general success rates achieved with medical therapies do not adequately improve PD, investigators have sought new treatment alternatives such as pentoxyfilline (PTX) and phosphodiesterase type 5 inhibitors (Ferrini *et al.*, 2006; Gonzalez-Cadavid & Rajfer, 2010; Safarinejad *et al.*, 2010; Smith *et al.*, 2011). PTX nonspecific phosphodiesterase inhibitor has been used in a variety of clinical inflammatory and fibrotic conditions and induced a decrease in the expression of collagen type I and a smooth muscle actin (a myofibroblast marker) in human fibroblast cultures. Lue *et al.* investigated the effects of TGF-b1 and PTX on collagen metabolism and elastogenesis in TA-derived fibroblasts (Lin *et al.*, 2010; Shindel *et al.*, 2010). The authors found that PTX also had inhibitory effects on both elastogenesis and collagen fibre deposition. However, these effects occurred only when cells were incubated with PTX prior to exposure to TGF-b1.

Decorin might be another drug alternative to be used in the treatment for PD. Decorin, a member of the small leucine-rich ECM proteoglycans, interacts with a variety of proteins that are involved in matrix assembly and regulation of cellular attachment, migration, proliferation and differentiation (Schönherr *et al.*, 1995). Decorin has a high affinity for TGF-β. The decorin core protein binds to TGF-β, neutralises TGF-β activity and modulates TGF-β-dependent cell growth stimulation or inhibition. Aside from the regulation of TGF-β activity, enhanced levels of unsequestered decorin could increase the availability of this proteoglycan for binding to its receptor(s), which regulate a host of cellular processes. Lack of decorin increased the rate of apoptosis and induced the overexpression of the IGF-IR in tubular epithelial cells of diabetic kidneys (Merline *et al.*, 2009). *In vitro* experiments using human proximal renal epithelial cells showed that recombinant decorin binds to the IGF-IR and protects these cells against high glucose-mediated apoptosis. Furthermore, overexpression of TGF-beta-1 and CTGF triggered by decorin deficiency resulted in enhanced accumulation of extracellular matrix in diabetic kidneys. Zhang *et al.* (2010) investigated the effects of decorin on kidney function in streptozocin (STZ)-induced diabetic rats and reported that renal protective effects of decorin in diabetic rats are at least partly due to downregulation of the TGF-1/Smad signalling pathway. Decorin also inhibits TGF-beta-1-induced alpha-smooth muscle actin (alpha-SMA), plasminogen activator inhibitor-1 (PAI-1) protein and mRNA expression in normal and hypertrophic scar fibroblasts. Decorin gene therapy decreased TGF-β levels, accumulation of extracellular matrix and proteinuria and also prevented the development of glomerulonephritis in rats (Zhang *et al.*, 2010). Pulmonary fibrosis induced by bleomycin in hamsters was prevented by antifibrotic effects of decorin, which led to recommendations favouring its usage in pulmonary fibrosis (Giri *et al.*, 1997). The purified recombinant human decorin (rhDecorin) significantly inhibited the proliferation of LX-2 cells, a human hepatic stellate cell line, after stimulation by TGF-beta-1 (Shi *et al.*, 2006). Furthermore, the protein expressions of smooth muscle-alpha-actin and collagen type III were significantly decreased in the presence of rhDecorin. Decorin also reduced fibrillogenesis of collagen type I in a dose-dependent manner (Merline *et al.*, 2009). In the present study, increased massive fibrosis in TA was detected in 87.5% of the TGF-β-injected group, while these changes were present in 37.5%

of the decorin-treated group. Moreover, in the TGF-β-injected group, calcification within the TA was detected in two (25%) rats. Furthermore, structural changes in TA of rats after intracavernosal injections of TGF-β were usually located at the sites of injection. Collagen bundles lost their normal wavy appearance within the TA in the majority of TGF-β-injected rats.

Intracavernosal decorin treatment administered for four consecutive days, starting from 2 days after TGF-β injections, elevated maximal and plateau erectile pressures elicited by electrical stimulation of cavernosal nerves, nearly up to mean penile pressures of the control group. Mean MIPs were significantly lower, and latent periods were more prolonged in TGF-β-injected groups when compared with control and decorin-treated groups. Mean duration of erection was lower in TGF-β-injected group 2, without any statistically significant difference between groups. These findings suggest that the process of blood pooling in the penis is slowed down because of tunical pathology, resulting in the prolongation of the latent period. Furthermore, impairment of subtunical venous occlusion mechanism associated with fibrosis, and increase in iNOS induced by TGF-β, might result in ICPs inadequate for satisfactory penile erection.

This study has some limitation. Firstly, it has relatively small number rats. Secondly, haemodynamic evaluation and histopathological examinations were performed but not Western blot analysis.

Conclusions

Our results indicate that decorin antagonises the effects of TGF-β in the rat model of PD and prevents diminished erectile response to cavernous nerve stimulation. Decorin is proposed to be a therapeutic option in a variety of human diseases such as pulmonary and hepatic fibrosis, and our preliminary results encourage the use of this naturally occurring proteoglycan in patients with PD.

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