Fibrin as an inducer of fibrosis in the tunica albuginea of the rat: a new animal model of Peyronie's disease

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Accepted for publication 25 January 2003

OBJECTIVES

To investigate the role of fibrin in inducing fibrosis in the tunica albuginea (TA) of the rat penis, to develop a new animal model for Peyronie's disease (PD).

MATERIALS AND METHODS

The TA of rats (five per group per period) were injected with either saline, fibrin, transforming growth factor-β1 (TGF-β1) or TGF-β1 plus fibrin; the rats were killed at 1, 3, and 6 weeks after injection. Images were analysed quantitatively from tissue sections stained for collagen (Masson trichrome), fibrin (Verhoeff's stain) and elastin (Hart's stain), and immunostained for TGF-β1, inducible nitric oxide synthase (iNOS), heme oxygenase 1 (HO1), α-smooth muscle actin (ASMA), apoptosis (TUNEL) and plasminogen activator inhibitor (PAI). Collagen fibre organization was characterized by electron microscopy. Human PD plaque tissue and normal human TA were assayed for fibrin by immunohistochemistry in nine samples.

RESULTS

At 1 week after injection of fibrin into the rat TA, only oedema was present; at 3 weeks, the oedema developed into a characteristic fibrotic PD-like plaque. The injection of TGF-β1 into the TA also induced oedema in the TA at 1 and 3 weeks but there was very little evidence of a recognisable plaque at either time. Injection with TGF-β1 plus fibrin resulted in oedema at 1 week but at 3 weeks there was a smaller plaque than with fibrin only. At 6 weeks the induced plaques in the fibrin-only and fibrin + TGF-β1 groups persisted, and were comparable with those elicited at this time by TGF-β1 alone. The control animals showed no pathology at any of the sample times. At 3 weeks the PD plaque induced by injection with fibrin alone had not only greater expression of TGF-β1 than the TA of the animals receiving TGF-β1 alone, but also greater levels of other markers of fibrosis, e.g. HO1 (reactive oxygen species), ASMA (presence of myofibroblasts), apoptosis, and PAI (inhibitor of fibrinolysis). iNOS, a known antifibrotic agent, was also increased. In human PD plaque tissue, fibrin was detected by immunohistochemistry in all nine specimens.

CONCLUSIONS

These results suggest that fibrin, when introduced into the TA of the rat penis, acts as a potential profibrotic protein, possibly via the local release of TGF-β1, and induces a plaque not only histologically similar to that induced by TGF-β1 but to that of the human condition. Because fibrin can extravasate from the blood into the human TA after an injury to the TA, and because fibrin persists in the plaque tissue, we hypothesise that fibrin may play a key role in the pathogenesis of human PD.

KEYWORDS

iNOS, penile trauma, TGF-β, plasmin activator inhibitor, collagen, reactive oxygen species

INTRODUCTION

Peyronie's disease (PD) is an inflammatory process of the tunica albuginea (TA) of the penis characterized by: (i) an increase in and disorganization of collagen fibres; (ii) persistent fibrin deposition within the tissue; and (iii) elastin fragmentation. Clinically, this may lead to penile deformity and/or curvature characteristic of PD, and at times may be associated with pain and erectile dysfunction [1,2]. The aetiology of PD is thought to be multifactorial, but one of the most common associations is trauma to the penis during intercourse [3,4]. The lesion is characterized by proliferation of the tunical fibroblasts and their subsequent differentiation into myofibroblasts, with evidence that both of these cell types are involved in excessive deposition of collagen and other extracellular matrix components [5,6].

El-Sakka *et al.* [7] developed an animal model for PD which is based on the injection of cytomodulin (a heptapeptide with TGF-β1 like activity) into the TA of the rat. At 6 weeks after injection a chronic cellular infiltration developed, characterized by focal and diffuse elastosis, thickening and disorganization of collagen fibres, and elastin fragmentation, similar to that seen in the human PD plaque [8] except for the absence of fibrin, which is characteristically present within the human tissue. This TGF-β1-injected rat model, like human PD tissue, has within the TA an increase in myofibroblast content, endogenous TGF-β1 expression and collagen synthesis [7–11]. This is accompanied by an increase in reactive oxygen species (ROS), a well recognized profibrotic factor, and an increased induction of inducible nitric oxide synthase (iNOS), a recently recognized antifibrotic compound. We hypothesise that iNOS is induced as a defence mechanism against oxidative stress and fibrosis [9–12]. The NO from iNOS quenches ROS by forming peroxynitrite, a known inducer of fibroblast and myofibroblast apoptosis [9,11].

However, the main feature that differentiates the human condition from the TGF-β1-induced animal model is the presence of fibrin in the PD plaque [6]. It is possible that in the human, trauma to the TA allows fibrin from the blood to enter the TA and this blood-borne protein could be the initiator of the inflammatory process that leads to the

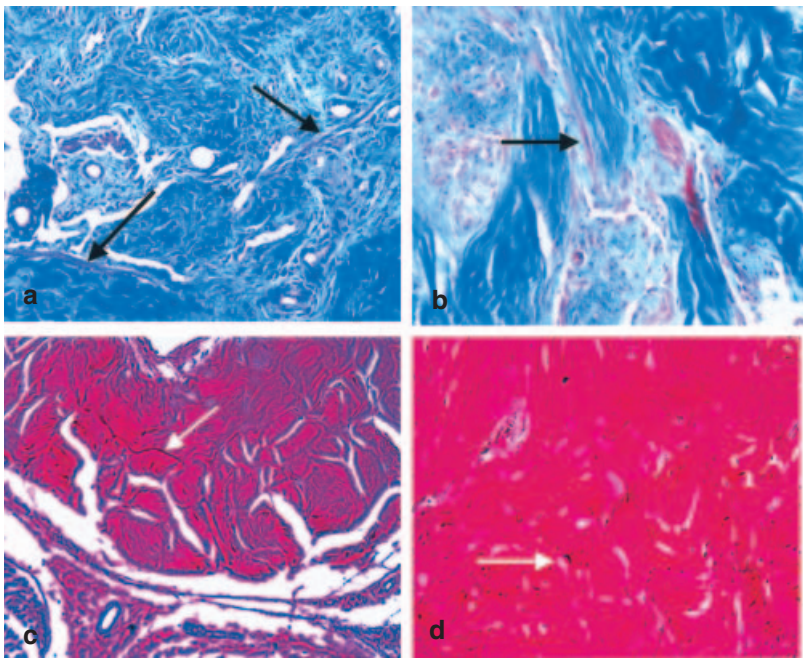
  doi:10.1046/j.1464-410X.2003.04224.x

TABLE 1 Image analysis of the areas of oedema and plaque, and the number of positive cells in the various treatments, as derived from the histology (indicated by the Figure). Images were analysed in five sections from each of the individual tissue specimens (five/group). The area of oedema was defined as the area encompassed by cell infiltration and tunical enlargement, and the area of fibrotic plaque as the area encompassed by collagen disorganization and tunical enlargement. Positive cells were counted in four microscope fields and averaged

Mean (SEM) variable	Figure	Saline (A)	TGFβ-1 (B)	Fibrin (C)	TGFβ-1 + fibrin (D)	P
Area (ImU) of:						
Oedema (pre-fibrotic)	2	345 (0.5)	865 (27)	6179 (381)	2318 (83)	A/D†; A/C‡; B/D†; B/C‡; C/D‡
Plaque:						
early fibrotic	3	317 (217)	1090 (146)	2600 (246)	3282 (190)	A/D†; A/C‡; B/D‡; B/C†; C/D*
late fibrotic	4	0	2072 (67)	2903 (298)	2624 (77)	A/D‡; A/C‡; A/B†; B/D‡; B/C†
Positive cells/field:						
TGF-β1	6	0	25 (2.3)	40 (3)	27 (2)	A/D*; A/C†; A/B*; C/D‡; C/B†
iNOS	7	20 (6)	–	51 (5)	–	A/C*
TUNEL	7	14 (1)	–	17 (2.5)	–	–
ASMA	7	2.5 (0.5)	–	26 (2.7)	–	A/C*
HO1	7	2.5 (0.5)	–	17 (6)	–	–
PAI-1	9	9 (1)	–	23 (6)	–	A/C*

*$P < 0.05$; †$P < 0.01$; ‡$P < 0.001$.

FIG. 1. Persistence of fibrin and disorganization of elastin in the PD-like plaque induced by fibrin injection in the tunica albuginea of the rat. Rats were injected in the TA with either TGFβ1 plus fibrin (a), or fibrin alone (b), and tissue sections in the site of injection obtained at 3 weeks (five/group), and stained with VMSB for fibrin detection. Tissue sections were obtained from the TA of rats injected with vehicle (saline) only (c), or with TGFβ1 plus fibrin (d), and stained with Hart for elastin detection (five/group, except saline, two rats). Black arrows indicate fibrin deposition and white arrows elastin filaments. ×200.



development of the plaque [4,6]. In the present study we aimed to determine whether an injection with fibrin into the TA of the rat can induce a lesion resembling that elicited by TGF-β1 injection, as has been previously described. We assessed, at several times after injection with either fibrin, TGF-β1 or both, the tunical thickness, arrangement of the collagen fibres, plaque size, fibrin deposition, elastin fragmentation, and expression of TGF-β1, iNOS, plasminogen activator inhibitor-1 (PAI-1), α-smooth muscle actin (ASMA), apoptosis, and ROS as determined by heme oxygenase-1 (HO1).

MATERIALS AND METHODS

ANIMAL TREATMENTS AND TISSUE PROCESSING

Male Sprague-Dawley retired breeder rats (9–11 months old; Harlam Sprague Dawley, San Diego, CA) were maintained under controlled temperature and lighting, according to NIH regulations. Animals received injections into the TA [9] (15/group) as follows: (a) with TGF-β1 (Biotech Diagnostic, Laguna Niguel, CA, 0.5 μg in 60 μL); (b) fibrin (Tisseel VH Sealer, Baxter, Glendale, CA, 30 μL each of human fibrin and thrombin solutions); or (c) TGF-β1 plus fibrin (as in a and b). Control rats received 60 μL of either (d) vehicle only (saline, nine/group) or (e) 1.2 mg of rat IgGs (three/group; Bethyl Laboratories Inc., Montgomery, TX) or (f) 1.2 mg of human serum proteins (Bethyl Laboratories, three/group). Groups a–c (five/group per period) and d (three/group per period) were killed at 1, 3 and 6 weeks after injection, and groups e and f only at 3 weeks (three/group).

Animals were perfused through the left ventricle with saline, followed by 4% formalin. Penile shafts were excised and transverse slices cut around the site of injection [9,10].

Tissues were post-fixed overnight in 4% formalin and stored at 4°C [9]. Paraffin-embedded serial sections (5 μm) were obtained for histochemistry and immunohistochemistry. Alternatively, additional rats (two/group) were treated with either saline or fibrin, as above, and tissue sections fixed in 2% glutaraldehyde overnight and stored in 2% sodium cacodylate buffer (pH 7.4), followed by embedding in Epon 812 resin [13,14]. Thin sections (1 μm) were cut on an ultramicrotome for electron microscopy.

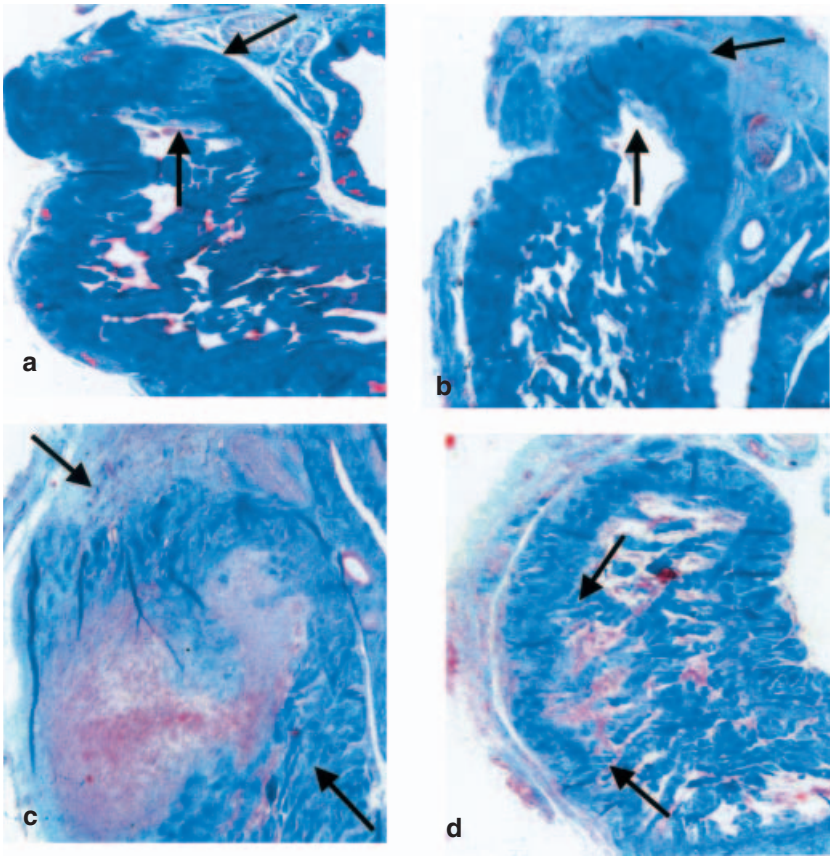
Human tissues were placed overnight in 4% formalin and stored at 4°C; paraffin-embedded sections (5 μm) were obtained for histochemistry and immunohistochemistry, as previously described [9]. These human samples were obtained only after written, informed consent was obtained from patients undergoing penile surgery. This research was approved by the institutional review board.

HISTOCHEMISTRY AND IMMUNOHISTOCHEMISTRY DETECTION

Sections were stained for: (a) collagen with Masson trichrome (Sigma Diagnostic, St. Louis, MO), which identifies collagen as blue and smooth muscle as red [10,11]; (b) Verhoeff's-Martius-Scarlet-Blue (VMSB) for fibrin, which stains mature fibrin as red [15], counter-staining with haematoxylin; the 6-week group was excluded, because 6-week-old fibrin stains blue and cannot be distinguished from collagen; and (c) Hart stain [8], which identifies the elastin fibres as black (counterstaining with Van-Gieson solution).

TGF-β1 was evaluated by immunohistochemistry [16,17] with anti-TGF-β1 polyclonal antibody (Promega, Madison, WI). Tissue sections were treated with proteinase K (20 μg/mL), quenched in 2% H_2O_2-methanol, blocked with goat serum (Vector Laboratories, Burlingame, CA) and incubated with primary anti-TGF-β1 IgG antibody at 1 : 100 dilution overnight at 4°C, followed by reaction with biotinylated antirabbit IgG (Vector Laboratories) for 30 min. Colour was developed with avidin-biotin (Vector Laboratories) followed by diaminobenzidine (Sigma) and haematoxylin. Negative controls omitted the first antibodies or were replaced by IgG isotype at the same concentration of the first antibodies. For HO1, iNOS, ASMA and PAI-1 the detection was based on a secondary antirabbit or antimouse, respectively, biotinylated antibody followed by the ABC complex and diaminobenzidine, as previously described [9]. Fibrinogen was detected with a polyclonal antibody (Dako Corporation, Carpinteria, CA) followed by a secondary antibody linked to alkaline phosphatase [18]. Apoptosis was determined by the TUNEL procedure [10].

FIG. 2. Induction by fibrin of a prefibrotic area in the TA of a rat assessed by collagen staining (Masson). a–d: oedema at the site of injection is indicated by the arrows. ×40.



QUANTITATIVE EVALUATION OF STAINED SECTIONS

Sections were quantified by computerized densitometry using the ImagePro 4.01 program (Media Cybernetics, Silver Spring, MD), coupled to a microscope equipped with a digital camera [9–11]. For Masson staining, the plaque was analysed by its diameter at the site of injection, tunical thickness, ratio between blue and white areas of the transverse section of the penis, and optical density/total area of the penis. For immunohistochemistry, the number of positive cells was counted in a computerized grid against the total number of cells determined by counterstaining, and results expressed as positive cells per field. Three fields were measured per section, with at least three anatomically matched sections per animal analysed in all determinations.

For electron microscopy, sections were mounted on 200-mesh copper grids and stained with uranyl acetate and lead citrate as contrast agent [13,14]; the sections were examined using a Philips 400 transmission electron microscope.

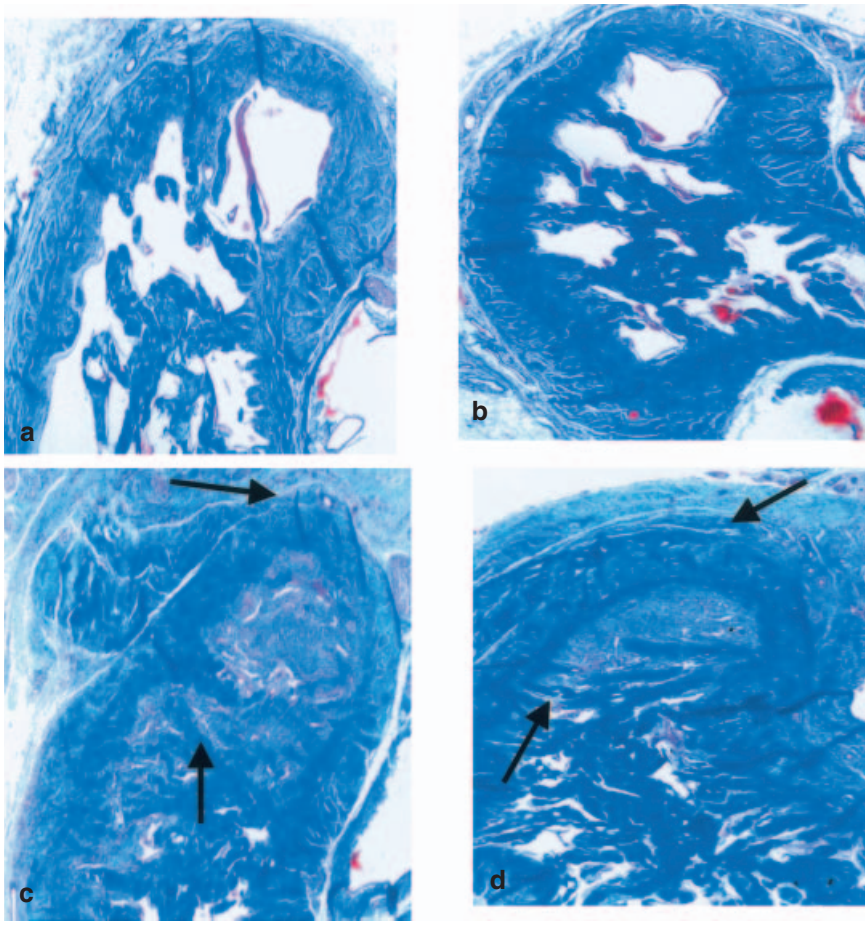
STATISTICAL ANALYSIS

Values were expressed as the mean (SEM), with the normality of the distribution established using the Wilk-Shapiro test. Multiple comparisons among the different groups were analysed by a single-factor ANOVA, followed by post-hoc comparisons with the Student-Neuman-Keuls test. Differences among groups were considered significant at $P < 0.05$.

RESULTS

To determine whether fibrin *per se* is able to elicit a fibrotic plaque in the rat TA, rats (five/

FIG. 3. Induction by fibrin of an early fibrotic PD-like plaque in the TA of a rat, assessed by collagen staining; sections were obtained from animals killed 3 weeks after injection and stained with Masson. a–d: the field around the area of injection; arrows indicate tunical thickening with an increase of collagen deposition (plaque). ×40.



group per period) were injected in the TA with either fibrin, TGF-β1 or both together, or saline, and killed at 1, 3 and 6 weeks afterward. The VMSB staining for fibrin was applied to the tunical tissue sections obtained from these animals at 3 weeks (Fig. 1). The animals that developed the largest fibrotic plaques after 3 weeks, as detected by Masson staining were those injected with either TGF-β1 + fibrin or fibrin alone, and they showed an intense staining of fibrin in the TA (Fig. 1a,b). As expected, animals injected with saline or TGF-β1 did not stain for fibrin (not shown).

Hart's staining for elastin on sections obtained at 3 weeks revealed fragmented and small elastin fibres within the TA (Fig. 1) for animals injected with TGF-β1 (not shown), TGF-β1 + fibrin (Fig. 1d) and fibrin (not shown), whereas animals injected with saline (Fig. 1c) had elongated and normal wavy elastin fibres interspersed with the collagen fibres. The pattern of fibrin staining and elastin fragmentation was replicated in all animals injected with TGF-β1 + fibrin or fibrin at 3 weeks, the stage where the plaque was fully developed in these animals.

Tissue sections from the TA obtained from the various treatment groups were stained for collagen with Masson trichrome. Figure 2 shows that after one week, injection of fibrin alone (Fig. 2c) had marked oedema and an inflammatory reaction in the TA, while in the saline-injected control there were minimal changes (Fig. 2a). At 1 week after injection of TGF-β1 alone (Fig. 2b), some oedema was seen, but when the TGF-β1 was injected together with fibrin (Fig. 2d) there was an inflammatory response but it was less severe than that seen with the injection of fibrin alone. By quantitative image analysis (Table 1) the area of oedema within the TA was significantly larger for both the fibrin and TGF-β1 + fibrin-treated animals than in rats injected with saline alone or with TGF-β1 alone. However, as expected so soon after injection, there was no increased deposition or disorganization of collagen in any of the 1-week treated groups.

At 3 weeks after injection (Fig. 3) a clear fibrotic plaque was identifiable in animals injected with either fibrin alone (Fig. 3c) or with TGF-β1 + fibrin (Fig. 3d). In the saline- (Fig. 3a) and TGF-β1- (Fig. 3b) injected groups, no plaques were evident. The size of the plaques induced by fibrin alone, compared with those induced by TGF-β1 + fibrin, was similar, as confirmed by quantitative image analysis (Table 1).

At 6 weeks (Fig. 4), plaque began to form in the TGF-β1-treated group (Fig. 4b), confirming previous results with this TGF-β1-injected model [8–10,12,15]. However, the plaques elicited by treatment with either fibrin alone (Fig. 4c) or TGF-β1 + fibrin (Fig. 4d) at 3 weeks were still present at 6 weeks after injection and appeared larger than those induced by TGF-β1 alone. Quantitative image analysis (Table 1) confirmed these histological observations. The control animals injected with either saline (Fig. 4a), human IgGs and albumin, or rat serum proteins developed no plaque at either 3 or 6 weeks after injection, thus highlighting the possible role of fibrin as an inducer of fibrosis in the TA of the rat.

Transmission electron microscopy of a tunical plaque induced by fibrin at 3 weeks after injection (Fig. 5) revealed an obvious disorganization of the collagen fibres (right) compared with the normal TA, where the collagen fibres were continuous, long, parallel and lay in a wavy pattern (left).

To determine whether fibrin could trigger the plaque in the TA by stimulating the expression of endogenous TGF-β1, sections were immunostained for TGF-β1 at 3 weeks (Fig. 6), when a plaque first became recognisable in the fibrin-only and TGF-β1 + fibrin-treated animals. Positive cells for TGF-β1 expression were abundant in rats injected with TGF-β1 alone (Fig. 6b), contrasting with the negligible expression seen in the saline-injected animals (Fig. 6a). Rats receiving fibrin alone (Fig. 6c), with a large 'early' plaque that was recognisable at 3 weeks, had considerable expression of TGF-β1, even higher than with TGF-β1 alone (Fig. 6b) or TGF-β1 + fibrin (Fig. 6d). Quantitative image analysis (Table 1)

showed that animals injected with TGF-β1 + fibrin (Fig. 6d) had a similar number of positive cells to that in rats injected with TGF-β1 (Fig. 6b). Fibrin alone (Fig. 6c) elicited the most TGF-β1-positive cells, not only at 3 weeks when compared with all other groups, but also at 6 weeks, including even the plaques that were induced by TGF-β1 at this time (not shown).

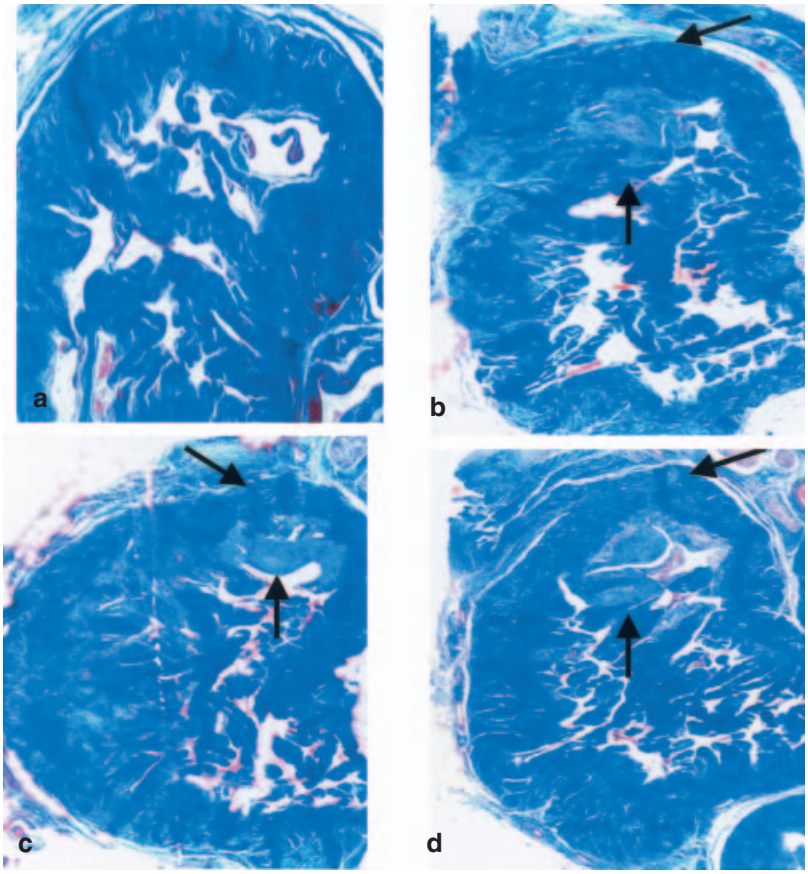
As iNOS is induced in both the human PD plaque and in the PD-like lesion elicited by TGF-β1 in rat model of PD [9,12], we investigated whether iNOS expression can also be detected in the plaque induced by fibrin in the rat TA, and whether this is accompanied by other processes described in the TGF-β1 rat model, i.e. oxidative stress, myofibroblast differentiation and apoptosis [9,19]. Separate immunohistochemical reactions in adjacent tissue sections from PD-like plaque elicited by fibrin and in normal TA from rats injected with saline were carried out for iNOS, HO1 as a marker of ROS (oxidative stress), ASMA as a marker of myofibroblasts, and TUNEL to determine the apoptotic index. Figure 7 shows typical staining in the PD-like plaque induced by fibrin for all these markers. The quantitative image analysis indicated a considerable increase in iNOS induction (Table 1) and the expression of HO1 and ASMA in the PD-like lesion, compared with the saline-injected TA. There was a moderate but insignificant increase in apoptosis.

The size of the individual plaque induced by fibrin appears to be a function of the number of fibrin filaments remaining in the plaque at 3 weeks after injection (Fig. 8a), whereas when the ratio of iNOS expression to plaque size was used, there was a negative correlation with the plaque area (Fig. 8b). As iNOS is postulated to be an antifibrotic factor [9], and fibrin a pro-fibrotic factor, the ratio between both values negatively correlated with the size of the plaque (Fig. 8c).

The persistence of fibrin at 3 and 6 weeks after its injection into the TA of the rat suggests an impaired fibrinolytic mechanism, possibly through an increase in the levels of the PAI-1, a well known blocker of plasmin activator and a known fibrinolytic factor [20]. This assumption is supported by the elevated levels of PAI on immunohistochemistry in the fibrin-induced PD-like plaque in the rat when compared to normal TA (Fig. 9) and by quantitative image analysis (Table 1).

As previous work showed the presence of fibrin in human PD plaque tissue using VMSB histochemical staining [6] we investigated whether fibrin could also be detected by an antibody against fibrinogen, the fibrin precursor sharing most antigenic determinants with it. Figure 10 shows one representative section of human PD with the typical collagen disorganization revealed by staining with Masson trichrome and the different morphological appearances, as compared to normal TA (top). Adjacent sections stained with VMSB showed a series of reddish fibrin filaments that is completely absent in the normal TA (middle). In contrast, the antifibrinogen antibody revealed large immunoreactive areas specific to the Peyronie's plaque zone, absent or scattered in the normal TA (bottom). This pattern of differential staining between PD tissue and normal TA was reproduced in all nine human PD specimens, compared with the three normal TA specimens, even in the chronic stage of the disease. The presence of some immunoreactivity in the normal TA is probably the result of contamination of blood that is omnipresent during surgery of the corpora cavernosa.

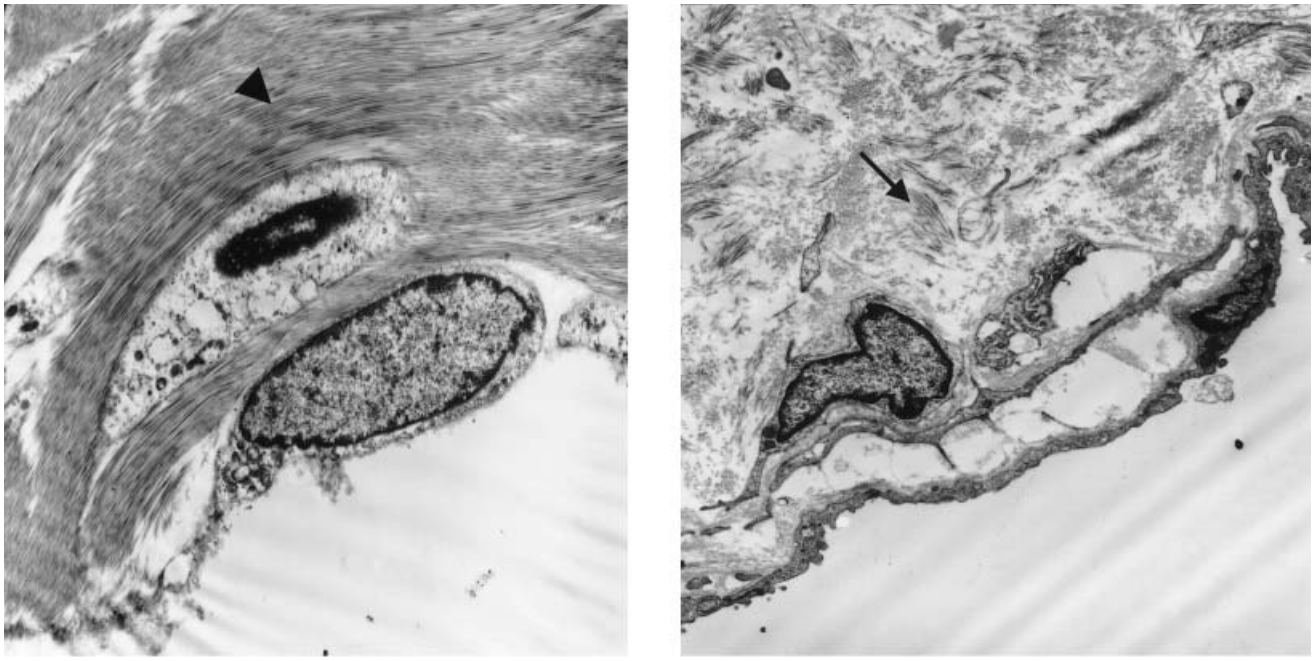
FIG. 4. Stabilization of the fibrotic PD-like plaque induced by fibrin in the TA of a rat, assessed by collagen staining, from five animals/group killed 6 weeks after injection. Panels as Fig. 2.



DISCUSSION

This study highlights the possible role of fibrin in the development of the inflammatory and fibrotic process of PD. The presence of fibrin in the human PD plaque even months after development of the plaque, as has been previously shown by histochemical staining [6], is very characteristic of the human condition and we confirm by immunodetection the presence of fibrin, or its precursor, fibrinogen, in the plaque. The injection of fibrin alone into the TA of the rat can elicit a lesion characterized by oedema and inflammation followed by tunical thickening, collagen disorganization, elastin fragmentation, and increased TGF-β1, ROS, ASMA and iNOS expression, as is seen in both human PD and the TGF-β1-induced animal model of PD [7–9,11–15]. This supports the hypothesis that fibrin deposition after trauma to the penis could be the primary event triggering the expression of pro-fibrotic factors like TGF-β1 that would lead to the development of a tunical plaque. This fibrin-injected animal model, when compared to

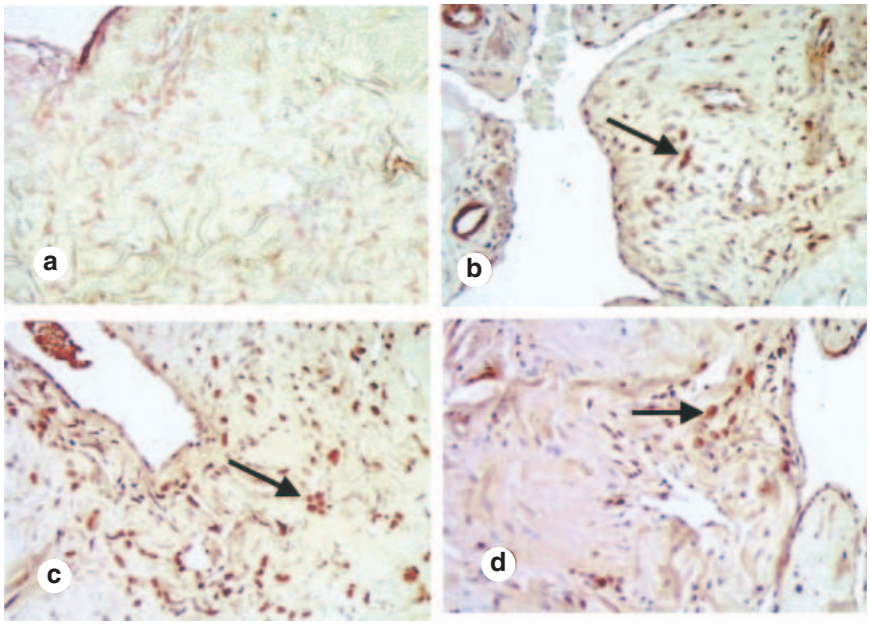
FIG. 5. Electron microscopy showing collagen disorganization in the PD-like plaque induced by fibrin in the TA of a rat. Thin sections were obtained 3 weeks after injection from one of the PD-like lesions induced by fibrin and one of the normal TAs examined (× 3000). Arrowhead: normal collagen fibres; black arrow: disorganized collagen fibres.



Normal tunica

Peyronie-like plaque induced by fibrin

FIG. 6. Induction of TGF-β1 expression in the Peyronie-like plaque induced by fibrin in the TA of a rat. a–d: Sections adjacent to those for plaques obtained 3 weeks after injection (Figs 3 and 5) immunostained with an antibody for TGF-β1 (five/group). Arrows indicate cells with intense staining. ×200.



the TGF-β1 animal model of El-Sakka *et al.* [7,8], more closely resembles the initial pathophysiological event after trauma (fibrin deposition) [4,6] and reproduces all the histological alterations (collagen disorganization, fibrin deposition, elastin fragmentation) seen in the human PD-plaque. The persistence of fibrin in the rat TA weeks after injection with fibrin could be associated with an increased expression of the inhibitor of fibrinolysis, PAI-1, in the extravascular system [20,21]. Furthermore, the fibrin-induced plaque develops more rapidly and the plaque is larger than its TGF-β1-induced counterpart in the rat [7–9,11,12]. These data in the fibrin-treated animals also suggest that iNOS may have an antifibrotic role in this rat model of PD, similar to that reported in the TGF-β1 rat model [9,11].

The fibrin preparation used in this study is a biologically derived, not cytotoxic, fully reabsorbable biomaterial that has been used routinely in human surgery for over two decades with no immunological reaction [22], and in a variety of experimental conditions

*FIG. 8. Relationship between fibrin filaments, iNOS levels and size of the plaque induced in the TA of the rat by fibrin injection. The number of fibrin filaments (**a**) or iNOS positive cells (**b**) in each PD-like plaque was plotted against the respective plaque areas, estimated by Masson staining as arbitrary imaging units (ImU) in the sections analysed in Figs 3 and 7. The expression of iNOS divided by the number of fibrin filaments in each plaque was also plotted against the respective plaque area (**c**). Each point corresponds to one plaque; the regression coefficients in a, b and c were 0.9449, 0.7968 and 0.8753, respectively*

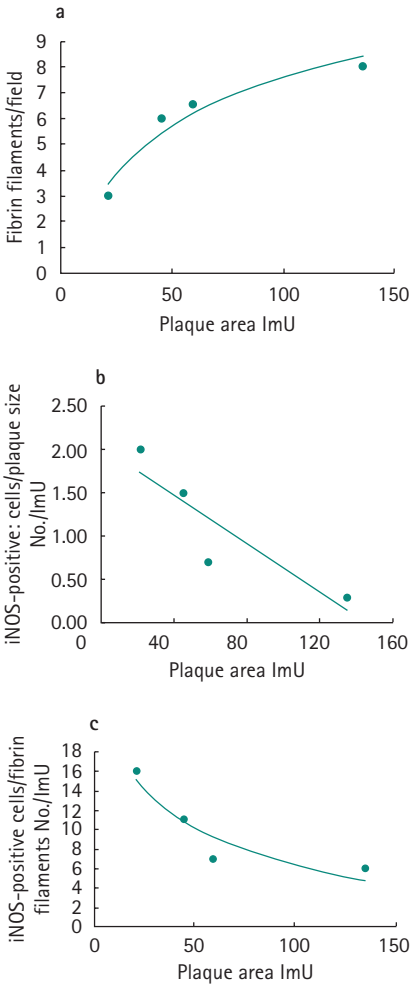


FIG. 7. Expression in the tunical PD-like plaque induced by fibrin of markers of iNOS, apoptosis, fibroblast differentiation and oxidative stress (HO1). a–d: Sections adjacent to those from plaques obtained at 3 weeks after injection (Figs 3 and 5) immunostained (four/group) with antibodies detecting iNOS (a), DNA breakdown (TUNEL) (b) for apoptosis, ASMA (c) for fibroblast/myofibroblast differentiation and ROS for oxidative stress (HO1) (d). ×200.

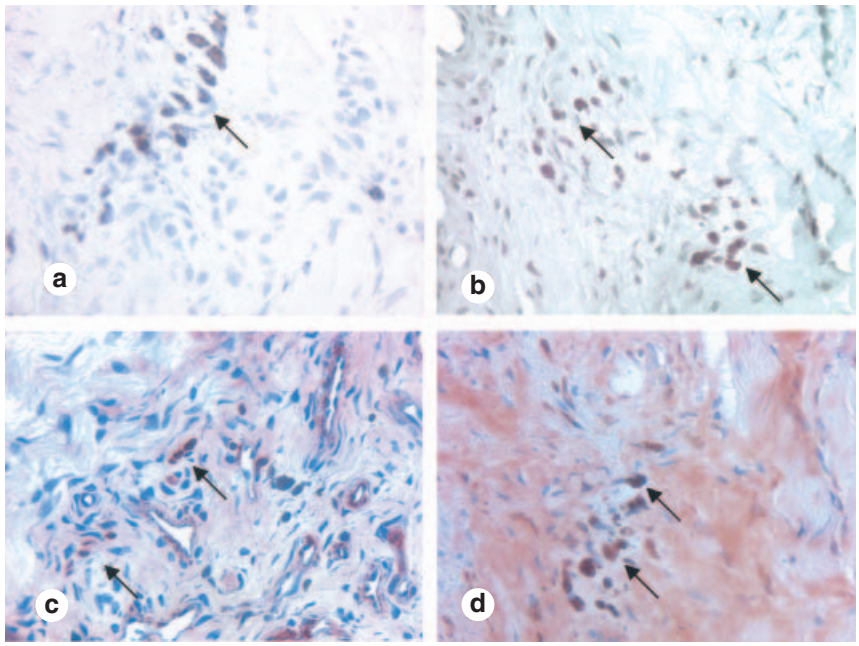
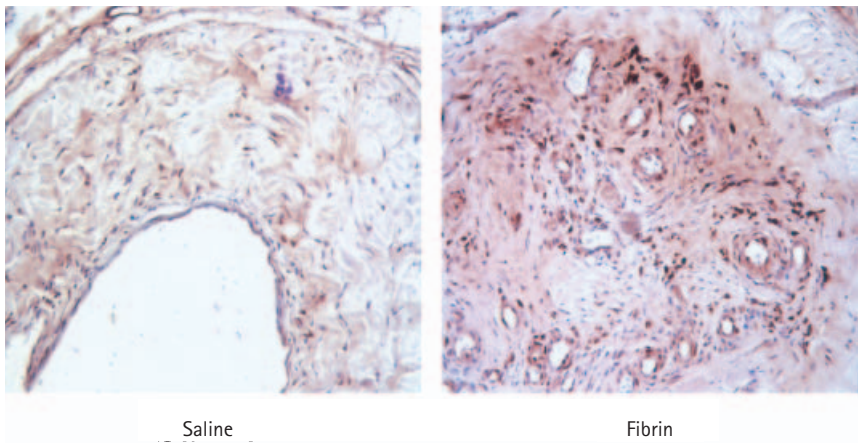


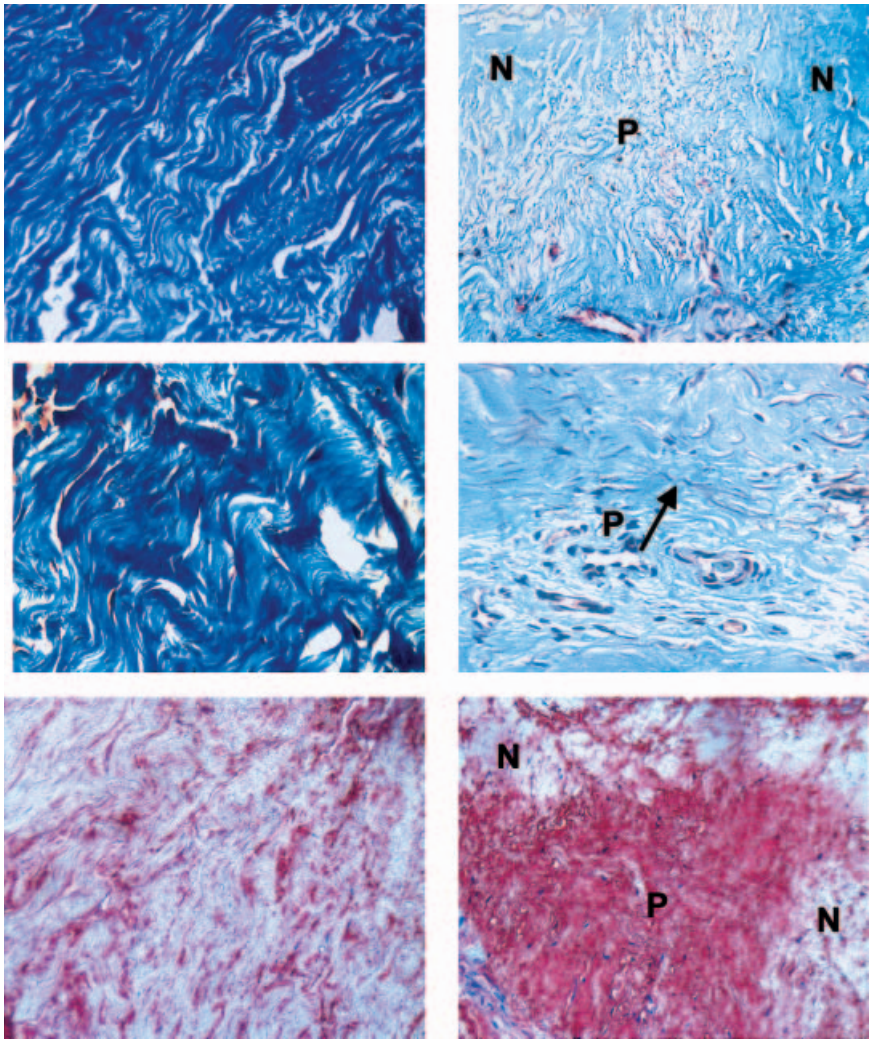
FIG. 9. Association of persistent fibrin with increased PAI levels in the plaque induced in the TA of a rat by fibrin injection. Tissue sections adjacent to those stained for fibrin in Fig. 1 were immunoreacted with an antibody against PAI.



[23–25]. It consists of fibrinogen, thrombin and calcium, which when mixed together simulate the last stages of the coagulation cascade [19]. Its injection into the TA reproduces experimentally what occurs clinically after trauma to the TA. The effects appear to be specific for fibrin because two different mixtures of other blood proteins, rat IgGs and human IgGs and albumin, caused no plaque formation or fibrotic reaction in the rat TA up to 6 weeks after injection of these proteins. In addition, there are many reports showing that injection of human fibrin in the rat causes no immunorejection even after long periods [26–31]. Therefore, it may be assumed that this process might not be important in the development of the PD-like plaque in the TA after fibrin injection. The profibrotic effects of this fibrin preparation in the TA are most likely the result of combining the deposit of fibrin into a hypovascular and fibroblast-rich tissue with the simultaneous trauma caused by the injection.

The present results suggest that fibrin is more effective than TGF-β1 in eliciting a plaque in the rat TA, and seems to trigger the expression

FIG. 10. Detection of fibrin in the human PD plaque. The top panels (×200) are serial paraffin-embedded sections from PD plaque and normal TA (control) stained for collagen with Masson trichrome; adjacent sections (middle panels) were stained for fibrin with VMSB (×400). Other adjacent sections were subjected to immunohistochemistry with a polyclonal antibody against fibrinogen that also detects fibrin, and a secondary biotinylated antibody linked to alkaline phosphatase (bottom panels, ×200; nine patients with PD, three with no PD and a normal TA). P, PD plaque; N, normal tissue around the plaque. Arrows denote fibrin filaments.



Normal tunica

Peyronie's plaque

of TGF-β1 [32], a key inducer of collagen synthesis and a major promoter of fibroblast differentiation into myofibroblasts [11,33,34]. At 1 week after injection, the initial oedema was greater in rats injected with fibrin than in those injected with TGF-β1 + fibrin or TGF-β1 alone, probably because of the anti-inflammatory effects of TGF-β1. This cytokine is known to decrease acute inflammatory reactions by reducing macrophage activation and inhibiting cytotoxic T cells [35,36].

However, with prolonged TGF-β1 secretion, this cytokine may then act as a pro-fibrotic agent by directly promoting collagen synthesis and inhibiting the matrix metalloproteinases that control collagen turnover, thereby enhancing collagen deposition [37]. This may explain the finding of a plaque at 6 weeks (through its profibrotic action) but not at 3 weeks (through its anti-inflammatory action) after injection with TGF-β1 into the TA.

Fibrin, once it extravasates into the TA, somehow resists degradation, possibly through the induction of PAI-1 that we have located in the plaque of the fibrin-injected rat PD model, and it is the persistence of this fibrin in the TA that could be the continuing pro-fibrotic trigger for the deposition of collagen and fragmentation of elastin that forms the characteristic plaque of PD. Possibly fibrin deposition not only causes the expression of pro-fibrotic factors, but could also impair the normal resolution of the wound-healing process. The induction of iNOS in the fibrin animal model may be an antifibrotic response to the production of ROS, as we have shown in the TGFβ1 rat model of PD [9,11], or may be indirectly through PAI-1 regulating the fibrinolysis [20]. The exact reason for the persistence of fibrin in the TA in PD is still unresolved, but the PD-like plaques in the fibrin-injected TA contained a high level of fibrin, and the same is seen in human plaques. These persistent fibrin filaments may be aetiologically significant and could be a target for potential therapeutic approaches to PD.

In conclusion, fibrin, possibly through the induction of the self-replicative pro-fibrotic factor TGF-β1, can trigger in the rat TA an inflammatory and fibrotic process resembling that of human PD. Because of its similarities to the human condition, including the persistence of fibrin within the plaque, this proposed animal model of PD has a faster onset than the existing TGF-β1-induced rat model and could contribute to the investigation of potential therapies for PD.

ACKNOWLEDGEMENTS

This study was funded by a grant from the Eli and Edythe L. Broad Foundation, and in part by NIH grants R01DK-53069 and G12RR-03026. We thank Ms. Natalie Simon (Baxter BioScience, Glendale, CA) for her kind supply of the fibrin preparation for the preliminary experiments.

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Abbreviations: **PD**, Peyronie's disease; **iNOS** or **NOS II**, inducible NOS; **TA**, tunica albuginea; **HOI**, heme oxygenase I; **ROS**, reactive oxygen species; **ASMA**, α-smooth muscle actin; **PAI–1**, plasmin activator inhibitor; **VMSB**, Verhoeff's-Martius-Scarlet-Blue.