Doxazosin Treatment Alters Stromal Cell Behavior and Increases Elastic System Fibers Deposition in Rat Prostate

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KEY WORDS doxazosin; prostate; extracellular matrix; elastin; smooth muscle cell

ABSTRACT Doxazosin (DOX), an α-adrenoceptor antagonist, induces the relaxation of smooth muscle cell tonus and reduces the clinical symptoms of benign prostatic hyperplasia (BPH). However, the effects of DOX in the prostate stromal microenvironment are not fully known. In a previous study, we showed that DOX treatment for 30 days increased deposition of collagen fibers in the three rat prostatic lobes. Herein, we investigated the effects of DOX on stromal cell ultrastructure and elastic fiber deposition. Adult Wistar rats were treated with DOX (25 mg/kg/day); and the ventral, dorsal, and anterior prostates were excised at 30 days of treatment. The prostatic lobes were submitted to histochemical and stereological-morphometric analyze and transmission electron microscopy (TEM). Histochemical staining plus stereological analysis of the elastic fiber system showed that DOX-treated prostatic lobes presented more elaunin and elastic fibers than controls, mainly in the ventral lobe. Ultrastructural analysis showed that fibroblasts and smooth muscle cells from DOX-treated prostates presented active synthetic phenotypes, evidenced by enlarged rough endoplasmic reticulum and Golgi apparatus cisterns, and confirmed the observation of thickened elaunin fibers. Our findings suggest that, under α-adrenergic blockade by DOX, the fibroblasts become more active and smooth muscle cells shift from a predominantly contractile to a more synthetic phenotype. The deposition of collagen and elastic system fibers in the prostatic stroma may counterbalance the absence of smooth muscle tone during α-blockers treatment. *Microsc. Res. Tech. 73:1036–1044, 2010.*

INTRODUCTION

The prostate is a major male reproductive system accessory gland, whereas benign prostatic hyperplasia (BPH) and prostate cancer (PCa) are the most common proliferative disorders affecting older men (Crawford, 2009; Rittmaster, 2008; Thomson, 2008). Functionally, BPH consists of two components: static (generalized epithelial gland enlargement regulated by androgens) and dynamic (contraction of stromal smooth muscle cells, mediated predominantly by α1-adrenoceptors) (Lacey, 1996).

The α1-adrenoceptor antagonists, such as doxazosin (DOX) and terazosin, have been widely used to target the stromal smooth muscle cells in the treatment of BPH (Kirby, 1996). The competitive inhibition of catecholamine prevents contraction of smooth muscles and reduces their tone, thus alleviating the lower urinary tract symptoms (Smith et al., 1999).

Recently, DOX has also been demonstrated to inhibit prostate growth by inducing apoptosis in stromal and epithelial cells, showing additional effects on long-term BPH treatment and emerging as a potential drug for the prevention and treatment of androgen-independent PCa (Anglin et al., 2002; Chiang et al., 2005; Kyprianou, 2003; Kyprianou and Jacobs, 2000; Kyprianou et al., 1998; Yang et al., 1997).

In a previous work, our group demonstrated for the first time that DOX treatment also induces a reduction in the epithelial cell proliferation rate (Justulin et al., 2008). Furthermore, we also showed that DOX reduces both absolute and relative glandular weights with a significant increase of the collagen fiber deposition in the prostate stroma (Justulin et al., 2008). More recently, Imamura et al. (in press) using clinical specimens found that patients orally treated with α1-blockers often exhibit accumulation of collagen fibers in prostatic stroma and suggested that this structural change could be one of the factors responsible for the development of resistance to this treatment.

In addition to collagen fibers, elastic system fibers are also an important component of prostate stroma (Carvalho et al., 1997). The number and thickness of elastic system fibers increase in the rat ventral prostate (VP) stroma during castration-induced involution (Carvalho et al., 1997), and these fibers also appear to be altered in BPH (Chagas et al., 2002; Costa et al., 2004; Sugimoto et al., 2008).

Moreover, Smith et al. (1999), using culture of prostatic smooth muscle cells, found that contractility of

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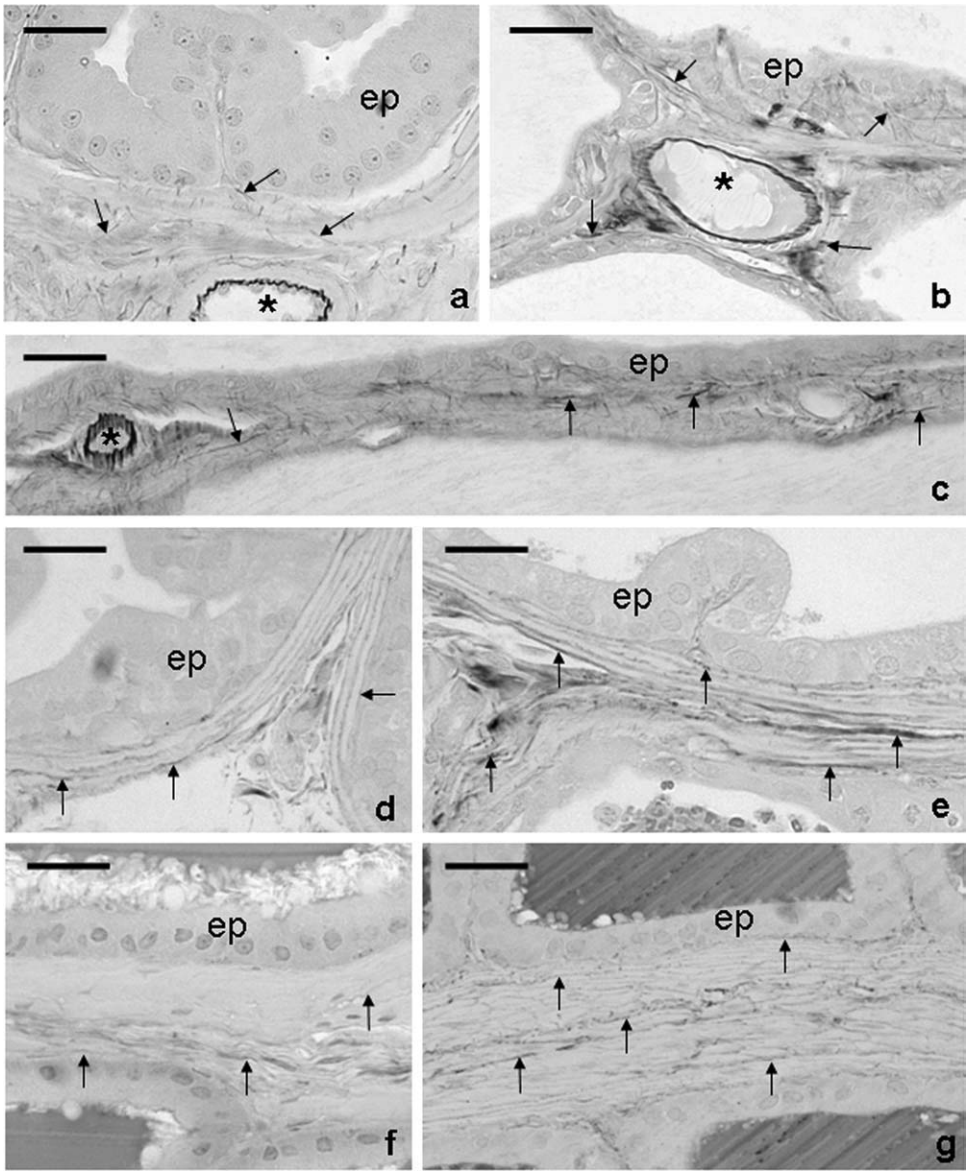

Fig. 1. Resin sections from rat prostatic lobes stained by Weigert's Resorcin–fuchsin. **a–c:** Ventral prostate; (**d**) and (**e**) dorsal prostate; (**f**) and (**g**) anterior prostate; (a, d, and f) control animals; (b, c, e, and g) doxazosin-treated animals. The elastin-containing elastic system fibers appear stained in dark (arrows). It can be observed that elaunin fibers are the main elastic system fibers in the control rat prostatic lobes and that 30 days of doxazosin treatment induced an increase in the number and thickness of these fibers in the three prostatic lobes. Epithelium (ep); blood vessels internal elastic laminae (*) were used as an internal positive control to the histochemical reaction. Bars = 20 μm.

smooth muscle cells decreased following DOX treatment. These authors also showed that DOX treatment reduced the expression of actin and myosin proteins, suggesting that α1-blockers induce dedifferentiation of smooth muscle into fibroblasts and myofibroblasts. A smooth muscle cell phenotype that is more synthetic than contractile also appears to play a major role in the process of depositing both collagen and elastic system fibers in the stroma during castration-induced prostate involution (Antonioli et al., 2004, 2007; Carvalho et al., 1997; Corradi et al., 2004; Vilamaior et al., 2000, 2005). Thus, in this study, we evaluated the effects of DOX treatment on stromal compartment of the ventral (VP), dorsal (DP), and anterior (AP) lobes, giving special attention to the phenotypes of the fibroblasts and smooth muscle cells and to elastic system fibers deposition.

MATERIALS AND METHODS

Animals

Adult male 3-month-old Wistar rats were maintained in a controlled environment with free access to food and water. The experiment was performed according to the *Guide for Care and Use of Laboratory Animals.* The animals were divided into two groups: control (CT) and the DOX-treated group. DOX-treated animals received daily doses of DOX (25 mg/kg of body weight)

(Doxazosin mesylate, Pfizer, Galena Pharmaceutical and Chemistry, SP, Brazil) dissolved in corn oil as vehicle, and administrated by oral gavage. The dosage and duration of treatments were based on consultation with Pfizer and in a previously published work (Yono et al., 2007). CT animals received only the vehicle. After 30 days of the DOX treatment, 10 animals from each group were killed by an overdose of pentobarbital. The VP, DP, and AP were excised, and alternate lobes were left and right assigned and processed for transmission electron microscopy (TEM) or for conventional microscopy. In our study, most of the lateral lobes presented acute prostatitis and, in this sense, it was excluded of the analysis.

Histochemistry and Stereological-Morphometric Analysis

Five lobe pairs of VP, DP, and AP from control and DOX-treated animals were immersed in 4% paraformaldehyde dissolved in phosphate buffer saline for 24 h. Fixed samples were washed in PBS for 24 h, dehydrated in a graded ethanol series, and embedded in glycol metacrylate resin (historesin embedding kit, Leica Heidelberg). Semiseriated resin (4 μm) sections were obtained and stained with Weigert's resorcin–fuchsin for elastic system fibers analysis (Carvalho et al., 1997).

The sections were analyzed with a Leica DMLB 80 microscope connected to a Leica C300FX camera. The digitalized images, obtained by using image analyzer Leica Q-win software Version 3 for Windows, were used for stereological-morphometric analysis.

The mean volume fraction of the elastic system fibers in the prostatic lobes was determined according to the Weibel system of point counting (Weibel et al., 1966) and using a 168-point grid test. Twenty-five microscopic fields were chosen at random from five different individual prostatic lobes from each experimental group. The volume fraction of elastic system fibers was expressed as percentage (%) after counting the number of points that coincided with elastic system fibers and the total number of points that coincided with the stromal area. All the measurements were taken from the intermediate regions, which represented the major portions of the prostatic lobe alveoli/tubules (Nemeth and Lee, 1996).

Transmission Electron Microscopy

Tissue fragments of five animals from each experimental group were immersed in 2.5% glutaraldehyde, plus 0.25% tannic acid solution in Millonig's buffer for 2 h (Cotta-Pereira et al., 1976), washed, and postfixed in 1% osmium tetroxide in the same buffer for 1 h, washed again, dehydrated in graded acetone, and embedded in Araldite. Semithin sections were stained with Toluidin Blue and analyzed under light microscopy. Ultrathin silver sections were obtained with a diamond knife and contrasted with 2% alcoholic uranyl acetate and then with 2% lead citrate in 1 N sodium hydroxide solution for 10 min. Grids were examined under a Phillips transmission electron microscope, operating at 80 kV.

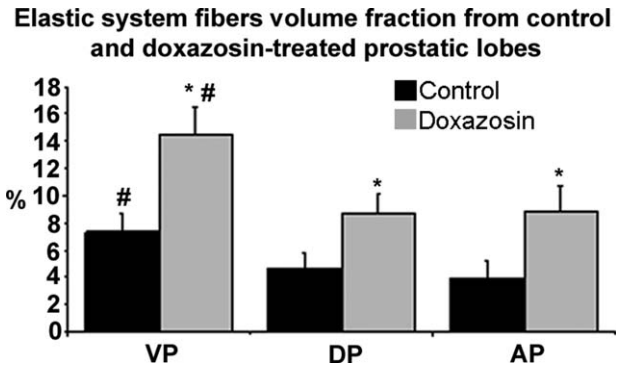

Fig. 2. Doxazosin effect on elastic system fibers volume fraction in the rat prostatic lobes. The values represent mean ± SD. In both control and treated groups, the ventral lobe (VP) had the highest elastic system fiber volume fraction among the prostatic lobes ($^{\#}P < 0.05$). Doxazosin treatment significantly increased the volume fraction of the elastic system fibers for all prostatic lobes ($^{*}P < 0.01$). Dorsal prostate (DP); anterior prostate (AP).

RESULTS

Histochemistry and Stereological-Morphometric Analysis

Histological analysis after Weigert's resorcin–fuchsin staining revealed a small number of delicate elaunin fibers in the control VP, DP, and AP lobes. These fibers were distributed mainly around smooth muscle cells, but also above the epithelium and in the interstitial space between the prostatic alveoli/tubules associated with collagen fibers (Figs. 1a, 1d, and 1f). DOX treatment promoted a visible increase of the elaunin fiber thickness and distribution in the three rat prostatic lobes (Figs. 1b, 1c, 1e, and 1g).

The measurement of Weigert's resorcin–fuchsin-stained areas by stereology showed that VP exhibited higher elastic system fiber volume fraction than dorsal and anterior lobes ($P < 0.05$) and confirmed the observation of increased elastic system fibers volume fraction in the three prostatic lobes after 30 days of DOX treatment, mainly in the VP ($P < 0.01$) (Fig. 2).

Transmission Electron Microscopy

The analysis of the control VP, by transmission electron microscopy (TEM), presented a thin layer of quiescent phenotype fibroblasts and smooth muscle cells that exhibited few protein synthesis-related organelles (Figs. 3a and 3b). Elaunin fibers, which were typically constituted by microfibril groups associated with an irregular elastin deposition and collagen fibrils were sparsely distributed between cells (Fig. 3a and 3b). DOX-treated VP stroma showed gross bundles of collagen fibrils and thickened elaunin fibers distributed among stromal cells (Figs. 3c–3e).

The stroma of control DP presented a dense and compact smooth muscle cell layer intermingled with thin bundles of collagen fibrils and thin elaunin fibers (Fig. 4a). DOX-treated DP presented less compact smooth muscle cells layer, thickened elaunin fibers, and an increased number of collagen fibrils (Figs. 4b and 4c).

The control AP presented a thick and compact smooth muscle cell layer intermingled with thin bundles of collagen fibrils and dispersed thin elaunin fibers

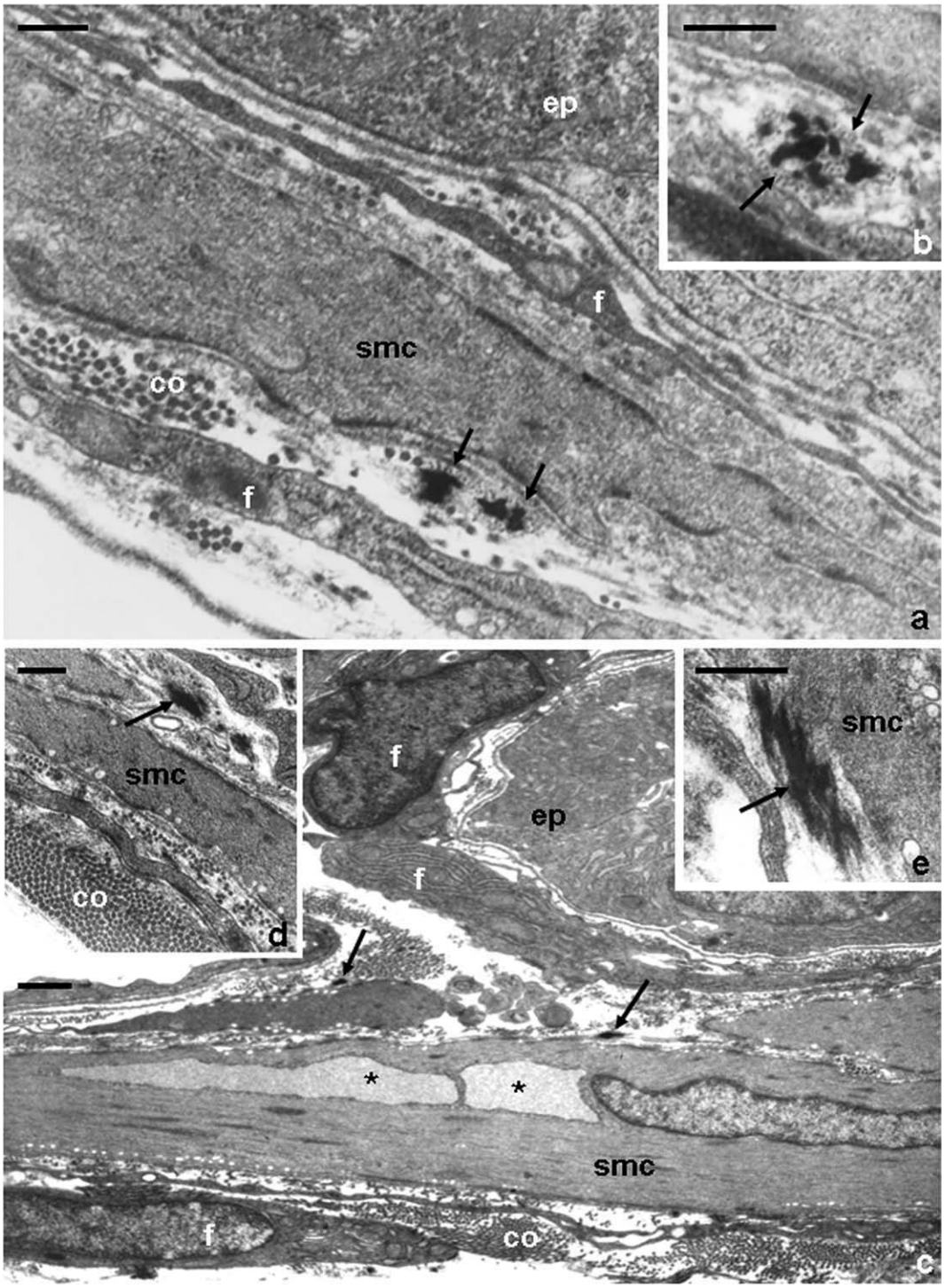

Fig. 3. Transmission electron microscopy of the control (**a** and **b**) and doxazosin-treated ventral prostate (**c**, **d**, and **e**): (a) ventral prostate stroma from control animals presents a thin layer of fibroblasts (f) and smooth muscle cells (smc) that exhibits few synthesis-related organelles. Collagen fibrils (co) are sparsely distributed between cells and elaunin fibers (arrows) are rare. b: Detail of an elaunin fiber, with groups of microfibrils (arrows) associated with an irregular elastin deposition. c, d, e: Doxazosin-treated ventral prostate stroma shows active smooth muscle cells and fibroblasts with visible rough endoplasmatic reticulum cisterns (asterisk). Gross bundles of collagen fibrils (co) and thickened elaunin fibers (arrows) can be observed among stromal cells. Epithelium (ep). Bars = 1 μm.

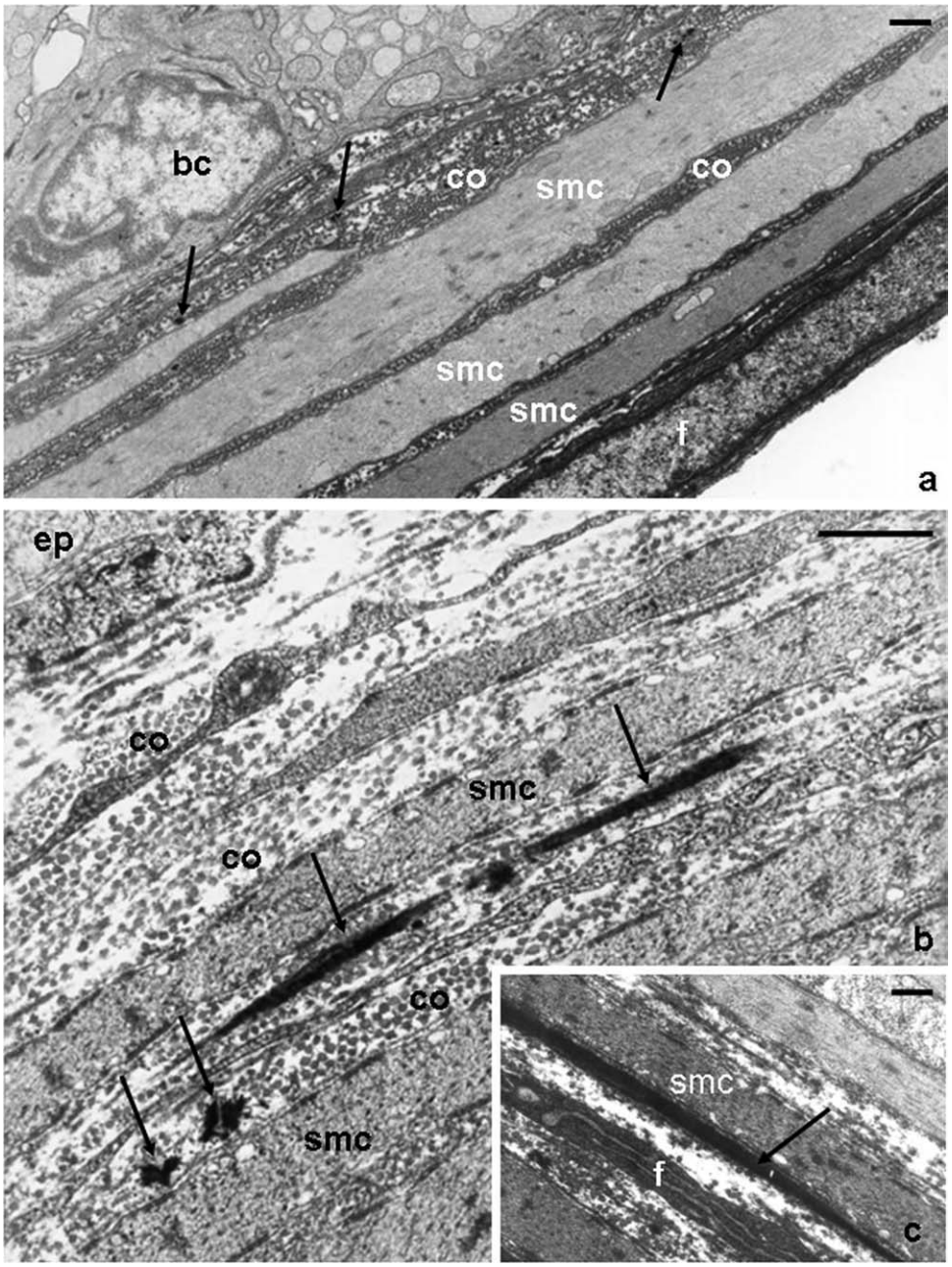

Fig. 4. Transmission electron microscopy of the control (**a**) and doxazosin-treated (**b** and **c**) dorsal prostate: (a) the stroma of control dorsal prostate presents a dense and compact layer of smooth muscle cells (smc) intermingled with thin bundles of collagen fibrils (co) and thin elaunin fibers (arrows). b, c: After doxazosin treatment, the smooth muscle cell layer appears less compact, whereas an increased amount of collagen fibrils (co) and thickened elaunin fibers (arrows) can be observed among these cells. Some fibroblasts (f) show enlarged rough endoplasmatic reticulum cisterns. Epithelium (ep); basal cell (bc). Bars = 1 μm.

(Fig. 5a). After Dox treatment, the distribution of collagen fibril bundles and elaunin fibers increased among stromal cells (Figs. 5b–5d).

The DOX-treated prostates exhibited smooth muscle cells and fibroblasts with active protein synthesis organelles, such as visible rough endoplasmic reticulum cisterns and well-developed Golgi apparatus cisterns (Figs. 3c, 4c, 5b, and 6a–6e).

DISCUSSION

In addition to androgen regulation, normal prostate physiology is maintained by a complex mechanism of epithelium-stroma crosstalk. Changes in the prostate stroma composition can alter epithelial homeostasis, leading to glandular dysfunction with possible emergence of diseases (Chung, 1995; Chung and Davies,

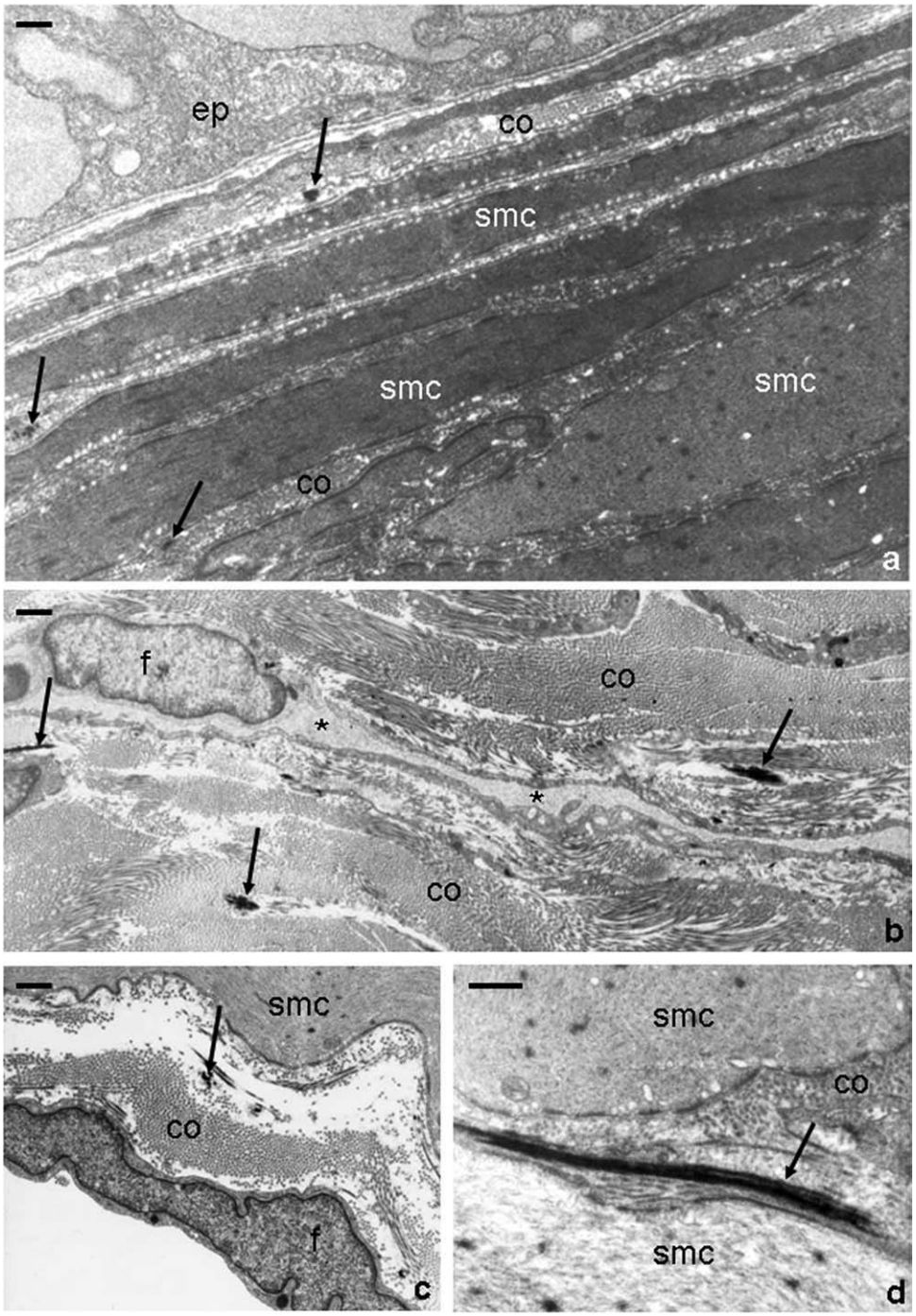

Fig. 5. Transmission electron microscopy of the control (**a**) and doxazosin-treated (**b**, **c**, and **d**) anterior prostate: (a) the stroma of control anterior prostate presents a thick and compact layer of smooth muscle cells (smc) intermingled with thin bundles of collagen fibrils (co) and dispersed thin elaunin fibers (arrows). b, c, d: Dox treatment promoted an increase in the quantity of collagen fibrils bundles (co) and elaunin fibers (arrows). Some fibroblasts (f) show enlarged rough endoplasmatic reticulum cisterns (asterisks). Epithelium (ep). Bars = 1 μm.

1996; Cunha et al., 2004; Wong and Tam, 2002). For example, the localized proliferation of the fibromuscular stroma is accepted to be the first step in BPH development (Pradhan and Chandra, 1975). Furthermore, alterations in the extracellular matrix components—collagen, elastic system fibers, and glycosaminoglycans—have also been observed in BPH (Cardoso et al., 2000; Chagas et al., 2002; Costa et al., 2004; Taboga and Vidal, 2003). However, little attention has been given to the effects of the drugs currently used in BPH treatment on prostate stromal cells and extracellular matrix.

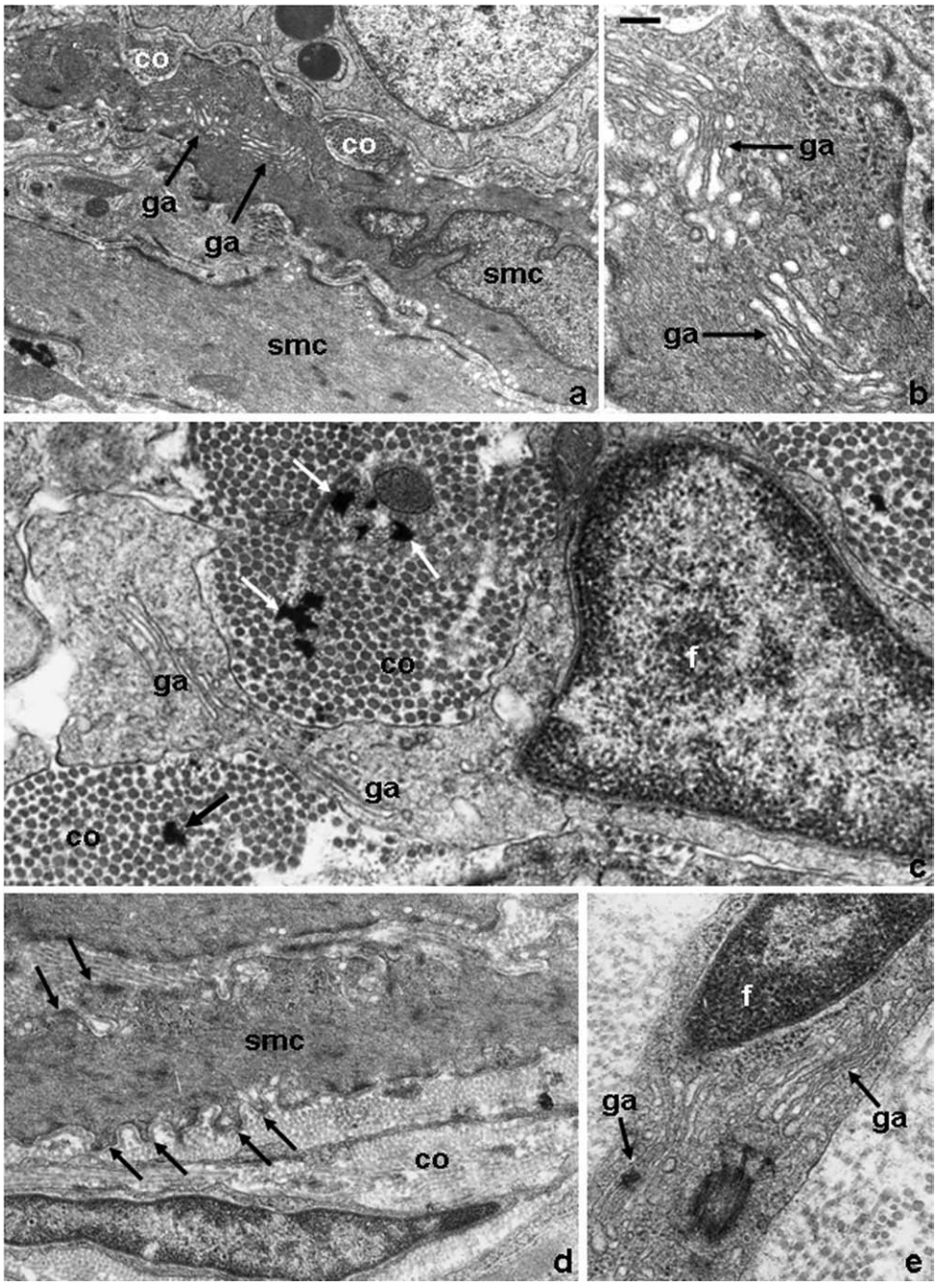

Fig. 6. Transmission electron microscopy of doxazosin-treated prostatic lobes. **a:** Ventral lobe showing smooth muscle cells (smc) with irregular outline associated with bundles of collagen fibrils (co) and well-developed Golgi aparatus (ga). **b:** Detail of (a) showing the Golgi aparatus cysterns and vesicles (ga). **c:** Dorsal lobe showing fibroblast (f) with loose chromatin and evident Golgi apparatus (ga). Note several elaunin fibers (arrows) intermingled with bundles of collagen fibrils (co). **d:** Anterior lobe showing smooth muscle cells with irregular outline (arrows) and associated with bundles of collagen fibrils (co). **e:** Anterior lobe showing a fibroblast (f) with well-developed Golgi apparatus (ga). Bars = 1 μm.

Changes in the prostate extracellular matrix induced by α-1 blockade were recently described. Terazosin treatment for 12 months altered glycosaminoglycan content and the activity of MMP-2, an enzyme involved in the degradation of extracellular matrix components, such as basement membrane, in the rat VP (Mitropoulos et al., 2007).

A previous work by our group demonstrated that DOX treatment increases the collagen fiber volume fraction in the stroma of rat prostatic complex, with a major enlargement of the anterior lobe and smaller augmentations of the ventral and dorsolateral lobes (Justulin et al., 2008). Recently, this effect was also observed in human prostates by Imamura et al. (2009).

In this study, we evaluated, histochemically and ultrastructurally, the changes in the prostate stroma induced by DOX treatment, with special attention to the content and distribution of elastic system fibers. Our results show that 30 days of DOX treatment elevated both the quantity and thickness of elastic system fibers in the three rat prostatic lobes, mainly in the VP. The VP also exhibited the highest values of elastic system volume fraction in both control and treated groups. This aspect may be related with the glandular structural differences found between the prostatic lobes, such as the number of smooth muscle cells layers around the tubules. The anterior lobe presents the thickest layer of smooth muscle cells, followed by DP and VPs (Roy-Burman et al., 2004). In this sense, as the muscular tonus of VP is maintained by a reduced number of smooth muscle cells, this lobe appears to be more affected by the α-adrenergic blockade requiring more intense stromal remodeling and elastic fiber system deposition.

The mechanism responsible for the increased deposition of elastic system fibers during DOX treatment remains to be determined. However, many studies have demonstrated that the apoptotic effect of DOX on prostate cells is mediated through the upregulation of TGF β-1 (Anglin et al., 2002; Glassman et al., 2001; Ilio et al., 2001; Justulin et al., in press; Kyprianou, 2003; Partin et al., 2003). Because TGF β-1 is known as a growth factor that augments the deposition of extracellular matrix proteins (Roberts et al., 1990) including elastin (Davidson et al., 1993; Kothapalli et al., 2009; Shanley et al., 1997), it is possible that this factor participates in the increase of elastic system fiber content observed in DOX-treated prostatic lobes.

Moreover, our ultrastructural analysis reveals that stromal cells from DOX-treated prostate exhibited a more secretory phenotype. This observation corroborates the study by Smith et al. (1999) who, using culture of prostatic smooth muscle cells, found that contractility of smooth muscle cells decreased following DOX treatment. These authors also showed that DOX treatment reduced the expression of actin and myosin proteins, suggesting that α1-blockers induce dedifferentiation of smooth muscle into fibroblasts and myofibroblasts.

Together, these observations suggest that smooth muscle cell relaxation induced by DOX promotes changes in the prostatic tissue biomechanical dynamic. The newly established tissue homeostasis, without the muscular tonus, may require stromal remodeling and reinforcement with consequent increase in deposition of collagen and elastic fibers by fibroblasts and smooth muscle cells.

In conclusion, this work demonstrated that stromal cells and elastic system fibers undergo modifications following DOX treatment and may contribute additional data on the effect of this drug during α-adrenergic blockade therapy.

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