ORIGINAL RESEARCH

Terazosin treatment suppresses basic fibroblast growth factor expression in the rat ventral prostate

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Abstract

Purpose: Alpha1-adrenergic receptor antagonists may not act solely on smooth muscle contractility. We evaluated the in vivo effect of the alpha1 blocker, terazosin, on the expression of basic fibroblast growth factor (bFGF) in the rat ventral prostate.

Methods: Wistar rats were treated with terazosin (1.2 mg/kg body weight, po, every second day) for 120 days. The expression of bFGF was assessed immuno-histochemically in tissue sections and by Western blotting in whole tissue preparations.

Results: Terazosin treatment did not affect prostate weight or histomorphology. In the control group, epithelial and stromal cells demonstrated positive staining for the anti-bFGF antibody. In contrast, the same staining in terazosin-treated specimens was either absent or extremely weak. An analogous difference was observed among the corresponding immunoblots.

Conclusions: These findings implicate the reduction of bFGF expression by terazosin as a potential additional molecular mechanism of its action that may include alterations in peptide growth factor mediated prostate homeostasis.

Drugs that block the action of sympathetic neurotransmitters on $\alpha 1$ -adrenergic receptors ($\alpha 1$ -ADRs) are widely used for the treatment of patients with be-

nign prostate hyperplasia (BPH)-related lower urinary tract symptoms. The rationale for their use stems is that they effectively relax prostate smooth muscles,¹ which represent approximately 40% of the cellular volume in hyperplastic glands.² The biological effects of extrinsic factors such as hormones and other endocrine-related agents on the prostate are mediated by various peptide growth factors produced by the gland and influence prostate growth, differentiation and function by promoting inter- and intracellular signaling between and within cell populations, through paracrine, autocrine and intracrine effects.³

Data from several studies indicate that $\alpha 1$ -ADR antagonists may not act solely on smooth muscle contractility. Intact autonomic innervation of the rat ventral prostate is necessary to maintain its structural and functional integrity.⁴⁻⁶ Administration of the sympathomimetic phenylephrine induces ventral prostate hyperplasia associated with reduced rates of cellular apoptosis, but not with increased rates of cellular proliferation.⁷ Moreover, rapid proliferation of prostatic epithelial cells has been seen in the spontaneously hypertensive rat,⁸ an animal model with in-

creased prostatic norepinephrine levels.⁹ Autonomic innervation is also implicated in the differentiation of stromal cells; norepinephrine stimulation directly modulates BPH-derived prostatic stromal cells towards a differential smooth muscle phenotype,¹⁰ while treatment of cell cultures with doxazosin, an $\alpha 1$ -ADR antagonist, inhibits this procedure.^{11,12} In vivo $\alpha 1$ -ADR blockade in either man or rats result in decreased smooth muscle myosin heavy chain gene expression.¹³ Prostatic cell apoptosis has been identified as an additional mechanism of long term action for doxazosin and terazosin,¹⁴⁻¹⁷ while it has been postulated that the apoptotic effect is probably quinoxaline nucleus directed rather than $\alpha 1$ -ADR mediated.¹⁸ The apoptotic effect of $\alpha 1$ -ADR antagonists has been attributed to transforming growth factor $\beta 1$ (TGF- $\beta 1$) since in vitro treatment of primary human prostate cell cultures with doxazosin¹⁹, as well as in vivo treatment with terazosin²⁰, resulted in enhanced TGF- $\beta 1$ expression. This results in upregulation of p27^{kip-1}, a downstream intracellular effector of TGF- $\beta 1$ apoptotic signaling²¹ and, possibly, activation of the caspase cascade.²²

In a previous study we showed that terazosin may also exert its apoptotic action through a differential effect on the glycosaminoglycans and matrix metalloproteinase 2 of the rat prostate extracellular matrix (ECM).²³ ECM regulates, among others, the trafficking of growth factors.²⁴ This prompted us to explore terazosin's mechanism of action by studying its effect on the expression of basic fibroblast growth factor (bFGF) in stromal cells which are known to be the main promoters of cellular proliferation inducing epithelial growth functioning via enhanced expression of growth factors^{25,26} especially those of the FGF family.²⁷⁻²⁹

Methods

Principles of laboratory animal care were followed and the study complied with European Union regulations for the care and use of laboratory animals. Wis-

tar rats were housed in a climatized environment (temperature 21°C, humidity 55±5%) with 12-hour light/dark cycles. Rats were fed a standard laboratory diet and water ad libidum. Body weight was determined once a week.

Study design

Thirty, 100±5 day-old, male rats were randomly allocated into two groups. One group received terazosin hydrochloride (Abbott Laboratories) (1.2 mg/Kg body weight every second day) dissolved in water for injection. Treatment was given, through a specially modified oesophageal catheter, for 120 days. Control animals received the same amount of distilled water. At the end of the experiment, rats were killed with an overdose of ethyl ether, and the ventral prostate glands were dissected, and weighed. Half of each gland was stored at -70°C and the other half was fixed in 10% buffered formalin. The fixed tissue was embedded in paraffin and serial sections 5 to 6 µm thick were either stained by Harris's hematoxylin-eosin for standard microscopic evaluation or used for immunohistochemical staining.

Western blotting and immunohistochemistry

Tissue samples were weighed, diced into very small pieces and homogenized in NP-40 lysis buffer (50 mM Hepes, 0.05 mM ZnCl₂, 2 mM EDTA, 1% NP40) in the presence of proteinase inhibitors (1 mM PMSF, 0.5 mg/ml leupeptin, 50 mg/ml pepstatin A, 15mg/ml benzamidine, 2 mg/ml aprotinin). The temperature was maintained at 4°C through all the procedures. Samples were sonicated (Soniprep 150, MSE) prior to centrifugation at 5,000 rpm for 30 min at 4°C. The protein concentration of supernatants was determined by the bicinchoninic acid method (Sigma-Aldrich).³⁰ One mg of total protein was immunoprecipitated with 5 µl rabbit polyclonal antibody against bFGF (Santa Cruz Biotechnology, Inc). The mixture was incubated overnight at 4°C on a rotary mixer. Next morning,

TABLE 1. Body weight, (BW), absolute prostate weight (APW), and relative prostate weight (RPW) of terazosin-treated (Group A) and control (Group B) rats (Mean $\pm$ Standard Deviation)

	BW (g)		APW (g)	RPW (mg/g)
	start	end		
Group A	430 $\pm$ 39	459 $\pm$ 42	0.61 $\pm$ 0.11	1.28 $\pm$ 0.29
Group B	418 $\pm$ 19	490 $\pm$ 27	0.52 $\pm$ 0.02	1.07 $\pm$ 0.05

30 μ l of protein A–Sepharose CL-4B beads (Pharmacia) were added to each sample and the mixture was incubated for 3 hr at 4°C on a rotary mixer. Immuno-precipitates were collected by centrifugation at 2,500 rpm for 5 min at 4°C. The pellet was washed three times with 0.5 ml wash buffer for beads (50 mM Hepes, 1% Triton X, 0.1% SDS, 150 mM NaCl, 100 mM NaF, 2 mM NaVO₄, pH 7.4) and resuspended in 40 μ l Laemli buffer. Samples were boiled and loaded on 10% SDS polyacrylamide gel.³¹ Electrophoresis was performed using the method of Laemli,³² following which the gels were equilibrated in Towbin's transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol, pH 8.3).³³ Proteins were transferred to a nitrocellulose membrane (0.45 μ m) (Amersham) by electroblotting at 15 V for 50 min using a BioRad semi-dry system. The membrane was briefly washed twice with TBS (Tris buffered saline, pH 7.5) and blocking of the non specific binding of antibody was performed by incubating the membrane for 1½ hr at room temperature with blocking solution (50 mM Tris, 150 mM NaCl, 0.2% Tween 20 pH 7.4, 5% w/v skim milk powder). The blots were probed with 2 μ g/ml primary antibody solution (as determined by dot blot analysis) by incubating overnight at 4°C. The membrane was then washed four times with TBST (TBS-Tween 20). bFGF protein levels were detected by the alkaline phosphatase-labeled antibody mixture.³⁴ The dot blot analysis used a reaction of rabbit immunoglobulins and incubation with the ready to use substrate according to manufacturer's instructions (Boehringer Mannheim).

Immunohistochemistry for bFGF was performed on formalin-fixed, paraffin-embedded tissue sections. The sections were deparaffinized in xylene and rehy-

drated with graded alcohols. Endogenous peroxidase activity was inactivated by incubating in 3% H₂O₂ for 10 min. Antigen retrieval was performed by immersing the tissue sections in 0.1 M citrate buffer (pH 6.0) and microwaving at 800 W for 15 min. The tissue was then incubated with the primary antibody against bFGF at 1:100 concentration in a humidified chamber at 4°C overnight. After applying the secondary antibody, reactivity was visualized with an avidin-biotin complex peroxidase system (Dako). Non-immune normal IgG at equivalent dilutions to the primary antibodies were used as negative controls.

Results

All rats, treated with either terazosin or sterile water, remained healthy throughout the course of the treatment. All animals showed the expected continuous increase in their body weights as in previous studies.³⁵ At sacrifice body weight, absolute and relative (defined as absolute prostate weight to body weight) and ventral prostate wet weights were not affected by terazosin treatment (Table 1). Moreover, terazosin treatment did not result in any macroscopic or microscopic alterations in prostate morphology.

Western blot analysis of immunoprecipitates from prostate homogenates revealed the presence of a major band of intense bFGF expression. Considerably lower bFGF expression was evident in samples derived from terazosin-treated animals (Figure 1).

FIGURE 1. Representative immunoblots for bFGF in ventral prostate specimens from control (7A,8A,10,11,12,13) and terazosin-treated (25,26,27,30) rats. Lane M is the size marker.

Stromal elements and the majority of prostatic epithelial cells in untreated animals demonstrated positive staining for the anti-bFGF antibody, this being consistent with previous studies.³⁶ In contrast, terazosin treatment resulted in considerable decrease of bFGF expression in both epithelial and stromal cells (Figure 2).

Discussion

The objective of this study was to determine whether terazosin, a commonly used α 1-ADR antagonist, affected expression of bFGF in the rat ventral prostate in vivo. Terazosin treatment (1.2 mg/Kg body weight every second day) was well tolerated and no toxic effects were observed. The terazosin LD₅₀ in rats following oral administration ranges from 5.5 to 6.6 g/Kg, while the "no-toxic-effect" dosage and the maximum-tolerated dose are 60 mg/Kg/day and 150 mg/Kg/day, respectively (Abbott Laboratories, data on file).

Our results showed that systemic terazosin treatment for four months did not affect prostatic growth or morphology. Terazosin administration in humans

results in the same mean change in prostate volume from baseline observed in the placebo group.³⁷ In a mouse model of prostatic hyperplasia, administration of doxazosin in dosages well above those used to achieve complete blockade of α 1-adrenoreceptors in rodents resulted in reduction in the wet weight of prostate reconstitutions with retroviral transduction of TGF β 1.¹⁴ In this model, weight reduction was attributed to increased apoptosis induced by doxazosin treatment. Conversely, exposure of spontaneously hypertensive rats to doxazosin did not result in reduction of the glandular epithelium volume but rather contributed to protecting against caspase-induced apoptosis.³⁸ Both doxazosin and terazosin have proved their in vivo effect on hyperplastic prostate tissue in humans by inducing cell apoptosis with microscopically apparent severe stroma degeneration and collagen accumulation^{15,16} We did not observe any changes in histomorphology, but this may represent a different reaction of normal rat tissue from that of human prostatic hyperplasia. Support for this notion stems from observations in the mouse in which doxazosin treatment induced apoptosis in prostate reconstitutions but did

FIGURE 2. Immunohistochemical expression of bFGF in the prostates from (a) control and (b) terazosin-treated rats. In controls bFGF is mainly expressed in epithelial cells (arrows) and scantily in stromal elements (short arrow). Terazosin treatment resulted in considerable decrease of bFGF expression in both epithelial and stromal cells.

not affect the glandular epithelia of the ventral prostate of the engrafted mice.¹⁴

The FGF family members are mitogenic to both prostatic epithelial and stromal cells.³⁹ bFGF is produced mainly by stromal and, to a lesser extent, by epithelial cells^{36,40} and participates in prostate growth regulation through an autocrine effect on stromal cells and a weak paracrine effect on epithelial cells.⁴¹ Although FGF's role in BPH development is indisputable, it is still unknown at which stage(s) of BPH development bFGF exerts its effects. However, novel reports demonstrated stromal production of bFGF and subsequent induction of angiogenesis in parallel with disease progression.⁴² The present Western blot results revealed considerably lower bFGF expression in samples derived from terazosin-treated animals compared to controls. Similarly, the immunohistochemical study showed that terazosin treatment resulted in decrease of bFGF expression in both epithelial and stromal cells. All FGF family members are protected from degradation by proteases through binding to heparan sulfate.⁴³ In our previous study,²³ we noticed a decrease in the relative content of heparan sulfate following terazosin treatment that could be responsible for excessive degradation of bFGF and could explain its reduced expression.

Although bFGF is not the only mitogenic factor in the prostate gland, its reduced expression could, at least, result in loss of signals contributing in constitutive cellular proliferation. However, the action of quinazoline-derived α 1-ADR antagonists on the human hyperplastic prostate has been reported to be proapoptotic without affecting cell proliferation,¹⁶ and the apoptotic effect has been attributed to enhanced TGF- β 1 expression.^{19,20} Data opposing the activation of the TGF- β transduction pathway being responsible for quinazoline-derived α 1-ADR antagonists-induced apoptosis have been published recently.⁴⁴ Instead, molecular targets consistent with tumour necrosis factor (TNF)- α -related activity were identified.^{44,45} In the mouse prostate reconstituted model, doxazosin in-

duced apoptosis and increased TGF- β protein expression with no effect on cell proliferation.¹⁴ However, in spontaneously hypertensive rats, terazosin suppressed cellular proliferation with prominent effects on prostate histology that were abolished one month following treatment discontinuation.⁴⁶ Finally, our findings support reports of the anti-angiogenic effect of terazosin on the prostate,⁴⁷ since bFGF is a known potent angiogenic factor.⁴⁸

In conclusion, our study suggests that reduction of bFGF expression by terazosin could be one of the underlying molecular mechanisms contributing to the overall action and long-term efficacy of the drug. Furthermore, it provides further insight into the ability of quinazolines to affect prostate homeostasis by interfering with growth factor expression that could be potentially independent of α 1-ADRs. Given the anatomical and functional differences between the human and the rat prostate, further documentation in the human hyperplastic prostate besides the normal, albeit ageing rat ventral prostate is needed.

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