Doxazosin mesylate affects localization of endothelin-1 in prostatic tissues

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KEY WORDS

endothelin-1 ◗ benign prostatic hyperplasia ◗ prostatic epithelium ◗ alpha-adrenergic blockers ◗ doxazosin mesylate

ABSTRACT

Introduction. Data suggest that not only alpha-adrenergic factors contribute to contractility of smooth muscles of the prostate. The authors determined the influence of doxazosin mesylate on localization of endothelin-1 in prostatic tissue in treated and non-treated patients.
Material and methods. Seventy patients with symptoms of prostate enlargement: 40 patients preliminary treated with doxazosin mesylate and 30 non-treated patients. All underwent prostate biopsy due to elevated PSA level (mean 5.85 ng/ml). Specimens were H&E stained for histopathology which confirmed diagnosis of benign hyperplasia and immunohistochemically stained for ET-1 in epithelium and prostate stroma. Authors calculated ET-1 mean optical density ratios (epithelial ET-1/stromal ET-1) for treated and non-treated patients. Statistical analysis was performed. Ratios of ET-1 in epithelium and stroma of both groups differed statistically.
Conclusions. Obtained data indicate that relocation of endothelin-1 from prostate epithelium to stroma is altered in the treated group in comparison with the non-treated group. Doxazosin mesylate might have an influence on ET-1 redistribution from the epithelial compartment to the non-epithelial environment. This could be an additional mechanism of action of alpha-adrenergic blockers in BPH patients.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a chronic disease affecting men over the age of 50. BPH leads to organ enlargement and results in bladder outlet obstruction. Increased prostatic volume is not the only component of obstruction. Voiding disturbances result from two mechanisms – a static one (gland enlargement) and a dynamic one [1]. The dynamic component depends on tonus of smooth muscle fibers in the prostatic stroma and capsule. Increased tonus intensifies urethral narrowing, essentially deteriorates symptoms, and significantly determines outflow resistance [2]. This tonus mainly depends on stimulation of alpha1-adrenoreceptors on smooth muscle cells (SMCs) located predominantly in the prostatic stroma. Estimations indicate that over 90% of alpha1-receptors are located there [3]. Until now, alpha-antagonists served as the most effective drugs in treatment of symptoms related to BPH. This inhibition decreases urethral resistance and may relieve the obstruction and BPH symptoms [1, 4].

There are some newer data that other factors may also contribute to contractility of SMCs. Among others, endothelin-1 (ET-1) exhibits potent activity on smooth muscle contractility [5, 6]. The presence of ET-1 in prostate was reported by several authors [7, 8]. ET-1 was located in prostate epithelium and in small amounts in stroma as well as in the vessel wall [7, 9]. On the other hand, some data indicate an increased ET-1 receptor density in hyperplastic prostate [10].

Endothelins comprise the family of 21-amino acid peptides. Endothelin-1 (ET-1) is the predominant isoform, synthesized mainly by epithelial cells [11, 12]. This peptide could also be synthesized by macrophages, fibroblasts, myofibroblasts, SMCs, and a number of cancer cells types [12, 13]. ET-1 acts in paracrine and autocrine mode and may express multiple functions: vasoconstriction, regulation of renal function, and SMC contractility as well as having an influence on the production of collagen, fibronectin, and other extracellular matrix proteins. Moreover, ET-1 regulates cells growth by stimulating the secretion of cytokines and growth factors, influencing proliferation and adhesion, promoting angiogenesis, embryogenesis, and neurogenesis as well as the inhibition of apoptosis of vascular SMCs, endothelial cells, and fibroblasts [11, 12, 13, 14, 15].

ET-1 is also synthesized by urothelium and detrusor SMCs and controls urinary bladder wall tone. It elicits potent contractile activity in prostate stroma and capsule which is not mediated by the alpha-adrenergic mechanism. Additionally, it is involved in prostate growth through a mitogenic effect on SMCs and fibroblasts [7, 11].

ET-1 is also present in ejaculate [4]. There are data on the correlation between prostate specific antigen (PSA) level and ET-1 [4].

ET-1 has been shown to stimulate contractibility and proliferation of prostatic elements [1, 11]. Moreover, some symptoms of BPH are related to the tension of prostate SMCs and the degree of gland enlargement/hyperplasia. It seems reasonable to study the endothelin system as a possible new target for BPH treatment.

Doxazosin mesylate is a widely used drug in pharmacotherapy of BPH – related obstructive symptoms [16, 17]. It is a quinazoline derivative (1-(4-amino-6,7-dimethoxy-2-quinazolinyl)-4-(1,4-benzodioxan-2-ylcarbonyl) piperazine methanesulfonate) and has properties of selective inhibition of the alpha-1 subtype of alpha adrenergic receptors. This receptor subtype is thought to be the predominant functional type in the prostate. *In vitro* doxazosin antagonizes contractions induced by phenylephrine and binds with high affinity to alpha-1c adrenoreceptors [18]. Besides its antiadrenergic activity, doxazosin has additional positive effects on other symptoms. For example, through its 6- and 7-hydroxyl metabolites it inhibits oxidation of LDL cholesterol and fatty streak formation in vascular walls [19, 20]. In urologic patients doxazosin

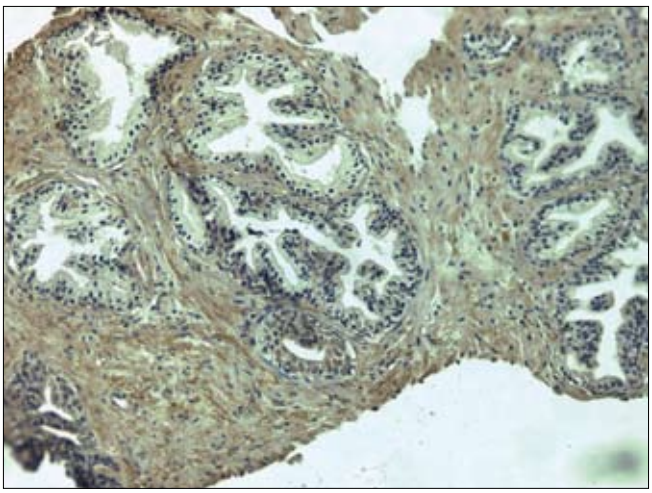

Fig. 1. Example of a prostate tissue section obtained from a non-treated patient. Immunohistochemical staining for ET-1 with additional Mayer's hematoxylin counterstaining. Immunoreactivity for ET-1 is localized mainly in the stromal (fibromuscular) part of prostate tissue. There is also less prominent ET-1 immunoreactivity in the glandular (epithelial) part. Primary objective magnification 40x.

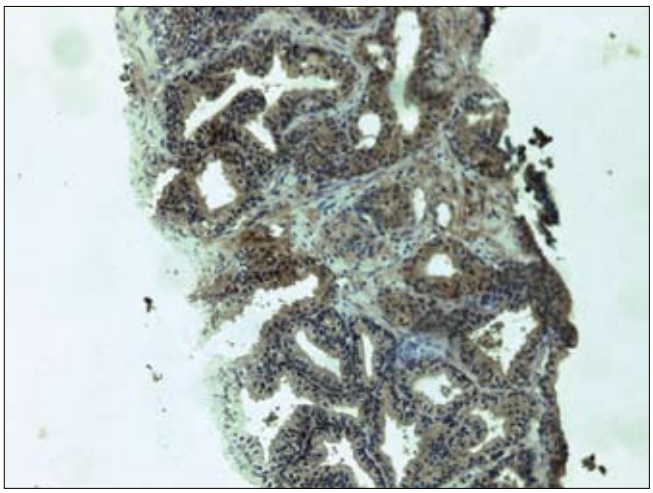

Fig. 2. Example of a prostate tissue section obtained from a doxazosin mesylate-treated patient. Immunohistochemical staining for ET-1 with additional Mayer's hematoxylin counterstaining. Immunoreactivity for ET-1 is localized mainly in the glandular part (epithelial cells) of prostate tissue. Less prominent ET-1 immunoreactivity is visible in the prostate stroma. Primary objective magnification 40x.

mesylate is indicated for the treatment of urinary bladder outlet obstruction and irritative symptoms associated with prostate hyperplasia.

The presented study is focused on determining the presence and distribution of endothelin-1 in prostates of patients receiving an alpha-blocker (doxazosin mesylate) due to bladder outlet obstruction symptoms resulting from BPH in comparison with non-treated BPH patients. Another objective of this study was to reveal the impact of the aforementioned mode of this treatment on tissue ET-1 distribution in order to assess possible alternative mechanisms of doxazosin action on prostatic SMCs contractility. Additionally, the authors were focused on assessing whether and how doxazosin can influence the interaction between epithelium (as its source) and SMCs (as its target/effector) in the ET-1 system in the prostate.

MATERIAL AND METHODS

Source of tissue

Two sets of paraffin embedded tissue specimens were obtained after sextant core prostate biopsy. Biopsies were performed to exclude carcinoma because of minimal coexisting PSA elevation (mean 5.85 ng/ml) or other indications including the results of digital rectal examination. One set of samples consists of tissues from 40 patients treated with doxazosin mesylate (4 mg/day, for at least one month) and the second one consists of samples from 30 patients who were not treated using the aforementioned therapy for obstructive symptoms. From each set of paraffin embedded tissue samples, one slide was stained with hematoxylin and eosin (H&E) for routine diagnostic procedure. Histopathological examination confirmed diagnosis of BPH and excluded the suspicion of prostate carcinoma. An additional slide, 4µm thick, was used for ET-1 immunohistochemical staining.

Immunohistochemistry staining for ET-1

All samples from both groups were used. We applied a standard protocol, briefly as follows: Immunohistochemistry Staining Kit LAB-SA System (Peninsula Lab. Inc. Cal., USA) utilizing biotin-streptavidin-horseradish peroxidase methodology was used for the immunohistochemistry procedure. The kit exhibits cross-reactivity with other peptides as follows: human ET-2 (91%), human ET-3 (0.05%), and human big ET (76%). Tissue sections, 4 µm thick, were

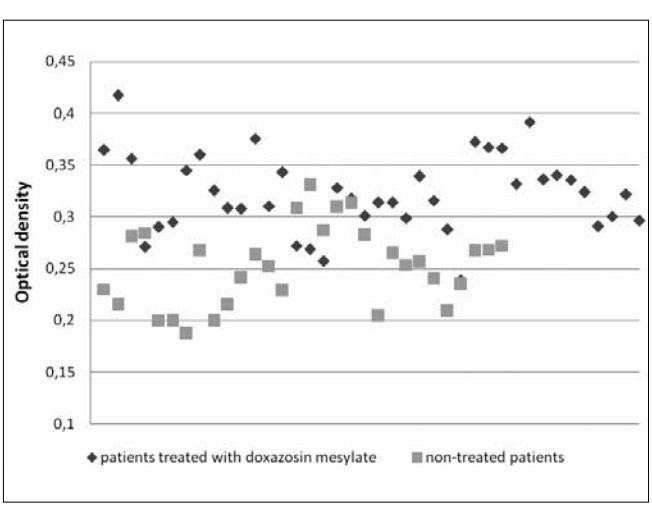

Fig. 3. Epithelial ET-1 mean optical density estimated for 30 non-treated patients and 40 treated patient. Each dot/element on the graph corresponds to results from one patient.

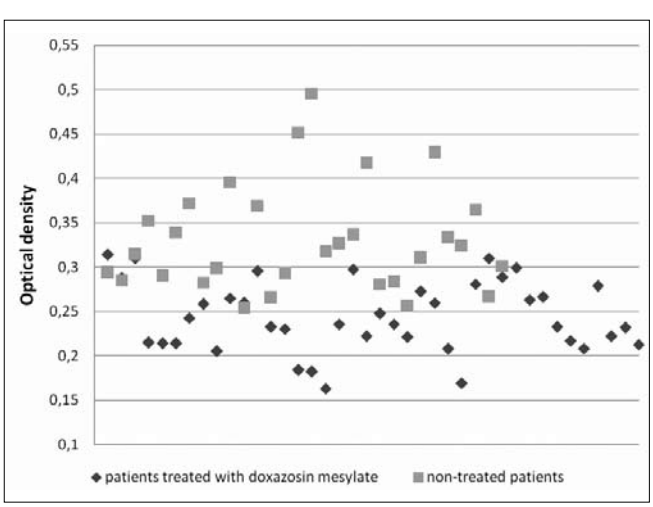

Fig. 4. Stromal ET-1 mean optical density estimated for 30 non-treated patients and 40 treated patients. Each square or rhombus corresponds to results of analysis from one patient.

deparaffinized with xylene and rehydrated in graded ethanol series and then pretreated with 3% H_2O_2 for quenching of endogenous peroxidase activity. Glass mounted tissue slides were rinsed three times with PBS and incubated for 20 minutes with normal goat serum solution to block nonspecific immunoglobulin binding. Each slide was then treated with primary rabbit anti-ET-1 antibody solution (60 min. in room temperature). After 3 washings in PBS, samples were incubated with biotinylated secondary goat anti-rabbit IgG antibody (60 min.). After careful washing with PBS, streptavidin-horseradish peroxidase conjugate was added to each sample and incubated for 30 minutes. Next, the slides were washed again 3 times with PBS. The reaction was developed with 100 µL of diaminobenzidine tetrahydrochloride (DAB) for 3 min. Finally, tissues were counterstained with Mayer's hematoxylin and mounted with coverglasses. Negative controls were carried out with normal rabbit serum.

Analysis of ET-1 immunohistochemistry staining patterns

Samples of examined prostatic tissue sections stained for ET-1 were studied using the Nikon Eclipse E800 microscope with built-in digital camera and Laboratory Universal Computer Image Analysis system (LUCIA DI).

Each histological image had a carefully selected area of interest (AOI) comprising of glandular and stromal elements. Each slide contained between 1 and 6 AOIs (mean 2.4 AOIs per slide) depending on the tissue sample size. Selected AOIs were captured, digitally converted, and stored as a digital picture readable by the LUCIA computer image analysis system. For final analysis we used 80 and 60 digital pictures from treated and non-treated patients, respectively.

From each digital picture, glandular and stromal elements were selected independently and the ET-1 staining patterns of their histological elements were evaluated. The image analysis software counted mean optical density of ET-1 immunostaining (ET1-MOD) in studied prostates for the epithelial and stromal compartment separately. The mean optical density value for stromal and epithelial components was estimated for each patient using an average value from separately evaluated regions.

To assess the differences of ET-1 expression in epithelium (eET1-MOD) and stroma (sET1-MOD) we determined eET1-MOD to sET1-MOD ratio for treated and non-treated patients.

Statistical analysis

For statistics, Statistica 5.1 (StatSoft Inc.) software was used. The Shapiro-Wilk W-test was performed to determine statistical data distribution. A T-test for independent samples with Levene's test for homogeneity of variances was performed for normally distributed data. Distribution of variables among treated and non-treated group of samples were compared using Mann-Whitney U-test for continuous variables (for samples with non-parametric distribution). A value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Products of immunoreactions with ET-1 were visualized in prostate epithelium and stroma (Figs. 1 and 2). In different cases the expression of ET-1 was variable. Using morphometric techniques and software, we found that epithelial ET-1 mean optical density for doxazosin mesylate treated patients was 0.32 (SD 0.037) and 0.25 (SD 0.038) for non-treated patients. Stromal ET-1 mean optical density was 0.24 (SD 0.039) and 0.32 (SD 0.06) for treated and non-treated patients, respectively. A detailed distribution of values for each patient is presented in figures 3 and 4.

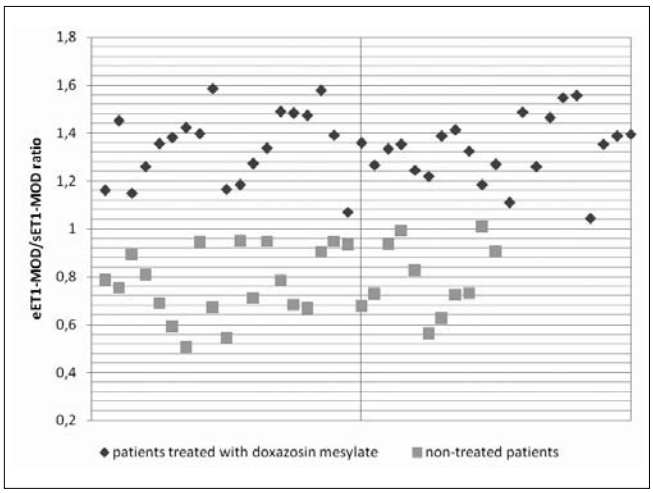

Fig. 5. Epithelial ET-1 mean optical density to stromal mean optical density ratio (eET1-MOD/sET1-MOD ratio) estimated for 30 non-treated patients and 40 treated patients.

The epithelial to stromal ET-1 mean optical density ratio (eET1-MOD/sET1-MOD ratio) for non-treated patients was 0.781 (SD 0.144) while for treated patients, the eET1-MOD/sET1-MOD ratio was 1.339 (SD 0.139).

Each patient's related data is presented in figure 5.

After statistical data analysis we found that all obtained data had normal distribution. The distribution of differences in ratios of eET1-MOD/sET1-MOD in doxazosin mesylate treated and non-treated patients was statistically significant ($p < 0.001$).

DISCUSSION

Symptoms of BPH are related to prostate enlargement and partially depend on the tonus of smooth muscle cells (SMCs) located within the stroma and its capsule. BPH, recognized as an organ enlargement, is caused by an increase in the number of glands and their size and an increase in the number of stromal elements such as SMCs, fibroblasts, and connective tissue fibers as well as proliferation of epithelial cells [1, 2, 3]. Proliferation of stromal cells is driven by various stimuli such as ET-1, a factor that acts through its mitogenic effect on target cells [11, 21]. As it was suggested in previous publications [22], this effect could be related to direct or indirect mechanisms. It was postulated that indirect effects are associated with ET-1-dependent stimulation of production and release of many additional growth factors such as: hepatocyte growth factor (HGF), epidermal growth factor (EGF), VEGF, PGE_2, IL-6 but not IL-1β, and TNF-α. There is also a possible correlation of ET-1 and the increased release of NO [4, 21, 22], but according to some published data such processes at least partially depend on "atypical" β-adrenoreceptors, namely β_3-adrenoreceptor [23]. Moreover, ET-1 increases the production of collagen, fibronectin, and other extracellular matrix proteins [24]. It also inhibits apoptosis of SMCs and fibroblasts and stimulates secretion of other cytokines and growth factors by stromal cells [11, 12, 13, 14, 15, 21, 22].

In addition to the proliferation of stromal and epithelial cells, we can expect that BPH related symptoms of bladder outlet obstruction (due to clinical significance recognized as aggravating effect) are related to the strong contractile response of SMCs to ET-1 [11]. Some authors regard endothelins, together with catecholamines, as the only mediators significantly contracting prostate tissue [25]. It was previously published that SMCs in various locations - including prostate - might activate and contract after ET-1-related stimulation [11, 21, 22, 26]. Recently, it was published that the action of

ET-1 on SMCs is related to increased levels of Ca^{2+} in the cytosol and nucleus. This process was related to ET-1-induced inositol triphosphate (IP_3) production, which in turn causes the release of $Ca2^{+}$ from a nucleoplasmatic reticulum [27]. Moreover, as it was already postulated, ET-1 might also play a role in mitogenesis and proliferation of SMCs [26].

Nowadays, doxazosin mesylate and other alpha-antagonists are very popular and effective drugs for the treatment of obstructive symptoms related to BPH. This is due to the relaxing activity of the aforementioned therapeutics on SMCs of the prostate [16, 17]. Therefore, in our opinion it is reasonable to investigate whether such drugs have any effect on ET-1 activity in prostate tissue and to consider endothelin-1 as a possible target in the treatment of BPH.

In human prostate, the main source of ET-1 is epithelial cell and its production depends on the level of cell activity [7, 8, 24, 26]. In this location, ET-1 is produced, stored, and released on demand as suggested by several previous papers [26]. However, it could be released from other cells, such as smooth muscle cells and stromal vascular endothelial cells [26, 28]. The stromal compartment is thought to be the main target for ET-1 action in the prostate [7, 8]. The differences of eET1-MOD/sET1-MOD ratios found in this study in both groups (treated and non-treated) indicate a greater ET-1 "efflux" from the epithelial to the stromal compartment of the prostate in the non-treated group than in the group treated with doxazosin mesylate.

CONCLUSIONS

Although this study does not reveal the pathways by which doxazosin mesylate can exert the before mentioned action, it is evident that alpha-adrenoreceptor blocking agents have an influence on ET-1 redistribution from epithelial elements to the surrounding environment. This finding can be related to the presence of doxazosin mesylate, but it is difficult to explain its mechanism of action in this phenomenon. One should keep in mind that ET-1 could act in a paracrine and autocrine manner by various cells [29]. As previously published, the source of ET-1 can be smooth muscle cells, endothelial cells, or epithelial cells and that the receptors for ET-1 (namely ET_A and ET_B) were found within the stroma on smooth muscle cells, fibroblasts, and myofibroblasts [21, 22, 24]. The data obtained in our study demonstrates that doxazosin mesylate affects ET-1 relocation (outflow) from the glandular part of the prostate (source) to the prostatic stroma (target). It could also serve as good background for the introduction of new therapies for BPH, considering that ET-1 and its receptors were suspected as playing a role in the development of prostate hyperplasia leading to the increased synthesis of ET-1 [26].

Because ET-1 is responsible for adrenoreceptor independent contractility of SMCs, we present the finding that doxazosin mesylate can alter endothelin-1 tissue distribution and, therefore, can be an additional mechanism of action of alpha-blockers in abolishing symptoms of bladder outlet obstruction in BPH patients.

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