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ANDROLOGY

Original Article

Effect of sildenafil citrate in testosterone induced benign prostate hyperplasia rat model

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ABSTRACT

Objective: Efficacy of treatments for benign prostate hyperplasia (BPH) is limited because the disease has complex etiopathogenesis. Recent studies have demonstrated the presence of phosphodiesterase-5 (PDE-5) receptors in prostate tissue. We investigated efficacy of sildenafil citrate in testosteron - induced BPH in rats.

Material and methods: The rats were divided into three groups. Each groups had 7 rats. Group 1 was control group. Testosteron propionate 3 mg/kg/day was injected subcutaneously for two weeks in Group 2. The same procedure was done for Group 3 and sildenafil citrate was added to water at daily doses of 2 mg/kg for two weeks. The rats were euthanized with intraperitoneal pentobarbital. The body weights were measured and the prostates were removed.

Results: The mean weights of rats were 288±31.93, 345±23.23 and 294±32.86 g in Groups 1, 2 and 3, respectively. The mean prostate weights of rats were 0.74±0.18, 1.3±0.13 and 0.72±0.24 g in Groups 1, 2, and 3, respectively. Group 2 had statistically significantly higher prostate weights than the other groups ($p<0.01$). Relative prostate weight is calculated with ratio of prostate weight to body weight. BPH group showed an increase in relative prostate weight compared with other groups with significant difference ($p=0.036$ and $p=0.040$). There was statistical difference for acinar area between Group 2 and the others, no significant difference of number of acini, interstitial space and epithelial thickness. Group 2 has more papillary projections per acini than the other groups.

Conclusion: Favourable effect of sildenafil citrate on dimensions of prostate but not all on histological parameters was observed. We expect that PDE-5 inhibitors might be a treatment option for BPH patients if the studies support our findings in the future.

Keywords: Hyperplasia; prostate; sildenafil citrate.

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Introduction

Prostate is a major accessory gland in the male reproductive system. Prostate cancer and benign prostate hyperplasia (BPH) are the most common proliferative disorders that affect elderly men.[1] BPH is an age-related disorder that consists nonmalignant enlargement of the prostate and results in unregulated growth of the prostate.[2] BPH may cause sepsis, renal failure, irreversible bladder damage and death in some cases. The etiopathologic mechanism of BPH has not been clearly understood. This mechanism is under hormonal control and involves changing in balance between androgens and estrogens. Most of the investigators think that androgens have an important role in the development and growth of the prostate.[3] Dihydrotestosterone (DHT) is converted from testosterone by 5-alpha-reductase activity and play criticial role for prostate growth. The increasing level of DHT with aging induces hyperplasia of the prostate. The other factor that influences the BPH progression is inflammation of the prostate.[4] Most of the patients (79%) with BPH have also chronic prostatic

inflammation. Inflammation associated-cytokines stimulate the cyclooxygenase-2 enzyme that increases proliferative rate and inhibits the cell death. Bcl-2 is an anti-apoptotic protein; up-regulation of bcl-2 protein and cyclooxygenase-2 decreases the apoptotic rate of the prostatic tissue.[2] Current guidelines do not recommend phytotherapeutic agents, because lack of long term studies and their unclear modes of action. Medical therapy is the first line treatment for symptomatic patients. Alpha adrenergic blockers and 5-alpha-reductase inhibitors are frequently used treatment agents. The phosphodiesterase-5 (PDE-5) inhibitors are used in recent years. PDE-5 inhibitors dose dependently decrease the contractions of prostate and bladder.[5] Mechanism of PDE-5 is supposedly mediated via cyclic guanosine monophosphate (cGMP), smooth muscle relaxation in response to nitric oxide. Bladder, urethra and prostate tissues have highest PDE-5 mRNA expressions in rat urinary tracts.[6] We evaluated the histologic effects of sildenafil citrate in testosterone induced benign prostate hyperplasia in rats.

Material and methods

This study was approved by the Ethics Committee in Marmara University Animal Experimentation (protocol number: 33.2011). The animals were handled in accordance with the guidelines of the National Institute of Health for the care and use of laboratory animals.

Sixteen week-old male Wistar rats were used in this study. The animals were housed in plastic cages (3 or 4 rats per cage). The rats were kept in the same room at a constant temperature of 22±2°C, 12 hour light/dark cycles under standard diet and drinking water. The rats were divided into three groups of seven rats each. Group 1 was control group which received standard diet and water. Group 2 was BPH group. In Group 2, testosterone propionate (3 mg/kg/day) was injected subcutaneously for 14 days at the inguinal region. Group 3 received sildenafil citrate (2 mg/kg/day) orally and testosterone propionate (3 mg/kg/day) subcutaneously for 14 days at the inguinal region. Twenty-four hours after the final treatment, weights of the animals' were determined and euthanized with an intraperitoneal pentobarbital administration. Prostate tissues of each groups were excised and weighted by an electronic scale. Prostate weight/ body weight of rats were calculated, and expressed as relative prostate weight.

Prostates of all groups were removed, and fixed in 10% neutral buffered formalin (NBF) for 24 hours and then washed under tap water for 2 hours. Thereafter, the prostates were dehydrated with subsequent 70%, 90%, 96% and 100% ethanol and cleared with toluene. After overnight incubation of paraffin in a 60°C incubator, prostates were embedded and blocked in paraffin at room temperature. Five μm paraffin sections were cut from these blocks and deparaffinized with toluene and rehydrated with ethanol series and stained with hematoxylin and eosin (H&E) for microscopic examination and histopathological analysis. The histological examination was performed according to the study of Fornari et al.[7]. The analysis consisted of viewing three fields of vision under 100 magnifications per slide and the number of acini, acinar area of the five most central acini, and interstitial area per field and the number of papillary projections per acini were determined. In addition, 400 magnifications were used for the purpose of measuring the epithelial thickness of the five most central acini.

Statistical analysis

Data were analysed with ANOVA for statistical significance. The level of $p<0.05$ was considered as statistically significant.

Results

The rats weighed between 270 and 370 gr. The mean weights of the rats in Groups 1,2, and 3 were 288±31.93, 345±23.23 and 294±32.86 g, respectively. Prostate weighed 0.74±0.18, 1.3±0.13 and 0.72±0.24 g in Groups 1,2, and 3. Group 2 had statistically significantly higher prostate weights than the other groups ($p<0.01$. BPH group showed an increase in relative prostate weight compared with other groups. Relative prostat weights were nearly similar in Groups 3, and 1, but lower than Group 2 (Table 1).

Normal acini, papillary projections, epithelial height and and thickness of epithelium are seen in Figure 1-3. Results of each group were expressed in Table 2. Acinar area was significantly higher in group 2 than the others. Group 2 presented less number of acini when compared with other groups. Interstitial space, thickness of the epithelium, and papillary projections/ acini were more numerous in Group 2 relative to Group 3.

Discussion

Benign prostate hyperplasia is histologically diagnosed approximetely in 80% of men over 80 years of age.[7] At least, half of these men are symptomatic and compromising their quality of life. Lower urinary tract symptoms (LUTSs) are a group of symptoms classified as storage, voiding and postvoiding symptoms.[8] LUTSs are seen in 30% of the men over the age of 65 years. Erectile dysfunction (ED) is another common disorder and is often seen with BPH and LUTS.[5] Mulhall et al.[9] reported that PDE-5 inhibitors improve not only symptoms of ED but also LUTS with relaxation of lower urinary tract tissues.

The phosphodiesterase-5 inhibitors metabolize the second messengers like cAMP and cGMP.[10] The 21 human PDEs genes are divided into 11 different classes according to their protein sequences, catalytic and regulatory considerations

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Table 1. Body weights, prostate weights and relative prostate weights

	Group 1	Group 2	Group 3	p	
BW (g)	288±31.93	345±23.23	294±32.86	0.017*	0.032#
PPW (g)	0.74±0.18	1.36±0.13	0.72±0.24	0.01*	0.01#
PW/ BW	0.0025±0.0004	0.0039±0.0006	0.0025±0.00125	0.036*	0.040#

*for Group 2 and 1, #for Group 2 and 3. BW: body weight; PW: prostate weight

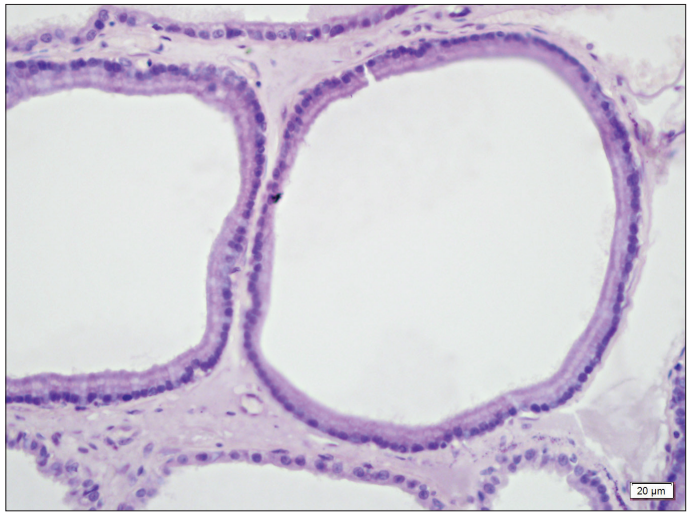

Figure 1. Normal acini was seen in Group 1, x40 magnification with H&E

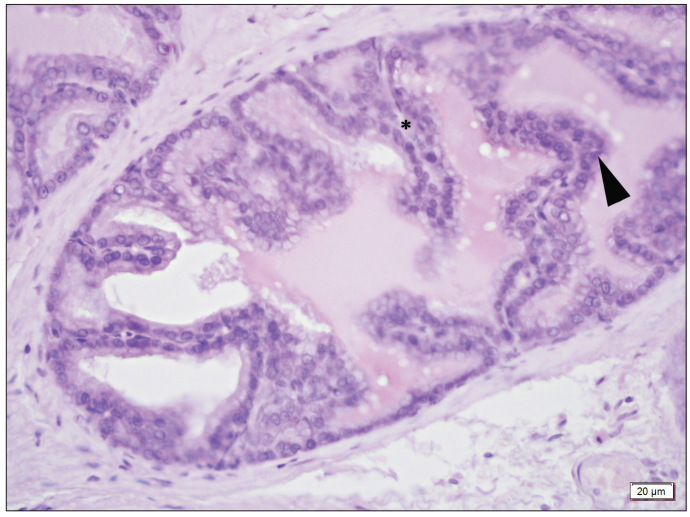

Figure 2. Multiple papillary projections in acini (*) and thickened epithelium (arrow) in Group 2, x100 magnification with H&E

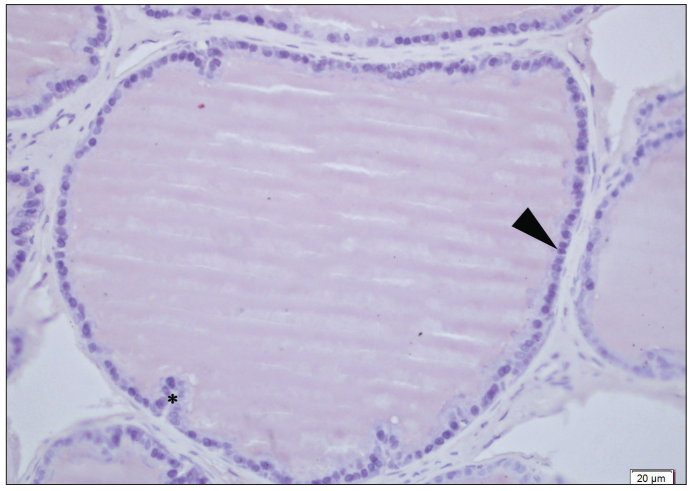

Figure 3. Fewer number of papillary projections (*) and normal epithelial height in Group 3 (arrow) x40 magnification with H&E

and sensitivity to the inhibitors (cAMP and cGMP affinity). Fifteen different PDEs have been demonstrated with molecular biology, biochemical approaches and inmunohistochemical studies in the human prostate.[11] Expression of PDE is especially seen in the transition zone of the prostate. Presence of PDE-5 and -11 in glandular and stromal subglandular areas of human prostate was demonstrated by Uckert et al.[12] Fibri et al.[13] demonstrated expression of PDE-5 in vascular smooth muscle, endothelial cells in prostate and showed the enzyme to be localized in smooth muscle of the prostatic urethra. NO is an important inhibitory neurotransmitter in smooth muscle of the urethra and increase the level of cGMP that exerts a relaxant effect. [14,15]

Cyclic adenosine monophosphate is more important than cGMP for detrusor function, hence the cAMP pathway in the bladder has been more thoroughly investigated than cGMP. Five isoforms of PDEs (1-5) were isolated and demonstrated in human and porcine detrusor in functional studies.[16,17] The effect of vardenafil on cyclic nucleotide levels and bladder contraction was investigated in rat detrusor by Werkstrom et al.[18] Relexation was accompanied with increased levels of both cAMP and cGMP, and it was proposed that vardenafil- induced relaxation of rat detrusor was mediated by cAMP through inhibition of cGMP dependent cAMP-PDE. Relaxation of bladder neck was achieved with high levels of sildenafil in contracted human bladder neck by phenylephrine which suggests that PDE inhibitors may have potential effect to relax the bladder outlet.[19]

Table 2. Histomorphometric evaluation of the prostate in each group

	Acinar area (μm^2)	Number of acini per field	Interstitial space (μm^2)	Epithelial thickness (μm)	Number of papillary projections per acini
Group 1	29859.80±15426.52	10±3.09	0.0025±0.00044	9.36±1.56	1.38
Group 2	60909.11±13758.77*	7.94±1.37	0.0039±0.00065	12.04±1.49	1.89
Group 3	38213.26±8042.77	9.06±1.23	0.0025±0.0012	11.83±2.36	1.18

*p<0.05 Groups 1,2, and 3

The studies demonstrated the presence of PDE-5 in several parts of lower urinary tract vasculature.[13,20] Expression of PDE-5 was shown in the endothelial cell and vascular smooth muscles of the human prostatic urethra, prostate and bladder neck in immunohistochemical studies.

Growth of prostate is a hormonal process regulated by androgens and estrogens.[21] It is known that estradiol acts synergistically with androgens to cause overgrowth of the prostate.[2] Etiopathological mechanism consists of control of endocrine processes and alterations in the balance between androgens and estrogens. Testosterone injection increases the prostate weights in rats.[11] Animals in Group 2 showed significantly higher prostate weight than the other groups. It is thought that sildenafil citrate could affect the prostate tissue in Group 3, because of smaller prostates in this group when compared with Group 2. Relative prostate weight can be calculated with ratio of prostate weight to body weight and it is an important indicator for BPH. This parameter was significantly smaller in sildenafil group than in BPH group.

Fornari et al.[7] demonstrated that acinar area was smaller in finasteride and finasteride +doxazosin groups than doxazosin group. Number of acini per field was significantly greater in finasteride +doxazosin group than other groups. Histologically, significant enlargement of the acini was also reported in glandular hyperplasia in rats.[22] We determined significantly greater acinar area in Group 2 than the other groups. Acinar area of sildenafil citrate group was less than BPH group without any significant difference with control group. Number of acini per field was greater in sildenafil citrate group.

Epithelial height is an indirect sign of secretory activity of the prostate gland.[23] Epithelial height is higher in testosterone or estradiol injected rats. Ejike et al.[2] reported that massive epithelial thickening was seen in DHT and estradiol injected rats. In another study, authors reported that, epithelial height was significantly reduced in finasteride group when compared with the control group.[7] Our study showed that epithelial height was less in sildenafil group than BPH group without significant intergroup difference. Papillary projections are usually seen in rats with testosterone- induced BPH.[24] The authors showed lesser number of papillary projections, and acini in the finasteride group than control and doxazosin groups.[5] We determined less number of papillary projections per acini in sildenafil citrate group than the others without significant intergroup difference.

Experimental studies with rat prostate tissue have a few limitations including difference of prostate tissue between human beings and rats, difficulty in evaluating symptoms and urine flow. Finally testosterone-induced BPH models in rats do not appear to fully mimic the epithelial and stromal changes in human prostate hyperplasia tissue. Consequently, sildenafil citrate was effective in some of the parameters with significant difference. We think that, if further animal studies support our findings, PDE-5 inhibitors may be the first line treatment in BPH.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Marmara University (Protocol number: 33.2011).

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References

1. Delella FK, Felisbino SL. Doxazosin Treatment Alters Stromal Cell Behavior and Increases Elastic System Fibers Deposition in Rat Prostate. Microsc Res Tech 2010;73:1036-44. [CrossRef]
2. Ejike CECC, Ezeanyika LUS. Inhibition of the Experimental Induction of Benign Prostate Hyperplasia: A Possible Role for Fluted Pumpkin(Telfairia occidentalis Hook f.) Seeds. Urol Int 2011;87:218-24.
3. Shin IS, Lee MY, Jung DY, Seo CS, Ha HK, Shin HK. Ursolic acid reduces prostate size and dihydrotestosterone level in a rat model of benign prostate hyperplasia. Food Chem Toxicol 2012;50:884-8. [CrossRef]
4. Sarbishegi M, Khajavi O, Arab MR. Withania coagulans Extract Induces Cell Apoptosis and Inhibits COX-2 Expression in

Turk J Urol 2017; 43(4): 434-8
DOI:10.5152/tud.2017.23356

a Rat Model of Benign Prostatic Hyperplasia. Nephrourol Mon 2016;5:e39284.

5. Lee JG, Moon DG, Kang SH, Cho DY, Park HS, Bae JH. Relaxation Effect of Phosphodiesterase-5 Inhibitor on the Animal Bladder and Prostatic Urethra: in vitro and in vivo Study. Urol Int 2010;84:231-5. [CrossRef]
6. Tinel H, Stelte-Ludwig B, Hutter J, Sandner P. Preclinical evidence for the use of phosphodiesterase-5 inhibtors for treating benign prostatic hyperplasia and lower urinary tract symptoms. BJU Int 2006;98:1259-63. [CrossRef]
7. Fornari A, Rhoden EL, Zettler CG, Ribeiro EP,Rhoden CR. Effects of the chronic use of finasteride and doxazosin mesylate on the histomorphometric characteristics of the prostate: experimental study in rats. Int Urol Nephrol 2011;43:39-45. [CrossRef]
8. Drake MJ, Bowditch S, Arbe E, Hakimi Z, Guelfucci F, Amri I, et al. A retrospective study of treatment persistence and adherence to α-blocker plus antimuscarinic combination therapies, in men with LUTS/BPH in the Netherlands. BMC Urol 2017;36:1-12. [CrossRef]
9. Mulhall JP, Guhring P, Parker M, Hoops C. Assessment of the impact of sildenafil citrate on lower urinary tract symptoms in men with erectile dysfunction. J Sex Med 2006;3:662-7. [CrossRef]
10. Andersson KE, de Groat WC, McVary KT, Lue TF, Maggi M, Roehrborn CG, et al. Tadalafil for the treatment of lower urinary tract symptoms secondary to benign prostatic hyperplasia: pathophysiology and mechanism(s) of action. Neurourol Urodyn 2011;30:292-301. [CrossRef]
11. Uckert S, Kuthe A, Jonas U, Stief CG. Characterization and functional relevance of cyclic nucleotide phosphodiesterase isoenzymes of the human prostate. J Urol 2001;166:2484-90. [CrossRef]
12. Uckert S, Oelke M, Stief CG, Andersson KE, Jonas U, Hedlund P. Immunohistochemical distribution of cAMP- and cGMP-phosphodiesterase (PDE) isoenzymes in the human prostate. Eur Urol 2006;49:740-5. [CrossRef]
13. Fibbi B, Morelli A, Vignozzi L, Filippi S, Chavalmane A, De Vita G, et al. Characterization of phosphodiesterase type 5 expression and functional activity in the human male lower urinary tract. J Sex Med 2010;7:59-69. [CrossRef]
14. Werkstrom V, Svensson A, Andersson KE, Hedlund P. Phosphodiesterase 5 in the female pig and human urethra: Morphological and functional aspects. BJU Int 2006;98:414-23. [CrossRef]
15. Andersson KE, Garcia Pascual A, Persson K, Forman A, Tøttrup A. Electrically-induced, nerve mediated relaxation of rabbit urethra involves nitric oxide. J Urol 1992;147:253-9. [CrossRef]
16. Truss MC, Stief CG, Uckert S, Becker AJ, Schultheiss D, Machtens S, et al. Initial clinical experience with the selective phosphodiesterase-I isoenzyme inhibitor vinpocetine in the treatment of urge incontinence and low compliance bladder. World J Urol 2000;18:439-43. [CrossRef]
17. Truss MC, Stief CG, Uckert S, Becker AJ, Wefer J, Schultheiss D, et al. Phosphodiesterase 1 inhibition in the treatment of lower urinary tract dysfunction: From bench to bedside. World J Urol 2001;19:344-50. [CrossRef]
18. Werkstrom V, Hedlund P, Lee T, Andersson KE. Vardenafil-induced relaxation and cyclic nucleotide levels in normal and obstructed rat urinary bladder. BJU Int 2009;104:1740-5. [CrossRef]
19. Bittencourt JA, Tano T, Gajar SA, Resende AC, de Lemos Neto M, Damião R, et al. Relaxant effects of sildenafil on the human isolated bladder neck. Urology 2009;73:427-30. [CrossRef]
20. Filippi S, Morelli A, Sandner P, Fibbi B, Mancina R, Marini M, et al. Characterization and functional role of androgen-dependent PDE5 activity in the bladder. Endocrinology 2007;148:1019-29. [CrossRef]
21. Gonzales C,Revilla JL, Rubio J, Gasco M, Gonzales GF. Effect of red maca(Lepidium meyenii) on prostate zinc levels in rats with testosterone induced prostatic hyperplasia. Andrologia 2012;44:362-9. [CrossRef]
22. Saha I,Chatterjee A, Mondal A, Maiti BRM, Chatterji U. Arecoline augments cellular proliferation in the prostate gland of male Wistar rats. Toxicol Appl Pharmacol 2011;255:160-8. [CrossRef]
23. Riberio DL, Pinto ME, Maeda SY, Taboga SR, Goes RM. High fat induced obesity associated with insulin resistance increases FGF-2 content and causes stromal hyperplasia in rat ventral prostate. Cell Tissue Res 2012;349:577-88. [CrossRef]
24. Ploumidou K, Kyroudi-Voulgari A, Perea D, Anastasiou I, Mitropoulos D. Effect of a Hypercholesterolemic Diet on Serum Lipid Profile, Plasma Sex Steroid Levels, and Prostate Structure in Rats. Urology 2010;76:515-9. [CrossRef]