



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

Benign prostat hiperplazisi oluşturulan ratlarda sildenafil sitratın etkinliği

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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 testosterone - induced BPH in rats.

Material and methods: The rats were divided into three groups. Each groups had 7 rats. Group 1 was control group. Testosterone 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.

ÖZ

Amaç: Benign prostat hiperplazisi (BPH) etyopatogenezinin kompleks olması nedeniyle tedavilerdeki etkinlik sınırlı olmaktadır. Son yıllarda çalışmalarda prostat dokusunda fosfodiesteraz tip 5 (PDE-5) reseptörleri gösterilmiştir. Çalışmamızda testosteron ile indüklenen BPH oluşturulmuş ratlarda sildenafil sitratın etkinliğini araştırdık.

Gereç ve yöntemler: Yirmi bir adet rat 3 gruba ayrıldı. Birinci grup kontrol grubuydu. İkinci grup 2 hafta boyunca testosteron verilerek deneysel BPH oluşturulan gruptu. Üçüncü gruba ise ikinci grup gibi BPH oluşturuldu ve beslenme sıvılarına sildenafil sitrat eklendi. Ratlara intraperitoneal 100 mg/kg pentobarbital ile ötenazi uygulandı. Vücut ağırlıkları ölçüldü ve prostat dokusu çıkartıldı.

Bulgular: Ratların ağırlıkları gruplara göre sırasıyla 288±31.93, 345±23.23 ve 294±32.86 gr idi. Prostat ağırlıkları sırasıyla 0,74±0,18, 1,3±0,13 ve 0,72±0,24 gr olarak bulundu. Grup 2 ile diğerleri arasında istatistiksel anlamlı fark bulundu (p<0.01) Prostat ağırlığı/vücut ağırlığında Grup 1 ve 3 ile Grup 2 arasında istatistiksel anlamlılık vardı (p=0,017 ve 0,032). Asinüs alanı incelemesinde Grup 2 ile diğerleri arasında fark varken, asinüs sayısı ve interstisyel alanlar arasından fark saptanmadı. Epitel yüksekliğinin değerlendirilmesinde gruplar arasında anlamlı fark saptanmadı.

Sonuç: İncelediğimiz tüm histolojik parametrelere istatistiksel olarak yansınasa da sildenafil sitratın prostat boyutlarına olumlu etkisi gözlemlendi. İleride yapılacak çalışmalarda bu etkinin desteklenmesi ile BPH tedavisinde PDE-5 inhibitörlerinin yerini alabilmesi beklenmektedir.

Anahtar Kelimeler: Hiperplazi; prostat; sildenafil sitrat.

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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- α -reductase activity and play critical 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; upregulation 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- α -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 $22\pm 2^{\circ}\text{C}$, 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 prostate weights were nearly similar in Groups 3, and 1, but lower than Group 2 (Table 1).

Normal acini, papillary projections, epithelial height 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.

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

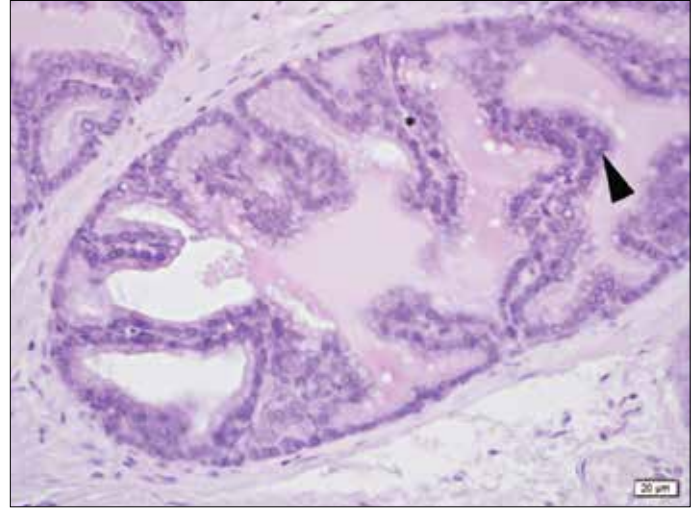


Figure 2. Multiple papillary projections in acini (*) and thickened epithelium (arrow) in Group 2, x100 magnification with H&E

Discussion

Benign prostate hyperplasia is histologically diagnosed approximately 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 (LUTS) are a group of symptoms classified as storage, voiding and postvoiding symptoms.^[8] LUTS 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 and sensitivity to the inhibitors (cAMP and cGMP affinity). Fifteen different PDEs have been demonstrated with molecular biology, biochemical approaches and immunohistochemical 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

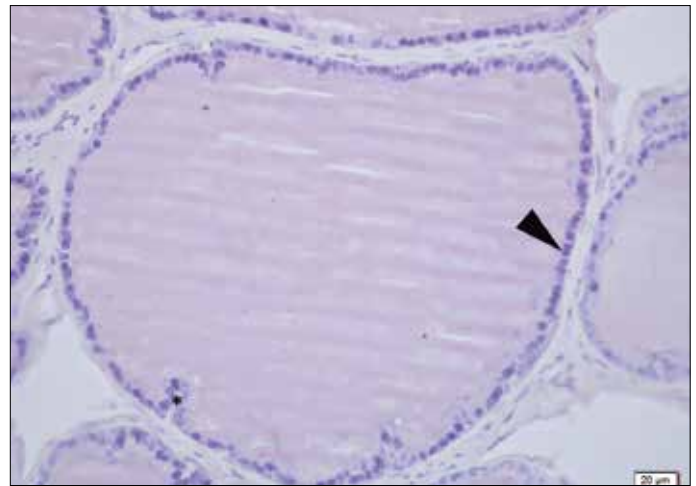


Figure 3. Fewer number of papillary projections (*) and normal epithelial height in Group 3 (arrow) x40 magnification with H&E

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

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

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] Relaxation 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]

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 hyper-

plasia 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.

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