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ORIGINAL ARTICLE / ÖZGÜN MAKALE

THE BENEFICIAL EFFECT OF INTRACAVERNOSAL INJECTION OF SILDENAFIL ON ERECTILE DYSFUNCTION IN DUTASTERIDE TREATED RATS

İNTRAKAVERNOSAL SİLDENAFİLİN SIÇANLARDA DUTASTERID TEDAVİSİ SONRASI GELİŞEN EREKTIL DİSFONKSİYON ÜZERİNE YARARLI ETKİSİ

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ABSTRACT

Objective: *Benign prostatic hyperplasia (BPH) and erectile dysfunction (ED) are the most common illnesses in aged male patients. 5α-reductase inhibitors (5-ARIs) are suggested for the treatment of BPH. Furthermore, the association of 5ARIs with ED has been indicated. This study aimed to investigate the effect of intracavernosal injection of sildenafil on ED in 5ARI treated rats.*

Material and Method: *Sprague-Dawley rats (n=30) were divided into three groups: Control; 10-week dutasteride treatment (0.5 mg/rat/day); and 6-week durasteride treatment followed by a 4-week washout period. In vivo erectile responses were assessed before and after intracavernosal injection of sildenafil (0.3mg/kg/rat). The relaxant and contractile responses of isolated corpus cavernosum were evaluated in in vitro organ bath.*

Result and Discussion: *Prostate weight decreased after 10-week dutasteride treatment. In vivo erectile responses, endothelial and nitrergic relaxation responses were decreased in dutasteride groups. The washout period moderately normalized erectile responses. The intracavernosal injection of sildenafil increased erectile function in treatment groups. Contractile responses were augmented in 10-week dutasteride treated rats. The cessation of the treatment did not alter erectile function as well as endothelial relaxation and nitrergic relaxation. Also, intracavernosal sildenafil caused an improvement in 5ARI treatment-induced ED.*

Keywords: *5-alpha reductase inhibitor, corpus cavernosum, dutasteride, erectile dysfunction, sildenafil.*

ÖZ

Amaç: *Benign prostat hiperplazisi (BPH) ve erektil disfonksiyon (ED), yaşlı erkek hastalarda en sık görülen hastalıklardır. BPH tedavisi için 5α-redüktaz inhibitörleri (5-ARI) önerilmektedir. Ayrıca, 5ARI'lerin*

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ED ile ilişkisi belirtilmiştir.Bu çalışmada, 5ARI tedavili sıçanlarda gelişen ED üzerine intrakavernozal sildenafil enjeksiyonunun etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntem: *Sprague-Dawley sıçanlar (n=30) üç gruba ayrılmıştır: Kontrol; 10 haftalık dutasterid tedavili (0.5mg/kg/gün); 6 haftalık dutasterid tedavili ve 4 hafta tedavisiz. İn vivo erektil yanıtlar, sildenafilin intrakavernozal enjeksiyonundan (0.3 mg/kg) önce ve sonra değerlendirilmiştir. İzole korpus kavernozum dokularının gevşeme ve kasılma yanıtları in vitro olarak organ banyosunda değerlendirildi.*

Sonuç ve Tartışma: *10 haftalık dutasterid tedavisinden sonra prostat ağırlığı azalmıştır. Dutasterid gruplarında in vivo erektil yanıtlar, endotelyal ve nitrerjik gevşeme yanıtları azalmıştır. Tedavinin kesilmesi azalan erektil yanıtları kısmen geri döndürmüştür. Sildenafilin intrakavernozal enjeksiyonu, tedavi gruplarında erektil yanıtları artırmıştır. 10 hafta dutasterid ile tedavi edilen sıçanlarda kontraktil yanıtlar artmıştır. Tedavinin kesilmesi, erektil fonksiyonun yanı sıra endotelyal ve nitrerjik gevşemeyi iyileştirmemiştir. Ayrıca, intrakavernozal sildenafil, 5ARI tedavisinin neden olduğu ED'de bir iyileşmeye neden olmuştur.*

Anahtar Kelimeler: *5-alfa redüktaz inhibitörü, dutasterid, erektil disfonksiyon, korpus kavernozum, sildenafil.*

INTRODUCTION

Benign prostatic hyperplasia (BPH), one of the most mutual conditions in aged men, is likely to induce lower urinary tract symptoms (LUTS) which is linked to a decrement in the quality of life [1, 2]. In addition, the prevalence of BPH rises with age that affects 75% of men over 50 years of age [2, 3].

5α-reductase inhibitors (5-ARIs), α1-adrenoceptor antagonists and phosphodiesterase-5 inhibitors (PDE5Is) are suggested for the treatment of BPH/LUTS [4]. Prostate growth is induced by dihydrotestosterone (DHT), which is converted from testosterone via 5-AR [5]. Dutasteride, a 5-ARI, is frequently used as a treatment option for BPH [6]. Dutasteride blocks 5-AR enzyme resulting in a decrement in serum, intraprostatic concentration of DHT and prostate volume [6-8]. Previous data have confirmed that 5-ARIs induce side effects on sexual function, especially erectile dysfunction (ED) [9, 10]. Furthermore, in preclinical data, dutasteride treatment induced ED in rats, even after withdrawal of dutasteride [11, 12]. In addition, PDE5Is are the preferred choice, even though there are conflicting results [13-15]. Concomitant treatment with 5ARI and PDE5Is prevented ED [14, 16]. Sildenafil is a highly selective PDE5Is and the most used drug for ED [17, 18]. On the other hand, a rat model of BPH treated with sildenafil displayed a decrease in corporal smooth muscle content compared to the BPH group [13].

To the best of our knowledge, studies assessing the acute effect of sildenafil on 5ARI-induced ED are lacking. The aim of this study was to examine the effect of intracavernosal sildenafil injection on ED after 6-week and 10-week dutasteride treatment, as well as to evaluate *in vitro* relaxant and contractile responses in the corpus cavernosum (CC).

MATERIAL AND METHOD

Drugs

All drugs were purchased from Sigma Chemical Co (St. Luis, MO) except dutasteride (Avodart,

GlaxoSmithKline) and sildenafil tablets (Viagra, Pfizer).

Animals

Adult male Sprague-Dawley rats (n=30, ten weeks old, 295.9±5.8 g) were purchased from Bilkent University (Ankara, Turkey). Rats were randomly divided into three groups; control (n=10), 10 weeks of dutasteride treatment (n=10), and 6 weeks of dutasteride treatment followed by 4 weeks of washout (n=10). As in previous studies [11, 12, 19], dutasteride was administered in drinking water (0.5 mg/rat/day). Dutasteride treatment was performed during 6 and 8 weeks in a previous study [19]. However, the alterations of erectile responses were partially restored in the rats treated with dutasteride after 2 weeks of washout period [19]. Therefore, we performed 4 weeks of washout period instead of 2 weeks.

All experimental procedure was approved by Ankara University Local Ethics Committee of Animal Experiments (approval no: 2015–16-185). The rats were housed individually in artificially lit rooms (from 7:00 a.m. to 7:00 p.m.) with food and water ad libitum under controlled temperature (22 ± 1 °C).

The body weights of all rats were calculated via a precision scale before the sacrifice of animals. The penis and prostate tissues were excised and weighted by an electronic scale after sacrifice.

***In vivo* erectile function measurement**

Erectile responses were measured by cavernosal nerve (CN) stimulation and monitoring electric stimulation-induced intracavernous pressure (ICP, mmHg) and main arterial pressure (MAP, mmHg). After ten weeks, in anesthetized rats (ketamine/xylazine; 100/10 mg/kg, i.p.) [20], polyethylene-50 tubing was inserted into the right crura of the penis and the carotid artery to measure ICP and MAP with a transducer (Statham, Oxnard, CA, USA) and a data acquisition system (Biopac MP 100 System, Santa Barbara, CA, USA). Following the detection of cavernosal nerve (CN), the CN was stimulated (2.5, 5, and 7.5 V, 15 Hz, 1 ms pulse width) with a stainless-steel bipolar-hook stimulating electrode and a square pulse stimulator (Grass Instruments, Quincy, MA) [12]. After intracavernosal injection of sildenafil (0.3 mg/kg) [21-23], the measurements were repeated in dutasteride-treated animals. Sildenafil was administered via intracavernosal injection to eliminate the blood pressure-lowering effect [23, 24].

Organ Bath Experiments

Organ bath experiments were performed to measure the relaxation and contractile responses in isolated CC. Following *in vivo* experiments, the rats were killed under anesthesia (ketamine/xylazine; 100/10 mg/kg, i.p.) [20], and CC tissues were isolated for organ bath experiments. After the isolation, the CC (1 × 1 × 8 mm) was placed under a resting tension (1g) within organ bath system containing Krebs-Ringer bicarbonate solution (containing mmol/L: NaCl 118.1, $CaCl_2$ 2.5, KCl 4.7, $NaHCO_3$ 25.0, $MgSO_4$ 1.0, KH_2PO_4 1.0, glucose 11.1) at 7.4 pH and 37 °C with 95%O_2/5%CO_2. The isolated strips

were attached to platinum electrodes (Grass Instruments, Quincy, MA, USA). Electrical field stimulation (EFS) of the autonomic nerves (15 seconds duration; amplitude 40-90 V; frequency 1-40 Hz; pulse width 5 ms) was executed with platinum electrodes (Grass Instruments, Quincy, MA). All tension alterations were noted via an isometric force transducer attached to a PC-based data acquisition system (Biopac System, St. Barbara, CA, USA). After 60 minutes, endothelium-dependent relaxant response to acetylcholine (ACh, 10^{-8}-10^{-3} M), nitrergic relaxant response to EFS (1- 20 Hz), endothelium-independent relaxant response to sodium nitroprusside (SNP, 10^{-8}-10^{-3} M) and a PDE5I, sildenafil (10^{-8}-10^{-4} M)-caused relaxant responses were obtained following precontraction of CC strips with phenylephrine (Phe, 10μM). Alpha- adrenergic agonist, Phe (10^{-8} to 10^{-3}M), EFS-induced neurogenic contraction (1–40 Hz), and KCl (60 mM)-caused contractile responses in CC strips were performed and standardized to tissue weight grams [20, 25].

Data analysis

The findings were analyzed by Prism v.4 (GraphPad Software, San Diego, CA, USA) and shown as mean± standard error of the mean (SEM). Multiple groups were compared via one-way analysis of variance (ANOVA) with post hoc Bonferroni analysis. A value of $p<0.05$ was considered to be statistically significant.

RESULT AND DISCUSSION

The weight of body and penis did not change in groups (Table 1). Furthermore, the weight of prostate in 10-week treated rats was lower ($p<0.001$) than in controls, which was partially returned after the discontinuation ($p<0.05$ vs. controls, Table 1). Similarly, previous studies have shown a decrease in prostate weight [26] as well as no alteration in body and penis weights [19].

Table 1. Body, penis and prostate weights of the control, 10-week and 6-week dutasteride groups

	Control	**Dutasteride-10 week**	**Dutasteride-6 week+4 week w/o**
Body weight (g)	418.3±13.6	401.2±10.9	414.1±7.9
Penis weight (g)	0.39±0.04	0.34±0.02	0.38±0.02
Prostate weight (g)	1.0±0.1	0.5±0.1 ***	0.8±0.1*

Values are the mean ± SEM from per group (n=10). *p < 0.05, ***p < 0.001 vs control.

Rats in the 10-week dutasteride group displayed reduced ICP/MAP ($p<0.001$ at 7.5V) and ICP ($p<0.01$ at 7.5V) values compared to controls (Figure 1A and 1B). In addition, the decrease in ICP/MAP and ICP was partially returned after the washout period, but there was no significant difference between the 10-week and 6-week dutasteride groups (Figure 1A and 1B). Likewise, earlier data demonstrated that 4 and 8 weeks of

dutasteride treatment induced a decline in erectile response, and also, the washout period did not alter the erectile response [11]. Furthermore, after intracavernosal injection of sildenafil, an increase in erectile responses was noted in all dutasteride treated groups (Figure 1A and 1B). PDE5Is have been involved in European Association of Urology (EUA) guidelines for the treatment for LUTS. The underlying mechanism of PDE5I on LUTS stays unclear, and also, it is likely to be dependent on increasing nitric oxide/intracellular cyclic guanosine monophosphate resulting in decreasing muscle tone of the detrusor, urethra and prostate as well as enhancing oxygenation and blood perfusion in the lower urinary tract [27]. Furthermore, PDE5I has good clinical outcomes for 5ARIs-linked ED [14, 16]. Moreover, Munk et al. indicated that the efficacy of the combined treatment of PDE5I plus 5-ARIs is unclear and needs further research [28]. However, other data reported PDE5Is were not sufficient to treat ED in hypogonadal men [29]. Testosterone is a critical component for maintaining erectile function. Indeed, castration caused ED and diminished the effectiveness of PDE5I in rabbits [30]. Also, hypertension-induced BPH and dutasteride treatment induced detrimental morphological changes in rat penis, which was not improved by coadministration of sildenafil [13]. Unlike the castrate model or different BPH models, dutasteride treatment may conserve both testosterone and DHT which can preserve the expression of PDE5 and the activity of PDE5Is.

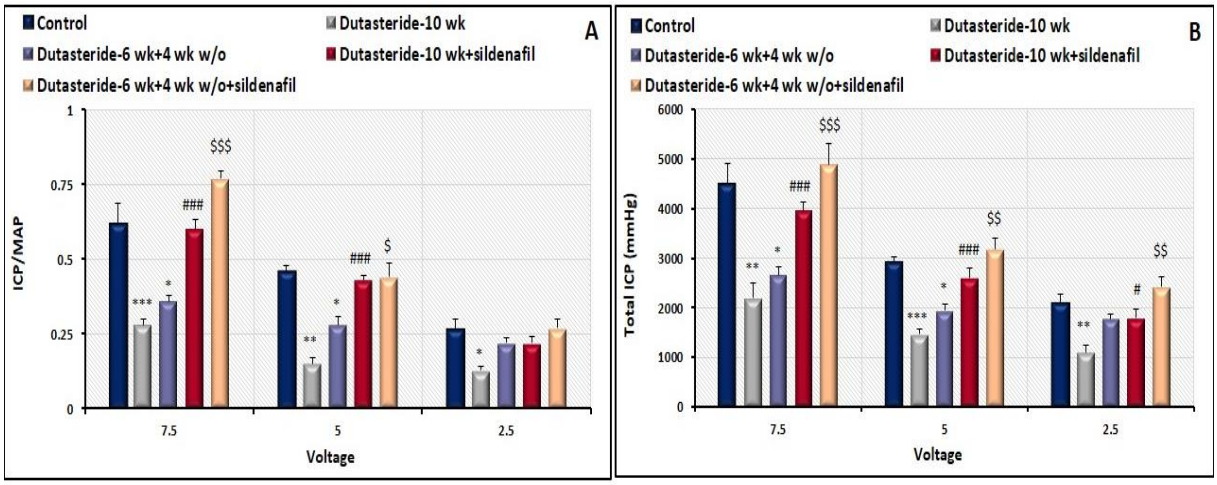

Figure 1. The effect of sildenafil on *in vivo* erectile function in all groups. Bar graphs are presenting ICP/MAP (A) and total ICP (B). Data are shown as the mean ± SEM (n=8-10). *p<0.05, **p<0.01, ***p<0.001 vs. control; # p<0.05, ###p<0.001 vs. 10-week dutasterid treated group; $ p<0.05, $$ p<0.01, $$$ p<0.001 vs. 6-week dutasterid treated group.

ACh-induced endothelium-dependent relaxation responses in both dutasteride treated rats were lower than in controls (p<0.001, Figure 2A). A previous study showed ACh-induced dose response curve shifted right without an alteration in the maximum response after 4-week dutasteride treatment [31]. In addition, the relaxation response to ACh was lowered in the 8-week dutasteride and 2-week

washout groups [19]. Furthermore, the endothelium-dependent relaxation responses were lower after both 8-week and 12-week dutasteride treatment [12]. According to the results, the reduction in the endothelium-dependent relaxation response is likely to be associated with a decreased in free and total T levels following 5-ARIs treatment [12].

EFS-caused relaxant response was considerably decreased in 10-week and 6-week dutasteride treatment groups ($p<0.001$ vs. controls), except 1 and 5V (Figure 2B). Similarly, earlier data indicated the reduction in both EFS-induced relaxation response and normal penile erection after 5ARI treatment [31]. Moreover, Oztekin et al. [19] showed that the neurogenic relaxant responses were decreased after dutasteride treatment.

The endothelium-independent relaxant response to SNP did not change in all groups (Figure 2C). Pinsky et al. [31] displayed no differences in SNP-induced relaxant responses between control and 4-week dutasteride treated rats. However, another study showed that relaxation responses to SNP were decreased after 12-week dutasteride treatment [12]. This difference was most probably due to the duration of dutasteride treatment.

Sildenafil-induced relaxation response did not change (Figure 2D). A previous study also indicated no differences in sildenafil dose-response curve between control and 4-week dutasteride treated groups [31]. Nevertheless, sildenafil-induced relaxation responses at 1 and 10 μM dosages in the 12-week dutasteride treated group was reduced compared to controls without alteration in the maximum relaxation response [12].

Phe-induced contractile responses at 100 μM and 1 mM were increased in 10-week dutasteride-treated rats (Figure 3A, $p<0.01$ vs. control). EFS-induced neurogenic contractile responses in 10-week dutasteride-treated rats were considerably increased at 15-40 Hz compared to controls ($p<0.05$, Figure 3B). Also, the increment in contractile responses was decreased after the washout period (Figure 3A-B). KCl-induced contractile responses were not different among groups (Figure 3C). Similarly, 4-week and 12-week dutasteride treatment regimens augmented α1-adrenergic agonist and neurogenic-induced contractile responses [12, 31]. Moreover, Phe-caused contraction in castrated rats was greater than in controls [32]. The current results can indicate that 5ARI treatment increased vasoconstrictor responses in the penile tissue resulting in ED.

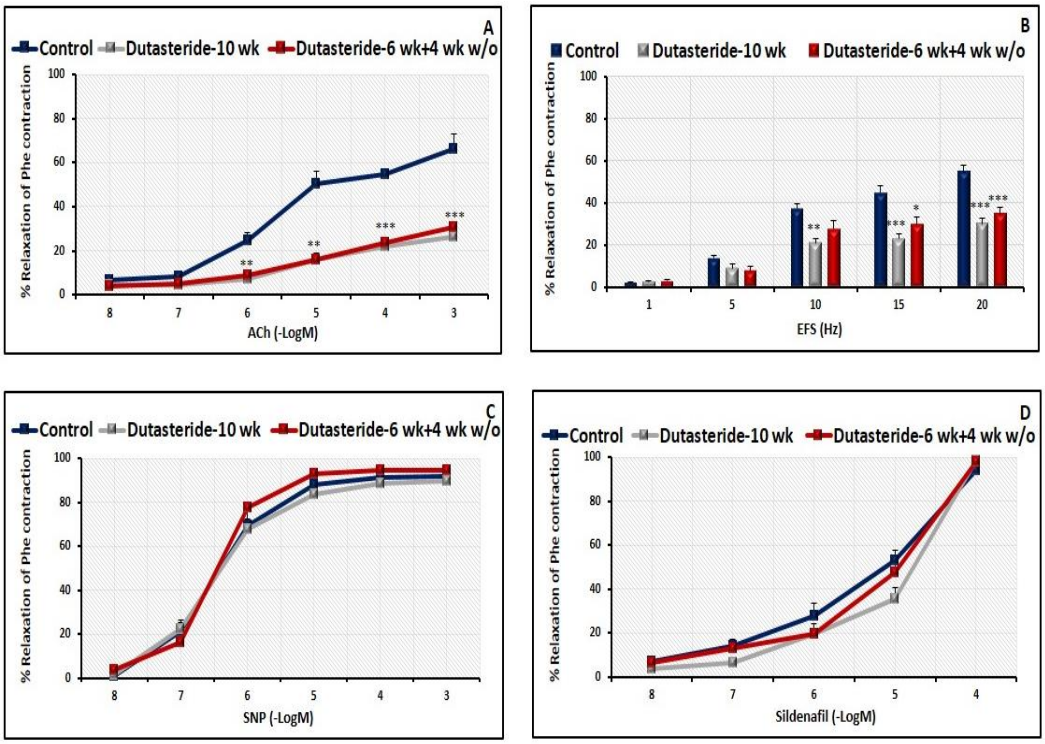

Figure 2. Dose-response curves in the isolated cavernosal strips to ACh (10^{-8}–10^{-3} M, A), EFS (1–20 Hz frequency, B), SNP (10^{-8}–10^{-3} M, C), sildenafil (10^{-8}–10^{-4} M, D)-caused relaxation responses. Data are mean ± SEM (n = 8-10). *p < 0.05, **p < 0.01, ***p < 0.001 vs. control group (ANOVA, Bonferroni post hoc).

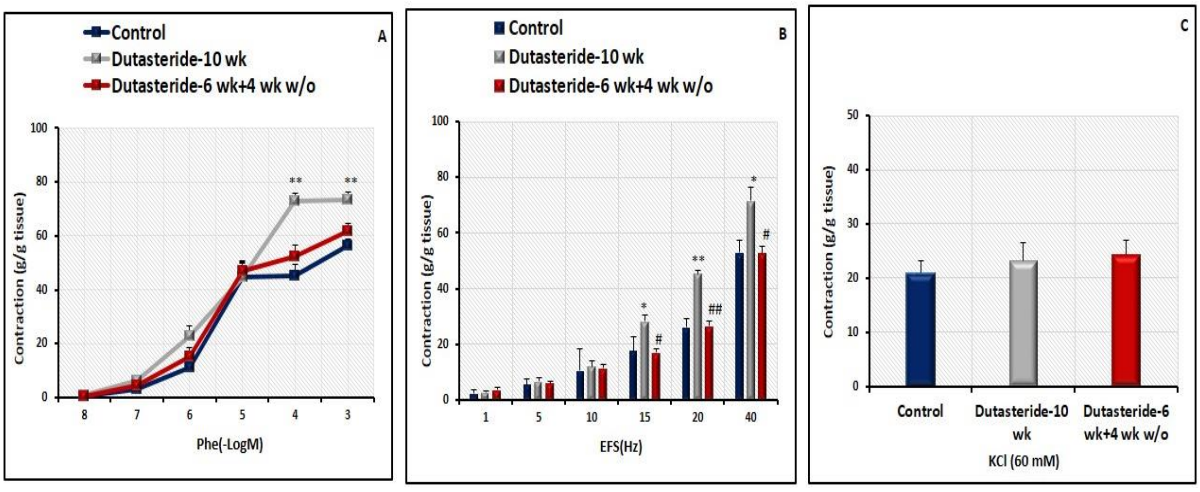

Figure 3. Dose-response curves in the isolated cavernosal strips to Phe (10^{-8}–10^{-3} M, A), EFS (1–40 Hz frequency, B) and KCl (60mM, C)-induced contractile responses. Data are mean ± SEM (n = 8-10). *p < 0.05, **p < 0.01 vs. control group; # p<0.05, ##p<0.01 vs. 10-week dutasterid treated group (ANOVA, Bonferroni post hoc).

In conclusion, the current study showed that treatment with dutasteride caused decreasing endothelial and nitrergic relaxant responses and increasing contractile responses resulting in ED in rats. The alterations in erectile function and relaxation responses except contractile responses were not ameliorated after the cessation. The cavernous injection of sildenafil improved erectile function, and induced the relaxation of the penile tissue in dutasteride treated rats. Further preclinical and clinical studies are needed to expand the knowledge of the combination treatment of sildenafil and 5ARIs which is a favorable alternative for the management of BPH and ED.

AUTHOR CONTRIBUTIONS

Concept: *D.Y.O., S.G.*; Design: *D.Y.O., S.G.*.; Control: *S.G., D.Y.O* ; Sources: *S.G., D.Y.O*; Materials: *D.Y.O., S.G.*; Data Collection and/or processing: *D.Y.O.*; Analysis and/or interpretation: *D.Y.O, S.G.*; Literature review: *D.Y.O, S.G.*; Manuscript writing: *D.Y.O, S.G*; Critical review: *D.Y.O, S.G.*; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

All experimental procedure of the animals was approved by the Ethics Committee of Ankara University (approval no: 2015–16-185).

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