The Effects of Oral 5-alpha Reductase Inhibitors on Penile Intracavernosal Pressures and Penile Morphology in Rat Model

Sahin Kilic¹*, Engin Kolukcu², Fikret Erdemir³, Ismail Benli⁴, Akgul Arici⁵

**Purpose:** Benign prostatic hyperplasia (BPH), and erectile dysfunction (ED) are urological diseases which affect more than 50% of men older than 50 years of age. It has been reported that 5-alpha-reductase inhibitors (5-ARIs) used in clinical studies for the treatment of BPH caused ED in 0.8-15.8% of the patients. The aim of this study is evaluation of the effects of oral finasteride and dutasteride on penile intracavernosal pressures and penile morphology in a rat model.

**Materials and Methods:** Thirty Wistar Albino strain male rats were randomized into control (n = 10), finasteride (n = 10), and dutasteride (n = 10) groups. After 8 weeks of treatment erectile responses were evaluated in all rats measuring intracavernosal pressure (ICP) changes during erectile responses to cavernosal nerve electrical stimulation. Serum hormone levels were studied and all rats underwent prostatectomy and penectomy. All tissue samples were examined histomorphologically and a semiquantitative scoring system was used for cavernosal tissue collagen density grading.

**Results:** Approximately 50% decrease was seen in mean ICPs in the finasteride and dutasteride groups compared to the control group for all voltages (2.5 V, 5 V, 7.5 V). Mean ICPs for 7.5 V were 62.17 ± 30.89 mmHg in control group, 35.27 ± 31.94 in the finasteride, and 36.01 ± 19.20 mmHg in the dutasteride group. But regarding ICPs there was no statistically significant difference between the groups (P > .05). The serum testosterone (T) concentrations were higher in treatment groups (P < .001). Serum dihydrotestosterone (DHT), luteinizing hormone (LH) and follicle stimulating hormone (FSH) concentrations were not significantly different between the groups. As a result of histomorphological studies, a statistically significant increase in cavernosal tissue collagen density, and marked atrophic changes in prostatic epithelial tissues were observed in the treatment groups.

**Conclusion:** Although 5-ARIs cause marked atrophic changes in prostatic epithelial tissues, and prominent collagen deposition in penile cavernosal tissues, no significant effect on penile ICPs was seen in this study. The failure to show a statistically significant difference was attributed to higher standard deviations of ICP values. The penile morphology evaluation results point to a negative effect of 5-ARIs on erectile function.

**Keywords:** dutasteride; erectile dysfunction; finasteride; intracavernosal pressure; penile morphology; prostate.

**INTRODUCTION**

BPH, accepted as a disease of advanced age, which impairs quality of life of the patients, is seen in approximately 50% of men between 51-60 years and approximately 90% over the age of 81 years.¹ In the etiology of BPH, androgens, estrogens, stromal-epithelial interactions, growth factors, and neurotransmitters can play a role alone or in combination.² Medical treatment methods of BPH include use of 5-ARIs, which target decrease in the prostatic volume. 5-ARIs ensure decrease in the size of prostate (nearly 30%) by preventing the production of dihydrotestosterone (DHT). Testosterone enters into prostatic epithelial cells and is converted into DHT through the action of 5-alpha reductase enzyme. DHT promotes the prostate enlargement by stimulating DNA synthesis in nuclei, and cellular growth.³ In animal models, androgen deprivation has been demonstrated to cause deterioration of dorsal nerve structure, and endothelial morphology, decrease in trabecular smooth muscle, increase in extracellular matrix, and penile tissue atrophy. Furthermore, androgen deprivation inhibits the production of protein, and enzymatic activities of endothelial nitric oxide synthase (eNOS), and neuronal nitric oxide synthase (nNOS)⁴. Androgen deprivation inhibits the production of protein, and enzymatic activities of endothelial nitric oxide synthase (eNOS), and neuronal nitric oxide synthase (nNOS)⁵. Still, as is understood from available data, androgen deprivation leads to a de-
crease in the smooth muscles of the corpus cavernosum, and the elastic fibres of the tunica albuginea, and an increase in collagen fibres\(^\text{40}\). ED is defined as the persistent inability of a male to achieve and/or maintain a penile erection sufficient to permit satisfactory sexual performance\(^\text{19}\). Its prevalence ranges between 30-52% in men aged 40-70 years, while it climbs to 80%, among men older than 70 years of age\(^\text{18}\). Psychogenic, hormonal, neurogenic, and arterial pathologies, iatrogenic causes, systemic, and chronic diseases, and drugs play a role in the etiology of ED\(^\text{29}\). As indicated in various studies, the use of 5-ARIs may cause loss of libido, ejaculatory disorders and erectile dysfunction\(^\text{19}\). However, most of the studies investigating the correlation between 5-ARIs and ED have consisted of clinical research, and an extremely limited number of experimental animal studies investigated cavernosal tissues. As far as we know in English medical literature, finasteride, and dutasteride have not been evaluated in combination in any animal studies. Accordingly, our study appears to be the first trial which evaluated the effects of 5-ARIs on rat cavernosal tissue, prostate morphology, and penile cavernosal response to electrical stimulation in rats. To this end in our study we aimed to evaluate the effects of 5-ARIs on penile cavernosal pressures, serum androgen levels, and penile tissue.

**MATERIALS AND METHODS**

A total of 30 10-week-old male Wistar Albino strain rats weighing 250-300 g (median, 270 g) were used. All procedures were realized in accordance with the stipulations of the 1986 Strasbourg Universal Declaration of Animal Rights in Gaziosmanpasa University Experimental Medicine Application Centre after the approval, and with the support of the Gaziosmanpasa University Animal Studies Ethics Committee (2012 HADYEK 036). The rats were housed in standard rat cages at 20-23 °C, and under 12-hour dark, and 12-hour light cycles. The rats were fed with special rat pellet, and water ad libitum.

The rats were randomized into 3 groups, each containing 10 rats with simple randomization. Group 1 was the control group which did not receive any drug therapy. Group 2 rats received daily doses of 0.5 mg dutasteride for 8 weeks according Table), and through midline scrotal incision of the left posterolateral prostate, and placed around the cavernosal nerve was stimulated with an electrical transducer (Biopac-MP 45 System, USA). While continuously measuring ICP, the cavernosal nerve was stimulated with an electrical nerve stimulator (STN 0211 Nerve Stimulator) for 30 seconds with 50 Hz, 2.5 V, 5 V, and 7.5 V at square wave times of 2 msec to achieve erection. ICP, measured before 2.5 V electrical stimulation, was considered as baseline, and maximum changes in ICP with reference to baseline ICP up to termination of 7.5 V stimulation were recorded for each voltage (2.5 V, 5 V, and 7.5 V, respectively).

Then intracardiac blood samples of 3cc were drawn from each rat to determine testosterone, DHT, FSH and LH levels. Subsequently the rats underwent prostatectomy, and penectomy through the previously made midline scrotal incision. At the end of the study all rats were sacrificed by cervical dislocation.

**Biochemical Measurements**

Blood samples drawn were centrifuged, and sera were kept at -80 oC pending biochemical analysis. Testosterone, DHT, FSH, and LH levels of samples were measured using the ELISA method (Organon Teknika Reader 2305, Austria). For the measurement of serum testosterone, FSH, and LH levels, Cayman (Cayman Chemical Company, MI, USA) brand, and for DHT levels General (WUHAN EIAAB Science CO., LTD, Wuhan, China) brand kits were used.

**Histopathological Evaluation**

Prostatectomy, and penectomy samples were fixated in a 10% buffered formaldehyde solution, then routinely embedded in paraffin blocks. Rat penises were cut in 5 µ sections, and stained with Masson trichrome dye so as to evaluate the collagen content in the tissue. Smooth muscle collagen ratios in penile cavernosal tissues were histopathologically evaluated. Rat prostates were cut in 5 µ sections, and stained with hematoxylin/eosine dye, and examined under a light microscope (Nikon ECLIPSE E600) at 100x magnification for the evaluation of muscular, and glandular structures. Microscopic images were obtained from a total of 4 serial sections, with 2 sections per slide of each rat. Then 16-20 areas were analyzed for each rat in total, with at least 4-5 areas on each section. Grading of tissue collagen density was performed based on scoring used by Erdemir et al\(^\text{11}\). The percentage of collagen concentration in each cut section, and field of examination was determined semiquantitatively and expressed in comparison with blue-stained collagen positive areas. Masson trichrome stained areas were graded on a scale between 1 and 4, based on percentages of penile cavernosal collagen content. Collagen percentages of < 30%, 30-50%, 51-70%, and > 71% were graded as 1, 2, 3, and 4, respectively.

**Statistical Analysis**

Descriptive analyses were performed so as to get information about the general characteristics of the groups. After assessing of the normality, one-way analysis of variance was used for intergroup comparisons of variables. Repeated measures analysis of variance was used for intergroup comparisons of variables. Repeated measures analysis of variance was used for comparisons based on changes in variables over time. For two group comparisons Tukey HSD test was used as post hoc test of one way analysis of variance. Data concerning continuous variables were expressed as mean ± standard deviation. \( P < .05 \) was accepted as the level of statistical significance. For statistical calculations commercially available software programs were utilized (IBM SPSS Statistics 19, SPSS inc., an IBM Co., Somers, NY).
RESULTS

**Penile cavernosal pressures**

Mean ICP values for electrical stimulation of cavernosal nerve with 2.5 V were calculated as 8.05 ± 14.58 mmHg in Group 1, 2.86 ± 3.97 mmHg in Group 2, and 2.06 ± 2.85 mmHg in Group 3. Intergroup differences were not statistically significant ($P = .278$). Mean ICPs for 5 V were estimated as 25.70 ± 30.30 mmHg in Group 1, 15.07 ± 15.08 mmHg in Group 2, and 15.15 ± 13.21 mmHg in Group 3 without any statistically significant intergroup difference ($P = .438$). Mean ICPs for 7.5 V were 62.17 ± 30.89 a mmHg in Group 1, 35.27 ± 31.94 c mmHg in Group 2, and 36.01 ± 19.20 c mmHg in Group 3. But intergroup differences were not statistically significant ($P = .062$). An approximately 50% decrease was seen in mean ICPs in the finasteride and dutasteride groups compared to the control group for all voltages in qualitative evaluation performed during the procedure. But in statistical analysis, no significant intergroups differences were seen.

In one-way repeated measures of variance analysis in intragroup comparisons of mean ICP values for all voltages (2.5 V, 5 V, 7.5 V), a statistically significant difference was detected in all groups ($P < .001$, Table 1). Generally, in all groups as the voltage of electrical stimulation of the cavernosal nerve increased, increases in mean ICP values were seen (Figure 1).

**Serum Hormone Levels**

The mean serum testosterone level in Group 2 was significantly higher relative to Group 1 ($P < .001$). The mean serum testosterone level in Group 3 was significantly higher relative to Group 1 ($P < .001$). However no significant intergroup difference was detected for other serum hormone levels (Table 2).

**Penile morphology**

When compared with Group 1, prominently increased collagen density was observed in Groups 2 and 3 (Figure 2). In Group 1 Grade 3, and 4 collagen densities were not seen, while Groups 2 and 3 had no Grade 1 collagen density. In Group 1, Grade 1, and Grade 2 collagen densities were observed in 80% and 20% of the specimens, respectively. In Group 2, Grade 2, Grade 3, and Grade 4 collagen densities were detected in 50%, 30%, and 20% of the specimens, respectively. In Group 3, Grade 2, Grade 3, and Grade 4 collagen densities were observed in 30%, 40%, and 30% of the specimens, respectively. As a result of histomorphological studies, a statistically significant increase in cavernosal tissue collagen density was observed in the treatment groups. (Table 3).

**Prostate Morphology**

In Group 1 high columnar epithelium, and papillary folds were markedly observed in prostatic glandular tis-
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Dihydrotestosterone (pg/mL) 2.57 ± 1.07 2.90 ± 1.23 2.43 ± 0.96 0.621
Testosterone (pg/mL) 308.97 ± 146.29 628.40 ± 153.97a 613.64 ± 179.24
LH (mIU/mL) 6.80 ± 0.39 6.80 ± 0.35 6.99 ± 0.31 0.377

**Table 2.** Mean hormonal values of all groups

<table>
<thead>
<tr>
<th>Control Group (n=10)</th>
<th>Finasteride Group (n=10)</th>
<th>Dutasteride Group (n=10)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH (mIU/mL)</td>
<td>1.29 ± 0.59</td>
<td>2.04 ± 1.43</td>
<td>1.68 ± 0.70</td>
</tr>
<tr>
<td>LH (mIU/mL)</td>
<td>6.80 ± 0.39</td>
<td>6.80 ± 0.35</td>
<td>6.99 ± 0.31</td>
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<td>2.43 ± 0.96</td>
</tr>
</tbody>
</table>

*One-way analysis of variance

a A significant correlation was found versus finasteride group.

b A significant correlation was found versus finasteride group.

Data are presented as mean ± SD

DISCUSSION

Effects of androgens on erectile function, libido, and sexual behaviour are very well known and are very important. Androgens exert their effects in erectile physiology by directly binding to their receptors or via conversion to their more active form of 5-alpha dihydrotestosterone which is mediated by the 5-alpha reductase enzyme. In experimental animal models androgen deprivation has led to morphologic deterioration in the dorsal nerve, endothelium; decrease in the content of trabecular smooth muscle; increase in connective tissue components, together with atrophy of the penile tissue. Androgen deprivation causes apoptosis of spongious, and cavernosal cells which can be prevented by androgen administration. It has been detected that DHT prevents disruption of erectile function in castrated rats, and this effect has been correlated partially with an increase in the levels of nitric oxide synthase (NOS). In the light of these data the effects of androgens on erectile physiology are apparently very important.

5-ARIs used in the medical treatment of BPH inhibit the conversion of testosterone to more potent androgen DHT. As a result of their effects, prostate volume decreases by 20%-30%, and within 6-12 months a nearly 50% decrease in prostate specific antigen (PSA) levels is induced. A decrease in the volume of prosthetic glandular tissue caused by 5-ARIs effects the static component of BPH, with a resultant increase in urine flow, and a decrease in BPH-related LUTS, acute urinary retention, and risk of surgery. Also 5-ARIs decreases the serum levels of DHT, which is the major androgen involved in erectile physiology, by 70-90 percent.

Many clinical, and experimental studies have revealed the correlation between 5-ARIs, and erectile dysfunction. The most frequently reported unwanted adverse sexual effect of 5-ARIs is ED, followed by ejaculatory disorder, and decrease in libido. Finasteride at a dose of 1 mg is frequently used in dermatology practice, and finasteride-related ED is reported in 0.8-3.8% of these patients, while ED is detected in 3.4-15.8% of the patients who used daily doses of 5 mg finasteride in the treatment of BPH. Findings in studies on dutasteride rates of ED are similar to those indicated above. In a double-blind, placebo-controlled study by Andriole et al. statistically significantly higher incidence rates of ED have been detected in the dutasteride group (4.7%) when compared with the placebo group (1.7%).

The effects of 5-ARIs on erectile function are clearly seen not only in clinical, but also in experimental studies. Within the last four decades, animal experiments have provided evidence demonstrating the key role of DHT in erectile physiology. Besides, an animal study has demonstrated that castration induces a 50% decrease in erectile response, and testosterone treatment reverses this effect. However this study has showed that in castrated rats when finasteride is given in combination with testosterone, the erectile response is not improve. When finasteride is administered with DHT, improvements in nNOS expression, and activity, and erectile response to electrical stimulation are detected. This finding underlines the important hormonal role of DHT in erectile physiology. In a different study the effects of finasteride (4.5 mg/kg/day for one month), and castration on serum hormone levels, cavernosal, and prostatic morphology, and intracavernosal pressure response to cavernosal nerve stimulation were evaluated. In the control, and castration group similar testosterone levels were observed, while significantly reduced DHT levels were detected in the finasteride, and castration groups. In the castration group the relative proportion of cavernosal smooth muscle markedly decreased, while the mass of connective tissue (collagen) increased prominently, and no change in the smooth muscle/collagen ratio was observed. In the castration group a decreased intracavernosal pressure response to cavernosal nerve stimulation was detected, while no difference was detected between control, and finasteride groups as for the intracavernosal pressure response. In another study the effects of long-term (16 weeks) use of finasteride on cavernosal tissues, and erectile response was evaluated...
in old (16 months) rats. Decreases in cavernosal smooth muscle ratio were detected. During in vivo evaluation a significant decrease in erectile response was observed. In our study, mean ICP values in the finasteride, and dutasteride groups are similar and decreased by nearly 50% when compared with the control group, without reaching any level of statistical significance. Generally, in all groups as the voltage of electrical stimulation of the cavernosal nerve increases, increases in ICP values are seen (P < .001). This indicates that the ICP measurements are done correctly. The failure to show a statistically significant difference was attributed to higher standard deviations of ICP values. This is in some rats maximum change in ICP values were reached after electrical stimulation with 2.5 V, while in some maximum change in ICP values were reached after electrical stimulation with 5 V or 7.5 V. We conclude that increasing the sample size and the duration of the treatment can give statistically significant results. When compared with the control group, increase in serum testosterone levels are detected in the finasteride group. However no significant intergroup difference was detected in serum DHT levels. In the semiquantitative histopathological evaluation, significant atrophic changes are detected in the prostatic tissues, and significantly increased collagen deposition is observed in the cavernosal tissues in the finasteride group versus the control group. Effects of 5-ARIs on penile cavernosal tissue, and prostate have been investigated in many experimental studies. Pinsky et al. evaluated the effects of dutasteride on cavernosal tissues, and erectile response in an animal study. They observed 86.5% suppression of mean serum DHT levels, increased collagen accumulation in cavernosal tissues, a decrease in nNOS activity, and in vivo assessments significant reduction in erectile response in the treatment group. Öztekin et al. evaluated the effects of long term dutasteride treatment and treatment withdrawal on cavernosal tissues and erectile responses. They observed that 8 weeks of dutasteride treatment significantly decreased erectile response in vivo assessments, when compared with the control group, and the group where the dutasteride treatment had been discontinued for 2 weeks after 6 weeks treatment. However erectile responses in the withdrawal group were not so strong as those seen in the control group. The researchers concluded that decreased erectile response caused by dutasteride could persist after its withdrawal.

In our study, the impact of dutasteride on mean ICP values is similar when compared with the finasteride group. Furthermore similar to the finasteride group, the serum DHT levels decreased relative to the control group without reaching any level of statistical significance. However, in our study, if the prostatic tissue hormone levels were measured instead of the serum hormone levels, it is possible that statistically significant results for DHT levels would be obtained. On the semiquantitative visual score, collagen accumulation in cavernosal tissues is more markedly observed in the dutasteride group, relative to the control, and finasteride groups. In the evaluation of prostatic tissues, atrophic changes in the dutasteride group are more prominent versus the control, and finasteride groups. These findings demonstrate that even though in clinical studies dutasteride decreases serum DHT levels, in the long term when compared with finasteride, in animal tissue studies they indicate that dutasteride can induce prostatic atrophy to a greater extent, and greater amount of collagen deposition in the cavernosal tissues. It should be noted that in clinical studies, similar incidence rates of sexual side effects related to finasteride or dutasteride treatment have been observed. Although 5-ARIs treatment results in marked atrophic changes in epithelial tissues, and prominent collagen accumulation in penile cavernosal tissue, a significant impact on penile cavernosal pressures is not detected. Even though 5-ARIs demonstrate similar efficacy, and side effect profiles, according to our study dutasteride induces more severe atrophic changes in the prostatic tissue, and more diffuse collagen accumulation in the cavernosal tissue compared with.

CONCLUSIONS
Androgens are critical for cavernosal smooth muscle integrity. Treatment with 5-ARIs decreases serum DHT levels up to 95%. Although 5-ARIs cause marked atrophic changes in prostatic epithelial tissues, and prominent collagen deposition in penile cavernosal tissues, they were not seen to produce a significant effect on penile ICPs in this study. Inability to demonstrate a statistically significant difference was attributed to the higher standard deviations of ICP values. This condition is a shortcoming of the study. Serum testosterone levels were observed to be statistically significantly higher in the treatment groups than in the control group. But there was no significant intergroup difference for serum DHT levels. Although dutasteride and finasteride have similar efficacy and side-effect profile in clinical studies, in our study dutasteride caused more atrophy in prostatic tissues and caused more intense collagen deposition in cavernosal tissues than finasteride. The penile morphology evaluation results are pointing to a negative effect of 5-ARIs on erectile function.

ACKNOWLEDGEMENTS
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CONFLICT OF INTEREST
The authors report no conflict of interest.

REFERENCES
5. Traish AM, Goldstein I, Kim N. Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insufficiency and erectile dysfunction. Eur Urol. 2007;52:54-70.
27. Pinsky MR, Gur S, Tracey AJ, Harbin A, Hellstrom JG. The Effects of Chronic 5-Alpha-
