The Effect of Different Doses of Finasteride on Epididymis and Testosterone and DHT Concentrations in Rats

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Abstract

Objective: Finasteride is a widely used in the treatment of alopecia and prostatic hyperplasia. Our study was conducted to assess the effect of different doses of Finasteride on epididymis, testosterone and dihydrotestosterone concentrations in male Sprague-Dawley rats.

Design, Material and Methods: This is experimental study conducted where thirty male rats are divided into three groups (10 rats in each group); high dose, low dose and control groups. During 2 months period, testosterone and DHT were being measured and at the end of the experiment, rats were killed by cervical dislocation. Weight of the organs and histopathological examination were done.

Results: DHT was found significantly reduced by finasteride administration. Testosterone was unexpectedly reduced in our study. Reduction in glandular epithelium and stromal component was clearly evident in histopathological examination of the prostate. Weights of seminal vesicles, dorsal prostate and ventral prostate were significantly decreased with finasteride administration. Sperm count and motility were not significantly changed in low-dose finasteride-treated group but there was a significant reduction in sperm counts and motility in high-dose group.

In Conclusion: Finasteride has significant effects on testosterone, DHT. Also, it can affect epididymal functions if administered in high dose. It is evident from our results that there is a need for further clarification of the relation between finasteride administration and specific parameters in epididymal functions.

Key Words: Finasteride – Epididymis – Testosterone – Dihydrotestosterone.

Introduction

ANDROGENS are necessary to maintain the size and function of the prostate and accessory sexual organs. Also, androgenic receptors were found in the hair follicles [1,2].

Testosterone is the principal androgen acting in tissues lacking 5 a-reductase. In tissues expressing 5 a-reductase, testosterone is converted to DHT and in those tissues, DHT is the principal androgen. 5 a-reductase is highly expressed in the prostate, but not in muscle or bone [3]. This observation led to the hypothesis that inhibitors of 5a-reductase might block the undesirable effects of testosterone on the prostate without blocking the desirable effects of testosterone on muscle, fat and bone. Also it is noted that conversion to DHT amplifies the effects of testosterone in several ways. First, DHT has a higher affinity for the androgen receptor than does testosterone [4]. Second, binding of DHT stabilizes the androgen receptor, slowing the rate of receptor degradation [5]. Third, conversion of testosterone to DHT prevents its conversion to androstenedione, a much weaker androgen [6].

Epididymis:

The epididymis consists of a single long convoluted tubule through which spermatozoa must pass following their release from the testis. Testicular spermatozoa are nonfunctional in that they lack progressive motility and the ability to fertilize and only acquire these functions after undergoing sperm maturation in the epididymis [7].

It is well known that the microenvironment in which spermatozoa undergo their maturation in epididymis is constantly changing in a carefully orchestrated sequence. Whether the constituents of the epididymal secretions are soluble [8], contained in large dense granules [9] or exosome-like vesicles [10], they must support and possibly induce the acquisition of sperm function. Spermatozoa seem to depend on such constantly changing microenvironment because spermatozoa are largely devoid of cytoplasm and heavily dependent on the extracellular milieu provided by the epididymis for protection from various kinds of attack, including infection and oxidative stress [8].
It is reported that the competence for movement is fully acquired as spermatozoa enter the corpus epididymidis. However, other sperm functions, including hyperactivation, zona binding and acrosomal exocytosis do not reach optimal levels until the spermatozoa had reached the cauda and vas deferens [11].

The caput epididymidis, the region the spermatozoa first enter coming from the testis/efficient ducts, is the most metabolically active region, secreting 70-80% of the total overall protein secretion in the epididymal lumen [12].

Moreover, within this same region, 99% of the fluid accompanying the testicular sperm is resorbed, resulting not only in a profound concentration of spermatozoa but luminal components as well [13].

Also, microenvironmental pH is known to play important role during sperm maturation in the epididymis. The pH regulation of luminal fluid in the male reproductive tract has been studied only in animals, since its examination in men is difficult. In rats, approximately 96% of the fluid discharged from testis with spermatozoa is reabsorbed in the efferent ducts [14]. This is thought to be important for the completion of sperm maturation and storage in the epididymis [15-17].

There is some acceptance that the cauda epididymidis of scrotal mammals plays a role in sperm competition because it is adapted to store sperm [18]. This ensures that, during several days of intense mating, daily sperm output by a male can greatly exceed daily sperm production by the testes [19,20]. Although there is little understanding of the mechanism of sperm storage there is some knowledge of how the specialization is achieved. The cauda epididymidis provides an environment lower than testicular temperature [21] and a unique milieu [22] that keeps the sperm at a metabolic rate one-third to one-fifth that of diluted epididymal spermatozoa [23]. There is also significant circumstantial evidence showing the evolution of the cauda epididymidis for sperm storage [24,25].

Many structural modifications occur during spermatozoa maturation process in association with the acquisition of functional competence [26]. The sperm plasma membrane undergoes extensive remodeling in terms of its surface glycosylation characteristics, fatty acid composition and cholesterol: Phospholipid ratio. Maturing epididymal spermatozoa also exhibit changes in intracellular calcium concentration, cAMP content and protein phosphorylation pattern that are pivotal to the development of coordinated movement [27-29].

Finasteride is a specific inhibitor of the prostatic (type II) steroid 5 a-reductase [30,31]. Such effect on 5 a-reductase has made finasteride a useful drug for the treatment of benign prostatic hyperplasia (BPH) [32,33] with a resulting improvement in urodynamic pattern [2]. Also, finasteride is used for prostate cancer chemoprevention [34]. Thus decreasing the risk of cancer prostate [35]. The empirical formula of finasteride is C23H36N2O2 and its molecular weight is 372.55. Its structural formula is.

Most of the adverse effects of Finasteride are reversible such as erectile dysfunction, loss of libido, a small volume of ejaculate and gynecomastia [36]. Some hypothesized that perhaps finasteride does not dramatically change the spermatogenesis process in healthy men as shown by Overstreet et al. [2], but in patients with other problems contributing to infertility, the negative influence of finasteride noted by others might be amplified [36].

This study was conducted to measure the effect of different doses of Finasteride on prostatic tissues and also its effects on the concentrations of testosterone and dihydrotestosterone in both serum and prostate.

Material and Methods

Type of the study:

This was an experimental study to assess the effect of finasteride (2mg and 0.2mg) on spermatogenesis and ROS formation of male Sprague-Dawley rats.

Animals:

30 healthy male Sprague-Dawley rats, 12 weeks old were purchased from Harlan (USA) and then they were kept in the Animal Facility in Case Western Reserve University to be acclimatized to
their new place and until they reached 16 weeks of age, their weight at the beginning of the study was 200-250gm.

Groups studied:
**Sprague-Dawley rats purchased from Harlan. Rats were divided into three groups:**
- High dose group (10 rats) where 2.5mg of finasteride was administered.
- Low dose group (10 rats) where 0.25mg of finasteride was administered.
- Control group (10 rats) where no finasteride was administered.

**Chemicals:**
Finasteride was purchased from Sigma-Aldrich, USA as 100mg bottles. Finasteride was dissolved in alcohol and it was added to distilled water and was put in bottles from which the rats drink (average 400ml each day). The dose was adjusted for the high and low dose groups.

**Duration of the study:**
The duration of the study was 2 months. The animal facility in case Western Reserve University was taking care of the animals and feeding them.

**Study design:**
The animals were divided randomly into three groups:
- Group 1 comprised 10 rats that received 1mg/kg/day of finasteride (Proscar®, Merck-Sharp Dohme, USA), diluted in 2mL of saline solution, administered intraperitoneally, once a day for 5 days/week and 2 consecutive months.
- Group 2 comprised 10 rats that received 0.2mg/kg/day of finasteride.
- Group 3 comprised 10 rats that received 2mL of saline solution in the same regimen and for the same period as in group 1.

**Estimation of the equivalent dose:**
The equivalent doses were 2.5mg/kg/day and 0.25mg/kg/day as the high and small doses respectively; this is equivalent to 20mg/day and 200mg/day in human.

**Animal weighing:**
Specific gown and gloves had to be put on before entering animal facility then the hood was sprayed with clidox and swiped then an autoclaved towel was spread on the hood and gloves were sprayed too with clidox and animals were weighted using an animal scale.

**Anesthesia:**
The rats were anesthetized with a cocktail of ketamine solution (100mg/ml), xylazine (20mg/ml) and Acepromazine (10mg/ml) intraperitoneally.

**Blood sampling:**
Blood samples were collected from the retro-orbital Space of the albino rats.

The anesthetized rat was laid on its side on a table or was hold in our hand with its head pointing down. The skin was pulled away from the eyeball with our first finger and thumb (finger above and thumb below the eye), above and below the eye, so that the eyeball was protruding out of the socket as much as possible with taking care not to occlude the trachea with our thumb. The tip of a microhematocrit blood tube was inserted into the corner of the eye socket underneath the eyeball, directing the tip at a 45-degree angle toward the middle of the eye socket. The pipette was rotated between our fingers during forward passage; we didn’t move it from side to side or front to back. Gentle downward pressure was applied and then released until the vein was broken and blood was visualized entering the pipette. When a small amount of blood was beginning filling the pipette, withdrawal was done slightly and the pipette was allowed to fill with taking care that the pipette didn’t come out of the eye socket. If the pipette was not withdrawn slightly, it could occlude the vein and blood was not going to flow freely. The open end of the pipette was covered with the tip of our finger before removing it from the orbital sinus to prevent blood from spilling out of the tube. Bleeding usually stopped immediately and completely when the pipette was removed. Sometimes it was necessary to apply gentle pressure on the eyeball for a brief
moment by closing the skin above and below the eye using our first finger and thumb. It was recommended that sample collection not be repeated on the same eye for at least two weeks.

**Euthanasia:**

Animals were killed by cervical dislocation. The animal was held by its tail and placed on a surface that it can grip, and then it would stretch itself out so that a pencil or similar object was placed firmly across the back of the neck. A sharp pull on the base of the tail then dislocated the neck. Dissection was made and the organs were got out.

**Assessment of DHT:**

DHT measurement kits were supplied by Alpha Diagnostic International.

**Measuring testosterone level:**

Testosterone EIA kits were supplied by Cayman Chemicals.

**Histological evaluation:**

At the end of the period of treatment the rats were anaesthetized using the same cocktail mentioned above intraperitoneally and sacrificed using neck dislocation. Through a median incision in the ventral walls of the scrotum, the testes with the epididymes were carefully removed and immediately weighed on electronic scales (Analytic Balance Marte, precision 0.01g), after which they were both preserved in Bouine’s solution and then processed for histological analysis.

**Assessment of sperm motility:**

Each cauda epididymis was removed from the transport medium, slashed twice through its thickness and placed in 1ml of pre-incubated cultured medium (T6) overlaid with mineral oil, in a culture plate well and incubated for 30 minutes in a 37°C water bath. After incubation a 1:10 dilution of sperm was prepared and an epidydymal sperm count was done using hemocytometer. Frequency of motile spermatozoa was determined [37].

**Ethical considerations:**

The animals were treated with Proper care and human treatment. Principles were applied when dealing with the laboratory rats as possible as it can be.

The male rats were selected and maintained in the same laboratory at a controlled temperature (22±2°C) and light exposure (07.00 a.m. to 19.00 p.m.), receiving standard food and drinking water ad libitum. The animals were kept in plastic cages (47x18x40cm) with four animals per cage.

**Results**

**Testosterone levels:** The results showed that testosterone levels were reduced after administration of finasteride in both low-dose and high-dose groups in both plasma and prostate measurements. The following table shows the mean testosterone measurements in control, low-dose and high-dose groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Low-dose group</th>
<th>High-dose group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma testosterone levels (in Pg/100ul)</td>
<td>156</td>
<td>115.2</td>
<td>89.6</td>
</tr>
<tr>
<td>Testosterone in Dorsal prostate (Pg/ug protein)</td>
<td>3.62</td>
<td>2.28</td>
<td>2.028</td>
</tr>
<tr>
<td>Testosterone in ventral prostate in (Pg/ug protein)</td>
<td>5.68</td>
<td>4.2</td>
<td>2.588</td>
</tr>
</tbody>
</table>

The differences of testosterone measurements were found to be significant compared to the control where $p$ value was <0.001.

**Mean DHT levels in serum, dorsal and ventral prostate:**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>Low-dose group</th>
<th>High-dose group</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHT (serum level)</td>
<td>1260</td>
<td>286.4</td>
<td>246</td>
</tr>
<tr>
<td>DHT (dorsal prostate)</td>
<td>1.08</td>
<td>0.704</td>
<td>1.046</td>
</tr>
<tr>
<td>DHT (ventral prostate)</td>
<td>2.832</td>
<td>2.048</td>
<td>2.26</td>
</tr>
</tbody>
</table>

$p$ <0.001, compared to control.
Weight of the organs at termination:

<table>
<thead>
<tr>
<th>Organs</th>
<th>Control</th>
<th>Low-dose finasteride group</th>
<th>High-dose finasteride group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>14.47±1.8</td>
<td>14.58±1.6 (NS)</td>
<td>14.64±2.0 (NS)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>1.36±0.2</td>
<td>1.27±0.2 (NS)</td>
<td>1.36±0.2 (NS)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.788±0.06</td>
<td>0.742±0.05 (NS)</td>
<td>0.721±0.05 (NS)</td>
</tr>
<tr>
<td>Testis</td>
<td>1.9±0.1</td>
<td>1.91±0.1 (NS)</td>
<td>1.99±0.1 (NS)</td>
</tr>
<tr>
<td>Epididymis</td>
<td>0.23±0.002</td>
<td>0.27±0.002 (NS)</td>
<td>0.22±0.002 (NS)</td>
</tr>
<tr>
<td>Seminal Vesicle</td>
<td>1.57±0.16</td>
<td>0.53±0.11 (**)</td>
<td>0.51±0.12 (**)</td>
</tr>
<tr>
<td>D-L Prostate</td>
<td>0.232±0.001</td>
<td>0.135±0.001 (**)</td>
<td>0.127±0.001 (**)</td>
</tr>
<tr>
<td>Ventral Prostate</td>
<td>0.744±0.008</td>
<td>0.402±0.006 (**)</td>
<td>0.375±0.005 (**)</td>
</tr>
</tbody>
</table>

Weight expressed in grams.
NS = Not significant compared to control.
*"p<0.001, from control.

Sperm count and motility:

<table>
<thead>
<tr>
<th></th>
<th>Sperm count in control group</th>
<th>Sperm count in low-dose group</th>
<th>Sperm count in high-dose group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total sperm count</td>
<td>138</td>
<td>139.375</td>
<td>104.25</td>
</tr>
<tr>
<td>Motile sperms</td>
<td>8.214286</td>
<td>8.450893</td>
<td>6.03125</td>
</tr>
</tbody>
</table>

*p<0.001, from control.

Histopathological examination of the epithelium in the epididymis of low-dose group showed no difference. Also, tubules are populated with sperms (normal findings compared to the control group).
Histopathological examination of the epithelium in the epididymis of high-dose group showed no difference. On the other hand, tubules are not populated with sperms—sparsely populated (lower number of sperms were seen compared to the control and low-dose group).

Discussion

Administration of finasteride resulted in significant decrease of DHT and such decrease is due to the action of finasteride as specific inhibitor to type II 5 alpha reductase enzyme, thus preventing the conversion of testosterone to DHT \[^{38}\]. Decrease of DHT was proportional to the dose of finasteride administered in the studied rats. More significant decrease of DHT was seen in rats where high-dose of finasteride was administered.

Significant reduction of DHT levels in serum corresponds with studies done by Gormley et al. \[^{2}\] and Rittmaster et al. \[^{39}\]. From their results it was found that finasteride reduces circulating serum concentrations of DHT by approximately 70%.

It was seen that reduction of DHT level was also significant in the prostate and such reduction was inversely proportional to the dose of finasteride administered in the studied rats. The higher the dose, the lower is the level of DHT measured. Such results goes with the fact that finasteride works specifically on inhibiting type 2 5a-reductase and that enzyme predominates in the reproductive tissues, genital skin and epididymis. Also, our results goes with the findings that in the prostate type II 5 alpha-reductase is the isoform primarily responsible for DHT formation \[^{40}\].

Following inhibition of 5 alpha reductase by finasteride administration, it was expected that testosterone level to increase or at least to stay within normal range simply because a fraction of the testosterone is not being converted to dihydrotestosterone. On the contrary, in our study, testosterone level was seen to decrease significantly with finasteride administration. And testosterone level was inversely proportional to the dose of administered finasteride. Such decrease could be explained by strong negative feedback effect following short-term increase in testosterone level after finasteride administration. Short period of finasteride administration in the present study could be a reason behind our results of low testosterone levels. We did not study the chronic effect of finasteride on testosterone level which could have resulted in the expected high or at least normal testosterone levels. In that aspect, our results contradict the results obtained by Amory et al. \[^{41}\] where serum Testosterone levels were nonsignificantly elevated throughout the treatment period with finasteride and then returned to baseline.

Also, reduction in DHT resulted in a decrease in both glandular and stromal components of the prostate as expected as those organs depend on DHT as the main androgen. Reduction in glandular epithelium and stromal component was clearly evident in histopathological examination of the prostate of sacrificed rats in our study. Our results correlates to morphologic changes seen with finasteride administration in other studies where a significant decrease was seen in absolute volume of both glandular epithelial and stromal compartments of rat prostate with a subsequent reduction in prostate secretions \[^{42,43}\]. Such findings are also in agreement with Rittmaster et al. \[^{44}\] who showed similar apoptotic events induced by finasteride administration.
Prostate involution in the finasteride-treated animals was significant. Weights of seminal vesicles, dorsal prostate and ventral prostate were significantly decreased with finasteride administration. Such statistically significant decrease was inversely proportional to the increase in finasteride dosage. High-dose group showed the maximal decrease. Similar findings were noted by Rittmaster et al. [44] and Shibata et al. [45] where prostatic involution was noted following finasteride administration. It was demonstrated that such involution is partly due to apoptosis and to the significant regression of prostatic vascularization [44,45]. Finasteride also was found to inhibit the expression of insulin-like growth factor (IGF)-I and IGF-I receptor genes in the ventral prostate. And such effect could further explain its effect on ventral prostate.

There is an evidence for lobe-specific regulation of androgen receptor expression. It was found that in the absence of testicular androgen, androgen receptor levels in the ventral and dorsal lobes, but not the lateral lobe of the rats were down-regulated [46]. This could explain our findings that the weights of ventral and dorsal lobes were decreased and such decrease is inversely proportional to the dose of administered finasteride. And also, our results go with the fact that proliferation and survival of cells within the prostate gland depends on DHT [46]. Shao et al. [47] also showed similar dramatic decrease in ventral prostate weight in finasteride-treated young adult male rats.

Reduction in the weight of prostatic and seminal vesicles in the finasteride-treated groups could also be attributed to a decrease in DHT as these organs are partially dependent on DHT for their homeostasis as shown by Steers [48]. Several studies have proposed that one of the earliest changes leading to ventral prostate shrinking following finasteride treatment is a decrease of prostate vascularization [49,50], with subsequent hypoxia [51].

In spite of non-significant changes noted in the weight of epididymis in both low and high dose groups, the function of epididymis as indicated by sperm motility was affected significantly in high-dose group. Sperm motility is developed in the epididymis as indicated by Robaire and Henderson [52] and it is worth noting that the development of sperm motility is considered a key element of epididymal sperm maturation [53]. By the time sperm reach the distal regions of the epididymis, maximum progressive motility is achieved that enables sperm to reach and penetrate the egg [53,54]. Such development of progressive motility needs interactions between sperm and normally functioning epididymis with suitable surrounding epididymal luminal environment [53,55,56].

It is known that in reproductive toxicology studies, altered sperm motility is a valuable indicator of toxicity arising from an exclusive effect on the epididymis or sperm within the epididymis [57]. While the characteristic head, mid piece and tail structures of spermatozoa are already present before sperm leave the testis, further morphological changes occur during sperm transit through the epididymis [58,59].

Conclusion:
Further studies clarifying the exact site and mechanism of action underlying the effects of 5 alpha-reductase inhibitions on epididymis and its functions are needed.

References


30- STONER E.: 5α-Reductase inhibitors/fas nestra. Prostate, 6: 82-87, 1996.


38- SPAN P.N., VOLLE M.C., SMALS A.G., SWEEP F.G., SCHALKE J.A., FENELEY M.R. and KIRBY R.S.: Selectivity of finasteride as an in vivo inhibitor of 5α-


