The Effect of Different Doses of Finasteride on Sperm Morphology and Motility and Reactive Oxygen Species Concentrations in Rats

CHERRY NASR KAMEL SERGA, M.D.

The Department of Pharmacology, Faculty of Medicine, Suez Canal University.

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 sperm morphology and motility and Reactive Oxygen Species ROS 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, Glutathione assay was done in the prepared blood samples the end of the experiment; rats were killed by cervical dislocation. Sperm counts and morphology were assessed.

Results: Glutathione were found to be decreased significantly with increasing dose of administered finasteride which indicate statistically significant increase in the ROS levels. On the other hand, sperm motility was negatively affected only in high dose group. Abnormal sperm forms were also significant in the high-dose group. Sperm motility and morphology were not significantly changed in low-dose finasteride-treated group.

In Conclusion: Finasteride has significant effects on ROS production. Also, it can affect sperm motility and morphology negatively in high doses. Further studies clarifying the exact site and mechanism of action underlying the effects of 5 alpha-reductase inhibition on spermatogenesis are needed.

Key Words: Finasteride – Sperm – ROS.

Introduction

Reactive oxygen species (ROS):

ROS are highly reactive oxidizing agents belonging to the class of free radicals. A free radical is any compound (not necessarily derived from oxygen) containing one or more unpaired electrons. The most common ROS that have potential implications in reproductive biology include superoxide (O2–) anion, hydrogen peroxide (H2O2), peroxyl (R00) radicals and the very reactive hydroxyl (OH) radicals say [1].

In general, reactive oxygen species are formed by several different mechanisms; unavoidable
A hydroxyl radical removes a hydrogen atom from one of the carbon atoms in the fatty acid chain (only a portion of which is shown) forming a molecule of water and leaving the carbon atom with an unpaired electron; thus now a radical. One of the most likely is to react with a molecule of oxygen (O₂) forming a peroxyl radical. This might then steal a hydrogen atom from a nearby side chain making it now a radical which interact with other molecules to gain a stable configuration of electrons, they convert that target molecule into a radical. So a chain reaction begins that will propagate until two radicals meet each other and each contributes its unpaired electron to form a covalent bond linking the two [2].

Membranes of sperm cells contain highly specific lipidic composition, high content of polyunsaturated fatty acids, plasmalogens and sphingomyelins. Such structure is responsible for membrane flexibility and the functional ability of sperm cells. They are also involved as intermediates in the cell fusion [11]. On the other hand, such lipids are the main substrates for peroxidation, which may provoke severe functional disorder of sperm. The ability of human spermatozoa to interact with zona pellucida is enhanced by physiological low level of lipid peroxidation caused by Reactive Oxygen Species (ROS) accordingly unbalanced oxidative stress may lead to pathological lipid peroxidation which leads to defective sperm function [12]. Also, such high lipids content make sperm cells are particularly susceptible to the damage induced by excessive ROS release [13,14].

**Physiological functions of free radicals:**

The generation of ROS occurs physiologically during normal cell metabolism. Mitochondrial respiration is the main biological source of superoxide anion radicals under physiological conditions. During the tetravalent reduction of oxygen to water by the mitochondrial cytochrome c oxidase, these radicals can leak to the cell. At low concentrations reactive oxidants have a biopositive physiologic effect and act selectively [15]. They act on the metabolism of prostanoids, in gene regulation or in the regulation of cellular growth, intracellular signaling and the other types of signal transduction. Moreover, oxygen free radicals play an important role in regulation of vasotonus and in antimicrobial defense. Limited amounts of ROS can also interfere physiologically in the regulation of sperm functions. It has been observed that low amounts of free radicals in human semen enhance spermatozoa ability to bind zona pellucida. In addition, the incubation of sperm cells with low concentrations of hydrogen peroxide was found to stimulate sperm capacitation, hyperactivation, acrosome reaction and oocyte fusion [16-19].

**Bionegative effects of lipid peroxidation on sperm functions:**

Excessive generation of ROS in semen, mainly by neutrophils but also by abnormal spermatozoa, could be a cause for infertility [17]. High concentrations of hydrogen peroxide induce lipid peroxidation and result in cell death. Also, Increased ROS production by spermatozoa is associated with a decreased mitochondrial membrane potential (MMP). The patients with abnormal semen parameters had a significantly lower MMP [21].

Excess of free radical generation frequently involves an error in spermiogenesis resulting in the release of spermatozoa from the germinal epithelium exhibiting abnormally high levels of cytoplasmic retention. Redundant cytoplasm contains enzymes that fuel further generation of ROS by the spermatozoa's plasma membrane redox systems. The consequences of such oxidative stress include a loss of motility and fertilizing potential and the induction of DNA damage in the sperm nucleus. The loss of sperm function is due to the peroxidation of unsaturated fatty acids in the sperm plasma membrane, as a consequence of which the latter loses its fluidity and the cells lose their function [9].

It was found that H₂O₂ directly affects sperm functions critical at fertilization process in a dose- and time-dependent fashion. Low concentrations maintain capacitation, whereas high concentrations have deleterious effects. These effects are probably dependent on modifications of plasma membrane and intracellular homeostasis by the oxidative process [22]. Also, it was found that oxidative stress induced by white blood cells has a damaging effect on the polyunsaturated fatty acids of sperm phospholipids which may result, among the other effects, in decreased membrane fluidity [23].

**Finasteride:**

In man, 4-8% of testosterone (T) undergoes 5a-reduction, resulting in the formation of the more potent androgen, dihydrotestosterone (DHT). DHT plays a vital role in the development of the male reproductive tract during embryogenesis [24]. Dihydrotestosterone (DHT) acts as the primary androgen in the prostate and hair follicles. Such actions tend to accelerate benign prostatic hypertrophy (BPH) and androgenic alopecia. Two isoforms of 5a-reductase have been identified in humans.
Type 1 is found mainly in the skin, liver and testes while Type 2 5a-reductase predominates in the reproductive tissues, genital skin and epididymis. Finasteride (synthetic 4-azasteroid compound) is 5a-reductase competitive and specific inhibitor and effectively inhibits Type 2 5a-reductase and thereby reduces circulating serum concentrations of DHT by approximately 70% without harmful effects on androgen responsive endpoints, such as lipid metabolism, bone mineral density or general health [25,26]. As a result, finasteride shows detectable efficacy and a high safety profile in large numbers of men for several years for the treatment of BPH.

The empirical formula of finasteride is C23H36N2O2 and its molecular weight is 372.55. Its structural formula is:

```
Finasteride
```

Finasteride has no affinity for the androgen receptor and has no androgenic, antiandrogenic, estrogenic, antiestrogenic, or progestational effects. Inhibition of Type II 5a-reductase blocks the peripheral conversion of testosterone to DHT, resulting in significant decreases in serum and tissue DHT concentrations. On the other hand, Mean circulating levels of testosterone and estradiol were increased by approximately 15% as compared to baseline, but these remained within the physiologic range [27].

Finasteride is widely used in the treatment of alopecia and benign prostatic hyperplasia.

It has also been demonstrated that rat prostate involution observed under finasteride treatment is partly due to apoptosis and to a dramatic regression of prostate vascularization [28]. In finasteride-treated rats, morphometric analyses revealed a significant decrease in absolute volume of both glandular epithelial and stromal compartments of rat prostate with a subsequent reduction in prostate secretions [26,29,30].

Interestingly Tam et al. [31] have shown recently that androgen deprivation in castrated rats clearly induces oxidative stress in the regressing prostate epithelium and that testosterone replacement partially reduces this oxidative stress.

As shown by Takano et al. [32], oxidative stress induces the activation of multiple signaling pathways related to various cellular responses such as mitotic arrest, apoptosis and necrosis, depending on the H2O2 concentrations. This might explain the aggravation of prostate tissue injury upon exposure to increasing doses of antiandrogen. Also, it was shown that a decrease in ventral prostate (VP) epithelial cell proliferation under finasteride treatment that also could be a consequence of H2O2 accumulation [33].

This study is conducted to clarify the relation between the effect of different doses of finasteride on spermatogenesis, testosterone, DHT and ROS.

**Material and Methods**

**Type of the study:**

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

**Animals:**

30 healthy male Sprague-Dawley rats, 12 weeks old were included in the study and kept in the Animal Facility 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.

**Sample size calculation:**

The number of rats included in the study was determined by the following equation:

\[
N = 2 \frac{K_s^2}{\gamma^2}
\]

Where:

- \( N \) = The number of the rats in each group.
- \( K \) = Constant \((Z_a + Z_B)^2 = 7.8\).
- \( Z_B \) = The value of a standard normal distribution for \( p \) value 5% for two sided tests and \( Z_B \) = the value of a standard normal for the desired statistical power 90%.
- \( s \) = The within group standard deviation.
- \( \gamma \) = The detectable difference between the means of two groups.
Groups studied:

Sprague-Dawley 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.

Duration of the study:
The duration of the study was 2 months.

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.

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

Euthanasia:
Animals were killed by cervical dislocation. The animal was held by its tail and placed on a surface that it can grip, 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.

Glutathione assay:
Whole blood samples were diluted 20-fold with water prior to the assay then the procedure was done using 96 well plates as follows: First 100 \( \mu \)L water and 100 \( \mu \)L calibrator were transferred into wells of a clear-bottom 96 well-plates. 200 \( \mu \)L reagent A mixture was transferred into wells of the 96 well-plate and 100 \( \mu \)L reagent B was added and plate was tapped lightly to mix and then they were incubated 25min at room temperature and at last it was read using specific software.

Assessment of sperm count:
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 37C water bath. After incubation a 1:10 dilution of sperm was prepared and an epididymal sperm count was done using hemocytometer. Frequency of motile spermatozoa will also be determined.
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 albino 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

Glutathione evaluation:

The following graph shows the plasma glutathione levels during the study in control, low-dose and high-dose groups:

Time-dependant plasma glutathione levels.

Plasma glutathione level is inversely proportional to the Finasteride dose and also to the duration of Finasteride administration. Such relation was found to be significant.

Plasma Glutathione levels at termination.

Fig. (1): Sperms in control group.

Fig. (2): Sperms in low-dose group (normally shaped sperms).

Fig. (3): Abnormal sperms as seen in high-dose group. Double-headed sperms are seen in this slide.
Fig. (4): Abnormally-shaped sperm heads as seen in high-dose group.

Fig. (5): Abnormally-shaped sperms in high-dose group. This slide shows fragmented sperms.

Fig. (6): Abnormally-shaped sperm as seen in high-dose group (deheaded sperms were seen).

Fig. (7): This slide show Sperm count in control group and as it is shown the number of sperms is quite high, when compared with the high dose finasteride group it was shown that the number of sperms was lower as shown in slide.

Fig. (8): This slide show Sperm count in high dose group. High-dose of finasteride administered was associated with decreased number of sperms. High dose of finasteride administered was found to have deleterious effects on sperm count. No such effects were noted in the low-dose group.

Discussion

Finasteride is widely used in the treatment of alopecia and benign prostatic hyperplasia. Finasteride was found useful as chemo preventive drug in prostate cancer [35]. Using finasteride can reduce the risk of cancer prostate [36]. It acts on prostate through inhibition of the (type II) 5 alpha-reductase which converts testosterone into dihydrotestosterone (DHT). DHT is a potent androgen [37,38].

Adverse effects of finasteride are a concern especially in young patients. It was stated that finasteride has no dramatic effect on spermatoge-
ness in young healthy men [39] and most of its adverse effects are reversible once finasteride treatment is stopped. Erectile dysfunction, loss of libido, a small volume of ejaculate and gynecomatia are examples of the reversible adverse effects [40]. On the other hand, finasteride can have a negative influence on spermatogenesis in patients with other factors contributing to infertility [40].

Reactive Oxygen Species (ROS) production has been correlated with infertility. It was found that ROS are seen in up to 40% of infertile men and ROS production was correlated with defective immature sperm [41]. It was suggested that ROS could contribute to the deleterious effect of anti-androgen like finasteride.

The relation between finasteride administration in low and high doses and ROS production was examined in the present work. Our results show that glutathione levels were inversely proportional to dosages of finasteride. Such relation was found to be statistically significant. Increase in ROS (as evident by decreasing glutathione levels) in the finasteride-treated rats in our study correlates with the findings of Tam et al. [31] who showed that androgen deprivation clearly induces oxidative stress in the regressing prostate epithelium. Also, reduction in prostate epithelium and subsequently reduction in prostate weight in finasteride-treated rats could be a consequence of ROS accumulation as stated by Cayatte et al. [33]. They used tyrosine phosphorylation as an indirect reporter of H2O2 production and they discovered that finasteride treatment resulted in a dose-dependent increase of protein tyrosine phosphorylation which indicates increase in H2O2 production. This might explain the aggravation of prostate tissue injury and inflammation noted upon exposure to increasing doses of finasteride which could be linked enzymatic H2O2 production. Further studies are needed to examine the extent of finasteride-induced ROS production and its relation to the adverse effects of finasteride administration.

Similarly, Takano et al. [42] showed that oxidative stress induces the activation of multiple signaling pathways related to various cellular responses such as mitotic arrest, apoptosis and necrosis, depending on the H2O2 concentrations. This might explain the aggravation of prostate tissue changes upon exposure to increasing doses of finasteride.

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 [43]. While the characteristic head, midpiece and tail structures of spermatozoa are already present before sperm leave the testis, further morphological changes occur during sperm transit through the epididymis [44,45].

On the other hand, rats treated with high-dose of finasteride in our study showed significant reduction in spermatogenesis as assessed by reduction of the number of seminiferous tubules with and with no spermatozoids inside. These results correspond to the reported cases by Liu et al. [46]. They report two cases of infertile patients with azoospermia or severe oligospermia who showed significant improvements in sperm concentrations 6 months after the discontinuation of finasteride. Stopping finasteride in the infertility cases may improve semen parameters and may allow for less invasive fertility treatments. Such adverse effects of finasteride seem to be reversible as indicated by Glina et al. [40]. Genetic differences between subjects, such as polymorphisms in 5 alpha-reductase, which have recently been associated with sperm concentration, may also contribute to the intersubject variability [47].

Sperm morphology and motility in our study was not changed significantly in low dose of finasteride group compared to the control group. But significant abnormal morphology was noted in rats treated with high-dose of finasteride. The differential effects on sperm motility and morphology suggest that finasteride has no effect on sperm formation but a possible effect on the epididymis in which sperm motility is developed [48].

On the other hand, Cukierski et al. [49] found that fertility was reduced by 30 to 40% in chronically finasteride-treated rats but could not identify qualitative changes in the semen of the animals. Such reduction was attributed to a deficiency in forming the copulatory plug. Cukierski et al. [49] also noted the lower weight of the seminal vesicles and prostate, suggesting that the changes provoked by finasteride on fertility are probably related to a specific effect on the species, in which it is essential that the copulatory plug is formed, but is probably of minor importance in species in which this stage is not essential [49]. It could be concluded that finasteride has no significant effects on spermatogenesis in spite of its significant effect on prostatic secretions. Some hypothesized that perhaps finasteride does not dramatically change the spermatogenesis process in healthy men as shown by Overstreet et al. [39], but in patients with other problems contributing to infertility, the negative
influence of finasteride noted by others might be amplified [40].

In general studies of drug effects on spermatogenesis in men are difficult for several reasons including: 1) the 72-d maturation period of sperm, from spermatogonium to ejaculated sperm so that onset of injury and time to recovery can both be delayed; 2) the inherent, large variability in semen parameters, even for a single individual; 3) the large range of normal semen values; 4) the variability of technique and consistency of analyses of semen parameters among different laboratories; and 5) the lack of specific guidelines for what constitutes clinically significant changes in a particular semen parameter or threshold values for impaired fertility [50,51]. Further studies clarifying the exact site and mechanism of action underlying the effects of 5 alpha-reductase inhibition on spermatogenesis are needed.

References


