ORIGINAL ARTICLE

Role of Flutamide on Acinar Diameter in Testosterone Induced Prostatic Hyperplasia

Md. Atiar Rahman[1], Humaira Naushaba[2], Md. Nahid Ahmed Khan[3], Md. Maksudur Rahman[4]

Abstract :

Testosterone is the male sex hormone responsible for growth of secondary sexual characters and accessory sex organs. Despite the effectiveness as a male sex hormone, testosterone causes benign prostatic hyperplasia (BPH) resulting in urinary dysfunction. On the other hand, flutamide is a pure antitestosterone, which blocks the effects of dihydrotestosterone (DHT) at the testosterone receptor and prevents BPH. The objective of the study was to observe the effects of flutamide on testosterone induced prostatic hyperplasia in Long Evans rats. This experimental study was carried out in the Department of Anatomy, Sir Salimullah Medical College, Dhaka from January to December 2006. Forty five matured male Long Evans rats of age 8-10 weeks and weighing 200-300 gms were used in this study. They were divided into three equal groups. Group A was vehicle (olive oil) control group, Group B was testosterone treated group and Group C was testosterone and flutamide treated group. Comparative study in different groups were done microscopically. There was significant reduction ($p<0.001$) of prostatic hyperplasia. The mean diameter of acini of prostate in flutamide treated rats was lower than the testosterone treated rats. It can be concluded from this study that flutamide is an effective drug against testosterone induced prostatic hyperplasia.

Introduction:

The prostate is an accessory gland of male reproductive system that surrounds the neck of male urinary bladder and the proximal portion of the urethra. The prostate consists of branched tubuloacinar glands embedded in a fibromuscular stroma[1]. Clinically, prostate is an important pelvic organ for its affinity to diseases like benign prostatic hyperplasia. Testosterone is responsible for development of accessory sex organs and secondary sexual characteristics[2]. In the target tissue, testosterone is not the active form of the hormone, it is reduced to dihydrotestosterone (DHT) by an enzyme 5, α-reductase and is 10 times more potent than testosterone because it dissociates from the cellular testosterone receptor more slowly[3,4].

Growth of the prostate, normal and abnormal, is mediated by testosterone. Benign prostatic hyperplasia (BPH) is a non-malignant enlargement of prostate gland that commonly develops in the aging male. It is a hyperplastic process of the stroma and epithelial tissues of

1. Associate Professor, Department of Anatomy, Holy Family Red Crescent Medical College, Dhaka.
2. Professor and Head, Department of Anatomy, Sir Salimullah Medical College, Dhaka.
3. Lecturer, Department of Anatomy, Holy Family Red Crescent Medical College, Dhaka.
4. Lecturer, Department of Anatomy, Holy Family Red Crescent Medical College, Dhaka.

the prostate gland. DHT binds to cytoplasmic receptor protein forming a complex, then migrates to the nucleus and binds to the nuclear testosterone receptor and induces the DNA-RNA transcription process which is mitogenic and leads to hyperplasia of the target organ[4].

Flutamide is a pure antitestosterone without significant progestogenic actions, which blocks the effects of DHT at the testosterone receptor and prevents BPH[5,6]. Mechanism of action of flutamide is the competition with testosterone for the testosterone receptor[7]. Flutamide is an effective drug in the treatment of BPH, which reduces hyperplasia of the prostate rapidly[8].

Materials and method:

Forty five adult male rats of Long Evans strain weighing between 200-300 gms of age 8-10 weeks were used in the present study. All the rats were divided into three groups and were sacrificed on the eleventh day of the study by decerebration under ether anesthesia.

Group A: This group served as the vehicle control group and comprised of 15 rats and each rat received an injection of 0.2 ml of olive oil (vehicle) daily for 10 days.

Group B: This group served as the testosterone control group and comprised of 15 rats and each rat received 0.32 mg testosterone propionate in 0.01 ml of suspension daily subcutaneously for 10 days.

Group C: This group served as the flutamide treated rats and comprised of 15 rats and each rat received 0.32 mg testosterone propionate in 0.01 ml of suspension and flutamide 2.00 mg in 0.08 ml of suspension daily subcutaneously for 10 days.

Table I: Grouping of animals, doses of drugs and sacrifice schedule

Groups	Number of rats	Feeding status	Drug	Dose/kg/ body wt	Dose/rat/ day	Duration of admin-istration (in days)	Day of sacrifice (in days)
A	15	Normal food and water	Vehicle		0.2 ml	10	11
B	15	,,	Testosterone	1.6 mg	0.32 mg at 0.01 ml	10	11
C	15	,,	Testosterone + Flutamide	1.6 mg + 10 mg	0.32 mg at 0.01 ml + 2 mg at 0.08 ml	10	11

Procedure for preparing histological sections:

Out of 15 prostates in each group, histological studies were carried out on six randomly selected specimens.

The ventral prostate was fixed in 10% formal saline solution and processed following routine histological procedure. The tissues were dehydrated in ascending concentration of alcohol, cleared in xylene, infiltrated and embedded in paraffin. Sections of the tissues of 6 µm thickness were made by a rotary microtome and were stained with haematoxylin and eosin (H&E).

Measurement of the average diameter of the acini at low power field (X10 objectives x 10 eyepiece) of microscope:

Three different fields were chosen from each slide for measuring acinar diameter. In each field, three acini were chosen at 10, 2 and 6 o'clock positions for their diameters to be measured. As the sectioned acini were not perfectly round in shape, three measurements were taken from each of those acini. The first one was maximum diameter along the long axis of the acini. Two other measurements were taken perpendicular to the first one, dividing the first measurement line into three equal parts. From the latter two, the average vertical diameter was calculated. Then, a transvertical diameter was calculated by taking the mean of the longest and the vertical diameters. From the three transvertical diameters, the average transvertical diameter for each slide was calculated. From the six mean transvertical diameters, the mean diameter for each group was calculated. All these diameters were measured with the help of an ocular micrometer adjusted to a stage micrometer. The readings were converted to the values by procedure described in standardization of microscopic measurements and expressed in micrometer (µm).

Standardization of microscopic measurements:

(Using an ocular micrometer and stage micrometer from Carl-Zeiss)
100 divisions of the stage micrometer = 1000 µm
So, 1 division of the stage micrometer = 1000÷100 = 10 µm.
For acinar diameter in µm under x10 objectives with a x10 eyepiece, 96 ocular micrometer divisions corresponded to 100 stage micrometer divisions
So, 1 ocular micrometer division corresponded to
= 100 ÷ 96 stage micrometer division
= 1.04 stage micrometer division
Because, 1 stage micrometer division = 10 µm.
Therefore, 1 ocular micrometer division
= 1.04 x 10 = 10.4 µm

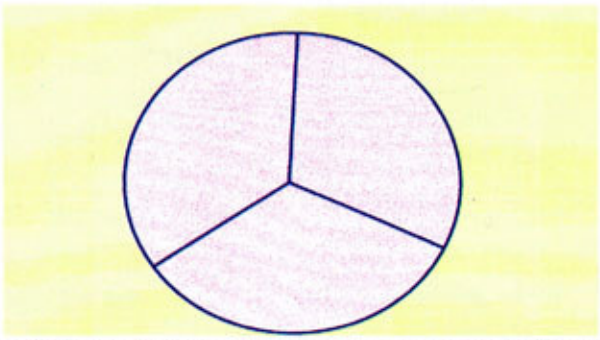

Figure-1: Three different fields on the slide to measure the diameter of the acini.

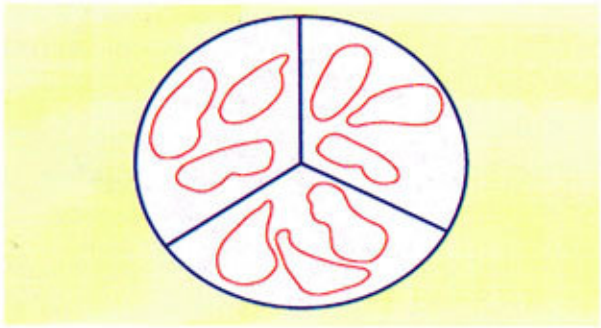

Figure-2: Three acini in each field to measure their diameters.

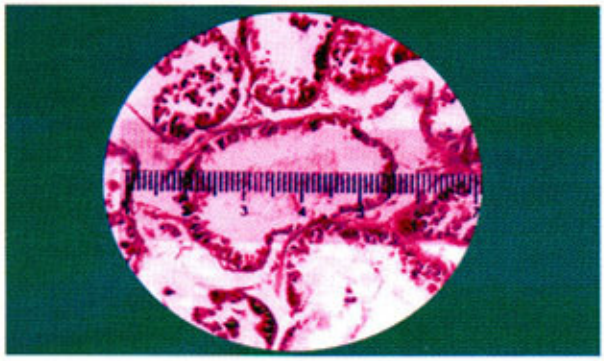

Figure-3: Photomicrograph showing procedure of measuring transverse diameter of the acini of prostate of rat.

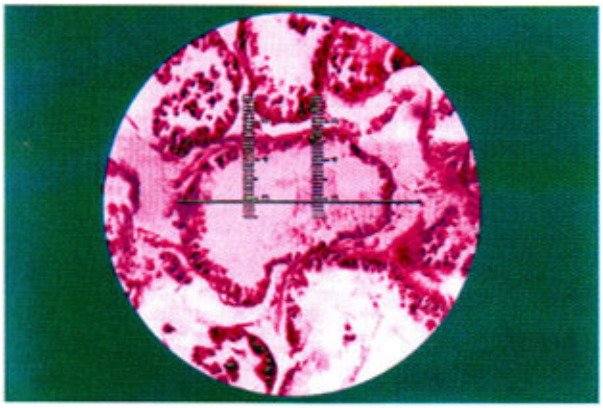

Figure-4: Photomicrograph showing procedure of measuring vertical diameter of the acini of prostate of rat.

Results:

Table-II and Figure-5 show the comparison of diameter of acini between different groups of rats. The mean diameter of acini in the vehicle control rats was 403.17 49.66. The mean in the testosterone treated rats was 527.67 36.30 which was higher than the vehicle treated rats. The mean in flutamide treated group was 416.17 9.58. The value was lower than that of testosterone treated rats. The mean difference was highly significant (p<.001) when compared between the groups.

Table-II: Comparison of diameter of acini between different groups of rats

Diameter of acini (μm)		
Groups	Range	Mean ± SD
A	370 – 492	403.17 ± 49.66
B	495 – 577	527.67 ± 36.30
C	401 – 425	416.17 ± 9.58

A vs B	p <0.001 s
A vs C	p <0.10 ns
B vs C	p <0.001 s

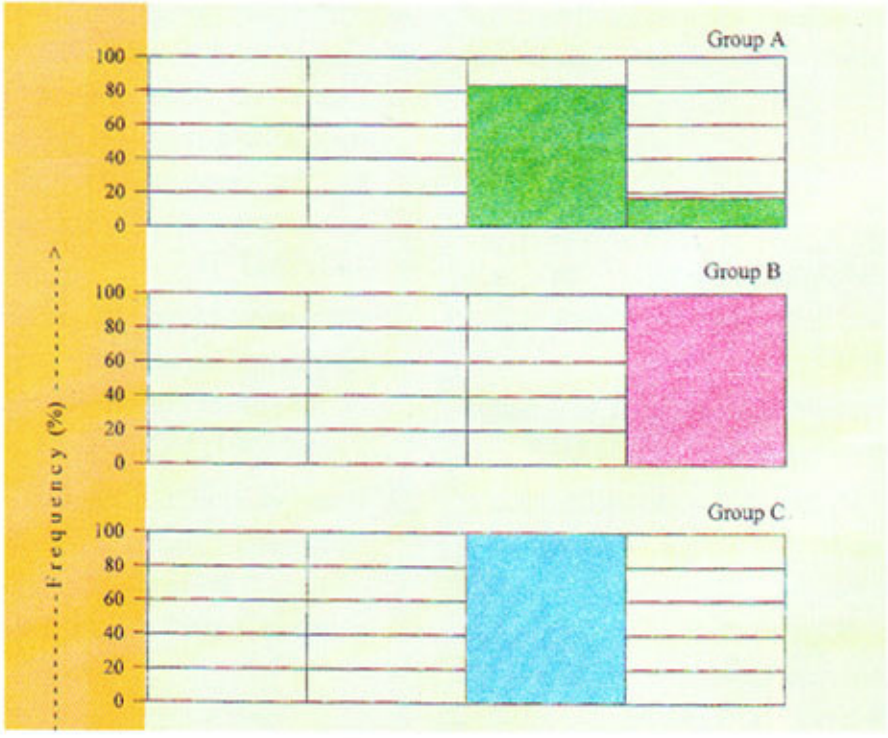

Figure-5: Histogram showing frequency distribution of diameter of acini in different groups.

Discussion:

The increase in values were due to hyperplasia of the acini and stroma of the prostate in testosterone treated rats. Shapiro et al[10], Handlsman[12] also stated that relative increase in diameter of acini and proportion of stroma were related to the effects of testosterone on prostate. In flutamide treated rats the diameter of acini of the prostate was lower than that of testosterone treated rats and the difference was significant ($p<0.001$). Wilson[4] and Niu et al[9] found in their studies the same effectiveness. Flutamide is a nonsteroidal antistestosterone used in the treatment of BPH and prostatic cancer. It effects DHT at the testosterone receptor. The trend of antitestosterone effects of flutamide against testosterone induced hyperplasia is also observed in the present study. Results indicate the effectiveness of flutamide against hyperplastic effect of testosterone.

References:

1 Bruchovsky N, Lesser B, Van Doom E, Cravens. Hormonal effects on cell proliferation in rat prostate. Vitam Horm 1975; 3: 61-102.

2. Davies P, Eaton CL. Regulation of prostate growth. J Endocrin 1991; 131: 5-17.

3. Elias H, Hyde DM. An elementary introduction to stereology. Physiol Rev 1980; 159: 412-23.

4. Wilson JD, Walsh PG. Recent studies on the mechanism of action of testosterone. N Engl J Med 1996; 21: 1284-94.

5. Labric F. Mechanism of action and pure antiandrogenic properties of flutamide. Cancer 1993; 72: 3816-27.

6. Cordero RS, Scarano WR, Goes RM, Tabga SR. Tissue alterations in the guinea pig lateral prostate following antiandrogen flutamide therapy. Biocell 2004; 28: 21-30.

7. Goto K, Koizumi K, Ohta Y, et al. Rats treated with flutamide during late gestation. J Toxicol Sci 2005; 30: 249-59.

8. Kang IH, Kim HS, Shin JH, et al. Comparison of antiandrogenic activity of flutamide, vinclozolin, procymidone and linuron. Toxicology 2004; 199: 145-59.

9. Niu YJ, Mz TX, Zhang J, et al. Androgen and prostatic stroma. Asian J Androl 2003; 5: 19-26.

10. Shapiro E, Becich MJ, Hartanto MJ, Lepor H. The relative proportion of stromal and epithelial hyperlasia is related to the development of symptomatic benigh prostatic hyperplasis. J Urol 1992; 147: 1293-97.

11. Huttunen E, Romppanen, Helminen HJ. A histoquantitative study on the effects of castration on the rat ventral prostate lobe. J Anat 1981; 132: 357-70.

12. Handlsman DJ. Testosterone and other androgens. In: Endocrinology, Third edition. Philadelphia: WB Saunders Company, 1995. pp-2351-61.