HISTOMORPHOMETRIC STUDY OF EFFECTS OF BICALUTAMIDE ON SPERMATOGENESIS IN MALE RATS

Atika Khursheed*, Liaqat Ali Minhas*, Waqar Azim Niaz**

*Army Medical College Rawalpindi, **Armed Forces Institute of Urology

ABSTRACT

Objective: To study the effects of Bicalutamide on spermatogenesis in male rats.

Study Design: Laboratory based randomized controlled trial.

Place and Duration of Study: Anatomy Department, Armed Forces Postgraduate Medical Institute (AFPGMI), Rawalpindi in collaboration with National Veterinary Laboratories (NVL), Islamabad from April 2008 to May 2008.

Material and Methods: Forty adult male Sprague Dawley rats weighing 200-300 grams were randomly divided into two groups, Group A and Group B, each consisting of 20 animals each. Group A was taken as control group and was administered 5 cc of distilled water orally daily for 24 days while group B (Experimental group) was given 5 cc of distilled water daily containing bicalutamide 10mg/ kg/ day for 24 days. All the animals were sacrificed on the next day after the last dose. The testes were removed and fixed in 10% formalin and then processed for paraffin embedding. Five micron thick sections were made. Haematoxylin, Eosin and PAS stains were used. Histomorphometric analysis was done and parameters, including the tubular diameter, height of seminiferous epithelium and germ cell count were noted.

Results: Statistically significant differences were found in tubular diameter, height of seminiferous epithelium and germ cell count in testes of experimental group when compared with the control group

Conclusion: The results showed that the mean tubular diameter, the height of the germinal epithelium of the seminiferous tubules and the number of germ cells were significantly reduced in the experimental group showing that bicalutamide suppresses spermatogenesis in the Sprague – Dawley rats.

Keywords: Bicalutamide, Germ cell, Spermatogenesis

INTRODUCTION

Spermatogenesis is the formation of mature sperms from primitive germ cells occurring in the testis. This process is under hormonal control by hypothalamus-hypophysis -gonadal axis. Androgens are essential for male development and play an indispensable role in the process of spermatogenesis.

Androgens act on their target cells via an interaction with androgen receptors (AR) resulting in direct regulation of gene expression[1]. Antiandrogens block the androgen receptors competitively by producing a different conformational change avoiding participation of testosterone in the cellular processes. In an animal study on rats, the administration of antiandrogen such as flutamide, results in impaired spermatogenesis and dysfunction of accessory sex organs[2].

In rats, spermatogenesis takes place over a period of 48–53 days[3]. In seminiferous epithelium, developing cells are arranged in well defined stages which follow one another in a regular fashion. The time interval between the appearances of the same cell association at a given point of the tubule is called the cycle of seminiferous tubule[4]. It occurs in rats in a specific 12 – 13 days cycle which is divided into 14 stages[5]. Because the tubules are folded repetitively, in a cross section of seminiferous tubule, only one of these stages is recognized.

With the discovery that antiandrogens suppress spermatogenesis in male rats, studies were carried out on antiandrogens like flutamide. The receptors for androgens in the hypothalamus are blocked by flutamide, which interrupts the negative feed back for release of

Correspondence: Dr Atika Khurshid, Assistant Professor of Anatomy, Army Medical College Rawalpindi
Email: atika.waqar@live.com
Received: 31Dec 2010; Accepted: 31 May 2011

LH, resulting in a temporary rise in the secretion of LH[6].

Search of even more selective antiandrogens without any central effect led to the development of a new drug known as bicalutamide (Casodex). Bicalutamide is a peripherally selective non-steroidal antiandrogen used now-a-days in the treatment of prostate cancer. It does not influence release of gonadotrophins into the circulation contrary to flutamide since it does not cross the blood-brain barrier[7]. It is four times more potent than flutamide and has longer half life[8].

Review of published literature revealed some studies advocating no effect of bicalutamide on spermatogenesis in male rats[9], while others mentioning impairment of spermatogenesis in male rats after administration of bicalutamide[10]. Its role in impairment of spermatogenesis in male rats is still debatable. In the light of above mentioned, the present study was designed to see the histomorphometric changes produced in testes of rats after administration of bicalutamide.

MATERIAL AND METHODS

This laboratory based randomized controlled trial was carried out at Anatomy department, Armed Forces Postgraduate Medical Institute (AFPGMI), Rawalpindi in collaboration with National Veterinary Laboratories (NVL), Islamabad from April 2008 to May 2008. Forty adult male Sprague Dawley rats were selected and kept in the animal house of the National Institute of Health (NIH), Islamabad. The animals were divided into two groups of 20 animals each as group A (Control) and group B (Experimental). Control group was administered 5 cc of distilled water daily by oral gavage tube for 24 days while experimental group was administered 5 cc of distilled water daily containing bicalutamide 10mg/ kg/ day for 24 days. The animals were sacrificed 24 hours after the last dose and testes were taken out.

The specimens were fixed, processed and stained using Haematoxylin & Eosin (H&E) for routine histological study of testes and Periodic Acid Schiff (PAS) reagent, counter stained with Harris's haematoxylin, to demonstrate basement membrane of seminiferous tubules. Parameters, including tubular diameter of seminiferous tubules, height of germinal epithelium and germ cell count were noted as per procedure described by Brendtson[11] (measurement of seminiferous tubule diameter can be based on only 15 tubules per testis and counts of germ cells in tubule cross sections can be based on only three or four tubules). Three cross sections of seminiferous tubules (randomly selected) in different fields, were observed under 40X objective using ocular micrometer (Fig. 1).

Statistical Analysis

Data was entered in a database using SPSS version 13. Descriptive statistics were used to describe the data. The statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by independent samples, "t"-test. P-value <0.05 was considered as significant.

RESULTS

All the animals survived and remained active during the duration of experimental period. Each testis, when examined under the microscope, was found to be covered by tough translucent membrane, tunica albuginea. Extending from this capsule were connective tissue septa that divided the organ into compartments containing seminiferous tubules. Cross section of tubules showed germ cells in various stages of development arranged in the cytoplasmic processes of supporting cells resting on basement membrane

Comparison of study variables between both the groups is shown in table 1. The mean tubular diameter was 331.70 µm (SD± 4.68) in the control group while in the experimental group it was 307.29 µm (SD ±4.21), which was highly significant ($p < 0.001$).

The mean height of germinal epithelium was 94.45 µm (SD±2.546) in the control group while in the experimental group it was 78.54 µm (SD±2.46). Reduction in the mean epithelial height of the germinal epithelium in the

experimental group was highly significant ($p < 0.001$).

The mean germ cell count was 334.13 (SD 8.145) in the control group while in the experimental group it was 196.00 (SD 7.377). The count was markedly reduced in the experimental group as compared to the control group. The number of germ cells in the experimental group was almost half of the control group which was highly significant ($p < 0.001$).

DISCUSSION

In the current study, there was no change in colour, consistency or size of testis in both groups, however, the difference was seen in the tubular diameters, height of germinal epithelium and germ cell count.

The mean tubular diameter in the experimental group was markedly reduced in comparison with the mean tubular diameter in the control group. This reduction of tubular diameter in the experimental group was highly significant suggesting that bicalutamide reduces the mean tubular diameter of the seminiferous tubules. This finding is in contradiction to a similar type of study carried out by Bustos – Obregon in 2006[2]. They, however, used flutamide, another antiandrogen, to see the effects on the mouse spermatogenesis and on the functions of the seminal vesicles and the prostate. They observed no change in the tubular diameter of the seminiferous tubules in their experimental group. Similarly in another study conducted by Viguer – Martinez in 1983[6], to see the histological changes produced by flutamide on the testis of adult male rats, observed no change in the tubular diameter of the seminiferous tubules. Since the experiment in both of the studies were carried out for shorter duration of only up to 14 days, perhaps this could be the reason that no significant reduction in the tubular diameters of seminiferous tubules was observed in their results. In our study, the experiment was carried out for a longer duration taking care of overlapping of at least two cycles of spermatogenesis in adult male rats. Since one cycle of spermatogenesis takes 14 days in the adult male rats, the experimental group in our study received drug for 24 days ensuring that at least two cycles of spermatogenesis are observed at the end of the experiment. This fact may have resulted in observing the significant reduction in the tubular diameter of the seminiferous tubules in the experimental group that received bicalutamide in our study.

The present study showed marked reduction in the epithelial height of the seminiferous tubules in the experimental group of animals that received bicalutamide as compared to the control group (Fig. 2). This reduction in the epithelial height of the seminiferous tubules in the experimental group was again highly significant. This finding is in agreement with a similar finding in the study of effect of flutamide on mouse spermatogenesis done by Bustos – Obregon in 2006[2]. In their study, these authors had two subgroups of experimental group of animals. One group of experimental group was sacrificed after 48 hours of intraperitoneal injection of flutamide to the rats while second group of experimental group was sacrificed 72 hours after administration of drug. They found no change in the epithelial height of the seminiferous tubules in the subgroup of animals that were sacrificed 48 hours after drug administration while a significant reduction in the epithelial height of the seminiferous tubules was observed in the subgroup of animals that were sacrificed 72 hours after drug administration. It seems again, that the duration of exposure of animals to the drug was the main reason behind the observation of the reduction in the epithelial height of the seminiferous tubules.

A reduction in the total germ cell count was observed in some of the seminiferous tubules of the experimental group. This reduction of mean germ cell count was highly significant. A similar result of reduced germ cell count was observed by Chandolia et al[7] in March 1991[9], in their study of effects of bicalutamide on spermatogenesis in adult male rats, while in a later study conducted by the same author, in Nov. 1991, bicalutamide was found to have no effects on spermatogenesis in rats.

CONCLUSION

It is concluded that the mean tubular diameter and the height of the germinal epithelium of the seminiferous tubules were significantly reduced in the experimental group. Marked reduction in the number of germ cells in the experimental group was also seen, which was almost half of the control group. The present study thus concluded that bicalutamide suppresses spermatogenesis in the Sprague – Dawley rats.

REFERENCES

1. Brinkmann A.O, Blok LJ, de Ruiter PE, Doesburg P, Steketee K, Berrevoets CA, and Trapman J. Mechanisms of androgen receptor activation and function. J Steroid Biochem Mol Biol. 1999; 69(1-6): 307 – 13.
2. Bustos-Obregon E, Esponda P, and Sarabia L. Effect of flutamide in mouse spermatogenesis and on the function of seminal vesicle and prostate. Int J. Morphol. 2006; 24(2): 171 – 74.
3. Hess RA. Quantitative and qualitative characteristics of the stages and transitions in the cycle of rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic –embedded testes. Biology of Reproduction. 1990; 43: 525 – 42.
4. Parvinen M. Regulation of seminiferous epithelium. Endocr. Rev. 1982; 3: 404.
5. Leblond CP and Clemont Y. Definition of stages of the cycle of the seminiferous epithelium in the rat. Ann. NY Acad Sci. 1952; 55: 548 – 73.
6. Viguier –Martinez MC, Hochereau de Reviers MT, Barenton B and Perreau C. Endocrinological and histological changes induced by flutamide treatment on the hypothalomo-hypophyseal testicular axis of the adult male rat and their incidences on fertility. Acta Endocrinol. 1983; 104(2): 246 – 52.
7. Chandolia RK, Weinbauer GF, Behre HM and Neischiag E. Evaluation of a peripherally selective antiandrogen (Casodex) as a tool for studying the relationship between testosterone and spermatogenesis in the rat. J Steroid Biochem Mol Biol. 1991; 38(3):367 – 75.
8. Furr BJ, and Tucker H. The preclinical development of bicalutamide: pharmacodynamics and mechanism of action. Urology. 1996 Jan; 47(1ASuppl):13 - 25.
9. Chandolia RK, Weinbauer GF, Simoni M, Behre HM, and Nieschlag E. Comparative effects of chronic administration of the non-steroidal antiandrogens flutamide and Casodex on the reproductive system of the adult male rat. Acta Endocrinol (Copenh). 1991 Nov; 125(5):547 - 55.
10. Furr BJ, Valcaccia B, Curry B, Woodburn JR, Chesterson G, and Tucker H. ICI 176,334: a novel non-steroidal peripherally selective antiandrogen. J Endocrinol. 1987 June; 113(3): R7-9.
11. Brendtson WE. Comparative reliability and sensitivity of different methods for assessing treatment effects on sperm production. Anim Reprod Sci. 2008; 105 (1-2): 5 – 22.

Table: 1 Showing quantitative analysis of observed parameters in control and experimental groups

Observed Parameters	Groups	MEAN ± SE	P – value
Tubular Diameter (µm)	Control	331.70 ± 4.680	< 0.001
	Experimental	307.29 ± 4.208	
Epithelial Height (µm)	Control	94.45 ± 2.546	< 0.001
	Experimental	78.54 ± 2.460	
Germ Cell count	Control	334.13 ± 8.145	< 0.001
	Experimental	196.00 ± 7.377	

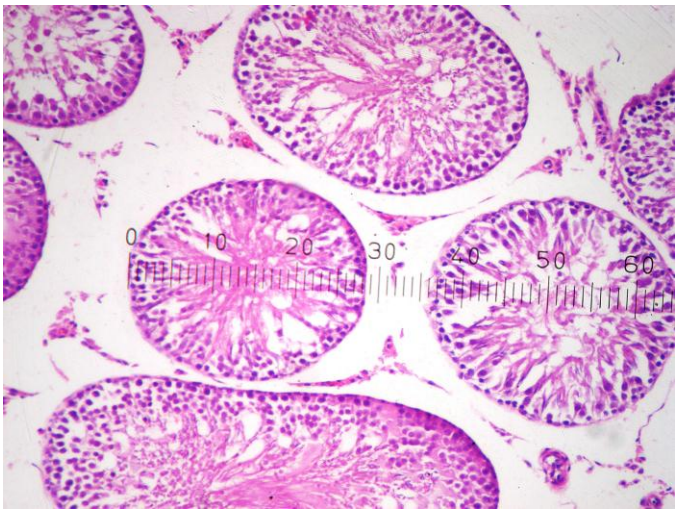
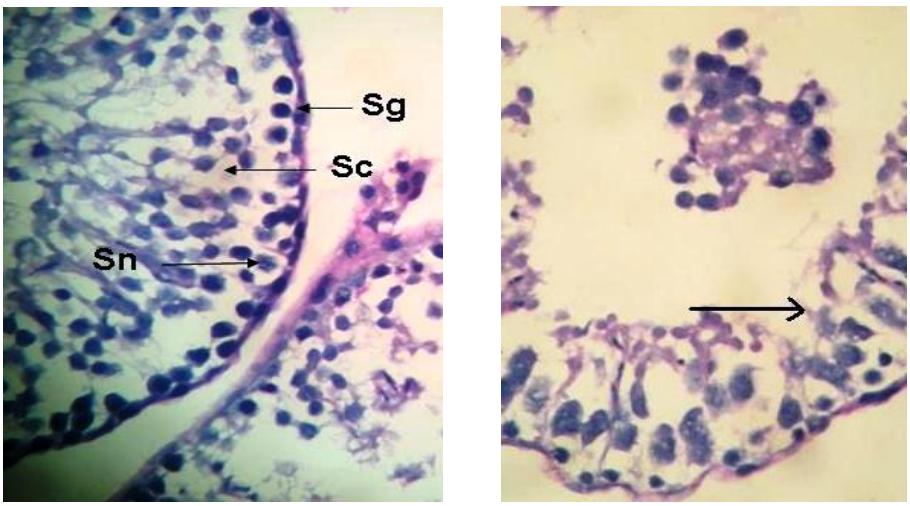


Fig1: Photomicrograph of testis (Control group) showing seminiferous tubules with ocular scale imposed on the tubule measuring tubular diameter. H&E stain. Photomicrograph. Approx. 105 X.



a **b**

Fig. 2 a. Section of testis of Control group (Group A) showing normal germ cell count and height of germinal epithelium. PAS stain. Photomicrograph. 420 X.

b. Section of testis of Experimental group (Group B) showing reduced germ cell count and reduced height of germinal epithelium (arrow). PAS stain. Photomicrograph. 420 X.