HISTOMORPHOMETRIC STUDY OF EFFECTS OF BICALUTAMIDE ON SPERMATOGENESIS IN MALE RATS

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 were used. Group A (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 measured.

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 germin al 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.

INTRODUCTION
Spermatogenesis is the formation of mature sperm 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. 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.

In rats, spermatogenesis takes place over a period of 48–53 days. 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. It occurs in rats in a specific 12 – 13 days cycle which is divided into 14 stages. Because of the tubular folding, 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 feedback for release of LH, resulting in a temporary rise in the secretion of LH.

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.

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 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 24 hours 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 measured.

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. The mean tubular diameter was 331.70 ± m (SD±4.68) in the control group while in the experimental group it was 196.00 (SD±7.37). 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 seminal vesicles and height of 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. 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, 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 our 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).

CONCLUSION

It is concluded that the mean tubular diameter and the height of 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.

Reference
