Journal of Infectious Diseases and Immunity Vol. 3(2), pp.17-23, February, 2011
Available online at http://www.academicjournals.org/JIDI
ISSN 2141-2375 © 2011 Academic Journals

Full Length Research Paper

Fertility in cimetidine and bromocriptine treated rats

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Accepted 23 December, 2010

The present study was designed to see the role of drugs affecting serum prolactin upon the morphology of the gonad of male rat by giving bromocriptine to one group of animals concurrently with cimetidine. This study was conducted at the Department of Anatomy, Army Medical College (AMC), Rawalpindi in collaboration with National Veterinary Laboratories (NVL), Chak Shahzad, Islamabad. Ninety adult young male albino rats between the ages of 60 to 120 days were selected. They were bred in the animal house of the National Institute of Health (NIH), Islamabad and were supplied with diet pellets supplemented with vitamins and water ad libitum. The animals were divided into three groups A, B and C. Each group consisted of thirty animals that were given intramuscular injection for two weeks and killed on the next day. Group A (Control), were given one millitre of normal saline. Group B, were given cimetidine in a dose of 200 mg/kg/bwt/day. Group C, were given cimetidine in a dose of 200 mg/kg/day and in addition, an injection of bromocriptine 2.5 mg/day was also given. The animals were sacrificed, the testis were removed, weighed, studied and then fixed. The spermatogenesis was normal in almost all of the tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like spermatogonia, primary spermatocyes, spermatids early and late, and spermatozoa were absent indicating total atrophy with both Sertoli cells and Leydig cells hyperplasia. It is concluded that the testicular atrophy as evidenced by decrease in diameter of tubules in case of group B and adverse effects on the qualitative changes such as cellular proliferation/spermatogenesis as well as quantitative morphometric parameters such as decrease in thickness of germinal epithelium in case of both group B as well as C could be due to the toxic effect of the drugs on the testes in general and seminiferous tubule in particular.

Key words: Cimetidine, bromocriptine, fertility.

INTRODUCTION

Cimetidine is a known reproductive toxicant as indicated by significantly reduced weight of accessory sex organs (França et al., 2000; Erfan, 2006). In case of men it is responsible for sexual dysfunction (Gill et al., 1991) such as impotence (Schroeder, 2005), modest decrease in sperm count, not enough to affect fertility, decrease in sex desire as well as drive. In woman, it decreases sexual desire causes pain and tenderness in the breast (Schroeder, 2005). Other well documented reports include hyperprolactinaemia (Bateson et al., 1977), galactorrhoea, gynaecomastia (Hugues et al., 2000) and a weak antiandrogenic (Erfan, 2006) effect seen in rats and dogs.

This drug causes tubular atrophy due to Sertoli cell damage (Leslie and Walker, 1977). The testes of high dose showed atrophy/shrinkage which was evidenced by increased number of smaller size semineferous tubules showing shorter height of seminiferous epithelium indicating the adverse effect of drug on spermatogenesis (Qamar and Khan, 2005; Sasso-Cerri et al., 2001). Loss of germ cells was also noticed (Sasso-Cerri and Cerri, 2008). There was a rise in plasma prolactin in patients treated with cimetidine (Park and Selman, 1991). The prolactin response in men was swift and only occurred in association with high circulating concertrations of cimetidine and was blocked by bromocriptine (Burland,

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1977). These observations have led to the proposal that cimetidine is acting directly or indirectly as a dopamine antagonist at the dopamine receptor sites in the anterior pituitary causing increased secretion of prolactin hormone (hyperprolactinaemia) (Brimbeclombe and Duncan, 1977) and since it has already been noted in male rats that there is inhibition of gonadotrophins (Center and Whitehead, 1981) by induced hyperprolactinaemeia, which is associated with hypogonadism in general and testicular atrophy/degeneration (Sasso-Cerri and Miraglial, 2002) in particular.

Objective

The present study was designed to see the role of drugs affecting serum prolactin upon the morphology of the gonad of male rat. It was done by giving bromocriptine to one group of animals concurrently with cimetidine to see whether the former drug (bromocriptine) blocks the effects of the latter drug (cimetidine) on the reproductive systems of rats. The morphology of the gross and microscopic structure of testes, in the male rat were studied and compared with another group of animals who was given cimetidine alone and also to a group of animals who was given just a placebo.

MATERIAL AND METHODS

This study was conducted at the Department of Anatomy, Army Medical College (AMC), Rawalpindi in collaboration with National Veterinary Laboratories (NVL), Chak Shahzad, Islamabad.

Ninety adult young male albino rats between the ages of 60 to 120 days were selected. They were bred in the animal house of the National Institute of Health (NIH), Islamabad and were supplied with diet pellets supplemented with vitamins and water *ad libitum*. The animals were divided into three groups namely:-

Group A

Twenty male rats were given injection of one millitre of normal saline intra- muscularly daily for two weeks and killed on the next day after the last injection. This group served as control for group "B" and "C" (Table 1).

Group B

Thirty male rats were given the injection of cimetidine intra muscularly in a dose of 200 mg/kg body weight daily for two weeks and killed on the next day after the last injection (Table 1).

Group C

Thirty male rats were given injection of cimetidine intra muscularly daily in a dose of 200 mg/kg and in addition, an injection of bromocriptine 2.5 mg was also given intramuscularly to each animal of this group for two weeks and was killed on the next day after the last injection (Table 1). The ocular micrometer scale was coincided with the stage micrometer scale, 40 divisions of the ocular micrometer coincided with 1 division of the stage micrometer. Since 1 division of stage micrometer scale was equal to 100 µm. Therefore, 1 division of ocular micrometer scale was equal to 2.5 µm under high power of the particular microscope used with (40 x objective and 10 x ocular).

The animals to be sacrificed were killed by an over dose of ether anaesthesia, cotton was soaked in ether and placed into the jar. The animal to be sacrificed was lifted by its tail and dropped into the jar and when it became unconscious it was taken out of the jar, placed on a clean sheet of paper on a dissecting board and while still keeping continuously anaesthetized by a bottle covering the head of the rat containing swabs soaked in ether. The chest was palpated for locating the heart. Once it was located, it was penetrated by the needle of a 5 to 10 ml plastic aseptic syringe to draw 2 to 10 ml blood directly from the heart. These collected blood samples were then immediately transferred into labeled plastic pipettes kept in a rack refrigerated at 2 to 8ºC for a few days till they were centrifuged later on to separate serum which was kept frozen at - C in labeled vancure bottles for enzyme immunoassays for the quantitative measurement of rat prolactin by an EIA kit catalog number 12-MKVRP1, size 96 Tests, version 2003-09-09 – ALPCO 10-02-03. 20º.

Statistical analysis of the data

The statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by "Student" "t" test (Glaser, 2001). The difference was regarded statistically significant if the "P" value was equal to or less than 0.05. "P" value was found by means of 't' distribution table with the help of which each 'P' value was read against the degree of freedom (d.f.) which in turn was determined by the formula:

$$d.f. = n_1 + n_2 - 2$$

To reduce the sampling error among the group for comparing mean weights, the pooled t test was applied. All calculations were done utilizing computer software, 'Microsoft Excel' in windows 2000 XP and SPSS version 10 (using one way ANOVA) followed by a post hoc test like LSD.

OBSERVATIONS AND RESULTS

The diameter of seminiferous tubules was measured (in micrometers under low power field) with ocular micrometer. The height of germinal epithelium was measured (in micrometers under high power field) with the same ocular micrometer.

Germ cells study:- Spermatogonia, spermotocytes, and spermatids were studied under oil immersion.

Qualitative findings of group A (control)

The seminiferous tubules did not show any disruption of the basement membrane/germinal epithelium/both. The basement membrane was not found discontinuous/thickened. The process of spermiogenesis proceeded normally as indicated by tails of sperms and residual bodies. Different types of spermatogonia A/B or intermedidate were seen near the basement membrane (Figure 1).

Table 1. Experimental schedule.

S. no	Groups	No. of animals	Treatment	Dose	Duration	Sacrificed
1.	A(Control)	20	Inj. normal saline (I/M)	1 ml (in 2 equal divided doses)	2 weeks	15th day
2.	B(Treated)	30	Inj. Cimetidine (I/M)	200 mg/Kg in 2 equal divided doses	2 weeks	15th day
3.	C(Treated)	30	Inj. Cimetidine (I/M) + Inj. Bromocripte (I/M)	200 mg/kg + 2.5 mg/day (in 2 equal divided doses)	2 weeks	15th day

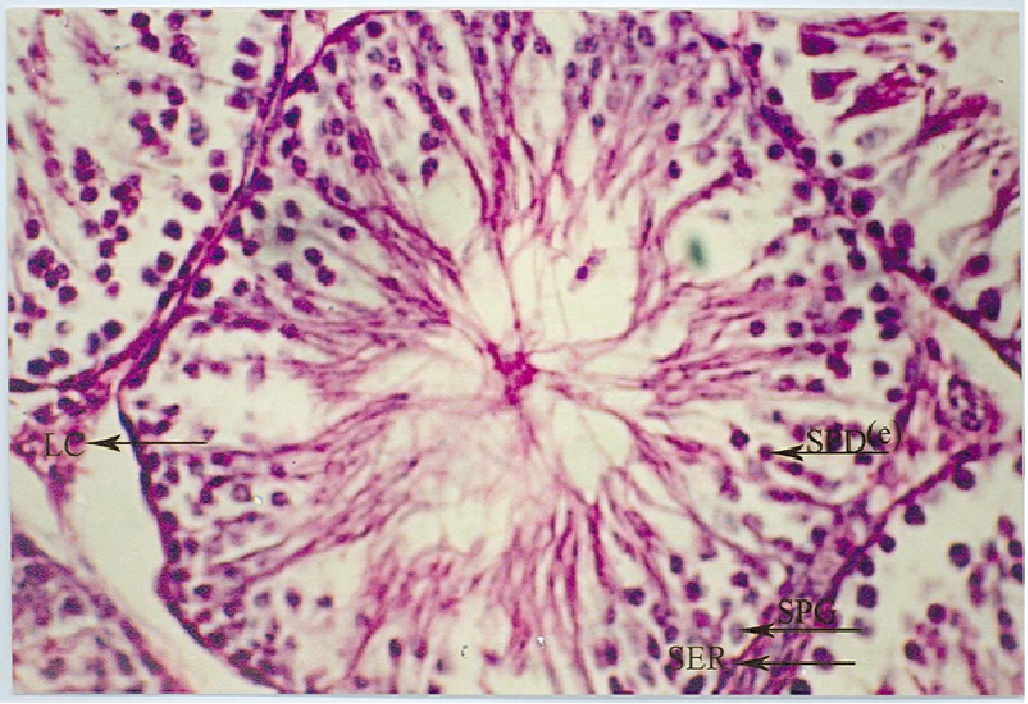

Figure 1. Section of testis Control (Group A) showing a number of seminiferous tubules. On the left of the central tubule could be seen Leydig cells (LC) in an interstitial space. The central tubule shows the lining cells of germinal epithelium. Spermatogonia (SPG) and sertoli cell (SER). Spermatid early SPD(e). Haematoxylin and eosin stain. (Photomicrograph x 400).

Quantitative findings of group A (control)

The mean serum prolactin was 19.38 + 0.85 ng/dl (Table 4). The mean diameter of tubules was 246.75 + 5.211 micrometer (Table 3).

Qualitative findings of group B

All tubules did not show disorganisation but a few of them displayed disruption and disorganisation of germinal epithelium or both (Figure 2). The spermatogenesis was normal in almost all of the tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like spermatogonia, primary spermatocyes, spermatids early and late, and spermatozoa were absent indicating total atrophy with both Sertoli cells and Leydig cells hyperplasia.

Quantitative findings of group B

The mean serum prolactin level was 18.37 + 0.88/ng/ml which was higher than that of the control but lower than that of group C. The mean diameter of seminiferous tubules was 229.33 + 3.57 micrometers which was lower than that of the control as well as group C (Table 3).

Qualitative findings of group C

Both normal and abnormal germinal epithelium was seen

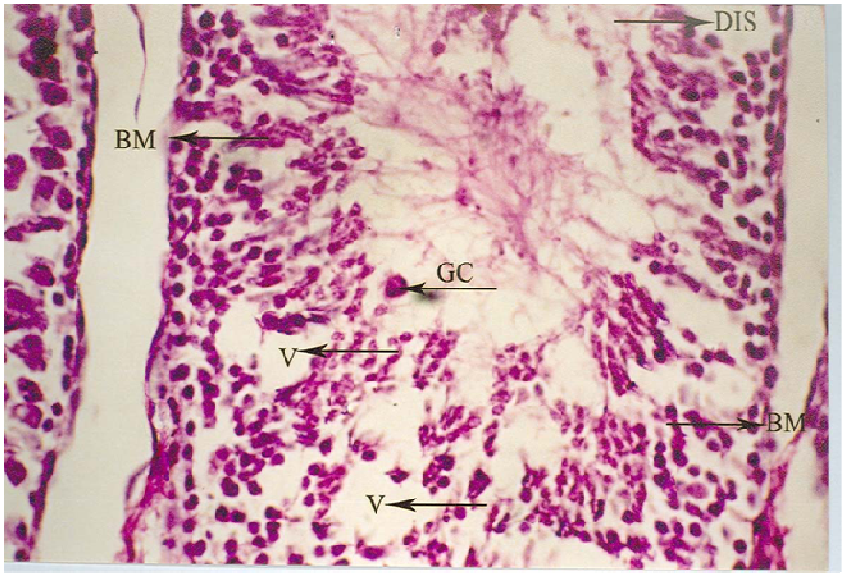

Figure 2. Section of cimetidine treated testis (group B), showing a part of seminiferous tubule with disrupted basement membrane (BM) disruption (DIS), and vacuolization (V) of germ cells. Near the lumen of the tubule could be seen a giant cell (GC) Haematoxylin and eosin stain. (Photomicrograph x 400).

Table 2. Mean* thickness of germinal epithelium (µm) of testes of animals of different groups.

Groups	Diameter of tubules (MM)
A n = 20	246.75 ± 5.211
B n = 30	229.33 ± 3.572
C n = 30	239.60 ± 3.417
* Mean ± SEM	

Statistical analysis of diameter of seminiferous tubules within groups	
Groups	**P- value**
A VS B	P < 0.004
A VS C	P > 0.2
C VS B	P < 0.05

(Figure 3) in same/different tubules but a few of them were seen lined with only Sertoli cells and all the other germ cells like spermatogonia, primary spermatocytes, spermatids early and late, and spermatozoa were absent (Figure 3).

Quantitative findings of group C

The mean serum prolactin level was shown to be 20.64 + 0.51 ng/dl which was found to be highest amongst all the three group that is, higher than the control as well as group B. The mean diameter of tubules was 239.6 + 3.41 micrometers which was found to be higher than group B but lower than control group A (Table 3). The mean thickness of germinal epithelium was 59.32 + 6.057 μm. It was lowest amongst all the three groups that is, AB and C. It was found to be lower than both B as well as control (Table 2).

DISCUSSION

Group B is supported by the following quote: “Histologically signs of degeneration of testicular elements appeared after administration of cimetidine significant decrease in tubular diameter and germinal epithelial cell height” (Mehmood et al., 1996; Gill et al., 1991). The former (morphometric histologic parameter) confirm the shrinkage or atrophy of testes while the latter

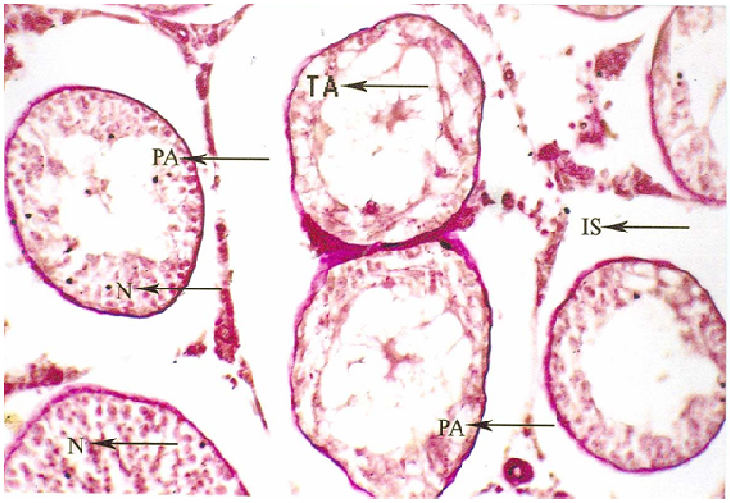

Figure 3. Section of cimetidine and bromocriptine treated testis, (group C), showing normal (N) and abnormal (PA) germinal epithelium in same or different seminiferous tubules. In the middle of the section one of the upper seminiferous tubule is showing total atrophy (TA) and a lower seminiferous tubule is showing partial atrophy (PA), There is a sparse tissue seen in the interstitial space (IS), PAS and Harris Haematoxylin stain (Photomicrograph x 400).

Table 3. Mean* diameter of seminiferous tubules (µm) of testes of animals of different groups.

Groups	Diameter of tubules (MM)
A n = 20	246.75 ± 5.211
B n = 30	229.33 ± 3.572
C n = 30	239.60 ± 3.417
* Mean ± SEM	

Statistical analysis of diameter of seminiferous tubules within groups

Groups	P- value
A VS B	P < 0.004
A VS C	P > 0.2
C VS B	P < 0.05

Table 4. Mean* final serum prolactin (NG/DL) of animals of different groups.

Groups	Serum prolactin (NG/DL)
A n = 20	19.38 ± 0.8512
B n = 30	18.37 ± 0.8877
C n = 30	20.64 ± 0.5160
* Mean ± SEM	

Statistical analysis of final serum prolactin within groups

Groups	P- value
A VS B	P > 0.3
A VS C	P > 0.2
C VS B	P < 0.02

finding probably indicates the adverse effect of cimetidine upon cellular proliferation/spermatogenesis. This is partly in accord with the morphometric findings of the high dose group of our previous study (Qamar and Khan, 2005) where an increase in the tubular count was also noted, while the above mentioned quantitative finding was not looked for in the present study. Qualitatively there was no abnormal finding in case of the high dose group as well as the low dose group of our previous study (Qamar and Khan, 2005) which is in complete disagreement with the qualitative findings of the data of the present study which varied from normal to total atrophy of germ cells. This qualitative finding is partly in accord with another study (França et al., 2000). Abnormal tubules exhibiting disorganization of cellular associations/components. This finding is consistent with the results of another study (Sasso-Cerri et al., 2001).

The seminiferous tubules appeared more compact at the periphery than at the centre of the section with sparse interstitial tissue a feature which could be due to damage done during processing/artefacts/species/strain specific. In case of group C treated with both 200 mg/kg of cimetidine and 2.5 mg/day of bromocriptine for 2 weeks. Though, there was an unexpected slight rise in serum prolactin which was not significant, all the parameters remained within normal limits except unexpected significant increase in the absolute weight of testis and significant decrease in height of germinal epithelium of its seminiferous tubules while prolactin was found elevated in case of group C which could probably be due to ineffective dosage/neutralization by bromocriptine but as we have already noticed in case of group B that there is an unexpected decrease instead of increase in the level of serum prolactin though not significant when compared with control therefore in case of group C perhaps due to some technical flaw.

Bromocriptine on the contrary has shown a paradoxical effect of acting synergistically/agonistically with cimetidine by increasing serum prolactin though not significant when compared with control and significant when compared with group B and as we already know that elevated PRL induces apoptosis. On the basis of the results of group C it is proposed that since both drugs, cimetidine as well as bromocriptine have acted synergistically/agonistically to raise the level of serum prolactin though insignificant which is not blocked by a meager 2.5 mg/day dose of bromocriptine. So the adverse effect on the height of germinal epithelium as well as qualitative testicular changes could be due to:

1. A low dose of bromocriptine.
2. Different species/strain of rat.
3. Resistance of the drug (bromocriptine) itself.
4. Ineffective/tolerant brand of bromocriptine.
5. Shorter duration of study.
6. Increased production of stress.

On the basis of the results of present experiment it is concluded that the testicular atrophy as evidenced by decrease in diameter of tubules in case of group B and adverse effects on the qualitative changes such as cellular proliferation/spermatogenesis as well as quantitative morphometric parameters such as decrease in thickness of germinal epithelium in case of both group B as well as C could be due to the toxic effect of the drugs on the testes in general and seminiferous tubule in particular. In the present study it has not been shown to be mediated through hormones which needs further research work.

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