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Full Length Research Paper

Influence of cimetidine and bromocriptine on weight of rats and its relation with fertility

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The present study was designed to see the influence of parenterally administered drugs cimetidine and bromocriptine on the weight of adult male albino rats and its relation with fertility. Ninety adult young male albino rats between the ages of 60 to 120 days were selected. The animals were divided into three groups. Cimetidine was administered in a dose of 200 mg/kg body weight to group B intramuscularly and in addition to cimetidine, bromocriptine in a dose of 2.5 mg/day intramuscularly was given to group C. Normal saline was administered intramuscularly to control group A. Spermatogonia, spermotocytes, and spermatids were studied under oil immersion. The difference between the initial and final body weight of group B was found to be highly significant statistically ($P < 0.001$) within the same group and also the mean final body weight was regarded highly significant statistically ($P < 0.005$) when compared with group C but when compared with control it was found insignificant. The difference between the initial and final body weights of group C was not significant within the group but it was definitely highly significant statistically ($P < 0.001$) and ($P < 0.005$) when compared with control group A and group B respectively. The spermatogenesis was normal in almost all of the tubules of group B 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. However, the seminiferous tubules of group C were showing disorganisation/ disruption or both at the level of basal compartment of germinal epithelium in small quadrants, a quarter, half or more than half of their tubules indicating partial atrophy. Both normal and abnormal germinal epithelium was seen 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. On the basis of the results of present study we could not exclude the possibility that besides the known anti-androgenic effect of cimetidine, a possible interference of cimetidine on the histoarchitecture of the seminiferous epithelium, as well as lack of other biochemical factors essential for spermatogenesis could be involved in the testicular changes/ alterations of both groups B and C.

Key words: Fertility, cimetidine, bromocriptine.

INTRODUCTION

Cimetidine (Tagamet) has been widely prescribed for about 20 years worldwide. It is a potent histaminic H_2-receptor antagonist extensively prescribed for ulcers and is available without prescription (Gill et al., 1991). The major therapeutic indications for cimetidine is for promoting healing of gastric and duodenal ulcers and for prophylaxis of stress ulcers (Schupp et al., 2003) Cimetidine can be administered intravascularly for acute gastrointestinal disorders or can be taken orally for chronic gastrointestinal problems.

Cimetidine is also known reproductive toxicant as indicated by significantly reduced weight of accessory

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sex organs (Françe et al., 2000). H_2 receptors are found in vascular smooth muscle cells, but the presence of H_2-receptors in the testis has not been demonstrated (Albrecht et al., 2005.).

The data strongly point to cimetidine as a testicular toxicant and that the peritubular myoid cells are a target for cimetidine action (Sasso-Cerri and Cerri, 2008). The data suggest that more studies in the human are necessary to determine if adverse cimetidine-related reproductive consequences may occur (Takeshi et al., 2002).

A well known side effect of cimetidine is its ability to competitively block dihydrotestosterone by occupying androgen receptors, making it a weak anti-androgen for tissues requiring dihydrotestosterone.

Peripheral accessory organ weights are reduced in rodents, probably due to dihydrotestosterone deficiency. The consequences of the loss of dihydrotestosterone activity in the testis are not known since the role of dihydrotestosterone is currently under investigation in regards to its ability to support spermatogenesis (Gill et al., 1991).

A significant decrease in absolute and relative testicular size of high dose group has also been reported (Sasso-Cerri et al 2001).

This finding is partly in accord with the similar finding reported earlier.

According to the former author, the testes of high dose group showed atrophy/shrinkage which was evidenced by the statistically significant histologic findings of increased number of smaller size seminiferous tubules showing shorter height of seminiferous epithelium indicating the adverse effect of drug on cellular proliferation/spermatogenesis.

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) (Knigge, 1990) and since it has already been noted in male rats that there is inhibition of gonadotrophins by induced hyperprolactinaemeia (Watanobe and Takebe, 1987), which is associated with hypogonadism in general and testicular atrophy-/degeneration, in particular. A decrease in testicular weight is also reported in rats treated with a high dose of 950 mg/kg/day.

The reduction in testicular size is also supported by other statistically significant parameters determined by morphometry.

As the above noted side effects could be due to raised levels of prolactin occurring by administration of cimetidine which is known to cause elevation of serum prolactin (hyperprolactinaemia) (Okada et al., 1996) and which could be blocked by a prolactin depressing drug such as bromocritpine, therefore the present study was designed to see the influence of parenterally administered drugs cimetidine and bromocriptine on the weight of adult male albino rats and its relation with fertility.

RESEARCH DESIGN AND METHODS

Animals

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 ethical committee of Army Medical College approved the protocol.

Treatment

The animals were divided into three groups. Out of them thirty male rats was in group A given injection of one ml of normal saline intra-muscularly daily for two weeks. This group served as control for group “B” and “C”. Thirty male rats were in group B given the injection of cimetidine intra muscularly in a dose of 200 mg/kg body weight daily for two weeks. Thirty male rats were in group C 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. All the animals were killed on the next day after the last injection. Two drugs cimetidine and bromocriptine were administered mainly the ulcerex brand (SAMI PHARMACEUTICALS) cimetidine ampoules containing 200 mg in 2 ml solution were used undiluted in these experiments. Two ml/kg body weight of the drug was injected intra muscularly twice a day at an interval of six hours, one injection at 9.00 a.m. and another at 3.00 p.m. for a period of two weeks to group “B” and “C”. Bromocriptine is available in the form of tablets of 5 mg with the trade name of ‘Parlodel’, manufactured by Sandoz Pakistan. Fifteen 5 mg tablets of bromocriptine were crushed, powdered and mixed with thirty ml of distilled water to make a suspension. Out of this solution 0.5 ml which contained 1.25 mg of bromocriptine was injected intra muscularly twice a day at an interval of six hours immediately after injecting cimetidine as mentioned above to rats of group “C” only for a period of two weeks. The site of injection had to be changed frequently because of local swelling and induration caused by repeated injections with one ml tuberculin plastic syringes having 0.01 ml graduations for cimetidine and 3 ml plastic syringes for injecting bromocriptine suspension as it was quite thick as compared to cimetidine solution.

The animals used in the present study were numbered and weighed initially before starting the experiment and again finally at the end of the experiment (Table 1). 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 scrotal sac was then opened with the help of forceps and scissors. Testes were removed, weighed and then put into 10% formalin for fixation. The relative weight of testes is also known as gonado-somatic index (GSI) was calculated with the help of following formula:-

$$GSI = 100 \times \frac{\text{weight of testes (g)}}{\text{body weight (g)}}$$

Table 1a. Mean*body weights (g) of animals of different groups.

Groups	Initial	Final
A n = 20	224.55 ±8.615	241.05 ± 9.881
B n = 30	221.13 ± 9.389	257.13 ± 6.913
C n = 30	268.86 ± 4.652	283.5 ± 4.709

*Mean ± SEM.

Table 1b. Statistical analysis of the difference in mean body weights within groups.

Groups	P-value
Ai Vs Af	P > 0.2
Bi Vs Bf	P < 0.0001
Ci Vs Cf	P > 0.1

Table 1c. Statistical analysis of final body weights within groups.

Groups	P-value
Af Vs Bf	P > 0.1
Af Vs Cf	P < .001
Cf Vs Bf	P < .005

Table 2a. Mean* absolute weights (g) of testes of animals of different groups.

Groups	Absolute weight of testes (G)
A n=20	1.044 ± 0.0492
B n=30	1.008 ± 0.0254
C n=30	1.151 ± 0.0217

* Mean ± SEM.

Table 2b. Statistical analysis of absolute weights of testis within groups.

Groups	P- value
A VS B	P> 0.4
A VS C	P< 0.02
C VS B	P< 0.001

Table 3. Mean* relative weights (mg/g) of testes of animals of different groups

Groups	Relative weights of testes MG/G
A n=20	4.365 ± 0.0225
B n=30	3.946 ± 0.0151
C n=30	4.010 ± 0.0117

* Mean ± SEM.

Table 3b. Statistical analysis of relative weights of testis within groups.

Groups	P- value
A VS B	P > 0.07
A VS C	P > 0.1
C VS B	P > 0.7

Besides weights (initial and final) of animals, weights (absolute and relative) of testes (Tables 2 and 3), Spermatogonia, spermotocytes and spermatids were also studied under oil immersion. Stages of spermatogonia were designated accordingly to Leblond and Clermont (Leblond and Clermont, 1952).

Statistical analysis

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.

The results are presented as mean ± SEM, the statistical significance of the difference of various quantitative changes between the experimental and control groups was evaluated by "Student" "t" test The difference was regarded statistically significant if the "P" value was equal to or less than 0.05.

RESULTS

The weights of the animals at the beginning and at the end of the experiment were noted in Table 1.

Absolute and relative weights of the testes, gross and microscopic examination was carried out.

The color, consistency and appearance of testes were noted. The testes of both right and left sides were studied separately and as the difference in the observations between the two sides was not significant statistically, the data for both right and left testes was pooled together.

Quantitative and qualtitative findings of group a (control)

Initially we started with thirty rats but since ten of them died on the very same date due to change of place and bad weather conditions prevailing in our laboratory as there was some fault in the cooling system.

Therefore we were forced to proceed with only twenty animals as controls in the present study as compared to thirty animals in both experimental groups B and C and

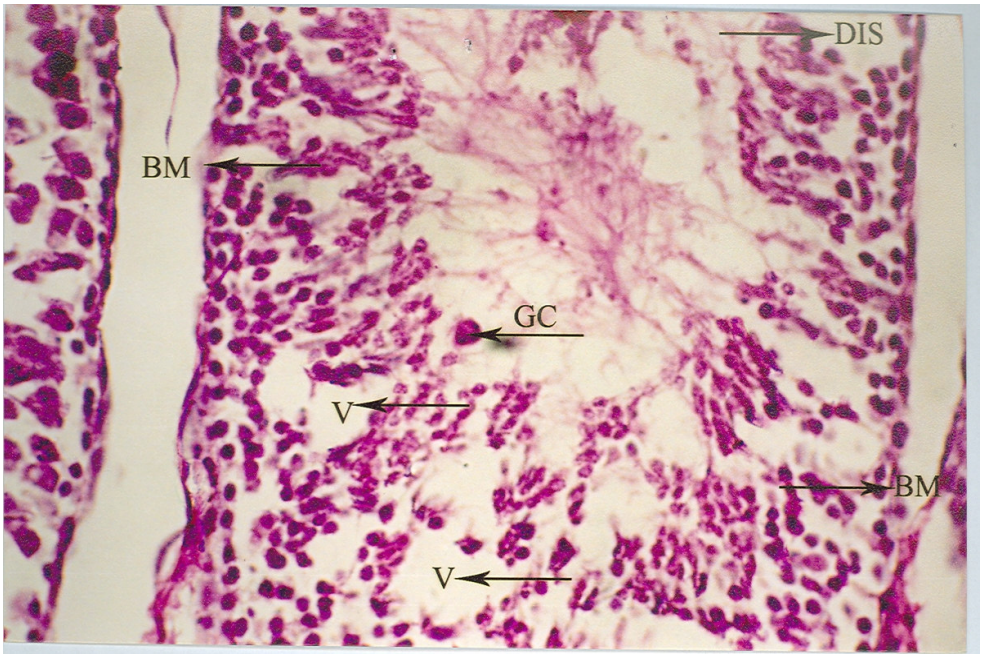

Figure 1. Section of cimetidine treated testis (group B), showing a part of seminiferous tubule with disrupted basement membrane (BM) disruption (DIS), and vacuolization (V) of germ cells. Near the lumen of the tubule could be seen a giant cell (GC) Haematoxylin and eosin stain. (Photomicrograph x 400).

also since ten adult male rats of the same strain were not available at the present centre.

The testes were pink, firm almost uniform size except one, rat number five (5) who showed abnormally small testes weighing 0.35 g each as compared to the majority of others who were mostly greater than 1 g gross structure of the testes did not reveal any thing abnormal most of the tubules did not show disorganization of germ cells as indicated by study carried out under oil immersion. Central/peripheral degeneration or hyalinization was not seen.

Quantitative and qualtitative findings of group B

A few seminiferous tubules were visible with basement membrane which was disrupted/ discontinuous at places (Figure 1), while thickened/hyalinised in atrophic tubules. 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 and qualtitative findings of group C

Most of the seminiferous tubules were showing disorganisation/disruption or both at the level of basal compartment of germinal epithelium in small quadrants, a quarter, half or more than half of their tubules indicating partial atrophy (Figure 2).

Both normal and abnormal germinal epithelium was seen 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. The process of spermatogenesis was variable and appeared to be normal in most but in some it was found to be suppressed.

The process of spermiogenesis was variable appeared to be normal in most but in some it was again found reduced/suppressed. There was a focal disruption of germinal epithelium as well as disorganization with disruption of basement membrane/both.

DISCUSSION

Role of drugs upon the morphology of the gonad of male rat by giving bromocriptine to one group of animals concurrently with cimetidine to see whether the former drug (bromocriptine) block the effects of the latter drug (cimetidine) on the reproductive systems of rats by studying the morphology of the gross and microscopic structure of testes, in the male rat and to compare it with another group of animals who was given cimetidine alone and also to a group of animals who was given just a

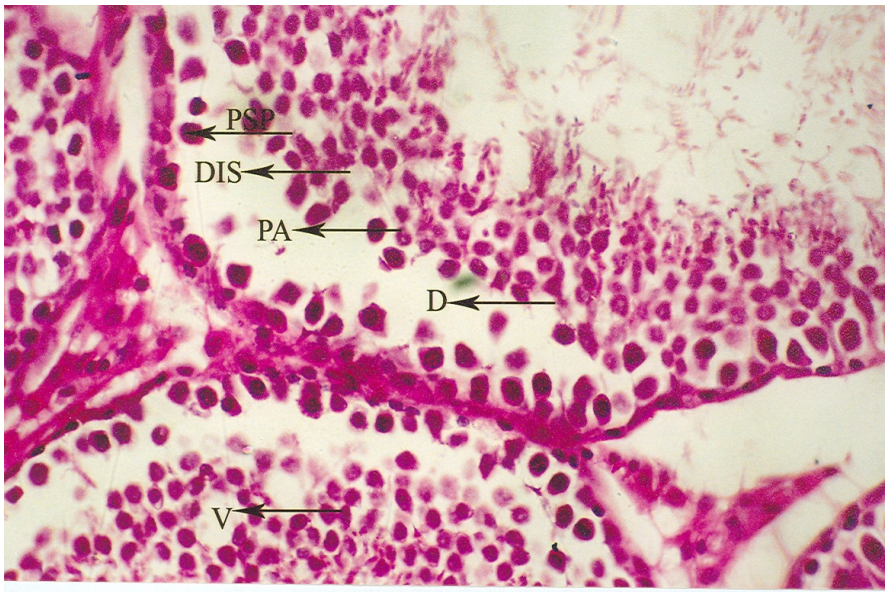

Figure 2. Section of cimetidine and bromocriptine treated testis, (GROUP C), showing a number of seminiferous tubules with vacuolization (V), disorganisation (D) and disruption (DIS) of germinal epithelium/ or both indicating partial Atrophy (PA) Primary spermatocyte (PSP) is also seen in a right upper seminiferous tubule. PAS and Harris Haematoxylin stain (Photomicrograph x 400).

placebo. In the present study we used intra- muscular administration since it was the most convenient route for a rodent model. Furthermore, our treatment extended approximately two weeks since the most common use of cimetidine is over a long duration. Regarding the results of group B the testes were reduced in size as indicated by their lowered mean absolute testicular weight which was statistically significant ($P < 0.001$) when compared with group C but it was not significant statistically when compared with control group A.

This finding is partly in accord with a similar study reported earlier (Sasso-Cerri et al., 2001) where 50 mg/kg cimetidine was given to male Wister rats over 52 consecutive days and it produced significant reduction of testicular weight. This finding is partly in disagreement with another study reported earlier (França et al., 2000) where testes weight was not significantly reduced with a high dose of 250 mg/kg/bwt of cimetidine.

Paradoxically as this decrease was associated with a significant increase in the mean final body weights of group B so the non-specific effect of cimetidine was excluded which could have been the cause of reduction in the testicular weight.

The results of the present study indicated a decrease in the relative testicular weight of group B as compared to control group A.

Though the decrease in the relative testicular weight of group B as compared to control group A was not found statistically significant. This finding is partly in accord with the similar finding reported earlier where a high dose of 950 mg/kg of cimetidine was given for one, three, six and twelve months respectively.

As quoted by them (Leslie and Walker), the reduction in the testicular size was noticed after a prolonged treatment of six and twelve months and not after a very short period of one and three months respectively (Leslie and Walker, 1977).

Initially we started with thirty rats but since ten of them died on the very same date due to change of place and bad weather conditions prevailing in our laboratory as there was some fault in the cooling system. Therefore we were forced to proceed with only twenty animals as controls in the present study as compared to thirty animals in both experimental groups B and C and also since ten extra adult male rats of the same strain were not available at the present centre.

In case of present study, the decrease in testicular size was noticed comparatively earlier after a very short period of two weeks. This is partly in agreement with our previous study (Qamar and Khan, 2005) where a significant decrease in both absolute and relative weight of the testes of high dose (950 mg/kg/bwt) occurred after a very short period of just ten days.

The difference in the period of occurrence of reduction of testicular weight and size could be attributed to (different routes of administration used in the two experiments). In a report of number of previous studies (Leslie and Walker 1977; Erfan, 2006) “oral route” was employed while in case of present experiment “parenteral route” was opted for which is partly consistent with previous studies reported earlier (França et al., 2000; Sasso-Cerri et al., 2001) and also in agreement with the

low dose of our pre- vious study (Qamar and Khan, 2005) where 150 mg/kg body weight of cimetidine was given intra muscularly for three weeks, also in agreement with the high dose of our previous study (Qamar and Khan, 2005) where 950 mg/kg body weight of cimetidine was given intramuscularly for ten days only.

Since both the routes have their peculiar merits and demerits, parenteral (intra-muscular) route was preferred in case of both group B as well as group C of present study as well as in the high and low dose of our previous study (Qamar and Khan, 2005) because it ensures "better absorption" and "lesser dependency" on gastrointestinal tract which is not predictable in case of oral route employed by previous workers (Leslie and Walker, 1977; Erfan, 2006).

While considering intra-muscular route of administration 15, one of the factors responsible for producing physical stress in case of present study and as acute release of both pituitary prolactin and ACTH occurs following a variety of stressful situations (Harms et al., 1975).

The possible mechanism which could be responsible for reduction in testicular weight could be attributed mainly to release of prolactin and since there is ample evidence of rise in plasma prolactin in patients treated with cimetidine orally (Kruss and Littman, 1978), intravenously (Nelis and Van de meene, 1980) or by intraventricular infusion (Reynolds et al. 1980) (ICV).

From the above discussion it is concluded that the testes have reduced in size in case of group B.

This atrophy could be an antiandrogenic effect of the drug cimetidine on testes in general and seminiferous tubules in particular or according to our hypotheses that cimetidine, a dopamine antagonist, is responsible for increased production of prolactin which in turn is responsible for hypogonadism/gonadal dysfunction/ decreased size of testes.

In case of group C since the absolute size of testis has not decreased rather on the contrary/paradoxically it has shown a significant increase which indicates enhanced spermatogenesis at the level of testis which is further supported by the work (Jabbour et al., 1998) which detects localization of expression of the prolactin receptor gene in the seminiferous tubule compartment and in the interstitial Leydig Cell compartment implicates prolactin as a possible gonadotrophic hormone regulating the process of both spermatogenesis and steroidogenesis in the (ungulate speces) red deer testis.

The pattern of expression of the prolactin receptor gene on germ cells imply a role for prolactin in the differentiation of spermatocytes or the regulation of the cell division cycle, or both, by acting as a meiotic inducer. In concert with gonadotrophins, prolactin may be an important factor in enhancing the efficiency of spermatogenesis.

The results of the present study indicated insignificant reduction in relative weights of testes of group B as well as group C.

While statistically significant increase in the absolute weight of testes of group C but insignificant reduction in the absolute weight of testes of group B.

Though the rate of increase in mean final body weights of groups B and C was not affected by the treatment. Qualitative analysis of the histology of testis of both groups B and C revealed a high degree of variability with most tubules appearing normal while abnormal tubules exhibiting disorganization of their cellular association with/without disruption of basement membrane On the basis of the results of present study we could not exclude the possibility that besides the known anti-androgenic effect of cimetidine, a possible interference of cimetidine on the histoarchitecture of the seminiferous epithelium, as well as lack of other biochemical factors essential for spermatogenesis could be involved in the testicular changes/ alterations of both groups B and C while increase in the absolute weight of testes of group C indicates enhanced spermatogenesis which could be a direct stimulatory effect of bromocriptine on spermatogenesis through follicle stimulating hormone. Since the dose of bromocriptine was not enough to neutralize/ counteract the anti-androgenic effect of cimetidine, the former drug was unable to show improvement in the morphometic parameters of the seminiferous tubules of group C for which further research work using low dose and longer duration is required.

Therefore, due to the limitations of the former study exact mechanism involved in testicular atrophy could not be determined. Hence, further research is required to elaborate the factor/factors responsible for decrease in weight/size of testes.

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