STUDY OF THE EFFECTS OF CIMETIDINE UPON RAT TESTIS

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ABSTRACT

The effect of parenterally administered high (950 mg/kg) and low (150 mg/kg) dose of cimetidine on germinal epithelium of seminiferous tubules of testis in adult albino rats was studied. Cimetidine in high doses produces reduction in testicular weight and size, increase in tubular count per low power field and decrease in diameter and thickness of germinal epithelium of seminiferous tubules. The testicular changes observed could be due to toxic affect of drug on the body in general and testis in particular or it may have been mediated through hormones.

Keywords: Cimetidine, testis, spermatogenesis

INTRODUCTION

Cimetidine, a histamine H_2 receptor antagonist [1] is a potent inhibitor of gastric acid secretion [2] and thus an effective anti-ulcer drug [3] in general and regarding its beneficial effect on survival after gastric [4] and colorectal [5] cancer in particular. As recently reported in literature, it

a) blocks cancer metastasis by inhibiting cancer cell adhesion to endothelial cells [5,6]
b) decreases the bioavailability of albendiazole [7] (metabolised by mucosal CYP 3A4 enzymes in intestinal mucosa).
c) exerts its effect on oxidative metabolism of oestradiol & thus increases serum oestradiol [8] concentration in men
d) causes an increase in plasma diazepam [9] (in diazepam treated patients)
e) enhances anti-bacterial function of gingival crevicular neutrophil leukocyte [10] by its 0.5% oral rinse.

As far as the possible sexual side effects are concerned in women, it decreases sexual desire [11,12] causes pain and tenderness in the breasts [11]. In case of men it is responsible for sexual dysfunction [8] such as impotence [11], modest decrease in sperm count not enough to affect fertility [11], decrease in both sex desire [11,12] as well as drive [13] and it can also affect sexual erections [13]. Its other well documented reports include hyperprolactinaemia [14,15], gynaecomastia [8,16,17] & anti-androgenic [18] effect resulting in the reduction in the size of testes [18].

The possible effect of cimetidine on seminiferous tubules has not been studied previously. This study was undertaken to study the effects of cimetidine on the qualitative [19] and quantitative [20] changes in the size of testes in general and seminiferous tubules in particular.

MATERIAL AND METHODS

Forty adult male albino rats between the ages of 90 and 150 days weighing between 150g and 300g were taken for the present study. They were bred in the animal house of the Jinnah Post Graduate Medical Centre Karachi and were supplied with gram supplemented with vitamins and water ad libitum. Tagamet brand of cimetidine was administered undiluted parenterally in two different dose regimens, one as high dose [18] (950 mg/kg body weight) for a short term of 10 days and another as low dose [18] (150 mg/kg body weight) for a relatively long term of 21 days. Normal saline was given to the animals of the control groups. The animals were divided into four groups (table-1).

The animals of groups-A and B were sacrificed on the 11th day and groups-C and D on the 22nd day. Weights of animals and weights of

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Table–1: Grouping of animals

Groups	No. of animals	Treatment	Dose	Duration	Sacrificed
A (high dose) (short term)	10	Inj. Cimetidine (IM)	950 mg/kg. (in 2 equal divided doses)	10 days	11th day
B (control) (for short term)	10	Inj. Normal saline	1ml. (in 2 equal divided doses)	10 days	11th day
C (low dose) Long term	10	Inj. Cimetidine (IM)	150/mg/kg (in 2 equal divided doses	21 days	22nd day
D (control) (for long term)	10	Inj. Normal-saline	1ml (in 2 equal divided doses)	21 days	22nd day

Table–2: Quantitative and qualitative observations

Parameters	A (High Dose)	B (Control) For A	C (Low Dose)	D (Control) For (C)
No. o f Animals	10	10	10	10
Mean Body Weights (Initial) (in G) Mean ± SD	236.2 ± 10.46	175.8 ± 13.3	217.7 ± 12.3	194.0 ± 13.9
Mean Body Weights (Final) (in G) Mean ± SD.	*** 214.8 ± 10.15	180.3 ± 29.7	228.3 ± 12.4	217.4 ± 14.3
Mean Absolute Weight (in g) ± S.E.M.	* 1.11 ± 0.07	1.34 ± 0.08	1.37 ± 0.08	1.42 ± 0.07
Mean Relative Weight (In mg/G Body weight)	*** 4.9 ± 0.21	7.6 ± 0.05	6.4 ± 0.3	6.75 ± 0.46
Mean Tubular Count (Per Low Power Field)	**** 16.8 ± 0.43	13.7 ± 0.49	14.0 ± 0.3	14.44 ± 0.45
Mean Diameter of Tubules (inμM)	* 207.1 ± 3.9	239.8 ±12.53	251.5 ± .04	242.4 ± 3.1
Mean Thickness of Germinal Epithelium (inμM)	** 80.1 ± 2.6	87.9 ± 1.37	93.6 ± 1.82	89.64 ± 2.78
Spermatogenesis (Oil Immersion)	Normal	Normal	Normal	Normal
Multinucleated Giant Cells	Nil	Nil	Nil	Nil

Statistical analysis. Value of 'P' as compared to the respective control
**** = P < .05 ** = P < .02 *** = P < .01 **** = P < .001***

testes were recorded at autopsy. Microscopic study of the testis included tubular count per low power field, measurement of diameter of tubules and thickness of germinal epithelium, germ cell study, presence or absence of morphological change in the cells and lastly presence or absence of abnormal cells. This study was carried out on 5μm thick sections which were stained with P.A.S. reagent and counterstained with iron haematoxylin.

RESULTS

The results of the present study indicate no significant difference between the qualitative and quantitative changes of low dose (group-C fig. 2) and its control (group-D, table-2 fig. 4). In case of the quantitative change of the high dose (group-A) there was a statistically significant decrease in the

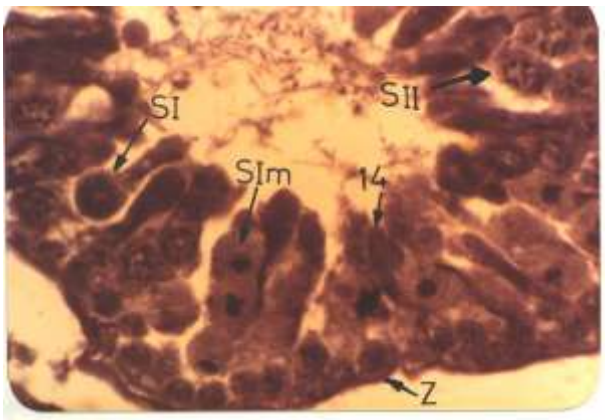

Fig. 1: Section of testis, high dose (group-A), showing seminiferous tubules at stage XIV of normal spermatogenesis. Seen in the section are Primary spermatocytes (SI) at zygotene stage (Z), and metaphase (Sim), secondary spermatocyte (SII) and spermatid (14) at step 14. P.A.S. Iron-haematoxylin stain. (Under high power.)

body weights as well as absolute and relative weights of testes with concomitant increase in tubular count (per low power field) and simultaneous decrease in diameter of tubules and thickness of germinal epithelium (table-2).

As far as the qualitative changes of (group-A) are concerned the microscopic picture of high dose (group-A, fig. 5) did not reveal obviously different findings from control (group-B, fig. 3), as well as from the low dose (group-C, fig. 2), and its control (group-D, fig. 4). The spermatogenesis seemed to be normal. The spermiogenesis did not show arrest at any stage of the cycle. No necrotic cells were visible. Staging was not difficult (group-A, fig.1). Atrophy of interstitial tissue was not detected. Though the Sertoli cells have a central role in spermatogenesis [21] they were found within normal limits in the present study.

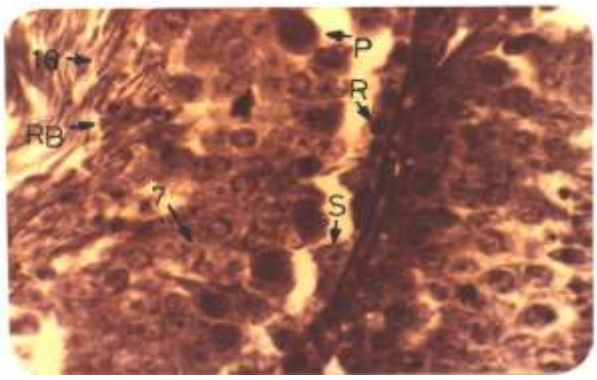

Fig. 2: Section of testis, low dose (group-C), showing two seminiferous tubules in different stages of the normal spermatogenesis Tubule, on the Left is in stage (VII) showing Resting (R) and Pachytene Primary spermatocytes (P) also residual bodies (RB) and younger spermatid (7) at step 7, older spermatid (18) at step 18, Right tubule is in an advanced stage. P.A.S. Iron-haematoxylin stain. (Under high power).

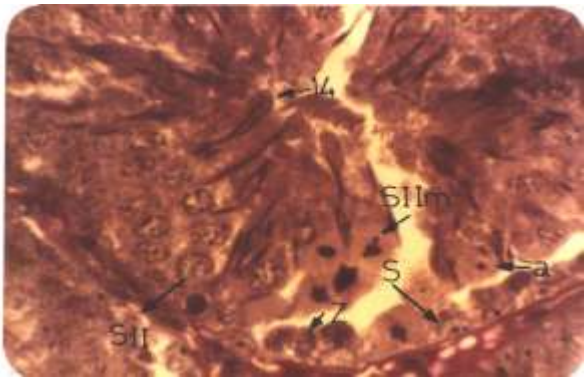

Fig. 3: Section of normal testis (group-B), showing section of a number of tubules. The upper and right one at stage (XIV showing zygotene primary spermatocyte (z), Secondary spermatocyte (SII), Secondary spermatocyte at meta phase (SIIm), one secondary spermatocyte at anaphase (a) is also seen. Apart from step 14 of spermatid (14), sertoli cells (S) is also visible in the section. P.A.S. Iron-haematoxylin stain. (Under high power field.)

DISCUSSION

The results of the present study indicate significant decrease in relative testicular size of high dose (group-A) as compared to Control (group-B). This finding is partly in accord with the similar finding reported earlier [18]. As this decrease in testicular size was noticed comparatively earlier after a very short period of 10 days, as compared to the previous study [18], where oral route of administration was employed while in case of present experiment parenteral route [22,23] was opted for which may account for the early effect.

Since there is ample evidence of rise in plasma prolactin in patients treated with cimetidine, orally [24] or intravenously [15], the reduction in testicular size could be attributed mainly to release of prolactin [25]. In acute dose studies cimetidine does not enter rat brain but is localised in pituitary gland. The prolactin response in man is swift and only occurs in association with high circulating concentrations of cimetidine and is blocked by bromocriptine [26].

These observations have led to the proposal that cimetidine is acting directly or indirectly at the dopamine receptors in the anterior pituitary to cause hyper-prolactinaemia [27] and since it has already been noted in male rats that there is inhibition of ganodotrophins by induced hyper-prolactinaemia [28,29].

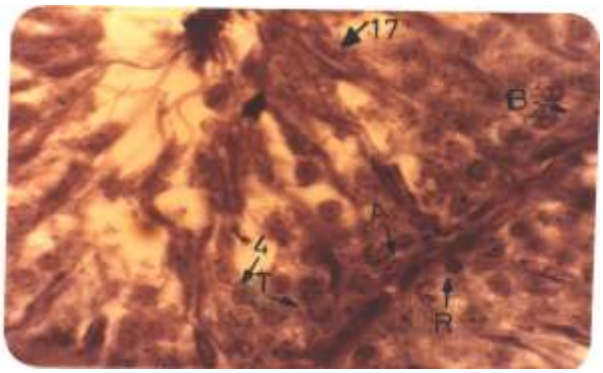

Fig. 4: Section of normal testis (group-D), showing two tubules in different stages of the cycle. Tubule on the left showing type A spermatogonia (A), primary spermatocyte at transition (T), younger generation of spermatids (4) at step 4, type B spermatogonia (B) and step 17 of older spermatid (17) Tubule on right showing resting primary spermatocyte (R). P.A.S. Iron Haematoxylin stain. (Under high power).

The reduction in testicular size in case of high dose study (group-A) is also supported by other statistically significant parameters such as increased tubular count per low power field, reduced diameter of tubules, and decreased thickness of germinal epithelium (table-2). The former two histologic findings confirm the shrinkage or atrophy of testes, while the latter finding probably indicates the adverse effect of the drug on cellular proliferation. However, qualitatively there was no abnormal finding in case of high dose (group-A, fig.1)

In case of low dose (group-C, table-2) treated with 150 mg/kg for 21 days, all the parameters remained within normal limits and no significant differences were detected when compared with control (group-D, table-2). The testicular size was unaffected which is in accord with the previous study [18]. However, as quoted by them the weight of prostate was decreased in animals treated for 12 months, while the anti-androgenic effect was not looked for in the present study.

From the above discussion it is concluded that the testes have reduced in size in case of high dose (group-A). This atrophy could partly be due to a non specific effect of the drug on the body as a whole including the testes and partly a direct or indirect effect of pituitary hormones such as prolactin particularly on the seminiferous tubules. Due to the limitations of the present study exact mechanism involved in testicular atrophy could not be determined. Further research is required to elaborate the factor/factors responsible for the qualitative and quantitative changes in the size of testes in general and seminiferous tubules in particular.

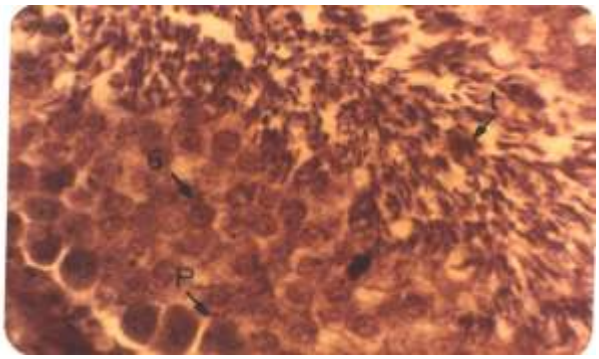

Fig. 5: Section of testis, high dose (group-A) showing normal spermatogenesis stage (VI) of the tubule, indicating tail of sperm (t), Pachytene Primary Spermatocyte (P) and step 6 of younger spermatid (6) P.A.S. Iron-haematoxylin stain. (Under high power).

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