

EFFECT OF IMATINIB ON HISTOLOGICAL PARAMETERS IN MALE SWISS ALBINO MICE

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ABSTRACT

Cytotoxic chemotherapy for malignant disease has markedly improved the chances of long-term remission or cure in young patients who have not yet started a family. Germ cells are important targets of many chemicals. Germ cell mutagens irrespective of their chemical nature and properties, affect population of cells in the gonads, especially of testis. There is paucity of reports on planned study of imatinib on testicular function. Hence a study was planned to assess the effects of imatinib on testicular functions in male swiss albino mice. Male swiss albino mice were treated with imatinib and sacrificed at the end of 1st, 2nd, 4th, 5th, 7th and 10th week after the last exposure to imatinib. The testis were removed, weighed and processed for histological analysis. Qualitative analysis of the testis showed sloughing of the germ cells, reduction in the number of sperm cells and variation in the size and shape of the seminiferous tubules. There was not much change in the diameter of the seminiferous tubules in the treated groups except for the higher dose group. The epithelial height was reduced significantly in all treated groups. Imatinib does affect the histopathology of mice testis significantly, but this effect is reversible once the drug is withdrawn. Imatinib had demonstrated high levels of efficacy in gastrointestinal tumors and also chronic myeloid leukemia. This finding may help the clinicians to plan and address the fertility related issues in young patients of reproductive age who are being treated with imatinib for gastrointestinal tumors and chronic myeloid leukemia.

Keywords: Imatinib, testis, sloughing, seminiferous epithelial height.

INTRODUCTION

Cytotoxic chemotherapy for malignant disease has markedly improved the chances of long-term remission or cure in young patients who have not yet started a family. Thus chemotherapy-induced impairment of fertility has gained increasing clinical importance. Approximately 1,400 of these patients are subjected to polychemotherapy and irradiation at doses sufficient to induce prolonged azoospermia. The impact of the chemotherapy treatment on fertility and gamete quality is of concern as a number of these patients are of reproductive age¹. Infertility is a common permanent sequel in adult cancer survivors who undergo the currently available treatments, which include surgery, radiation therapy, and systemic chemotherapy². Nowadays, an important percentage of infertility problems affects to males.

Germ cells are important targets of many chemicals. Germ cell mutagens irrespective of their chemical nature and properties, affect population of cells in the gonads, especially of testis³. For many years it has been recognized that radio and chemotherapy adversely affect the rapidly proliferating germinal epithelium in the pre-pubertal and adult testis^{4,5,6}. Histopathology of the testis has been shown to be the most sensitive method for the detection of effects on spermatogenesis⁷. Hyperplasia of Leydig cells associated with germ cell loss was also reported in mouse. This is important because control of spermatogenesis is mediated by Sertoli and Leydig cells⁸. Many histopathological evaluations of the testis only detect lesions if the seminiferous epithelium is severely

depleted, degenerating cells are obvious, or sloughed cells are present in the tubule lumen⁹. Histopathological evidence of increasingly severe and dose-dependent damage to the seminiferous tubules was reflected in the estimation of the survival of stem cells, which exhibited significant dose-related reductions. This observation confirmed the subjective estimate of dose-related damage of seminiferous tubules.

Imatinib mesylate, a first synthetic tyrosine kinase inhibitor, inhibits proliferation and induces apoptosis in Bcr-Abl positive cell lines as well as fresh leukemic cells from Philadelphia chromosome positive chronic myeloid leukemia. It is indicated for the treatment of patients with chronic myeloid leukemia. Imatinib (formerly known as STI571) has demonstrated high levels of efficacy in gastrointestinal stromal tumors⁹. There is a report of gastrointestinal stromal tumor (GIST) patient with male gynecomastia and testicular hydrocele after treatment with imatinib mesylate¹⁰. Also reports are available for gynecomastia in men with chronic myeloid leukaemia after imatinib¹¹. But the report on effect of imatinib on reproductive function is scanty. There is paucity of reports on planned study of imatinib on testicular function. Hence a study was planned to assess the effects of imatinib on reproductive functions in male swiss albino mice.

MATERIALS AND METHODS

Male Swiss albino mice (9-12 week old) were used. Animals were bred in the central animal house of Manipal University, Manipal. Breeding and maintenance of



animals were done according to the guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA) and Animal Welfare Division, Government of India for the use of laboratory animals. This work was carried out after obtaining approval from the institutional animal ethical committee. All the animals were housed in polypropylene cage using paddy husk for bedding at $28\pm 1^\circ\text{C}$ temperature and $50\pm 5\%$ humidity. Five animals were housed in each cage to prevent overcrowding. Animals were fed on laboratory feed (Gold Mohur; Lipton India Ltd) and water *ad libitum*.

The animals were segregated into 24 groups containing 6 animals in each group. Eighteen groups were injected with Imatinib at the dose levels of 50, 75 and 100 mg/kg body weight (6 groups for each dose) intraperitoneally for a continuous period of five days with an interval of 24 hours between two injections. Remaining six groups served as controls, which received distilled water. After the last dose, the animals were sacrificed on 1, 2, 4, 5, 7 and 10 weeks sample times by overdose of anesthesia (Pentobarbital sodium, 40mg/kg, Sigma Chemicals Co). These sample time (1-5 weeks) establishes the treatment of spermatozoa in the epididymis and testis, spermatids, primary spermatocytes, secondary spermatocytes, spermatogonia and 7,10 week sample time represents treatment as stem cells respectively^{12,13,14}.

Histological and Histopathological Evaluation of the Testis

In all drug treated groups, gonadotoxic effect is evaluated by using histopathology of the testes. For histopathology, tissue is collected from the animals employed in the sperm morphology essay. Animals are sacrificed by overdose of anesthesia, laparotomy is done and testes are removed, weighed and fixed in Bouin's fluid for 10 h. The tissue is then transferred to 70% alcohol from the fixative and washed for seven days with daily change of alcohol. Tissue is further transferred to 90% alcohol and maintained for 16 h, and then to the absolute alcohol with three changes at 3 h intervals. The tissue is kept in xylol overnight for clearing and transferred to paraffin with three changes at intervals of 1h and finally embedded in fresh paraffin wax. Five micron thick paraffin sections are obtained and mounted on clean glass slides and labeled.

Qualitative Analysis

Testes from the control and treated animals are analyzed for the presence of vacuoles, gaps and abnormal cells in the seminiferous epithelium and sloughing of epithelium.

Quantitative Analysis

Seminiferous Tubular Diameter (STD)

The diameters of 10 transversely cut round seminiferous tubules randomly selected per animal is measured using ocular micrometer calibrated with stage micrometer. In each tubule two measurements are made one perpendicular to the other and their average is taken.

Seminiferous Epithelial Height (SEH)

The epithelial height is measured in 10 tubules from its basement membrane to the surface of the epithelium at two different regions and the mean is taken.

Photography

The photomicrograph will be taken in Leica microscope with camera attached using planapochromatic objectives. The magnifications of photomicrograph will be indicated with the legends for the photograph.

Statistical Analysis

For each group six animals were used and mean \pm SD (standard deviation) was calculated. Results obtained from the present study were correlated and analyzed by one way Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test. Values of $P < 0.05$ were considered statistically significant.

RESULTS

Qualitative Analysis

The cytoarchitecture of the testes was intact in control as well as the treated groups. However, sloughing of epithelial cells in testes was observed in the mice treated higher dose of the drug (Fig 1C). There was a reduction in the number of sperm cells in the treated groups when compared to the control groups. This was more evident in the high dose group (Fig 1B). We also noted variation in the size and shape of the seminiferous tubules of the treated groups when compared to the control. Few of the high dose group slides showed interstitial edema (Fig 1D). Few of the seminiferous tubules showed vacuoles during the 4th and 5th week sampling time with the higher dose. Atrophy of the tubules was not seen and multinucleated cells were absent.

Quantitative analysis

Seminiferous tubular diameter

There was not much significant change in the diameter of the seminiferous tubule in the treated groups except on 5th week sampling time. The lower dose groups (50mg/kg and 75mg/kg) showed the values closer to the control group in all sampling weeks except on week 5. However the tubular diameter was significantly increased in mice treated with the higher dose (100mg/kg) during the 4th and 5th week sampling time (Table 1). Even at the 7th week sample time there was significant increase in tubular diameter in mice treated with the higher dose. Complete recovery of the tubular diameter was seen only at the 10th week sample time except the higher dose which showed an increase in the tubular diameter (Fig 2).

Seminiferous Epithelial Height

The epithelial height of the tubules was significantly reduced at all sampling weeks except for the 10th week. The epithelial height was reduced regardless of the dose. Maximum reduction of the epithelial height was observed



during the 5th week in all doses of the drug. Recovery period for high dose was long and normal height was attained only by the 10th week (Table 2). In lower dose treated groups, the epithelial height came closer to the

control values by 7th week sampling time. Imatinib decreased the seminiferous epithelial height in a time dependent manner as indicated by the time response graph (Fig 3).

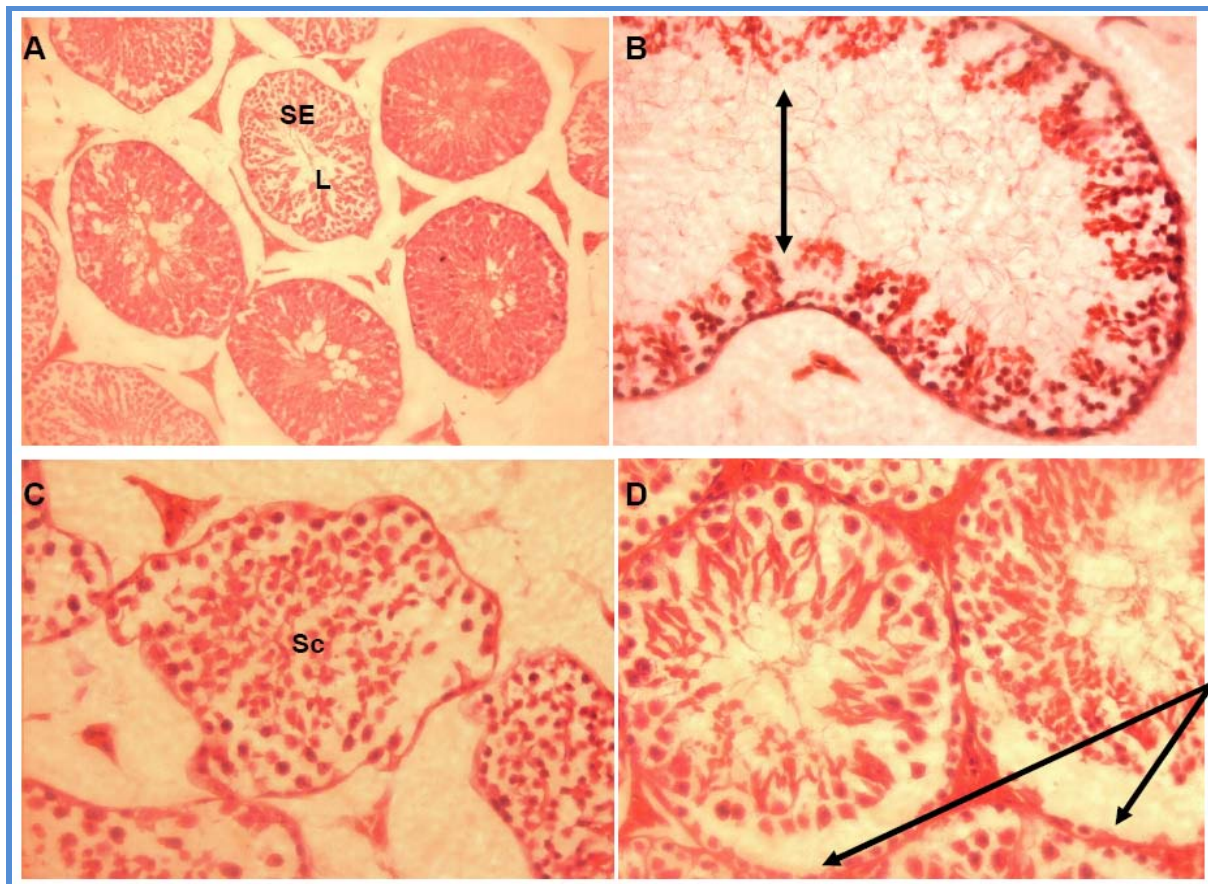


Figure 1: Testicular structure in control and imatinib treated mice.

A) A photomicrograph of testicular section from a control mouse showing normal seminiferous epithelium (SE) and normal lumen (L), B) A photomicrograph of testicular section from a mouse treated with 100mg of imatinib with the arrow indicating the increased diameter in the lumen of the seminiferous tubule (5th week) and also showing the reduced number of germ cells, C) Photomicrograph of testicular section of mouse treated with 100mg of imatinib showing the degenerated tubules with the presence of sloughed cells in the lumen of the seminiferous tubules (5th week) and D) A photomicrograph of a testicular section showing the presence of interstitial edema (indicated with arrows) in mouse treated with imatinib (5th week).

Table 1: Effect of imatinib on seminiferous tubular diameter of the testis (micrometer). Each dose from particular time represents mean ±SD from 6 animals

Dose	Sampling Time					
	1w	2w	4w	5w	7w	10w
Control	185.6 ± 4.30	188.8 ± 3.43	199.6 ± 3.6	207.8 ± 3.13	192.8 ± 2.91	188 ± 4.43
50mg/kg	184.6 ± 2.21	190.5 ± 2.36	204.3 ± 4.34	223.1 ± 4.05 ⁺⁺	192.5 ± 5.43	187.1 ± 4.81
75mg/kg	186.8 ± 4.25	195.5 ± 3.73	210.5 ± 3.40 ⁺⁺	226.3 ± 5.46 ⁺	198.6 ± 5.5	193.8 ± 2.33
100mg/kg	190.1 ± 5.95	201.5 ± 6.39 ^{bb}	266 ± 6.63 ^{bc}	243.6 ± 5.46 ^{bcc}	208.1 ± 4.7 ^{bccc}	204.8 ± 5.20 ^{bcc}

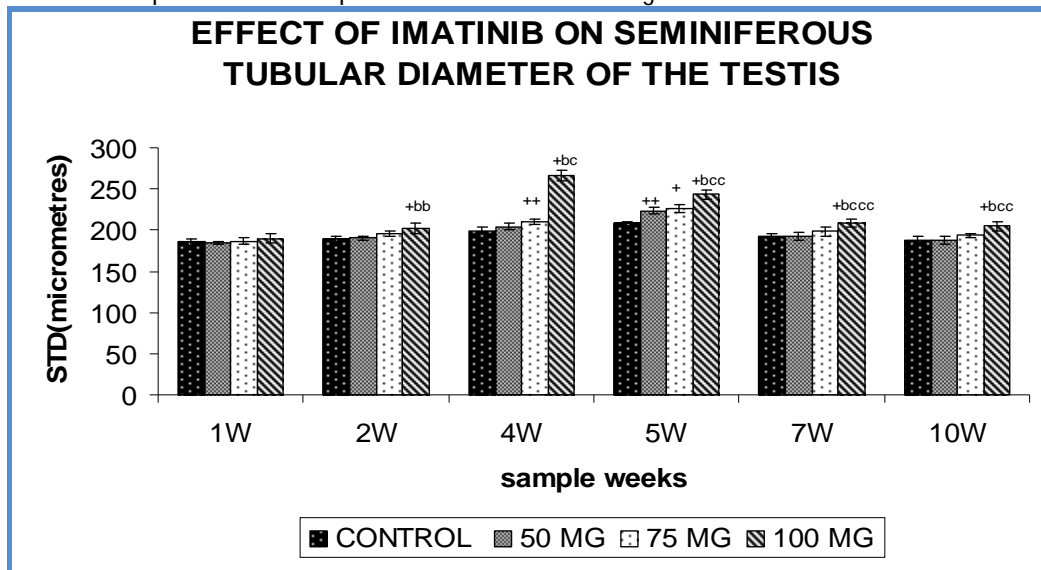
Significant values are control vs treated, ++<0.01 +<0.001; 50 mg / kg vs 100 mg/kg, bb<0.01, b<0.001; 75 mg / kg vs 100 mg/kg, ccc<0.05, cc<0.01, c<0.001. w=weeks.

Table 2: Effect of imatinib on epithelial height of seminiferous tubule (micromter). Each dose from particular time represents mean \pm SD from 6 animals.

Dose	Sampling Time					
	1w	2w	4w	5w	7w	10w
Control	63.9 \pm 1.26	63 \pm 0.77	59.4 \pm 0.95	59.5 \pm 0.70	60 \pm 0.78	61.4 \pm 1.07
50mg/kg	62.1 \pm 1.02	57.6 \pm 0.87 ⁺	52.2 \pm 0.63 ⁺	51.1 \pm 1.10 ⁺	57.5 \pm 0.55 ⁺	63 \pm 1.75
75mg/kg	55.7 \pm 1.23 ^{+a}	51.8 \pm 1.26 ^{+a}	52.2 \pm 0.94 ⁺	44.9 \pm 0.99 ^{+a}	55.4 \pm 0.89 ^{+aa}	59.8 \pm 0.62 ^{+aaa}
100mg/kg	56.7 \pm 0.69 ^{+b}	51.9 \pm 1.5 ^{+b}	45.8 \pm 1.86 ^{+bc}	42.2 \pm 1.07 ^{+bcc}	52.2 \pm 0.82 ^{+bc}	61.2 \pm 1.90

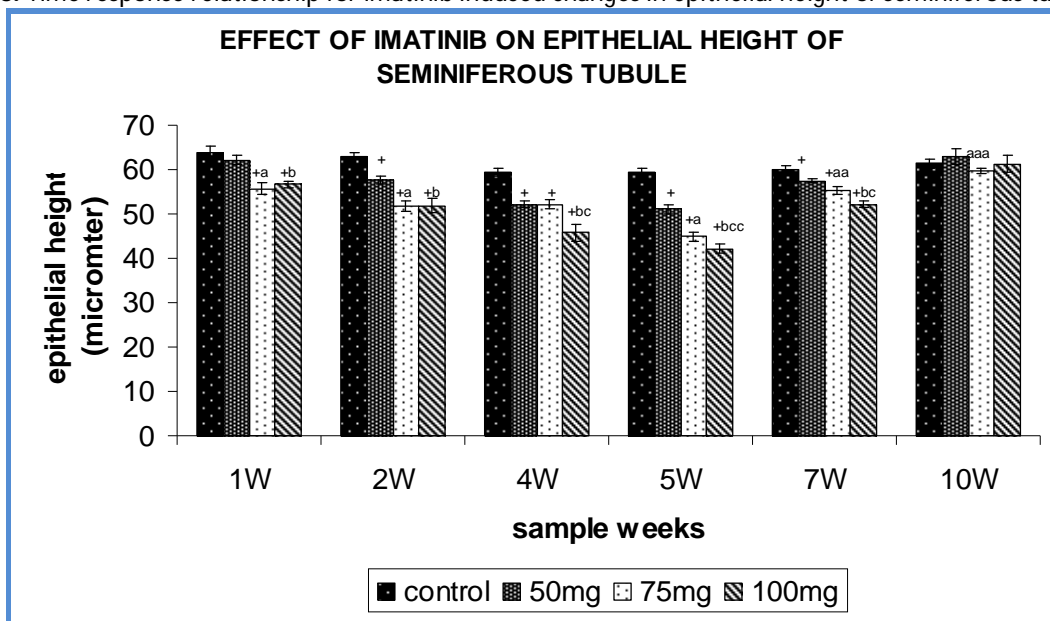
Significant values are control vs treated, +<0.001; 50 mg/kg vs 75 mg/kg, aaa<0.05, aa<0.01,a<0.001; 50 mg / kg vs 100 mg/kg, b<0.001; 75 mg / kg vs 100 mg/kg, cc<0.01, c<0.001. w=weeks

Figure 2: Time response relationship for imatinib induced changes in seminiferous tubular diameter(STD).



Each time at particular dose represent mean +SD from 6 animals. Significant values are control vs treated,++<0.01,+<0.001; 50 mg / kg vs 100 mg/kg,bb<0.01, b<0.001; 75 mg / kg vs 100 mg/kg, ccc<0.05, cc<0.01, c<0.001. w=weeks

Figure 3: Time response relationship for imatinib induced changes in epithelial height of seminiferous tubule(SE).



Each time at particular dose represent mean +SD from 6 animals. Significant values are control vs treated, +<0.001; 50 mg/kg vs 75 mg/kg, aaa<0.05, aa<0.01,a<0.00150 mg / kg vs 100 mg/kg, b<0.001; 75 mg / kg vs 100 mg/kg,cc<0.01, c<0.001. w=weeks

DISCUSSION

The histopathological changes caused by imatinib showed that the drug is a gonadotoxic agent. The major changes observed were, increase in the seminiferous tubular diameter (STD), decrease in the seminiferous epithelial height (SEH), sloughing of seminiferous epithelial cells and dilation of seminiferous tubular lumen. The epithelial height of the tubules was significantly reduced at all sampling weeks except for the 10th week. The epithelial height was reduced regardless of the dose. Thinning of the seminiferous epithelium and subsequent decrease in seminiferous tubular diameter was due to sloughing and cell death of germ cells¹⁵. These findings support our study that the sloughing of germ cells in the seminiferous epithelium resulted in the decreased epithelial height. Reduction of intratesticular testosterone to low enough levels results in germ cell loss^{16,17,18}. In response to decrease in intratesticular testosterone concentration, the Sertoli cell presumably communicates a signal to the attached and developing germ cells, which lack the androgen receptor, resulting either in the loss or restoration of germ cells¹⁹. It is now established that lowering of intratesticular testosterone concentration results in the apoptotic death of some germ cells (spermatocytes)²⁰. However, other germ cells (e.g., round spermatids) undergo a loss of adhesion to the Sertoli cell, slough into the lumen of the seminiferous tubules²¹ and are phagocytized by Sertoli cells and this apoptotic cell death is mitochondrial pathway related²². The decreased testosterone level in the triclosan treated rats led to degenerative changes and atrophy of the testis¹⁶. These reports suggest that the decreased testosterone level led to germ cell loss thus leading to a decreased seminiferous epithelial height. This is in agreement with our findings that the reduced testosterone level (result not shown) and the histopathological changes caused by the imatinib treatment resulted in the decreased seminiferous epithelial height and also a decreased testicular weight.

The histopathological evaluation showed that there was not much significant change in the diameter of the seminiferous tubule in the treated dose except on 4th and 5th week sampling time. However, the tubular diameter was significantly increased in mice treated with the higher dose (100mg/kg) during the 4th and 5th week sampling time. The increase in tubular diameter with the higher dose of imatinib is possibly due to the induction of secretion of seminiferous tubular fluid. Another possibility would be the extensive sloughing that was seen during 4th and 5th week sampling time and according to Nakai et al²³ the sloughed cells can block the efferent ductules and hence increase the diameter of the tubules. Chemicals, which affect the microtubules in Sertoli cells, induced epithelial sloughing²⁴. Further, the destruction of vimentin filaments, which are required as an anchoring device for the germ cells, also results in exfoliation of the germinal epithelium²⁵. Exposure to 1, 2, or 4 cycles of cisplatin at dose-levels of 1, 2.5 or 5 mg/kg resulted in disruption of the testis structure mainly due to germ cell

apoptosis, but also assisted by the Sertoli cell damage²⁶. Hence it can be concluded that the sloughing seen with Imatinib treatment is due to the decreased testosterone level which must have caused apoptic cell death of the germ cells. It could also be due to the deformity in the microtubules of the Sertoli cells as well as failure of the germ cell to remain adherent to the Sertoli cells. However, the spermatogonia were observed after the 7 week treatment period indicating these changes caused by imatinib treatment are reversible. Hence this may indicate that the fertility index of human subjects taking this drug for cancer treatment may be low and caution should be exercised so that the reproductive capacity of young cancer patients is not affected while they are on treatment with this drug.

CONCLUSION

Imatinib does affect the histopathology of mice testis significantly, but this effect is reversible once the drug is withdrawn. Imatinib had demonstrated high levels of efficacy in gastrointestinal tumors and also chronic myeloid leukemia. This finding may help the clinicians to plan and address the fertility related issues in young patients of reproductive age who are being treated with imatinib for gastrointestinal tumors and chronic myeloid leukemia.

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