

Effect of Imatinib on the Oogenesis and Pituitary -Ovary Hormonal Axis in Female Wistar Rat

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Abstract

Background: Imatinib mesylate, a small-molecular analog of adenosine triphosphate (ATP) that potently inhibits tyrosine kinase activities of Bcr-Abl, PDGFR- β , PDGFR- α , c-Fms, Arg and c-kit, is one of the novel molecularly targeted drugs being introduced into cancer therapy. We tested the effect of imatinib on the ovarian histological structure and the concentration of estrogen and progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) in the serum of female Wistar rats.

Materials and Methods: Two groups of rats (180 ± 15 grams) were gavaged with doses of 50 and 100 mg/kg body weight imatinib dissolved in distilled water for 14 days. The control group received sterile water. On day 7, after termination of the treatment, blood serum concentration was measured with the radioimmunoassay (RIA) method. Also, sections (5 μ m thick) of ovaries stained with hematoxylin and eosin (H&E) were investigated histologically.

Results: Progesterone concentration in the experimental groups was increased ($p < 0.001$), estrogen and FSH concentrations were decreased ($p < 0.01$), and the LH concentration decreased but was not statistically different in comparison with the control group. The weight of ovaries and number of atretic follicles in the experimental groups was increased compared with the control group ($p < 0.05$). The diameter of corpus lutea were increased but the number of corpus lutea decreased in both experimental groups ($p < 0.01$).

Conclusion: These findings suggest that administration of imatinib may have profound effects on female fertility.

Keywords: Imatinib, Estrogens, FSH, Ovary, Rat

Introduction

Imatinib mesylate, a small-molecular analog of ATP that potently inhibits Bcr-Abl, PDGFR- α , PDGFR- β , c-Fms, Arg and c-kit tyrosine kinases, displays antitumor activity toward chronic myelogenous leukemia and Philadelphia-positive acute lymphoblastic leukemia by inhibiting Bcr-Abl, and against gastrointestinal stromal tumors (GIST) by inhibiting c-kit (1). The tyrosine kinase inhibitor imatinib (STI571), belonging to the 2-phenylamio pyrimidine class, selectively inhibits Bcr/Abl, platelet-derived growth factor receptor (PDGFR), c-kit, and c-fms (macrophage colony-stimulating factor receptor) kinase activity (2). Tyrosine kinases (TKs) are important for the regulation of growth, differentiation, survival and motility. Various tumors which overexpress TKs or harbor activating TK mutations lead to uncontrolled mitogenic signals to the neoplastic cells. Lately, highly potent and selective TK inhibitors have been developed by blocking TKs and serve

as an alternative to standard chemotherapy. Imatinib mesylate, as an example of this novel class of drugs, suppresses the TK activities of c-abl, Bcr-Abl, PDGFR and c-kit receptors (3). Imatinib acts through selective inhibition of tyrosine kinases, which are constitutively activated in a number of cancers. Some of the drug's adverse reactions are presumably caused by the inhibition of normal kinases in various tissues (4). FSH is required for follicular growth from the preantral to the preovulatory stage. This is clearly shown by the phenotype of FSH β -deficient mice, which are infertile owing to a block in folliculogenesis at the preantral stage (5). FSH and LH appear to act via similar pathways; granulosa cells need to be able to discriminate between an FSH and an LH signal on a biochemical basis as well as on a temporal basis for normal follicular growth and differentiation. Various growth factors have been proposed as agents that can influence granulosa cell functions (6). Recent studies have suggested

Received: 16 Mar 2009, Accepted: 10 May 2009

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Royan Institute
International Journal of Fertility and Sterility
Vol 3, No 1, May-Jun 2009, Pages: 11-16

that FSH activity in granulosa cells may be influenced by a tyrosine kinase-like activity that is affected by tyrosine kinase inhibitors, a class of compounds that have been shown to inhibit receptor tyrosine kinases (7). In theory at least, drugs could be targeted with a high degree of selectivity towards the tumor cells, thereby avoiding many of the common toxic side-effects associated with conventional cancer therapy. However, certain signaling pathways that are up-regulated in many types of tumor cells also perform critical functions in normal cells, especially during development (8). Nowadays, a high proportion of children who develop cancer survive into adulthood. Reduced fertility, which is especially common following abdominal radiotherapy and chemotherapy involving alkylating agents, is one of the major long-term sequelae that influence the quality of their lives (9). The present investigation was designed to determine whether a 14 day treatment of female rats with imatinib mesylate (50 and 100 mg/kg doses) later affects their oogenesis, the plasma level of sexual hormones (estrogen and progesterone) and gonadotropins (LH and FSH).

Materials and Methods

Animals

The experiment was conducted on Wistar breed rats, originally obtained from a commercial breeder (Pasteur; Tehran, Iran), with an initial body weight of 180 ± 15 grams. They were housed in standard laboratory cages (maximum of 10 rats per cage) at a room temperature of $20 \pm 3^\circ\text{C}$ on a daylight cycle (7 am - 7 pm). Food and water consumption were monitored daily. The animals received humane care. The experiments were approved by a state-appointed board on animal ethics and were performed according to international guidelines concerning the conduct of animal experimentation. The female rats were allocated into two experimental groups and one control group (ten animals in each group). Imatinib mesylate (Gleevec; Novartis, East Hanover, NJ) was dissolved in sterile water and rats were force-fed with a gastric tube. The suspension was administered orally, once daily between 8.30 am and 9.30 am. The experimental groups were force-fed with doses of 50 mg/kg and 100 mg/kg imatinib, for a period of two weeks while the control group received sterile water.

Hormone assays

The animals were killed by decapitation one week after termination of drug treatment. Blood samples were collected directly after decapitation, kept overnight at 4°C and centrifuged the following day

at 3000 rpm for 15 minutes at 4°C . Serum samples were stored at -20°C until assayed for FSH, LH, estrogen and progesterone. Serum concentrations of progesterone, estrogen, FSH and LH were measured by radioimmunoassay (RIA).

Histological analysis

After decapitation, the ovaries were removed, weighted and fixed in formalin. For histological examination formalin fixed ovaries were dehydrated, embedded in paraffin, and after routine histological procedures, $6 \mu\text{m}$ sections were mounted on slides and stained with hematoxylin and eosin.

Statistical analysis

Means were compared by one-way analysis of variance (ANOVA). Where a significant overall difference was detected among groups, differences between individual means were assessed by the Tukey – Kramer test. P values < 0.05 were considered significant.

Results

Treatment with imatinib for 14 days decreased the plasma levels of the gonadotropins FSH ($p < 0.01$) and LH, but the decrease in plasma levels of LH was not statistically significant ($p > 0.05$, Fig 1).

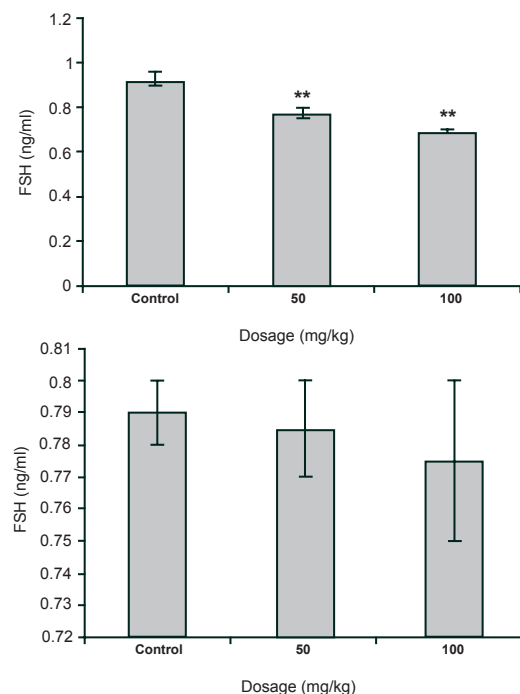


Fig 1: Plasma levels of FSH and LH in rats subjected to 14 day exposure to imatinib (50 and 100 mg/kg) and untreated (control) animals. The plasma levels of LH and FSH were determined by RIA. * $p < 0.05$ compared to control value. ** $p < 0.01$

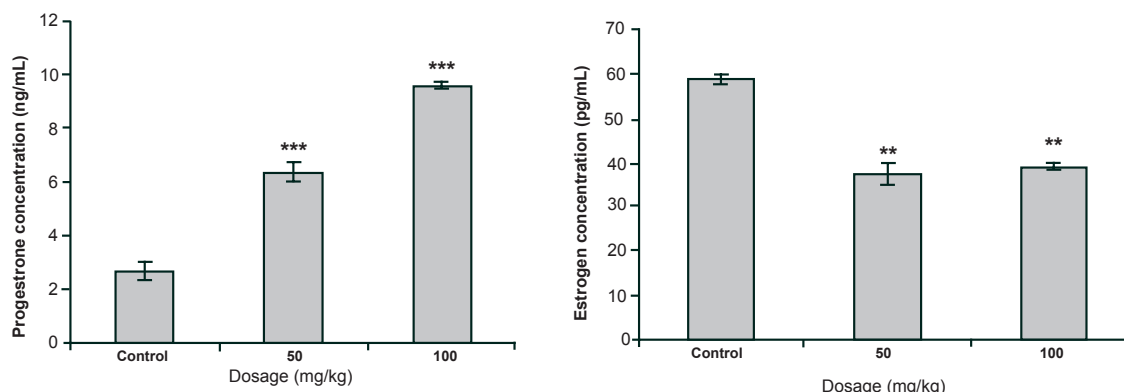


Fig 2: Plasma levels of progesterone and estrogen hormones in female rats treated with imatinib (50 and 100 mg/kg) and untreated (control) animals. Plasma levels of progesterone and estrogen hormones were determined by RIA. $p < 0.05$ compared to control value. ** $p < 0.01$, * $p < 0.001$**

Treatment with imatinib on a 14 day regimen significantly elevated plasma levels of the progesterone hormone and the plasma levels of estrogen decreased significantly ($p < 0.001$ and $p < 0.01$, respectively Fig 2).

The ovary weights of treated and untreated animals were measured one week after treatment termination. The ovary weights significantly increased ($p < 0.01$) compared with the control group (Fig 3).

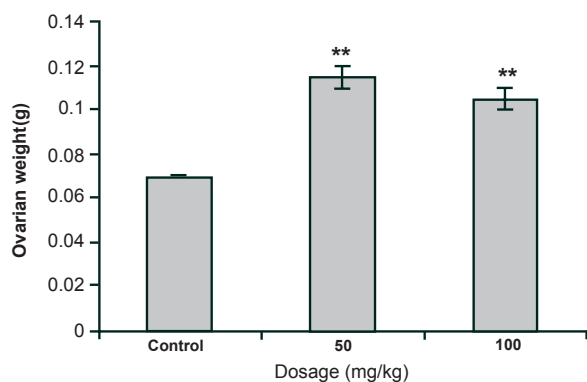


Fig 3: Ovarian weight in treated with imatinib (50 and 100) and untreated (control) group. Data represent the Mean \pm SEM. $p < 0.05$ compared to the control value. ** $p < 0.01$

The untreated ovaries contained primordial and primary follicles, developing Graafian follicles and developed Graafian follicles (Fig 4C); while in the ovaries of rats treated with imatinib, the number of follicles in different developmental stages was significantly decreased. Corpus luteum formation decreased significantly in both treatment groups ($p < 0.05$, Fig 5). A significant increase in the diameter of the corpus luteum in ovaries treated with imat-

inib as compared with the control group was observed (Fig 4A).

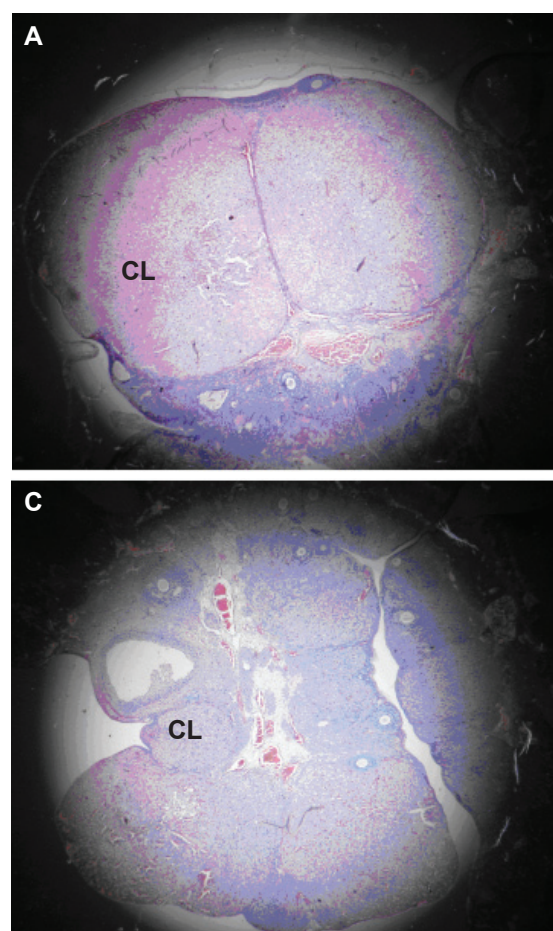


Fig 4: Photomicrographs of ovarian sections of imatinib treated (A) and untreated (C) female rats. In treated and untreated ovaries the corpus luteum (CL) is present. In untreated ovaries (C), follicles of different developmental stages are found. (A) Number and diameter of corpus luteum in ovaries treated. Magnification $\times 200$.

The numbers of atretic follicles significantly increased in rats subjected to 14day exposure to imatinib. Fig 6 shows the effect of imatinib on extravasation of ovarian blood vessels with varying penetration of effects.

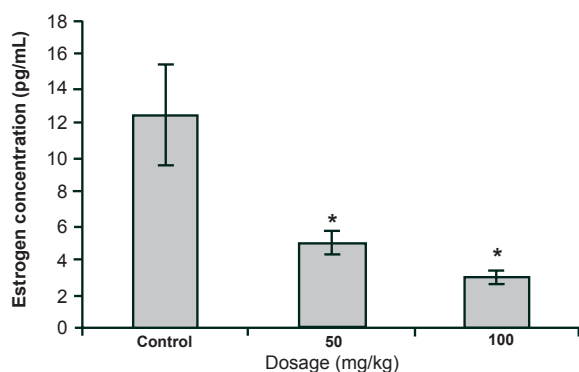


Fig 5: Decreased corpus luteal formation in rat ovaries treated with imatinib compared with the control. Figure represents the Mean \pm SEM. * Shows significant difference between the control group and experimental groups ($p < 0.05$).

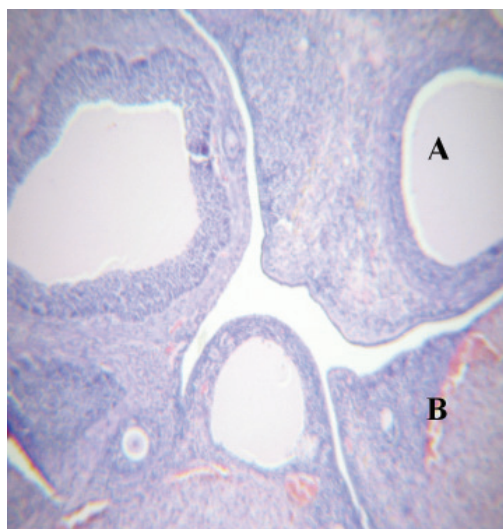


Fig 6: Increased atretic follicles in rat ovaries treated with imatinib, 50 and 100 mg/kg doses, (A). Extravasation of blood vessels (B). Magnification $\times 400$.

Discussion

Imatinib mesylate is a tyrosine kinase receptor inhibitor approved previously in 2001 by the United States Food and Drug administration for treatment of chronic myelogenous leukemia (10). The c-kit proto-oncogene receptor and its ligand stem cell factor (SCF) regulate the proliferation and survival of germ cells. In the adult rodent ovary, c-kit and SCF play important roles in follicular development (11). Paracrine signaling between the oocyte and its

surrounding somatic cells is fundamental to the processes of oogenesis and folliculogenesis in mammals.

The study of animal models reveals that the interaction of granulosa cell-derived kit ligand (KL) with oocyte and theca cell-derived c-kit which is important for multiple aspects of oocyte and follicle development; including the establishment of primordial germ cells within the ovary, primordial follicle activation, oocyte survival and growth, granulosa cell proliferation, theca cell recruitment and the maintenance of meiotic arrest. Though little is known about the specific roles of KL and c-kit during human oogenesis, the expression profiles for KL and c-kit within the human ovary suggest that they are also functionally relevant to female fertility (12).

In conclusion, our data show that imatinib decreased the number of primordial follicle, primary follicle, developing Graafian follicle and developed Graafian follicle significantly.

In several recent studies in granulosa cells, RG 50810 and genistein (a general, not EGF-specific tyrosine kinase inhibitor (TKI), have been shown to reduce levels of steroidogenic enzymes, or their mRNA; suggesting that tyrosine kinases influence FSH action in a selective manner (13). In this study, imatinib (as a tyrosine kinase inhibitor) significantly increased the plasma levels of progesterone, but the plasma levels of estrogen was decreased in rats subjected to 14 day exposure to this drug. FSH and LH are important regulators of ovarian follicular growth and differentiation. FSH and LH bind to adenylyl-cyclasecoupled receptors on granulosa cells within the follicle resulting in cAMP, progesterone (P4), and estradiol (E2) production (14). In the present study, imatinib in both dosage groups decreased plasma levels of FSH and LH.

Sleer et al. demonstrated that intra-ovarian injection of an inhibitor of PDGF receptor activity, the tyrphostin AG1295 (tyrosine kinase inhibitor), to the rats resulted a significant decrease in corpora lutea in treated ovary in comparison to the control ovary. In addition, the treated ovary in 3 of 16 rats showed widespread hemorrhage throughout the entire ovary, indicating a possible role for PDGF receptor activity in the maintenance of ovarian vasculature (15). Our study showed that treatment with imatinib in a 14 day regimen significantly decreased the number of corpus luteum and increased ovarian weight, compared with untreated animals.

According to the previous studies, imatinib can enlarge hemorrhagic ovaries in rats. These findings may be related to the effect of imatinib on c-kit, a tyrosine implicated in spermatogonial proliferation and ovarian follicle development (16).

In histological examination, ovaries treated with imatinib (both 50 and 100 mg/kg) showed pathological changes such as hemorrhage, atretic follicles increase, extravasation of blood vessels and increase in the diameter of corpus luteum and ovaries.

Christopoulos recently reported the development of ovarian insufficiency in a young woman who was treated with imatinib (600 and 800 mg/kg doses) for chronic myeloid leukemia (CML). Two years after the start of imatinib therapy and about six months after a dose increase, the patient noticed oligomenorrhea which subsequently evolved into amenorrhea (17). These findings suggest that prolonged administration of imatinib may have profound effects on female fertility. The significance of the relatively high dose that the patient received is not known, but a young man in whom oligospermia developed during imatinib treatment for hypereosinophilic syndrome was also receiving a high dose of the drug (800 mg daily) (18).

Conclusion

The true incidence, possible dose dependence and reversibility of imatinib-induced ovarian failure should be examined in future studies. Awareness of this potential complication will enable physicians to offer patients appropriate counseling and to consider strategies of preserving fertility and ovarian function before embarking on imatinib therapy.

Acknowledgements

There is no conflict of interest in this article.

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