

Effect of sorafenib on sperm count and sperm motility in male Swiss albino mice

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ABSTRACT

The issue of male germ line mutagenesis and the effects on developmental defects in the next generation has become increasingly high profile over recent years. Mutagenic substance affects germinal cells in the testis. Since the cells are undergoing different phases of cell division and maturation, it is an ideal system to study the effect of chemotherapeutic agents. There are lacunae in the literature on the effect of sorafenib on gonadal function. With background, a study was planned to evaluate the effects of sorafenib on sperm count and sperm motility in male Swiss albino mice. Male Swiss albino mice were used for the study. The animals were segregated into control, positive control (PC) and three treatment groups. PC received oral imatinib (100 mg/kg body weight) and treatment groups received 25, 50, and 100 mg/kg body weight of sorafenib orally for 7 consecutive days at intervals of 24 h between two administrations. The control group remained in the home cage for an equal duration of time to match their corresponding treatment groups. The animals were sacrificed at the end of 1st, 2nd, 4th, 5th, 7th, and 10th weeks after the last exposure to drug, respectively. Sperm suspensions were prepared and introduced into a counting chamber. Total sperm count and motility were recorded. There was a significant decrease in sperm count and sperm motility by sorafenib which was comparable with the effect of PC imatinib. Sorafenib adversely affects sperm count and sperm motility which are reversible after discontinuation of treatment.

Key words: Anticancer drug, cauda epididymis, fertility, spermatogenesis

INTRODUCTION

Chemotherapy is one of the most effective methods for the treatment of cancer; in most of the cases it is associated with several short- and long-term toxicities.^[1] Today, a high percentage of cancer patients are drastically cured by using chemotherapy, radiation, or their combination,

but side effects of antineoplastic agents and radiation have the temporary or permanent influence on fertility. The long-term treatments can cause infertility; semen analysis is the initial and most essential step of the infertility evaluation. The radiotherapy and chemotherapy have deleterious effect germ cells in both prepubertal and adult testis.^[2,3] Sorafenib is a multi-targeted kinase inhibitor. It inhibits the action of vascular endothelial growth factor and is an angiogenesis inhibitor. It is indicated for the treatment of patients with advanced renal cell carcinoma and hepatocellular carcinoma (HCC). Huang and Yang reported that sorafenib induced tumor lysis syndrome in advanced hepatocellular carcinoma patients.^[4] It is

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reported that synergistic interactions between sorafenib and bortezomib in HCC involves protein phosphatase 2A-dependent Akt inactivation.^[5] There are hardly any reports on the effect of sorafenib on male reproductive system. Hence, a study was undertaken to evaluate the effects of sorafenib on sperm count and sperm motility in male Swiss albino mice. Imatinib mesylate is a first synthetic tyrosine kinase inhibitor. Prasad *et al.*, proved that imatinib affects the spermatogenesis by causing depression in sperm count and motility.^[6] Hence, imatinib was used as positive control (PC) in this study.

MATERIALS AND METHODS

Experimental animals

Inbred male Swiss albino mice weighing 20–30 g were used in this study. Breeding and CPCSEA guidelines were used to breed and maintain the animals. The study was carried out after getting permission from Institutional Animal Ethical Committee (IAEC/KMC/56/2010-2011). A total of three mice only were kept in each polypropylene cage to prevent overcrowding. Animals were kept at $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$ temperature and $50\% \pm 5\%$ humidity and were fed on laboratory feed (VRK Nutritional Solutions, Pune, India Ltd.) and water *ad libitum*.

Experimental design

Animals were divided into five groups comprising six animals each (three animals in each cage): Group 1 –normal control (NC), Group 2 – PC (PC-treated with imatinib at the dose level of 100 mg/kg body weight), Group 3 – S1 (treated with sorafenib at the dose level of 25 mg/kg body weight), Group 4 – S2 (treated with sorafenib at the dose level of 50 mg/kg body weight), Group 5 – S3 (treated with sorafenib at the dose level of 100 mg/kg body weight).

Dose and treatment

A total of 180 mice were used in this study, they were divided into 30 groups (six animals per group). Six groups served as NCs, which received gum acacia and six groups served as PCs, which received imatinib 100 mg/kg body weight. Remaining 18 groups were given with sorafenib at the different dose levels of mg/kg body weight orally for a continuous period of 7 days with an interval of 24 h between two administrations. The mice were sacrificed on 1st, 2nd, 4th, 5th, 7th and 10th weeks sample times by overdose of anesthesia (pentobarbital sodium, 40 mg/kg, Sigma Chemicals Co.).^[7]

Epididymal sperm count and sperm motility

Mice were sacrificed by an overdose of anesthesia. The method described by Vega *et al.* was used for sperm count and sperm motility. In brief, sperm suspensions were prepared by mincing cauda in 2 ml of phosphate-buffered physiological saline (PBS, pH = 7.2). The suspension was pipetted and filtered through 80 μm nylon mesh

to remove tissue fragments. An aliquot (0.05 ml) from the sperm suspension (1 ml) was diluted with 1:40 PBS (PBS, pH 7.2) and mixed thoroughly. A sample of the diluted sperm suspension was put into a counting chamber after discharging few initial drops of the suspension. The total sperm count in 8 squares of 1 mm² each was determined and multiplied by 5×10^6 to calculate the number of sperms per epididymis. Sperm motility was also counted in same eight squares and percentage of motile sperm were recorded.^[8]

Statistical analysis

The data generated are analyzed by one-way analysis of variance followed by Bonferroni's *post-hoc* test. $P < 0.05$ were considered statistically significant.

RESULTS

Effect of sorafenib on sperm count

Sorafenib had a significant effect on the sperm count at each of the treated doses. On the 1st week sampling time, each dose of the sorafenib (25 mg/kg, 50 mg/kg and 100 mg/kg) considerably decreased sperm count in mice when compared to the control groups. Significantly decreased sperm count was also seen through the 2nd, 4th, 5th, and 7th week sampling time. For the mice treated with 25 mg/kg, 50 mg/kg, and 100 mg/kg sperm count was least during the 4th and 5th week sampling time. However, the sperm count returned closer to control levels in two dose groups (25 mg/kg and 50 mg/kg) by the 10th week [Figure 1]. The recovery was almost the same in both lower doses (25 mg/kg, 50 mg/kg), which was initiated in the 7th week and reached close to the control group by the 10th week. However, the higher dose group did not show complete recovery. PC imatinib had a significant effect on the sperm count in 1st, 2nd, 4th, 5th, and 7th week sampling time; sperm count returned closer to control group in 10th week sampling time.

Effect of sorafenib on sperm motility

Sperm motility was significantly decreased during the 1st, 2nd, 4th, 5th, and 7th week sampling time with all treated doses of the sorafenib (25 mg/kg, 50 mg/kg and 100 mg/kg). For the mice treated with sorafenib sperm motility was least during the 4th and 5th week sampling time [Figure 2]. By the 10th week, there was complete recovery and the sperm motility values reached the control values except the higher dose group (100 mg/kg). PC imatinib had a significant effect on the sperm motility in 1st, 2nd, 4th, 5th, and 7th week sampling time; sperm count returned closer to control group in 10th week sampling time.

DISCUSSION

Chemotherapeutic drugs can often cause severe alterations in spermatogenesis.^[9] Testicular dysfunction

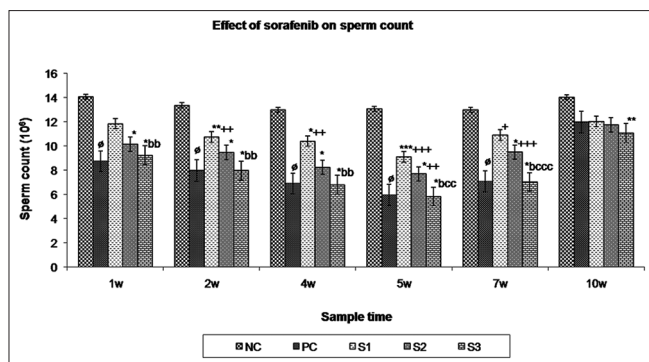


Figure 1: Effect of sorafenib on sperm count (10^6). Each dose group from particular time represents mean \pm standard deviation from six animals. P values are normal control versus treated, $***P < 0.05$, $**P < 0.01$, $*P < 0.001$; normal control versus positive control, $^{\circ}P < 0.001$; positive control versus treated, $+++P < 0.05$, $++P < 0.01$, $+P < 0.001$; S1 versus S3, $^{bb}P < 0.01$; $^bP < 0.001$; S2 versus S3 $^{ccc}P < 0.05$, $^{cc}P < 0.01$. w = weeks

is the most common long-term side effects of cytotoxic chemotherapy used in the treatment of many malignancies. Chemotherapeutic drugs cause physiological damage to male germ cells in the testis has been associated with fertility, which is assessed by parameters of semen quality. Cytotoxic drugs suppress the spermatogenesis in mammals by causing the death of developing germ cells in the seminiferous tubules.^[10] A strategy to decrease the incidence of serious side effects of anticancer drugs with preservation of their chemotherapeutic efficacy is necessary. The male germ cells are incredibly perfect and simple for the study of the genotoxicity of drugs, since they stay alive in different phases of cell development and differentiation.^[11] Male infertility is directly related to quality and quantity of sperm within the semen.^[12] A reduction in epididymal sperm count denotes altered spermatogenesis owing to the gonadotoxic substance.^[13] Male germ cells are incredibly perfect and simple for the study of the genotoxicity of drugs since they stay alive in different phases of cell development and differentiation.

In our study, sorafenib was given orally for 7 days to estimate the sperm count. Data generated indicates that sorafenib is cytotoxic to the sperms. From our findings, we can point out that the germ cells affected are approximately the spermatids, spermatocytes, and spermatogonia. Recovery was stated in the 7th week, and complete recovery was observed by the 10th week in the lower dose groups (25 mg/kg, 50 mg/kg). This study indicates that sorafenib affects spermatogenesis by causing a decrease in sperm count, but this effect is reversible once the drug is withdrawn, which indicates that the stem cells were not affected severely. The difference in sperm count between the treated and the control suggests reduced spermatogenic activity and enhanced apoptotic activity on the highly proliferative germ cells, namely spermatogonia and primary spermatocytes. Any agent that interferes with the mitotic division is also known

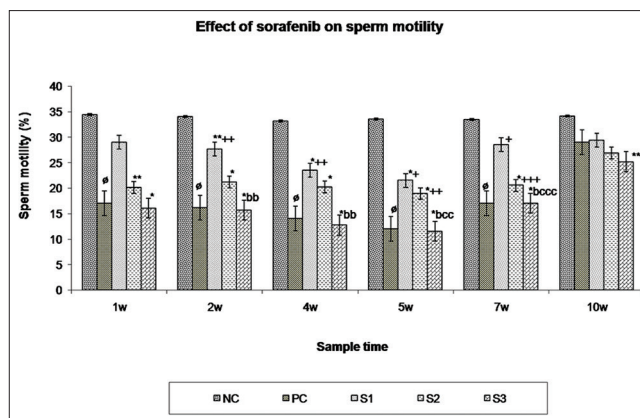


Figure 2: Effect of sorafenib on sperm motility. Each dose group from particular time represents mean \pm standard deviation from six animals. P values are normal control versus treated, $**P < 0.01$, $*P < 0.001$; normal control versus positive control, $^{\circ}P < 0.001$; positive control versus treated, $+++P < 0.05$, $++P < 0.01$, $+P < 0.001$; S1 versus S3, $^bP < 0.05$; $^{bb}P < 0.01$; S2 versus S3, $^{ccc}P < 0.05$, $^{cc}P < 0.01$. w = weeks

to reduce the sperm count.^[14] Sperm count reduction is an important indicator of male infertility.^[15,16] Altered function of the germ cell can also reduce the sperm count. Functional and fully differentiated sertoli cells are critical for the development of quantitatively and qualitatively normal spermatogenesis. They also provide structural and functional support to the developing and differentiating germ cells^[17] and each sertoli cell can support restricted germ cell.^[15] Degeneration in sertoli cells can directly affect sperm counts. More than 90% of cases of male infertility are because of low sperm counts and poor semen quality.^[18] Sperm motility has been considered as one of the most important predictors of fertility. Sperm motility may be affected by altered enzymatic activities of oxidative phosphorylation process. Oxidative phosphorylation process is required for ATP production, a source of energy for the forward movement of spermatozoa.^[19] In our result, sperm motility was significantly decreased during the 1st, 2nd, 4th, 5th, and 7th week sampling time with all treated doses of the sorafenib (25 mg/kg, 50 mg/kg and 100 mg/kg). The adverse effect on sperm motility and sperm count was completely reversible by 10th week. The least sperm motility was observed during the 5th sampling time in mice treated with all the doses. The sperm motility largely depends on the microtubular apparatus of the sperm tail.^[20]

Several reports have demonstrated the correlation of sperm motility with fertilization rates.^[21,22] The normal mitochondrial function is required for sperm motility.^[23] Sorafenib toxicity might have affected the mitochondrial function which may lead to decreased motility. During sperm, maturation spermatozoa acquire progressive motility and fertilizing capacity for the transport of spermatozoa during ejaculation. Any intervention at this site would result in either azoospermia

or inability of spermatozoa to initiate fertilization associated events.^[24] Spermatogenesis is regulated by testosterone hormone. Lack of testosterone level would have direct effects on the process of spermatogenesis. Decreased sperm count and motility was due to reduced supply of testosterone to the epididymis.^[25,26] Chemotherapeutic agents are extensively used for the treatment of various types of cancers, to some extent they cure the disease or at least increase the life expectancy of cancer patients.^[27] Present results thus indicate that sorafenib inhibited spermatogenesis is probably affecting the cell multiplication and differentiation. The difference in sperm count between the treated and the control suggests reduced spermatogenic activity and enhanced apoptotic activity on the highly proliferative germ cells, namely spermatogonia and primary spermatocytes. The spermatogenic stem cells differentiate through division of spermatogonia (mitotic divisions) to form spermatocytes (meiotic cells) that undergo two meiotic divisions to give rise to spermatids (haploid postmeiotic cells) that mature into functional sperm. The kinetics of spermatogenesis is well-established for men and several mammalian species.^[28] According to Prasad *et al.*^[6] higher dose of imatinib decrease the sperm count and motility. In our study, imatinib was used as PC, comparing with the result of control, PC and experimental groups, the result of 100 mg/kg body weight of sorafenib also similar to that of imatinib result. Hence, comparing with the result we can say that sorafenib also effects on sperm count and motility.

CONCLUSION

Sorafenib adversely affected sperm parameters of mice significantly, but this effect is reversible once the drug is discontinued. Outcome of the study may help the clinicians to plan and address the fertility-related issues in young patients of reproductive age who are treated with sorafenib for advanced renal cell carcinoma and HCC.

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Conflicts of interest

There are no conflicts of interest.

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