ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Sorafenib Induced Sperm Shape Abnormalities in Male Swiss Albino Mice

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ABSTRACT

Sorafenib is a multi-targeted kinase inhibitor. It inhibits the action of vascular endothelial growth factor (VEGE) and is an angiogenesis inhibition. Male gonadal toxicity is common complications of modern anti-cancer treatments. Anti-cancer drugs have adverse effects on spermatogenesis. This study was planned to assess the effects of sorafenib on sperm morphology assay. Male Swiss albino mice were segregated into control, positive control and three treatment groups. Positive control received imatinib (100 mg/kg body weight) and treatment groups received 25, 50 and 100 mg/kg body weight of sorafenib orally for seven consecutive days at intervals of 24 hours between two administrations. Control group remained in home cage for equiduration 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. Sperms from epididymis were stained as per standard protocol and 1000 sperms per rat were counted and analysed**.** There was significant increase in head and tail sperm abnormality. Sorafenib does affect on sperm morphology assay significantly, but this effect is reversible once the drug is withdrawn.
Keywords: Sorafenib, mice, cauda epididymis, sperm abnormality.

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ISSN: 0975-8585

INTRODUCTION

Chemotherapy is one of the most effective methods for the treatment of cancer, but is often associated with several short and long-term toxicities [1]. Among all the pharmaceutical agents, chemotherapeutic drugs are more toxic, the cytotoxicity of these drugs acts on both normal and cancerous cells [2]. In male cancer patients, surgery, radiotherapy and chemotherapy can be followed by transient or permanent infertility [3]. Since sperms exist in different stages of development, they are ideal for study of genetic toxicity [4]. Genetic abnormality can result in abnormal sperms, hence studying sperm morphology of sperm can indirectly tell about genotoxicity of the drug [5].

Sorafenib is a multi-targeted kinase inhibitor. It inhibits the action of vascular endothelial growth factor (VEGE) and is an angiogenesis inhibition. It is indicated for the treatment of patients with advanced renal cell carcinoma and hepatocellular carcinoma (HCC). Sorafenib has been demonstrated that effectively of radiation therapy in combination with multi-kinase inhibition based on sorafenib or sunitinib in progressive metastatic renal cancer [6]. There is a report on sorafenib used for advanced hepatocellular carcinoma [7]. It is also effective in treatment for metastatic renal cell carcinoma in a case with chronic renal failure [8]. Imatinib mesylate, a first synthetic tyrosine kinase inhibitor, used in chronic myeloid leukemia is known to cause sperm shape abnormality [9]. But there are no reports of sorafenib on sperm morphology. The sperm morphology assay is one of the most widely used genetic toxicology assays. The ability of the sperm to fertilize a functional ovum is considered as the ultimate criteria of its function [10]. We have undertaken a study to evaluate the effect of sorafenib on sperm morphology assay in male Swiss albino mice.

MATERIALS AND METHODS

Experimental animals

Inbred male Swiss albino mice weighing 20-30g 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. A total of 5 mice only were kept in each polypropylene cage to prevent overcrowding. Animals were kept at 28±1°C temperature and 50±5% humidity and were fed on laboratory feed (VRK Nutritional Solutions, Pune, India Ltd) and water ad libitum.

Dose and treatment

A total of 180 mice were used in this study. They were divided in to 30 groups(6 animals per group). Six groups served as normal controls, which received gum acacia and 6 groups served as positive control 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 seven days with an interval of 24 hours between two administrations. The mice were sacrificed on 1^{st}, 2^{nd}, 4^{th}, 5^{th}, 7^{th} and 10^{th} weeks sample times by overdose of anesthesia (Pentobarbital sodium, 40mg/kg, Sigma Chemicals Co).

Experimental design

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

Sperm morphology assay

The mice were sacrificed at different week samples and laparotomy was done. Method described by Wyrobek AJ et al was used for sperm morphology assay was used [11]. Briefly, testes were removed; cauda epididymis was separated (Testes were processed for histopathological sections and tissue homogenization). Sperm suspensions were prepared by mincing cauda in 2ml of phosphate buffered physiological saline (PBS, pH=7.2). Suspension was pipetted and filtered through 80 m nylon mesh to remove tissue fragments. A fraction of suspension was then mixed with (10:1) with eosin Y and 30 minutes later about one drop of stained

ISSN: 0975-8585

suspension was placed on the clean slide. It's dried, cleaned and mounted in DPX. Slides were looked for sperm shape abnormality. Slides were coded for blind analysis. From each suspension 1000 sperms were examined at 400X with blue-green filter. Abnormal sperms are classified as, I. Head abnormality- that included: hook less, banana shaped, double headed and amorphous. II. Tail abnormality- which includes the coiled and double tailed sperms [11].

Statistical analysis

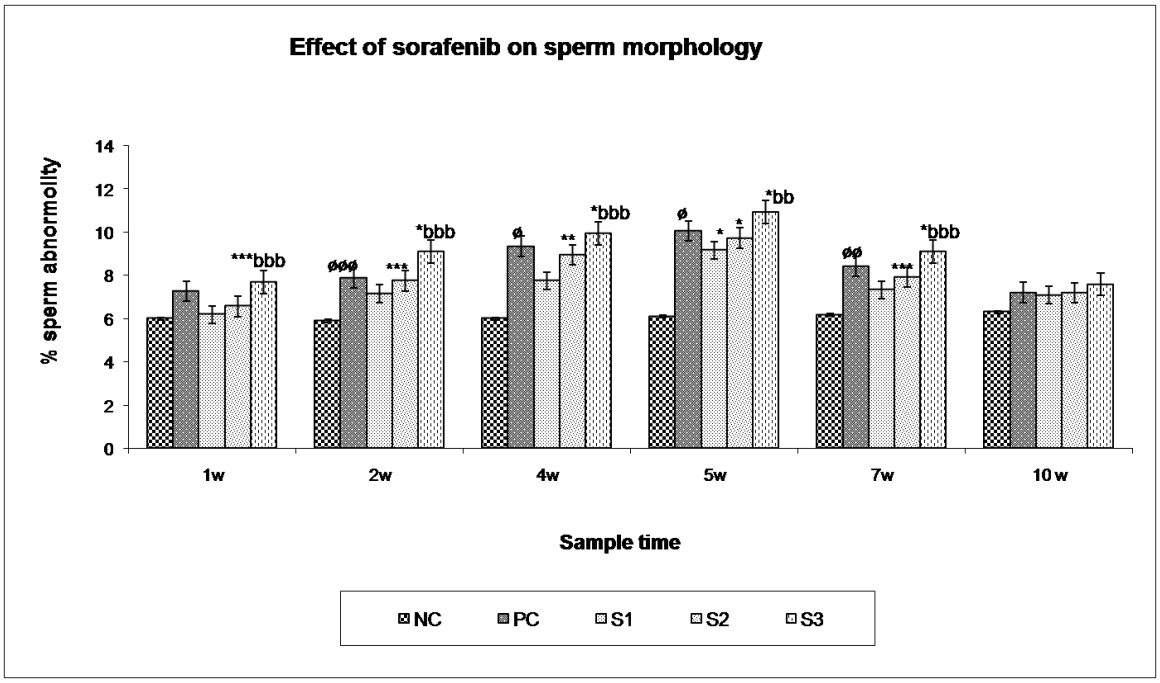
The data generated are analysed by one way Analysis of Variance (ANOVA) followed by Bonferroni's post hoc test. Values of $P<0.05$ were considered statistically significant.

RESULTS

Effect of Sorafenib on sperm morphology

Sorafenib induced the anomalies of head and tail of sperm. Head abnormalities were amorphous, hook less, banana shaped and the double headed sperm. Drugs caused the formation of coiled and double-tailed sperm. The incidence of abnormal sperms showed significant increase during the 1st week sampling time in mice treated with 100mg/kg body weight of sorafenib when compared to the control group. In mice treated with all the doses of sorafenib the percentage of abnormalities increased in a time dependent manner during the 1st, 2nd, 4th and 5th week sampling time. The maximum sperm abnormality was observed during the 5th week sampling time in mice treated with all the doses of sorafenib (Table 1 and Figure 1). The higher dose group showed the maximum percentage of abnormal sperms during the most week sampling time except week 10th which showed a value closer to the control group. Although maximum sperm abnormalities were seen during the 5th week sampling time in mice treated with 25mg/kg and 50mg/kg body weight of sorafenib, the percentage of abnormal sperms were still less compared to the mice treated with 100mg/kg body weight of sorafenib. The recovery period was similar for all the treated groups of mice. The percentage of abnormal sperms reached closer to the control values in mice treated with all the doses of the sorafenib during the 10th week sampling time. Positive control imatinib had a significant affect on the sperm morphology in 2nd, 4th, 5th and 7th week sampling time, sperm abnormality returned closer to control group in 10th week sampling time.

Figure 1: Effect of sorafenib on sperm morphology (%).



Each dose from particular time represents mean±SD from 6 animals. P values are NC vs treated, ***<0.05, **<0.01, *<0.001; NC vs PC, øøø<0.05, øø<0.01, ø<0.001; S1 vs S3, bbb<0.05; bb<0.01; w=weeks.

ISSN: 0975-8585

Table 1: Effect of sorafenib on sperm morphology (%)

Dose	Sampling time					
	1w	2w	4w	5w	7w	10w
NC	6 ±1.21	5.9 ±1.0	6.01 ±0.62	6.1 ±0.57	6.18 ±0.53	6.31 ± 0.60
PC	7.28±0.40	7.9±0.41^{øøø}	9.35±0.55^{ø}	10.06 ±0.60^{ø}	8.43 ±0.48^{øø}	7.21 ± 0.31
S1	6.2±0.71	7.16±0.92	7.76±1.22	9.18 ±0.88*	7.35 ±1.18	7.1 ±0.86
S2	6.58±0.40	7.76±0.95***	8.96±1.75**	9.73 ±0.87*	7.93±0.91	7.2 ± 0.94***
S3	7.7±1.09^{***bbb}	9.1±1.17*	9.96±1.01^{*bbb}	10.94 ±0.97^{*bb}	9.1 ±1.32	7.6 ±0.84^{*bbb}

Each dose from particular time represents mean±SD from 6 animals. P values are NC vs treated, ***<0.05, **<0.01, *<0.001; NC vs PC, øøø<0.05, øø<0.01, ø<0.001; S1 vs S3, bbb<0.05; w=weeks.

DISCUSSION

Sperm morphology is an important aspect in assessing sperm quality as well as a key index to evaluate reproductive toxicity and mutagenicity of exogenous chemicals. Spermatogenesis is a highly regulated differentiating system, both temporally and spatially. Germ cells, in particular, differentiating spermatozoa are extremely susceptible to cytotoxic agents because of their rapid proliferation. The non-proliferating Leydig cells and sertoli cells survive most cytotoxic therapies but could suffer functional damages [12]. The sperm morphology assay is one of the most widely used genetic toxicology assay. In the evaluation of chemical genotoxicity sperm head abnormality is the most reliable short term biological indicator [13]. The sperm head morphology gives a rough assessment of the functional capability of the spermatozoa and reveal the quality of the sperm DNA [14]. The structure of mature sperm consists of a head and a long flagellum [15]. The tail is divided into four distinct segments: the connecting piece adjacent to the head, the midpiece, and the principal and end pieces[14]. In our study sorafenib was given orally for 7 days to estimate the sperm morphology.

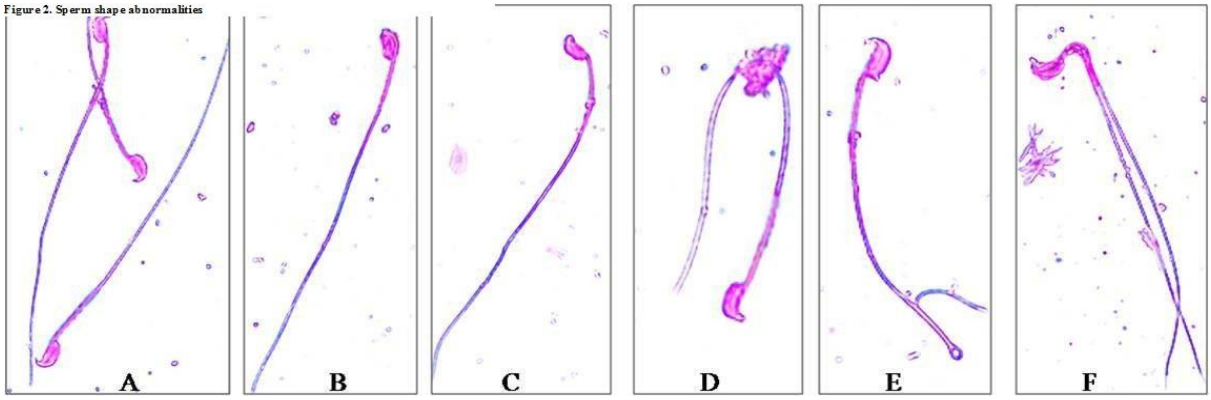


Figure 2: Sperm head & tail abnormalities: (A) Normal sperm; (B) amorphous; (C) hook less; (D) banana shaped; (E) coiled/folded tail; (F) double tail.

Our results show that sorafenib was cytotoxic to the sperm since the percentage of sperm abnormalities increased significantly. However the percentage of abnormalities increased in a time dependent manner during the 1st, 2nd, 4th, 5th and 7th week sampling time in mice treated with all the doses of sorafenib. The maximum sperm abnormality was observed during the 5th week sampling time in mice treated with all the doses of sorafenib. Sperm abnormalities are the resultant end points after point mutations or other chromosome variations [16]. It is possible that these changes in the sperm structure may be due to point mutation. In our results sperm of the sorafenib treated groups showed tail and head abnormalities. The percentage of abnormal sperms reached closer to the control values in mice treated with all the doses of sorafenib during the 10th week sampling time. Maximum sperm abnormalities were seen during the 5th week sampling time in the sorafenib treated groups, which indicates that spermatocytes might have been more susceptible to the toxic effect of sorafenib. The head abnormalities most probably reflect a change in DNA content [17]. Coiling of sperm tail mainly involves its orientation, which give an impression of a reduced sperm movement. Such limitation in sperm movement was reported to reduce fertility in both animals [18] and

humans [19]. Since Comet assay measures DNA damage in the sperm it can add further information on the quality of the sperm. There are reports that sperm comet assay and sperm head morphology are positively correlated [20]. An increase in number of abnormal sperms results in altered motility as normal intact sperm morphology is prerequisite for linear progressive motility [21, 22]. The sperm function is strictly correlated with sperm morphology and that sperm motility is the best predictor of fertility potential in man [23]. Sperm head abnormalities may arise due to small deletions endoplasmic or point mutations, physiological, cytotoxic or genetic mechanisms [24] or alteration in testicular DNA which in turn disrupts the process of differentiation of spermatozoa [25].

Sperm abnormalities are usually taken as characteristic criteria and as an applied test for monitoring the mutagenic potential for many chemicals [26]. Increased level of abnormal sperms is an indication of mutagenic potency of the test chemical. The drug that induces abnormal sperms can be expected to clearly interfere with the normal differentiation of germ cells [27]. The exact mechanism responsible for the decreased semen quality in cancer patients is not well established. Multiple factors are likely involved, including preexisting defects in germ cells and systemic effects of cancer [28]. The mouse sperm morphology test also has potential in identifying chemicals that induce spermatogenic dysfunction and perhaps heritable mutations [29].

Data generated shows that sorafenib significantly increased in number of abnormal sperms. There are reports that antineoplastic drugs induce the sperm abnormality, which reflects their genotoxicity to germ cells [5]. In results, spermatozoa end up with variety of abnormal morphologies in both heads and tails, and this increase in abnormalities has reached to maximum at concentration of 100mg/kg body weight of sorafenib. These results are in consisting with the suggestion of Mangelsdorf et al, [30] according to him the decrease in the total sperm count, increase in abnormal sperm shape, impair in stability of sperm chromatin or damaged in sperm DNA results in the disruption of spermatogenesis at any stage of cell differentiation. The morphological abnormalities could be the consequence of damage exerted on differentiated spermatogonia, or the step between differentiated spermatogonia to spermatocyte. In fact, there is general agreement that differentiated spermatogonia is the most sensitive spermatogenic cellular type to the action of various chemicals agents in the production of abnormal sperms [31]. According to Prasad et al higher dose of imatinib increase the sperm shape abnormality [9]. In our study imatinib was used as positive control, comparing with result of control, positive control and experimental groups, the result of 100mg /kg body weight of sorafenib also similar to that of imatinib effect.

CONCLUSION

Sorafenib does affect the shape of the head and tail of the mice sperm significantly, but this effect is reversible once the drug is withdrawn. 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 hepatocellular carcinoma.

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