

ORIGINAL ARTICLE

TOXIC EFFECTS OF 5-FLUOROURACIL ON SPERM COUNT IN WISTAR RATS

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The antimetabolite, 5-fluorouracil is widely used in the treatment of cancers. Although its toxic effects on testis causing germinal epithelial sloughing, tubular atrophy and generation of multinucleated cells were reported, its effect on spermatogenesis has not been studied. Hence the present study was conducted to evaluate the effects of 5-fluorouracil on epididymal sperm count. Male Wistar rats were employed in the study (n=5 per group). The animals were injected (i.p) with five consecutive doses of 5-fluorouracil (10, 20, 30mg/kg b.w) at an interval of 24h and the control with 0.1ml-distilled water. Samples were obtained at 14, 35, 42 and 70 days after injection. Rats were sacrificed, a laparotomy was performed and epididymes were collected in 1ml phosphate buffered saline (pH 7.2), minced, filtered and stained with 1% aqueous eosin Y. An aliquot was taken in leucocyte pipette, diluted with phosphate buffered saline and sperm count was done as per the standard procedure. Data were analyzed by Mann Whitney U test. The results of this study revealed that 5 - fluorouracil significantly decreased the sperm count in a dose- and time- dependent manner.

Key words : 5-fluorouracil, sperm count, epididymis

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Introduction

Antimetabolite, 5-fluorouracil (5-FU) has been effectively used for the treatment of cancers of head and neck, breast and alimentary system (1). Despite its therapeutic efficacy, it was found to be a mutagen, inducing chromosome aberrations and causing appearance of micronuclei (2). It was also known to induce testicular toxicity like germinal epithelial sloughing, cell killing, multinucleated cell formation and seminiferous tubular atrophy (3,4). Russell and Russell (5) had earlier reported that it also induces spermatogonial damage and arrest of spermatid development. Takizawa and Horii (6) reported a decline in serum prolactin and testosterone level in rats exposed to 5-FU. They also observed a decline in Sertoli cell function. However, it is not known, whether 5-FU has any effect on the number of spermatozoa as sperm concentration plays an important factor in fertility index. Since the life of

cancer patients is prolonged with treatment, the study on the effect of anticancer drugs on reproductive capacity need to be taken into consideration as there are many young subjects suffering from cancer who are under treatment with anticancer drugs like 5-FU. Hence this study was undertaken to investigate the effects of 5-FU on spermatogenesis by using a standard sperm assay procedure.

Materials and methods

Male albino rats of Wistar strain weighing 150-200g (11-13 week old) were maintained under standard laboratory conditions in polypropylene cages. They were fed with standard rat feed pellets and water ad libitum. Five animals were chosen for each group. Three different doses of 5-FU (fluracil; Biochem) viz. 10, 20 and 30mg/kg body weight were prepared and injected (i. p) for five consecutive days at the time interval of 24h and the control group

Table 1: Epididymal sperm count ($\times 10^6$) in control & 5-fluorouracil treated rats

Drug/dose	Sample time (days)			
	14	35	42	70
Control	58.70 \pm 2.30	59.12 \pm 1.60	61.40 \pm 2.14	60.68 \pm 2.46
5-FU 10mg/kg	54.48 \pm 1.08	46.42 \pm 1.62**	52.40 \pm 1.36**	59.38 \pm 1.81
5-FU 20mg/kg	55.20 \pm 0.86	35.89 \pm 1.88**	48.48 \pm 0.48**	60.20 \pm 0.82
5-FU 30mg/kg	44.60 \pm 1.18**	28.40 \pm 1.68**	44.82 \pm 1.28**	45.18 \pm 1.48**

**P<0.01 control versus treated

received 0.1ml distilled water. Animals were sacrificed by using anesthesia (Nembutal, 40mg/kg; Sigma Chemicals, USA) on the 14, 35, 42 and 70th day after injections and the epididymes were collected and minced in 1ml phosphate buffered saline (PBS, pH 7.2). The suspension was filtered through muslin cloth and filtrate was mixed with 1% aqueous eosin Y (10:1) and kept for 30 min for the staining of sperms. Then an aliquot of stained the filtrate was taken in white blood cell pipette up to the 0.5 mark and diluted further up to mark 11 with PBS. The mixture was shaken and charged into Neubauer's chamber and sperm count was performed as per standard procedure (7). The sperm count in 8 squares of 1mm² each area except the central erythrocyte counting area of Neubauer's chamber was performed and multiplied by 5 X 10⁴ factor to calculate the total number of sperms. Data were analyzed by Mann Whitney U test using SPSS software and P<0.05 was considered as the level of statistical significance.

Results

A significant decrease in the sperm count was noted in the epididymal suspension, which received 30mg/kg of 5-FU injection (Table 1). The highest fall in the sperm count was noted in the samples collected 35 days after 5-FU injection. The decline in sperm count was significantly different compared with the control group (P< 0.001). In the groups that received 10 and 20mg/kg 5-FU the reduction in sperm count was significant on 35 and 42nd days after injection. The lower two doses (10 & 20mg/kg) did not produce any significant decline in the sperm count on 70th day after injection compared to the control group. However animals that received a higher dose (30mg/kg) continued to show reduction in the count 70 days after injection with 5-FU. There

was a dose - dependent decrease in the number of spermatozoa whereas time response followed a parabolic pattern with maximum toxicity on the 35th day sample time

Discussion

Cytotoxic drugs depress spermatogenesis in mammals (8) by causing death of developing germ cells in the seminiferous tubules (9). This results in the elimination of active cells of spermatogenesis and thereby brings about a reduction in daily sperm production. The dose - and time - dependent decrease in the number of spermatozoa following 5-FU treatment noted in the present study is an indication of depressed spermatogenesis due to the inhibition of cell multiplication and interference by genetically controlled programme of sperm differentiation process. 5-fluorouracil also induces germinal cell sloughing and atrophy of the seminiferous tubules in rats, (4) which lends further proof for a decline in the sperm count noted in our study.

The fall in the sperm count as noted in 14th and 35th day sample exhibits a negative linear relation (Table 1), which indicates a rise in the toxicity of 5-FU over time. Among different sample time studies, the 35th day sample indicates a maximum toxic effect which points to the possibility that the cells exposed at this stage belong to spermatogonial cell population, which are most sensitive to 5-FU exposure. However the toxicity that is seen in other samples of different days exposure to 5-FU study, highlights the fact that toxic effect of this drug is maintained. Similar reports have been published for tamoxifen citrate, an antineoplastic agent which also produced a decline in sperm count following 5 weeks of treatment in mice (10). The exact mechanism underlying the sperm toxic effect of 5-FU is not known. This drug inhibits thymidylate synthetase enzyme (11) that also causes single strand DNA breaks and cell death (12). This is the likely reason for the cause of tubular atrophy, sloughing of germinal cells causing significant fall in the sperm density. This study indicates that 5-FU affects spermatogenesis by causing depression in sperm count. 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.

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