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EFFECT OF ACYCLOVIR ON THE SPERM PARAMETERS OF ALBINO MICE

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Abstract : A number of animal studies as well as human epidemiological studies have demonstrated that exposure of males to various agents could result in abnormal reproductive toxicity. Acyclovir (ACV) is known to be toxic to gonads, but fails to provide the in-depth analyses of timing of damage, the types of germ cells affected, dose and the duration of damage and timing of reversal of fertility. Hence this study on sperm morphology, sperm count and sperm motility.

Doses of 4 mg, 16 mg, 32 mg and 48 mg/kg body weight of ACV were administered to 9-12 weeks old male swiss albino mice by intraperitoneal route for 15 days continuously. One hundred and eighty animals were segregated into 30 groups (N=6). Twenty four groups were injected with acyclovir (4 mg, 8 mg, 16 mg and 32 mg/kg bodyweight) and the rest six groups served as control. After the last treatment, the animals were sacrificed on 7, 14, 21, 28, 35 and 70 days sample times and the sperm parameters were estimated.

ACV causes increased incidence of abnormal sperms on most of the dose ranges from day 21 to 35 indicating the effect on spermatocytes and spermatogonial cells. ACV is cytotoxic to the testis. It causes oligospermia from day 7 to day 35 after the last exposure. It also decreased the sperm motility on same time points. All these effects were reversible by day 70.

ACV exerts reversible genotoxic and cytotoxic effect on germ cells. ACV does not affect stem cell lines of spermatogenesis since all sperm parameters return to control level on day 70.

Key words : acyclovir, sperm morphology, sperm motility, sperm count

INTRODUCTION

With the increasing survival rate after chemotherapy over last few decades, long term side effect of chemotherapy on fertility is a principal social concern for the young men of reproductive age. A number of animal studies as well as human epidemiological studies have demonstrated that exposure of males to various agents could result in abnormal reproductive, pregnancy or progeny outcomes (1). Therefore it is particularly

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relevant to study the cytotoxic and genotoxic effects of various agents on germinal cells, because this is the only system in which transmissible genetic damage from one generation to another takes place (2). The high multiplication rate of germ cells makes the male gonad highly susceptible to the toxic effect of chemotherapy (3).

Most antiviral drugs are nucleoside analogues with potential teratogenic, embryotoxic, carcinogenic and antiproliferative activities. Therefore they must be administered with caution during pregnancy, because some are known teratogens (e.g. amantadine) and a similar propensity cannot be entirely excluded for others (e.g. ACV) (4). ACV, a synthetic acyclic purine nucleoside analogue, discovered and approved initially in 1982 is one such agent commonly used for the treatment of herpes simplex virus (HSV) and varicella zoster virus (VZV) (5).

ACV is known to be toxic to gonads, but available literature fails to provide the in-depth analyses of timing of damage, the types of germ cells affected, dose and the duration of damage and timing of reversal of fertility. Keeping these aspects in mind the effect of acyclovir on sperm parameters i.e sperm morphology, sperm count and sperm motility was investigated.

MATERIALS AND METHODS

Animals

Male Swiss albino mice (9–12 weeks) were used. Animals were bred in central animal house of the institution. 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 has been carried out under the supervision of institutional animal ethical committee. (Institutional animal ethical committee approval reference number (IAEC/KMC/91/2001-2002). All the animals were housed in polypropylene cages using paddy husk bedding at 28±1°C temperature and 50±5% humidity. Five animals were housed in a cage to prevent overcrowding. Animals were fed on laboratory feed (Gold mohur; Lipton India LTD) and water *ad libitum*.

Drug, dose and route of administration

Pure ACV powder obtained from Glaxo Wellcome operations, Green ford-UK, was used in this study. Random doses of 4 mg, 16 mg, 32 mg and 48 mg/kg body weight were used for the present study. These doses approximately correspond to $1/150LD_{50}$, $1/50LD_{50}$, $1/25LD_{50}$ and $1/10LD_{50}$. The acyclovir oral LD_{50} doses in rats and mouse are 20 and 10 mg/kg body weight respectively. One hundred and eighty animals were segregated into 30 groups (N=6). Six groups each (Total 24 groups) were injected with ACV at the dose of 4, 16, 32 and 48 mg/kg body weight intrapentoneally for a continuous period of 15 days with an interval of 24 hr between two successive injections. Remaining six groups served as control, which received only distilled water. After the last treatment, the animals have been sacrificed on 7, 14, 21, 28, 35 and 70 days sample times. These sample time establishes the treatment of spermatozoa in the epididymis and testis, spermatids, primary spermatocytes, secondary spermatocytes, spermatogonia and stem cells respectively (6, 7, 8).

Sperm morphology assay (7, 9)

After sacrificing the mice, testes were removed and cauda epididymis was separated. Testes were processed for tissue homogenization. Sperm suspensions were prepared by mincing cauda in 2 ml of phosphate buffered physiological saline (PBS, pH=7.2). Suspension was pipetted and filtered through 80 µm nylon mesh to remove tissue fragments. A fraction of suspension was then mixed (10:1) with eosin Y and 30 minutes later the smears were made, air dried and mounted. 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.

Epididymal sperm count and sperm motility (10)

After separating the cauda epididymis, sperm numbers per epididymis were determined by haemocytometer. Dilute sperm suspension was prepared with phosphate buffered saline and introduced into a counting chamber. The total sperm count in 8 squares of 1 mm^2 each was determined and multiplied by 5×10^4 to calculate the number of sperms per epididymis. Sperm motility was also counted in same eight squares and percentage of motile sperm was recorded.

Statistical analysis

Data obtained from the experiments were correlated and analyzed by one way "Analysis of Variance (ANOVA), followed by Bonferroni's post test wherever applicable by using statistical software package, Graph Pad In Stat (GPIS); 1990, version 1.13. Values of $P<0.05$ were considered as statistically significant.

RESULTS

Effect on sperm morphology (Fig. 1, Table I)

On day 7, abnormal sperms increased in number at all dose levels, but elevation was not significant as compared to the control. On day 14 also similar results have been obtained Unlike first 2 sampling days, on day 21, abnormal sperms increased at all dose levels ($P<0.05$), except at 4 mg/kg. On day 28, significant elevations in sperms with abnormal heads were seen at 32 mg and 48 mg/kg ($P<0.01$). On day 35 a significant elevation in the number of abnormal sperms was seen only at 32 mg/kg, and in other dose-levels even though the abnormalities were more, it was not statistically significant. On day 70, except at 48 mg/kg in which a higher incidence of abnormal sperms was seen, other doses did not cause any changes.

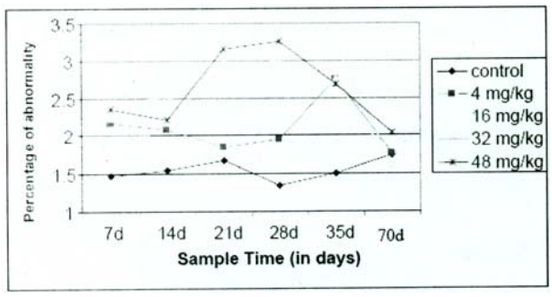

Fig. 1: The time-response relationship representing incidence of sperm shape abnormality (percentage) in acyclovir treated mice. Each time point at particular dose level represents mean from 6 animals per group.

TABLE I: Incidence of abnormal sperms (percentage) in control and acyclovir treated mice at different days after the last exposure.

Dose/kg body weight	*Sample time (in days)*					
	7 days	*14 days*	*21 days*	*28 days*	*35 days*	*70 days*
Control	1.48±0.51	1.55±0.68	1.68±0.34	1.35±0.48	1.51±0.64	1.75±0.85
4 mg	2.16±1.03	2.08±1.07	1.85±0.18	1.95±0.85	2.75±1.04	1.76±0.07
16 mg	2.23±0.89	2.18±0.68	2.98±1.16*	2.9±1.05	2.75±1.06	1.61±0.78
32 mg	2.58±0.89	2.46±0.94	2.86±0.95*	3.01±0.83*	3.23±1.02*	1.61±0.67
48 mg	2.35±0.93	2.2±0.93	3.16±0.87*[a]	3.25±0.95*	2.68±1.19	2.03±1.00

Data are represented as mean±S.D. from 6 animals per group (1000 sperms/animal).
Significance values are indicated as control v/s treated: *P<0.05, **P<0.01; ***P<0.001; 4 mg v/s other treated groups; [a]P<0.05, [aa]P<0.01, [aaa]P<0.001.
One-way ANOVA and Bonferroni post-test.

Effect on epididymal sperm count (Fig. 2, Table II)

On day 7, ACV caused significant decrease in sperm count (P<0.001) in a dose-dependent manner. On day 14, the effects were significant and the highest effect was found in 32 mg/kg group (P<0.001). On days 21, 28, and 35 similar effects were seen, but the sperm count returned to the control limit by day 70.

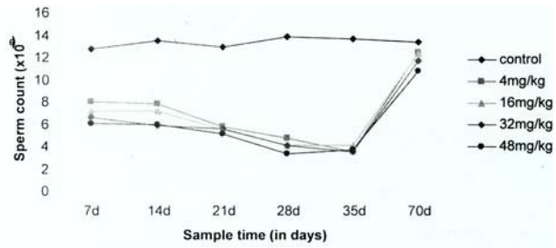

Fig. 2: The time-response relationship representing sperm count (per epididymis) in acyclovir treated mice Each time point at particular dose level represents mean from 6 animals per group.

Effect on sperm motility (Fig. 3, Table III)

After the treatment of acyclovir for fifteen consecutive days, the sperm motility decreased in all sampling days except on day 70 when the ability of the sperms was on par with that of controls to swim.

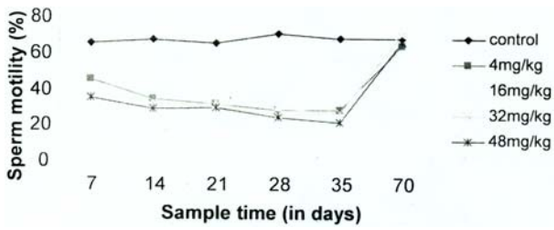

Fig. 3: The time-response relationship representing percentage of sperm motility in acyclovir treated mice. Each time point at particular dose level represents mean from 6 animals per group.

TABLE II: Effect of acyclovir on sperm count ($X10^6$) in control and drug treated mice at different sample times after the last exposure.

Dose/kg body weight	*Sample time (in days)*					
	7 days	*14 days*	*21 days*	*28 days*	*35 days*	*70 days*
Control	12.75±1.57	13.53±1.55	12.96±1.34	13.85±1.05	13.65±1.08	13.41±0.97
4 mg	8.00±0.91***	7.81±0.76***	5.80±0.86***	4.73±0.51***	3.51±0.85***	12.5±1.52
16 mg	7.16±1.69***	7.16±1.34***	5.46±0.83***	4.08±0.63***	4.08±0.53***	12.33±1.21
32 mg	6.61±0.74***	5.85±0.68***	5.57±1.24***	4.08±0.44***	3.5±1.38***	11.75±1.07
48 mg	6.06±1.05***	5.98±1.15***	5.07±0.47***	3.37±0.86***	3.71±0.67***	10.75±1.31*

Data are represented as mean±S.D. from 6 animals per group.
Significance values are indicated as control v/s treated: *P<0.05, **P<0.01; ***P<0.001.
One-way ANOVA and Bonferroni post-test.

TABLE III: Effect of acyclovir on sperm motility (in percentage) in control and drug treated mice at different sample times after the last exposure.

Dose/kg body weight	*Sample time (in days)*					
	7 days	*14 days*	*21 days*	*28 days*	*35 days*	*70 days*
Control	64.5±5.73	66.83±5.20	64.33±5.18	69.83±3.13	67±5.29	67.5±2.75
4 mg	44.16±5.45***	33.66±4.81***	30.83±4.74***	27.5±4.92***	27.5±4.11***	63.5±4.46
16 mg	36.66±3.90***	30.83±4.37***	30.83±4.37***	29.33±5.21***	24.16±4.41***	71.66±4.60
32 mg	37.00±3.82***	31.66±5.12***	29.0±5.59***	25.16±3.93***	26.0±5.80***	69.33±5.99
48 mg	34.00±2.51***^{a}	28.33±6.28***	28.66±6.04***	23.5±4.38***	21.0±3.26***	65.5±3.09

Data are represented as mean±S.D. from 6 animals per group.
Significance values are indicated as control v/s treated: *P<0.05, **P<0.01; ***P<0.001; 4 mg v/s other treated groups; ^{a}P<0.05, ^{aa}P<0.01, ^{aaa}P<0.001.
One-way ANOVA and Bonferroni post-test.

DISCUSSION

Our study shows that ACV caused an increase in the percentage of abnormal sperms up to day 35, and the effect was returned to the control levels by day 70. Sperm shape abnormality test is one of the most reliable, rapid methods used as an *in vivo* assay for genotoxicity (11, 12, 13). Acyclovir is reported to inhibit the thymidine kinase in the viral infected DNA. A similar mechanism of action in the non-infected cell is reported. Acyclovir is known to cause inhibition of cell division at a dose of 50–100 µm in human fibroblast cell (14). Jagetia and Aruna (15, 16, 17) reported that ACV is clastogenic to somatic cells. It causes induction of micronuclei in cultured HeLa cells. ACV, in the range of 5-600 mg/kg induced micronuclei in the polychromatic erythrocytes in mice bone marrow (18). By induction of micronuclei, it shows its efficacy to damage cellular DNA in the non-infected cells also. A significant increase in the chromosomal damage has been reported in the human lymphocyte (19). A borderline increase in the sister chromatid exchanges has been reported in V79-E cells exposed to ACV (20). All these studies support our finding that acyclovir is genotoxic to germ cells. Sperm shape abnormality caused by ACV may be due to interference with DNA synthesis during mitotic stage of spermatogenesis or interference with chromosome structure. But ACV does not affect spermatogonial stem cells, as it is evident by the returning of normal sperm shapes by day 70. Similar effect was observed in cases of ribavirin in rats in a dose-dependent manner (21, 22).

Sperm count is an important indicator of male fertility (23). Sperm count could be altered either because of change in the germ cell function or epididymal storage. Any agent that interferes with meiotic division is also known to reduce the sperm count (24). Acyclovir caused large reduction in the sperm count until day 35 after the last exposure and recovery of sperm count was observed on day 70 at all tested doses except at 48 mg/kg. The mechanism of action of acyclovir may be due to its effect on the Leydig cells which leads to decreased testosterone levels. Decrease in the intratesticular testosterone levels can cause sloughing of germinal epithelium hence declining sperm count. Narayana et al (21,

22) reported that antiviral drug ribavirin affects spermatozoa and declines sperm count. Faqi et al (25) reported that reduction in sperm count occurred in ganciclovir treated rats by reducing the number of spermatids, but the effect was reversed by 168 days after the cessation of treatment. Return of the sperm count to normal indicates that the effects of acyclovir on the germinal cells are reversible.

Defective sperm motility is one of the causes of untreatable infertility or sub fertility in men (26). Sperm motility defects (asthenozoospermia) may also result from swelling of mitochondria that lead to the discharge of a proton gradient across mitochondrial membrane (27). Aarnoud et al (24) reported that the irregular mitochondrial sheath, aberrant attachment of tails, or deviant head shapes impair the motility. Changes in the motility may be also due to changes in the adluminal components, i.e. accumulation of fluid observed on these sample times (from 7 d to 28 d) in the acyclovir treated mice. Acyclovir decreases sperm motility starting from day 7 until day 35 after the last exposure and motility returns to control levels by day 70. Highest dose caused large decline in the motility. Since acyclovir alters sperm shape abnormality from day 7 until day 35, here too it is thought that because of alteration in the structure, motility might be affected. Leandri et al (28) observed decreased sperm motility in rats treated with zidovudine due to sperm mitochondrial respiratory chain dysfunction. Huang et al (29) reported heat shock protein 90 (HSP90) may be associated with porcine sperm motility. An HSP90-specific inhibitor- geldanamycin (GA) was added to diluted semen (declined sperm motility.

The duration of spermatogenesis in human is about 74 days. Considering that the human germ cells have same sensitivity as that of mouse germ cells, the young patients undergoing one cycle chemotherapy with acyclovir type drugs must avoid conception for the period of about 150 days. If chemotherapy is repeated in cyclic fashion, long term cytotoxicity, genotoxicity and gonadotoxicity in human can be expected.

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