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Research Article

Evaluation Effect of Dacarbazine on Mice Testis Tissues and Sperm Parameters

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Abstract: Dacarbazine (DTIC) is widely used as anti-cancer drug and can result in severe cytotoxic effect and sometime this effect might be fatal. This study was carried to evaluate the effects of the DTIC drug on mice test tissue and some of sperm parameters. The evaluation of DTIC effects showed that 15 and 20 mg/kg produced significant alteration to some sperm parameters and the accumulated dose of 10 mg/kg for 3 days caused severe damage to the testis tissue of the treated mice..
Key words: Dacarbizine , DTIC, Test, Sperms, mice.

INTRODUCTION

Dacarbazine, 5-(3,3-dimethyl-l-triazeno)imidazole-4-carboxamide (NSC-45388, DTIC) is an anti-cancer chemotherapy drug used for metastatic malignant melanoma, Hodgkin's disease, soft tissue sarcomas, neuroblastoma, fibrosarcomas, rhabdomyosarcoma, islet cell carcinoma, and medullary carcinoma of the thyroid. DTIC is classified as an alkylating agent or cell-cycle non-specific and which is most active in the resting phase of the cell1. This drug has been approved by the Food and Drug Administration in 1975 for metastatic malignant melanoma treatment but the estimated cure rate achieved by this drug is 20% [2].

Doubts about DTIC began to appear as early as 1975, therefore, some studies have demonstrated that this agent is carcinogenic to experimental animals like rats, mice, and tumors might occur in the brain, spleen, lung, uterus[3-7]. Furthermore, some experiments proved that DTIC has a carcinogenic effect on the fetuses, as well as on the formation of sperms of rabbits and rats [8-9].

In spite of the existence of strict regulations how to deal with drugs in order to avoid their side effects the danger to still to excess. Many studies have demonstrated the seriousness of dealing with such drugs that are believed to be carcinogenic or mutagenic for the patient or the medical staffs or the people close to the patient, and precautions often did not work [10-20].

The study conducted by Al-Hawary and Al-Saleh in 1989 is considered the first study to prove that DTIC causes structural chromosome damages of mouse bone marrow cells [21]. In 2002, Alder and co-workers proved that DTIC induce chromosomal aberration in somatic and germinal cells of mice [22].

In 2006, Kumar evaluated the effect of DTIC on germinal cells of mice and found not only a reduction of testosterone hormone and induction of lactate dehydrogenase, but shape deformation and reduction of viability and total number of sperms [23].

The aim of this work is to study the effect of three different doses of DTIC on testis tissue and some of the sperm parameters of mice.

MATERIALS AND METHODS

Dacarbazine

DTIC was obtained from Security Forces Hospital in Riyadh in small vials, each vial contains 100 mg of white powder and provided with ampoules containing 10 ml of sterile distilled water for dissolving the powder.

Animals

Male Swiss albino mice weighing 25-30 g were used in all experiments. Animals were maintained under standard conditions of temperature & humidity with regular light/dark cycle and allowed free access to food and water. Animals were segregated into 4 groups of 10 of each. Group1 served as control and received sterile distilled water. Groups 2,3 and 4 were administered respectively 10, 15 and 20 mg/kg of DTIC intraperitoneal cavity injection.

Sperm collection

50 days later, the mice sacrificed with cervical dislocation and left epididymal sperms were collected by slicing epididymides in 5 ml of human tubal fluid containing 4 mg/ml bovine serum albumin and incubated for 5 min at 37 C to allow sperms to swim out of the epididymal tubules.

Sperm count

The sperm count was determined by hemocytometer as described in WHO manual [24]. A 5 ul aliquot of sperm fluid was diluted with 95 ul of diluents of 0.35% formalin containing 0.25% trypan blue. A small drop of diluted sperm suspension was transferred into the

chamber of the hemocytometer and allowed to stand for 5 minutes. The sperm heads were counted and expressed as million/ml.

Sperm morphology

One drop of 1% eosin Y was added to 1ml of sperm suspension and mixed by gentle agitation. Smears were prepared on clean slides and allowed to dry overnight. Sperms were examined with Carl Zeiss light microscope under 400X and following Wyrobek procedures [25-26].

Sperm viability

Sperm viability was evaluated by adding 20 ul of 0.5% trypan blue solution to 20 ul of sperm suspension and after 2 min of incubation; sperms were viewed by light microscope at 400X. Dead sperms appeared to be stained blue and live sperms were not stained. In each sample 500 sperms were counted and viability percentages were calculated [26].

Sperm motility

One drop of sperm suspension was placed on microscopic slide, covered with a coverslip and 10 random fields of view were examined at 400X. The number of motile and nonmotile sperms was counted. Motility was then expressed as percentage of motile sperm to the total number of sperm [24].

Histological study

Histological examination was performed on about 50% of randomized animals of each group. Animals were injected with 10 mg/kg for 3 days, one injection per day (accumulated dose). Testis samples were taken and fixed for at least 48 hours in 10% formalin. The samples were then embedded in paraffin, cut into 5 um sections, and stained with hematoxylin and eosin and examination by Carl Zeiss light micrograph.

Samples for electron microscope were fixed for 24 hours in 3% glutaraldhyde and prepared according the procedures of Hayat [27] . Sections were examined under Jeol JEM - 1011.

Statistical analysis

The data are presented as the mean ± SEM. Differences between groups were analyzed by One Way Analysis of Variance (ANOVA) followed by Tukey test using SPSS package, version 16 and level of significance was taken as $p<0.05$.

RESULTS

Sperm parameters

Table 1 shows that low concentration of DTIC (10 mg/kg) did not cause significant changes in total sperm count as compared to control. However, medium and high concentration of DTIC (15 & 20 mg/kg) treatment produced significant decrease in sperm count at the level of $P<0.01$. At the same time the percentage of sperm motility remain within the normal range when mice treated with 10 mg/kg of DTIC and the percentage decreased significantly when animals treated with 15 and 20 mg/kg of DTIC. The motility percentage dropped from 71% to 32% when mice treated with 20 mg/kg. The same effect appeared on the percentage of sperm abnormality which increased significantly when animals treated with the high dose (20 mg/kg) of DTIC. Finally, the percentage of live sperm expected to decrease dramatically from 73% in control animals to score 30% after the treatment with 20 mg/kg of DTIC.

Table-1: Effects of DTIC on sperm parameters in adult male mice

	Total sperm Count (10^6)	Motile sperm %	Abnormal sperm %	Live sperm %
Control	**4.2** ± 0.84	**71.21** ± 1.31	**10.12** ± 0.43	**73.26** ± 1.43
10 mg/kg	4.0 ± 1.00	66.37 ± 1.84	**12.25** ± 0.27	**67.95** ± 2.45
15 mg/kg	1.6 ± 0.89*	41.29 ± 1.72*	19.71 ± 0.68*	37. 57 ± 1.56*
20 mg/kg	1.2 ± 0.45*	31.51 ± 1.48*	27.13 ± 0.52*	**29.86**± 0.74*

*P<0.01

Light microscope histological studies

Figure 1 shows the effect of accumulated dose of DTIC at 10 mg/kg for 3 days on the testis tissue of the mice compared by the control sample as seen under the light microscope. It is clear that DTIC caused severe damage to the cell structure of the testis. There are very clear cell dropping and the lumen of the seminiferous tubules is wider. This indicates that DTIC definitely causes cell death of the active spermatogonia and spermatocytes of the test.

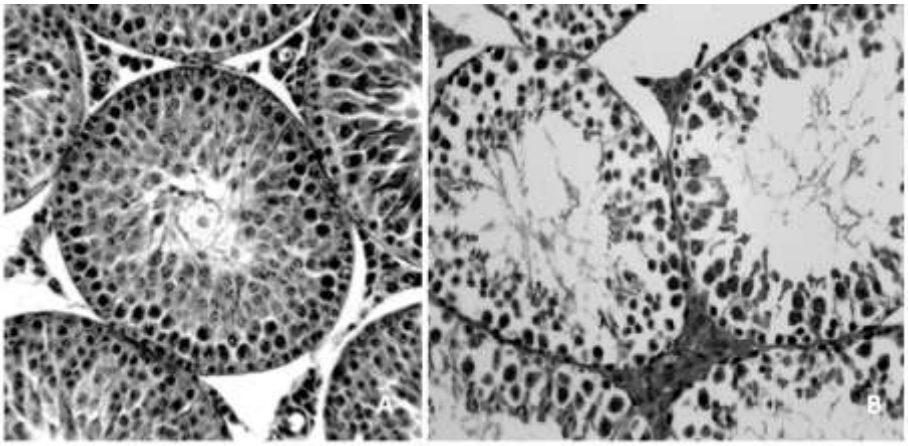

Fig-1: Shows a light microscope photomicrograph of a section in mouse testis. (A) Represents control sample and (B) represents treated sample with 20 mg/kg,

Electron microscope histological studies

Figure 2 shows the effect of accumulated dose DTIC at 10 mg/kg for 3 days on the test cells of mice compared with the control samples as seen under the electron microscope. The control photomicroscopic pictures (A-C) show normal images of spermatogonial stem cells. Picture (A) represents a nucleus contains only euchromatin material and surrounded with continuous intact nuclear membrane. Picture (B) is an intermediate stem cell which shows nucleus with euchromatin and few peripheral heterochromatin surrounded with continuous intact nuclear membrane. Picture (C) shows two bridged stem cells with clear nuclei full of Euchromatin and few heterochromatin and each was surrounded with a prefect nuclear membrane.

The treated photomicroscopic pictures (D-F) show defected images of spermatogonial stem cells. Picture (D) shows stem cell nucleus with groups of condensed chromatin surrounded with clean nuclear membrane which indicate to an apoptosis phenomenon. Picture (E) represents a stem cell nucleus with nuclear membrane damage and a vacuole near the broken membrane which look like a lysosome. Picture (F) is spermatogonial stem cell contains enormous vacuoles and most of those vacuoles are lysosomes which mains the cell is going to lyze itself.

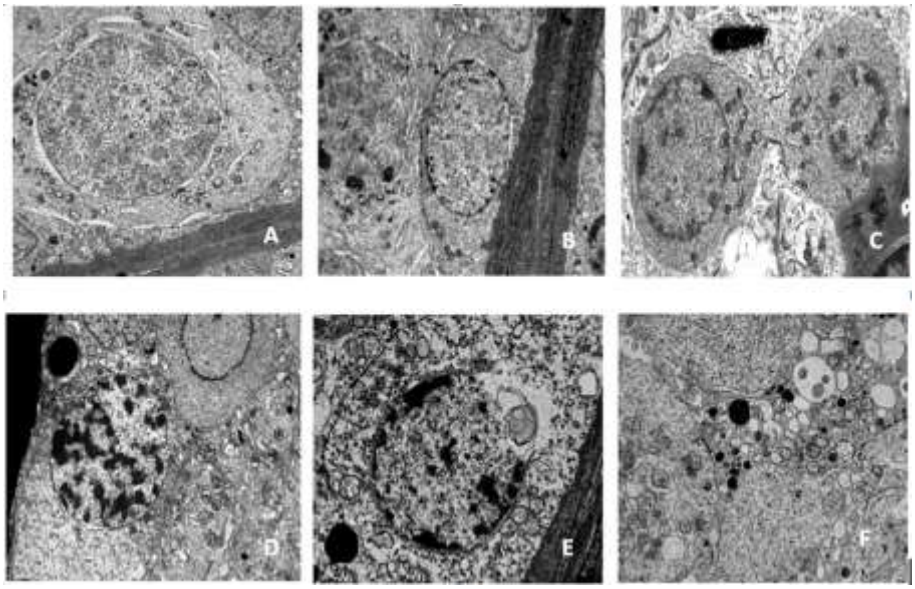

Fig-2:Shows an electron microscope photomicrograph of a section in mouse testis. (A-C) Represents control samples and (D-F) represents treated samples with 10 mg/kg accumulated doses for 3 days.

DISCUSSION

There is no doubt that infertility disease is unwanted and substances that lead to it are one of the most dangerous substances. In general most of the anti-cancer drugs cause a sort of infertility; therefore, patients taking such drugs must know that. In mostly, infertility can be presumed from the nature of the sperm of an organism and therefore, we used this path to study the influence of Dacarbazine on the nature of the sperms of mice at three different doses.

This study has shown that DTIC affect the nature of the sperms in terms of form, viability, motility and number and it is worth mentioning that the low dose 10 mg/kg did not have significant impact. But the medium and high doses 15 and 20 mg/kg have led to a decrease in the

percentage of sperm parameters as well as a decline in the average number of sperm count and this decrease was a highly statistical significance.

It is worth mentioning that we found only one study has evaluated the effect of DTIC on mice sperm parameters and it turned out that their results correspond with what was obtained in this study. This study carried out by Kumar & Associates, they found that DTIC at a dose of 100 mg / kg may cause severe decrease on the motility, shape abnormality and sperm number after 53 days of treatment [23.]

However, there are many studies have used other anticancer drugs such as bleomycin, busulfan, etoposide, deoxycytidine and vinblastin and their results were corresponded with the finding of this study[28-30]. Since DTIC was classified as an alkalyting drug then it is expected to effect on the spermatogenesis process and thus will be reflected on the fertility of the organism, and this will effect reversely on the descendants of this organism [8-9]. Furthermore, other studies also indicated that changes in sperm shape reflects the damage that might occurred in DNA of germinal cells, as a result of animal exposure to mutagens and because the DTIC is a toxic drug it is expected to cause deformities in the shape of sperms [26, 31-32].

This study proved for the first time that DTIC has effected on the cells of the seminiferous tubules after treatment with 10 mg/kg accumulative doses for 3 days. The influence was clear under the light microscope, which confined in the death of cells of seminiferous tubules. It is obvious that cell dropping and disintegration have occurred and cell spacing from each other has occurred, and with an abnormal breadth in the cavity area of tubules. At the same time DTIC has effected on the nuclei of mice testis stem cells and caused agglomeration of condensed chromatin and nuclear membrane burst a sign of cell apoptosis. Lysosome accumulation has been observed and this indicates to the cell suicide as a result of treatment with DTIC.

From here we can emphasize that DTIC works on cells that had dividing nature, and this is due to two main reasons. The first one is due to the DTIC mechanism work and as counterpart of purine bases DTIC enters and alters the purine bases of the DNA. This will disrupts DNA replication process and will causes DNA fragmentation and this ultimately will leads to cell death [33-37]. The second reason, as proposed by Bradly in 1987 which he considered DTIC might lead to an explosion of cell lysosomes and emancipation their enzymes which destroy the effected cells [38]. The results of this study indicate the occurrence of damage at the level of nuclear chromatin and at the cellular level and this corresponds with the two reasons mentioned above.

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