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ASSESSSMENT OF GENOTOXICITY OF FLUTAMIDE, AN ANTI-CANCER DRUG, USING THE SPERM-HEAD MORPHOLOGY ASSAY AND MICRONUCLEUS TEST IN SWISS ALBINO MICE

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ABSTRACT

Flutamide is a non-steroidal compound reported to have anti-androgenic properties, which appears to act by inhibiting the uptake and / or binding of androgens in target tissues. It is used in the palliative treatment of prostatic carcinoma. Flutamide exerts its potent anti - androgenic effect with little or no other hormonal activity. The present study was undertaken to fill in the void in knowledge on its cytotoxic effect by performing the sperm-head morphology assay and micronucleus test in swiss albino mice.

The sperm-head morphology assay is an in vivo cytogenetic assay which helps in the detection of agents responsible for inducing mutations or causing damages in genn cells. The micronucleus assay is also as in vivo assay which measures the clastogenicity. Experiments revealed that, sperm-head abnormalities and micronuclei increased directly in proportion to the concentration.

Key words : Flutamide, genotoxicity, micronuclei, sperm - head, mice.

INTRODUCTION

Among all the subspecialties of internal medicine, medecine oncology may have had the greatest impact in changing the practice of mocircine in the past three decades, as curative treatments have been identified for a number of previously fatal malignancies such as testicular cancer, lymphomas and leukaemia. New drugs have entered clinical use for disease presentations previously either untreatable or amenable to only local means of therapy, such as surgery and irradiation.

In the interval between World Wars I and II, extensive studies of the biological and chemical actions of the *nitrogen mustards* were conducted. The marked cytotoxic action on lymphoid tissue prompted Gilman, Goodman and Dougherty to study the effect of nitrogen mustards on transplanted lymphosarcoma in mice and in 1942, clinical studies were initiated. This launched the era of modorn cancer chemotherapy (Gilman, 1963) : [Hardman and Limbird, 1996].

Common cancers in man such as of thyroid, lung, stomach, colon, brain and breast are best treated by surgery. Radiotherapy, as a curative measure is useful against cancers of the skin, lip, oral cavity, soft palate, cervix and corpus uteri. tonsils, nasal cavity, larynx, thyroid, etc. Karnofsky (1959) & (Kothari and Mehta, 1973) defines cancer chemotherapy as the use of a systematically administered drug that will, while remaining relatively non-toxic to the host interfere with, favourably modify or destroy a cancerous growth or alleviate it's harmful effects on the host.

Chemotherapeutic agents exert, in varying digrees, cytostatic, cytolytic, cytotoxic and even mutagenic effects on cancer cells by interfering with the metabolic and / or mitotic activities of the cells by disturbing the fundamental mechanisms concerned with cell growth, mitotic activity, differentiation and function (Kothari and Mehta, 1973; Becker, 1977). Cytotoxic drugs can be classified biochemically into five main groups : alkylating agents, cytotoxic antibiotics, antimetabolites, vinca alkaloids and etoposide and other antineoplastic agents (Malik, 1998 ; Gulhati, 1999). As there is paucity of information on the genotoxic effects of Flutamide, the present investigations were undertaken.

MATERIALS AND METHODS

Swiss albino mice (*Mus.musculus*) of 6 - 8 weeks and weighing 23-29 gms were used as the test system in the present study. They were housed in polypropylene cages and fed on standard pelleted feed; water was given adlibitum.

Flutamide was administered orally in various doses ranging from 1.0 mg - 3.5 mg / mouse (body weight 23-29 gms) dissolved in 1ml distilled water, for a week. The control mice were fed with 1ml distilled water for 7 days. The treated as well as control mice were maintained on a regular diet during the recovery period for one month. They were then sacrificed by cervical dislocation and the following tests were performed.

(a) *Sperm - head morphology assay* : The cauda epididymis was dissected out and transferred to a petridish containing physiologial saline. It was teased as much as possible and the cell suspension was transferred to a small test - tube. Aqueous eosin was used for staining. A drop of suspension was taken on clean, grease - free slids and smeared. The slides were air-dried and mounted with DPX (Wyrobek and Bruce, 1975). 2000 sperms per animal werer scored for the incidence of sperm - head abnormalities.

(b) *Micronucleus assay in peripheral blood erythrocytes* : The peripheral blood was collected from the tail-tip of both, control and experimental animals and diluted with a few drops of saturated tri - sodium solution. The mixture was smeared on clean, dry slides and fixed in methanol for 5 mins. The slides were air-dried and stained with aqueous Giemsa and air-dried agtain (Mac Gregor *et al.*, 1980). 2500 peripheral erythrocyes per animal were screened for the presence of micronuclei.

OBSERVATIONS

It was observed that the number of sperm-head abnormalities and micronuclei increased directly in proportion to the dose. Sperm-head abnormalities such as : long hook, bent hook, hooklessness, triangular head, hammer-shaped head, etc., were observed.

The results of the above mentioned tests have been shown in tables 1 and 2. It was observed that upto dose - level of 2.0 mg, there was a constant increase in both, the number of sperm - head abnormalities and micronuclei. But from the dose - level of 2.5 mg, there was a sudden increase in their respective numbers with the effect almost leveling off at 3.0 mg concentration. In the sperm-head abnormality test, the value obtained at 1.5 mg concentration showed significance at $p < 0.1$ while, in the micronucleus test, the values at 2.5 mg, 3.0 mg and 3.5 mg dose levels, showed significance at $p < 0.05$.

DISCUSSION

The most important pharmacological actions of the alkylating agents are those that distrub the fundamental mechanisms concerned with cell proliferation, in particular DNA synthesis and cell division. The capacity of these drugs to interfere with DNA integrity and function in rapidly proliferating tissues provides the basis for their therapeutic applications and for many of their toxic properties.

The principal clinical application of flutamide to date is in the treatment of prostatic cancer. Flutamide also has been used experimentally in combination with an oral contraceptive for the treatment of hirsutism in women (Hardman and Limbird, 1996).

The mouse sperm morphology test is simple, inexpensive and relatively rapid when compared with other *in vivo* short - term tests. The sperm morphology protocol is adaptable to different species, dosage regimens, sampling times, routes of exposure etc. This versatility makes it a useful animal model for human exposure. In this assay, germ cells are exposed *in vivo*. So, a positive result in the test demonstrates an agent's ability to damage spermatogenesis. As such, it is a valuable tool in safety evaluation for assessing an agent's potential adverse effects on sperm production. The induction of abnormally shaped sperm in mice appears to be very sensitive to mammalian germ cell mutagens; this test may therefore be a valuable tool for identifying germ-cell mutagens.

The sperm-head morphology assay has been conducted on other anti-cancer drugs such as : Busulfan [Wyrobek and Burce, 1975; Bruce and Heddle, 1979 and : Wyrobek et al. 1983)], Cyclophosphamide (Wyrobek and Bruce, 1975) ; (Bruce and Heddle, 1979; Pomerantseva *et al.*, 1981) (Wyrobek *et al.*, 1983) ; Mitomycin C [Wyrobek and Bruce, 1975; Zimmerman *et al*, 1979 and : (Wyrobek *et al.*, 1983)]. Thiotepa (Wyrobek and Bruce, 1975), Vinblastine sulfate [Wyrobek and Bruce, 1975; Bruce and Heddle, 1979 (and : Wyrobek *et al.*, 1983)].

The bone - marrow micronucleus assay has been widely used as a screening test for detecting clastogenic and spindle - damaging effects of chemicals *in vivo* [Schmid, 1975; Heddle *et al.*, 1983; Mac Gregor *et al.*, 1987; Mavoumin et al., 1990; Miller *et al.* 1991; Gudi *et al.*, 1992

and : Cao et al., 1993). However, this test does not allow us to monitor the same treated animals continuously, because the animals have to be killed to obtain the bone-marrow samples. During the last two decades it has been shown that the analysis of micronucleus in polychromatic erythrocytes of mouse peripheral blood has the same sensitivity compared with the bone-marrow micronucleus test (Cao *et al.*, 1993).

The micronucleus test has been performed on other anti - cancer drugs such as Mitomycin C (Adler and Kliesch, 1990; Hayashi *et al.*, 1992), Cyclophosphamide (Adler and Kliesch, 1990), etc.

One of the advantages of the micronucleus test as an *in vivo* cytogenetic assay, compared with conventional metaphase chromosomal aberration analysis, is the simple and rapid scoring. At present, the micronucleus test is the most popular short - term assay for the *in vivo* detection of clastogens or spindle poisons (Hayashi *et al.*, 1992).

Table 1 : INCIDENCE OF SPERM-HEAD ABNORMALITIES IN MEIOTIC CELLS OF MICE

Dosage (mg)	Bent Hook	Hookless	Long Hook	Hammer-Shaped Head	Triangular Head	Banana-Shaped Head	Micro Head	Abnormal Head	Total No. of abnormalities	%
Control	72	44	60	24	35	0	15	41	291	2.4
1.0	114	64	128	60	107	57	13	20	563	4.7
1.5	135	140	105	23	34	52	38	57	584	4.9
2.0	155	98	815	62	184	80	61	150	1605	13.4
2.5	213	40	1124	80	110	22	20	133	1755	14.6
3.0	529	357	582	38	106	91	52	160	1783	14.9
3.5	107	112	1119	72	190	33	32	118	1915	16.0

12000 Sperms scored /group

t values for the incidence of sperm-head abnormalities.

Dosage (mg)	t values
Control	O
1.0	2.738
1.5	3.027*
2.0	1.553
2.5	1.098
3.0	1.150
3.5	1.71

* $p < 0.1$

TABLE 2

INCIDENCE OF MICRONUCLEI IN PERIPHERAL ERYTHROCYTES OF MICE

Dosage (mg)	Micronucleated peripheral erythrocytes/ 2500 cells						% of micronuclei / 15000 cells
	I	II	III	IV	V	VI	
Control	0.7	1.0	0.6	1.4	0.9	1.2	0.97
1.0	0.9	1.2	0.8	0.6	1.4	1.6	1.08
1.5	0.6	1.0	1.6	1.0	1.4	1.2	1.13
2.0	1.9	1.4	2.0	1.6	2.2	1.0	1.68
2.5	1.5	2.2	2.1	2.8	2.0	2.0	2.10
3.0	2.2	1.8	2.7	3.3	2.9	1.6	2.42
3.5	2.2	4.1	1.4	3.2	2.4	2.0	2.55

t values for the incidence of micronuclei in peripheral erythrocytes of mice

Dosage (mg)	t values
Control	O
1.0	0.63
1.5	0.85
2.0	2.67
2.5	9.42*
3.0	5.00*
3.5	4.65*

* $p < 0.05$

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