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Cisplatin induced genotoxicity in swiss albino mice.

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Summary

Cisplatin [cis-Diammine dichloro-platinum (ii)] is a divalent, inorganic, water-soluble, platinum-containing complex. It has broad activity as an antineoplastic agent and the drug is especially useful in the treatment of epithelial maligancies. To fill in the dearth of information on it's cytotoxicity, studies were conducted in swiss albino mice using the protocols of sperm-head morphology and micronucleus assays.

The sperm-head morphology assay in mice performed in this study is a time-tested in vivo cytogentic assay, which is imprtant in the detection of those agents inducing mutationa or causing damages in germ cells. The micronucleus test conducted in this study is also an in vivo assay, devised by Schmid, (1975), which measures the clastogenicity. Cumulative cytogenetic damages can be assessed because micronuleated erythrocytes persist in the circulation. It was observed that the number of sperm-head abnormalities and micronuclei increased with an increase in the dosage upto 60ml//ml after which there was a decline.

Keywords: Cisplatin, genotoxicity, micronucleus, sperm-head, mice.

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Introduction:

A wide range of activities occur in the cells of an organism. One of them is cell-division. A control mechanism exists in the cell which dictates when the cell should divide and when it must stop to divide. When a cell undergoes changes leading to certain physiological disturbances, the control mechanism fails and the cell repeatedly divides whtn it should not. This character of dividing at the wrong time is passed on to it's daughter cells which also become outlaw and thus repeated divisions of the progeny cells results in abnormal growth in the tissue forming neoplasms or tumours. This uncontrolled cell growth is termed as cancer (Hynes, 1979; Sarin, 1986).

Rgarding surgical treatment of cancer, Homburger remarks, is as old as surgery itself. In the *Ebers Papyrus* [{*circa* 1550 B.C.) cf : Kothari and Mehta, (1973)], there are references to the treatment of "tumours"

by surgery. Radiotherapy is used in some for, in the management of nearly 60% of all cancer cases. Karnofsky (1959), 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.

In routine practice, the drugs used fall into two main groups, hormonal and cytotoxic (Malik, 1998 ; Gulhati, 1999). Hormonal agents are generally much better tolerated than cytotoxic drugs. The latter can cause severe morbidity and even death. Attention to dosage, absorption, metabolism and excretion is particularly important with cytotoxic drugs.

Materials and Methods :

Swiss albino mice *(Mus musculus)* of 6 - 8 weeks and weighing 23 - 29 gms were housed in polypropylene cages. They were fed on standard pelleted feed and water was given *ad libitum*.

Cisplatin was adinistered intraperitoneally, in doses ranging from 40µl to 90µl mixed with 1ml saline once a week. The control group and the experimental groups cosisted of six mice each. After a recovery period of one month, the mice were sacrificed by cervical dislocation and the following tests were performed.

a) **Sperm-head abnormality assay :** The epididymis was dissected out and transferred to a petridish containing saline. It was teased as much as possible and the cell suspension was trasferred to a small test-tube. Staining was done with aqueous eosin. A drop of suspension was taken on a clean grease-free slide and smeared. The slides were air-dried and mounted with DPX (Wyrobek and Bruce, 1975). 2000 sperms per animal were 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 treated animals and diluted with a few drops of saturated tri-sodium citrate 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 later air-dried again (Mac Gregor, et. al., 1980). 2500 peripheral erythrocytes per animal were screened for the presence of micronuclei.

Observations :

It was found that the percentage of sperm-head abnormalities and micronuclei increased proportionately upto the dose-level of 60µl/ml, after which there was a decline in their respective percentages. This shows that 60µl/ml is the optimum dose at which maximum effect is observed. The decrease observed thereafter is interesting. Sperm-head abnormalities such as - long hook, bent hook, hooklessness, triangular head, hammer-shaped head, etc., were observed.

The result of the above mentioned tests have been shown in Tables 1 and 2. The number of sperm-head abnormalities and micronuclei was the highest at dose level 60µl/ml. The values obtained, showed

Table - 1

INCIDENCE OF SPERM-HEAD ABNORMALITIES IN GERM CELLS OF MICE

Dosage (µl)	Bent Hook	Hookless	Long Hook	Hammer-Shaped Head	Triangular Head	Banana-Shaped Head	Micro-Head	Others	Total No. of Abnormalities	%
Control	55	48	53	30	64	35	15	17	317	2.6
40	67	132	161	40	200	58	89	150	897	7.5
50	65	202	252	59	547	74	18	67	1284	10.7
60	83	335	322	51	1007	98	63	22	1981	16.5
80	95	264	327	77	895	86	51	6	1801	15.0
90	75	209	631	69	413	83	27	—	1507	12.6

12000 sperms scored / group

t values for the incidence of sperm-head abnomalities

Dosage (µl)	t values
Control	0.0
40	5.57*
50	1.74
60	1.48
80	1.51
90	1.63

* $p < 0.01$

significance at $p<0.01$ for sperm-head morphology assay, while for the micronucleus test they were significant at $p<0.05$, $p<0.01$ and $p<0.002$.

Discussion :

The platinum-coordination complexes were first identified by Rosenberg and co-workers as cytotoxic agents in 1965. They observed that a current delivered between

Table - 2

INCIDENCE OF MICRONUCLEI IN PERIPHERAL ERYTHROCYTES OF MICE

Dosage (µl)	Micronucleated peripheral erythrocytes / 2500 cells						% of micronuclei / 15000 cells
	I	II	III	IV	V	VI	
Control	0.8	0.7	0.5	0.6	1.0	1.0	0.77
40	1.7	1.4	1.4	2.2	1.6	1.4	1.62
50	2.9	2.4	1.8	2.0	1.5	2.1	2.12
60	3.1	1.8	1.9	2.4	3.0	1.8	2.31
80	2.6	1.5	2.4	1.6	3.0	2.3	2.23
90	2.4	1.4	2.0	0.5	1.0	2.0	1.53

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

Dosage (µl)	t values
Control	0.00
40	4.89**
50	6.11***
60	7.00
80	7.28
90	2.65*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.002$

platinum electrodes produced inhibition of *E. coli* proliferation. The inhibitory effects on bacterial replication were later ascribed to the formation of inorganic platinum-containing compounds in the presence of ammonium and chloride ions (Rosenberg *et. al.*, 1965, 1967. Hardman and Limbird, 1996). *cis*-Diamminedichloroplatinum (ii) (Cisplatin) was the most active of these substances in experimental tumor systems and has proven to be of great clinical value (Rosenberg, 1973 cf : Hardman and

Limbird, 1996).

Cisplatin has become the foundation for curative regimens for advanced testicular cancer and has notable activity against ovarian cancer and cancer of the head and neck, bladder, oesophagus and lung. Interestingly, the drug also sensitizes cells to the cytotoxic effects of radiation therapy (see Person and Raghavan, 1985 cf : Hardman and Limbird, 1996).

Spermatogenesis in mice produces highly structural sperm cells whose head-shapes have been shown to be under genetic control. Induced sperm-head abnormalities are due to altered function of the gene required for normal sperm development.

Sperm-head morphology assay in mice, performed in this study is an *in vivo* cytogenetic assay which is important in the detection of those agents which cause mutations in germ cells. A number of germ-cell mutagens have been reported by this test.

The micronucleus test conducted in this study is an *in vivo* bone-marrow test which measures the clastogenicity. The micronuclei are formed from chromatin and chromosome lagged behind during anaphase which could be detected easily in erythrocytes. Assessment of cumulative cytogenetic damage can be done because micronucleated erythrocytes remain in the circulation.

A steady increase in sperm-head abnormality and inductions of micronuclei is seen to be directly proportional to the concentration of the drug used upto 60µl/ml. The probable decline after 60µl/ml indicates that 60µl/ml is the optimum dose to bring in changes in gametic cells, after which, higher doses turn to be counter-productive. These observations are in accordance with the earlier observations made by Pohl and Rother (1953), who explained biphasic effect of growth regulators and Garg and Mehrotra (1977), who described the reason for such kind of biphasic action of a chemocal molecule.

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