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Genotoxicity Evaluation of Haloperidol on Human Lymphocytes *in vitro*

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ABSTRACT Haloperidol, a butyrophenone antipshchotic drug, was evaluated for its cytogenetic effects *in vitro* on human lymphocytes. Peripheral blood cultures were set up from three normal healthy donors for 72 hours. Haloperidol was added at the start (72 hr exposure), 24 hour (48 hr exposure) and 48 hours (24 hr exposure) after initiation of cultures. The concentrations tested at each exposure time corresponded to the plasma levels (5 and 25 ng/ml) after therapeutic dose and higher than that (100 and 500 ng/ml). All treatments for a donor were given at the same time. Untreated cultures served as concurrent controls. The parameters assayed were chromosome damage, mitotic index, cell growth kinetics and sister chromatid exchanges. Haloperidol was found to have a significant genotoxic potential at concentrations corresponding to upper plasma level, and higher than that in the chromosome damage assay and at all concentrations in the mitotic index and SCE assay compared to the controls. However, it did not have any significant effect on the cell growth kinetics at any of the doses tested. The present study points to a genotoxic potential of Haloperidol. Therefore, this drug should be used with caution.

Haloperidol is a butyrophenone antipsychotic drug used for long term therapy of psychiatric disorders. It has been evaluated for its genotoxic effects in various test systems.

In Ames / *Salmonella* test, Haloperidol was shown to be mutagenic in one study (Fey et al., 1982), but not in another (Balbi et al., 1980). In the DNA cell binding (DCB) assay, it was found to be non-genotoxic (Kubinski et al., 1981). However in *Allium cepa* assay, Haloperidol exhibited a mitodepressive and clastogenic action (Reddy et al., 1976). In Drosophila it caused increased sex-linked recessive lethals (Rapoport et al., 1971). A comparative mutagenic (*Drosophila melanogaster* sex-linked recessive lethals) and cytogenetic activity (human lymphocyte culture) evaluation demonstrated Haloperidol to induce gene mutations but no chromosomal aberrations (Filippova and Jurkov, 1973).

Haloperidol induced SCE *in vivo* in Swiss albino mice at clinical doses given intraperitoneally (Kitchin et al., 1987). It also showed leukemogenic and mutagenic activity in 2 strains of mice following intra-peritoneal administration (Fey et al., 1982)

Haloperidol has been investigated both *in vitro* and *in vivo* in human lymphocytes for its cytogenetic effects. It was found to be non - clastogenic *in vitro* as well as *in vivo* (van den Berghe, 1974). But it was found to increase the frequency of chromatid and isochromatid gaps (Wagner, 1971) as well as to inhibit mitosis and blast transformation *in vitro* (Tsancheva, 1976). It was also found to have clastogenic effect *in vivo* in patients on long term therapy (Ahuja et al., 1984).

Since reports on the genetic toxicology of Haloperidol are controversial, the present study was carried out to evaluate the genotoxicity of this drug *in vitro* on human lymphocyte chromosomes. The various end points assessed were chromosome aberrations, mitotic index, cell growth kinetics and sister chromatid exchanges (SCEs).

MATERIALS AND METHOD

Peripheral blood samples were taken from three normal healthy donors who had not undergone any radiation exposure and has not taken any drug therapy for at least 4 weeks before sampling. Cultures in duplicate were set up for each treatment. Whole blood (0.4ml) was inoculated into 4.5 ml of RPMI 1640 (GIBCO) medium

containing human AB serum, phytohaemagglutanin (PHA), Penicillin- streptomycin mixture and 5-Bromodeoxyuridine (BrdU) (20μM).

The drug Haloperidol (Serenace ampoule, Searle, Bombay, Lot 072B) was diluted with double distilled water and added at the start (72 hr exposure) 24 hours (48 hr exposure) and 48 hours (24 hr exposure) after the initiation of cultures.

The concentrations tested at each exposure time were 5, 25, 100 and 500 ng/ml. The first two concentrations corresponded to the plasma level range after therapeutic doses in man (Miyazaki et al., 1981; McBurney and George, 1984; Smith et al., 1982; Mavroides et al., 1983). Untreated cultures served as negative concurrent controls for the base line frequency of the parameters assayed.

Culturing was done for 72 hours. Harvest included colchicine (0.04 μg/ml) and hypotonic treatment (0.56% KCL) following by fixation in 3:1 methanol-acetic acid. The slides were prepared by flame drying and stained by the FPG technique (Perry and Wolff, 1974). All slides were coded before scoring and decoded at the time of statistical analysis. The following parameters were assayed:

1. Chromosome Aberration Frequency : Attempt was made to screen 100 well spread metaphase plates for each *in vitro* treatment and control. The aberrations recorded were chromatid and isochromatid lesions, fragments, exchange figures, pulverised chromosomes, endoreduplications and polyploidy.

2. Mitotic Index : The number of metaphases in 100 cells were counted in the 72 hours treatment and controls. The per cent mitotic index (MI) was calculated as

$$\text{Per cent MI} = \frac{\text{No. of metaphase}}{\text{Total cells scored}} \times 100$$

3. Cell Growth Kinetics : The frequency of metaphases after one replication (M_1), two replications (M_2) and three replication (M_3) based on the differential staining pattern following BrdU incorporation were recorded in over 100 metaphases for 72 hour treatment and control. The replication index (RI) was calculated as

$$RI = \frac{(1 \times M_1) + (2 \times M_2) + (3 \times M_3)}{M_1 + M_2 + M_3}$$

where M_1 is the number of metaphases showing all chromosomes darkly stained, M_2 with all chromosomes differentially stained, and M_3 with about half the chromosomes differentially stained and the rest lightly stained.

4. Sister Chromatid Exchanges (SCEs) : Attempt was made to score SCEs in over 20 well spread metaphase with differential staining. Centromeric exchanges were excluded because of difficulty in identifying true exchanges from twisted chromatids at the centromere.

Statistical Analysis : The chi-square test for homogeneity was done for each treatment as well as control cultures to determine the variations between donors for the effect of the drug. The chi-square contingency test was performed to compare total aberrant metaphases , the mitotic index and cell growth kinetics between each of the treatment and the control. Student's 't-test' was done to compare the frequency of chromosomal aberrations as well as sister chromatid exchanges between the treated and control cultures.

RESULTS

The chi-square homogeneity test indicated no significant difference between the response of different donors to the drug treatment. Hence the data were pooled from the donors for statistical analysis.

A significant increase in abnormal metaphases as well as chromosome aberrations was observed at concentrations of Haloperidol at upper plasma level (25 ng/ml) and higher concentrations (Table 1). This increase was observed for all exposure times (72, 48 and 24 hr). Chromatid gaps were the most predominantly observed aberrations.

Haloperidol was found to decrease significantly the mitotic index compared to the control at all concentrations evaluated (Table 2). There was a sudden decline in the mitotic index at concentrations higher than the plasma level. These results indicate that Haloperidol has a detrimental effect on the rate of mitosis in lymphocyte cultures and that higher concentration may even be cytotoxic.

The results of cell growth kinetics following Haloperidol exposure are given in table 3. The 2x3 chi-square contingency test indicated no significant deviation from the control culture.

Table 1: Analysis of chromosome aberrations after Haloperidol Exposure *in virto*

Treatment (ng/ml)	*Hours of exposure*	*No. of metaphases scored*	*No. of abnormal metaphases*	*Per cent abnormal metaphases*	*No. of Gaps*		*No. of Breaks*		*Other aberra-tions*	*Per cent aberra-tions*
					Chro-matid	*Iso-chro matid*	*Chro-matid*	*Iso-chro matid*		
500	72	211	12	5.68*	5	4	2	1		5.68*
	48	170	12	7.05*	6	6			P,ER	7.05*
	24	249	17	6.82*	14	3				6.82*
100	72	230	16	6.95*	7	3	3	1		7.82*
	48	300	22	7.33*	12	6	2		2P, PC 6.66*	
	24	300	24	8.00*	12	11	2		P	8.33*
25	72	288	17	5.90*	11	2	3	1	EX, P	6.59*
	48	247	19	7.69*	5	8	7		3P	8.09*
	24	200	14	7.00*	10	3			Ex	7.50*
5	72	300	15	5.00	7	3	1		4P	3.66
	48	265	13	4.90	7	2	1		AF, 2P	4.52
	24	217	9	4.14	8	2				4.60
Control		300	6	2.00	4	2			4P	2.00

* = P < 0.05 ; PC = Pulverised chromosomes; ER = Endoreduplication; Ex = Exchange figure; P = Polyploidy

Table 2: Analysis of Mitotic Index after Haloperidol exposure *in vitro*

Treatment (ng/ml)	*No. of cells scored*	*No. of Metaphases*	*Percent Mitotic Index*
500	3,000	32	1.06*
100	3,000	32	1.06*
25	3,000	43	1.43*
5	3,000	43	1.43*
Control	3,000	73	2.43

* = p < 0.05

Thus Haloperidol was found not to affect the cell turnover rate of lymphocytes in cultures *in vitro*.

A significant increase in SCE frequency was observed for all exposure times at concentration higher than plasma level (100 and 500 ng/ml) (Table 4). At concentrations corresponding to plasma level, however, some interesting observations were made. At the upper plasma level (25ng/ml) the SCEs were significantly increased following 48 and 24 hr exposure (prior to fixation) but not after 72hr exposure. At the upper plasma level (25ng/ml) the SCE frequency was significantly increased only following 24hr exposure but not 48 and 72 hr exposure.

These observations probably indicate that the SCE induction by Haloperidol is dependent not only on the concentration of the drug but also on the time and the number of cell cycles following exposure.

Table 3 : Analysis of cell Growth Kinetics (CGK) after Haloperidol exposure *in vitro*

Treat-ment (ng/ml)	*No. of cells scored*	*Per cent cells*			*Repli-cation Index*	*2 × 3 χ^2 test*
		M_1	M_2	M_3		
500	352	19.31	46.59	34.09	2.14	NS
100	396	28.53	35.60	35.85	2.07	NS
25	462	23.37	35.06	41.55	2.18	NS
5	297	23.58	43.77	32.65	2.09	NS
Control	460	23.04	38.91	38.04	2.15	

NS = Not significant at 5% level

Table 4: Analysis of Sister Chromatid Exchanges (SCE) after Haloperidol exposure *in vitro*

Treat (ng/ml)	*Hours of exposure*	*No. of metaphases*	*Total SCE*	*Range of SCE*	*SCE per cell*
500	72	81	373	2-9	4.60*
	48	37	185	2-11	5.00*
	24	81	409	1-10	5.04*
100	72	57	242	1-8	4.24*
	48	86	458	2-11	5.32*
	24	89	414	0-9	4.65*
25	72	76	301	1-9	3.96
	48	76	330	1-10	4.34*
	24	25	106	1-10	4.24*
5	72	89	347	1-9	3.89
	48	84	312	1-10	3.71
	24	75	329	1-9	4.38*
Control		102	362	1-8	3.55

* = p < 0.05

DISCUSSION

Haloperidol was found to cause chromosome damages at concentrations corresponding

to upper plasma level and higher than that. Maximum damage seen was in the form of gaps. The studies of Wagner (1971) who reported an increase in isochromatid and chromatid gaps in human chromosomes following Haloperidol exposure *in vitro*, and of Tsancheva (1976), who found a weak mutagenic/clastogenic activity of Haloperidol *in vitro*, support the present observations. However , the studies of van den Berge (1974) contradict the present finding.

The question whether gaps should be included in genetic toxicology studies is rather controversial. According to some workers gaps are merely artefacts of processing and staining (Schnitzel and Schmidt, 1976). On the other hand, many workers feel that the gaps represent a form of chromosome damage (Anderson and Richardson, 1981; Hsu, 1982; Hansteen et al., 1984: Gebhart, 1982; Saxena and Ahuja, 1988). Fragile sites, which are mainly expressed as gaps, have been shown to be targets of mutagens and carcinogens (Yunis, 1987). Therefore, the role of gaps in genetic toxicology cannot be ignored (Ahuja and Shobha Rani, 1985; Prasad et al., 1988). Perhaps Haloperidol may be considered as gap inducer rather than a clastogenic agent. Moreover, this drug may be classified as a weak mutagenic till the importance of gaps in genetic toxicology is verified.

The mitotic index analysis of the lymphocyte cultures following Haloperidol exposure indicated significant mitodepression as compared to the untreated controls (Table 2). The study of Tsancheva (1976) and Nahas et al. (1979) support our observation.

Cell growth kinetics (average lymphocyte division time) analysis is a sensitive method for detection of mitotic delay or stimulation of lymphocyte cultures (Tice et al., 1976; Crossen and Morgan, 1977; Morimoto and Wolff, 1980). The present study indicated no significant effect of Haloperidol on the cell growth kinetics of lymphocyte cultures *in vitro* (Table 3).

This probably indicates that Haloperidol inhibits the DNA synthesis and blast transformation of lymphocytes (as indicated by mitotic index) but does not significantly affect the cell cycle time of the transformed cells.

Sister chromatid exchanges are considered to be sensitive indicators of DNA damage with the frequency of SCEs significantly increased at non-clastogenic concentrations of known mutagens (Latt, 1974: Carrano et al., 1978). A similar trend was observed with Haloperidol which demonstrated an SCE inducing potential at all concentrations of the drug evaluated including the concentration at lower plasma level which did not induce any chromosome damage (Table 4). The *in vivo* induction of SCEs by clinical doses of Haloperidol in Swiss albino mice (Kitchin et al., 1987) supports our observations. On the contrary Lamberti et al. (1980) studied the SCE frequency in patients on long term therapy with Haloperidol, and found no increase in the mean SCE frequency between the treated and control subject, though they observed a great individual variability.

Variable results in the genotoxicity evaluation of Haloperidol could be explained on the basis of different experimental designs and different concentrations evaluated by different workers, as well as the individual variability in the subjects. Our results in the present study indicate the genotoxic potential of Haloperidol. Therefore it is advised that Haloperidol should be prescribed with caution.

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