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In vitro Effect of Monocrotophos on Human Lymphocytes

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Monocrotophos is an organophosphorous pesticide which is extensively used in the cotton fields in India. It has been shown that organophosphorous pesticides cause a significant increase in sister chromatid exchanges in mammalian cell lines (Chen et al.1981). A significant increase in exchanges has been reported in flouriculturists who were exposed to organophosphorous pesticides and carbamates (Dulout et al.1985). Workers occupationally exposed to pesticides showed a significant increase of chromosomal aberrations (Paldy et al.1987). Organophosphorous pesticides like methyl parathion and phorate showed mutagenic effect in rats (Malhi and Grover 1987). In the present study an attempt has been made to evaluate the chromosomal damaging effects of monocrotophos in human lymphocyte cultures.

MATERIALS AND METHODS

Intravenous blood was collected by heparinized syringe from healthy donor (male) under aseptic conditions. Lymphocyte cultures were initiated by adding 0.3 ml of wholeblood to RPMI 1640 medium supplemented with 25% human AB serum, 0.5% phytohemagglutinin, 0.25% dicrysticin. Monocrotophos (36%) was obtained from Khaitan & Co., Calcutta, India, and the cells were treated with 0.1, 0.2, 0.4 and 0.8 ug per 8 ml culture medium of monocrotophos for 24, 48 and 72 hrs of duration. Since monocrotophos is not soluble in water it was dissolved in 1% DMSO (Dimethylsulfoxide). Control cultures were maintained simultaneously with DMSO (control II) and without DMSO (control I). The cultures were terminated by adding 0.1 ug/ml of colchicine, two hours before harvesting the cultures, to arrest the cell cycle at metaphase. All the cultures were harvested by the method of Moorhead et al.(1960). A separate set of cultures were maintained for the study of sister chromatid exchanges (SCEs). 3 ug/ml of bromodeoxyuridine (BrdU) was added to the cultures at the time of initiation. The cells were treated with the test compound for 24, 48 and 72 hours duration. The cells were allowed to complete at least two cell cycles

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Table 1. Frequency of chromosomal aberrations in human lymphocytes treated with monocrotophos for 24, 48 & 72 hrs.

Treatment ug/culture	Chromatid type of aberrations							
	Gaps	Breaks	Deletions	Fragments	Exchanges	Dicentrics	Endo-reduplications	Total No.of aberrations without gaps
24 hours								
Control I	4(1.0)	3(0.75)	0.0	0.0	0.0	0.0	0.0	3(0.75)
Control II	4(1.0)	3(0.75)	0.0	0.0	0.0	0.0	0.0	3(0.75)
0.1	5(1.25)	5(1.25)	0.0	0.0	0.0	0.0	0.0	5(1.25)
0.2	9(2.25)	6(1.50)	0.0	0.0	0.0	0.0	0.0	6(1.50)
0.4	8(2.00)	8(2.00)	0.0	1(0.25)	0.0	0.0	0.0	9(2.25)*
0.8	11(2.75)	7(1.75)	1(0.25)	2(0.5)	0.0	0.0	0.0	10(2.50)*
48 hours								
Control I	4(1.0)	3(0.75)	0.0	0.0	0.0	0.0	0.0	3(0.75)
Control II	5(1.25)	4(1.0)	0.0	1(0.25)	0.0	0.0	0.0	5(1.25)
0.1	6(1.50)	5(1.25)	0.0	0.0	0.0	0.0	0.0	5(1.25)
0.2	9(2.25)	8(2.00)	0.0	0.0	0.0	0.0	0.0	8(2.00)
0.4	10(2.50)	8(2.00)	1(0.25)	1(0.25)	0.0	0.0	0.0	10(2.50)
0.8	11(2.75)	10(2.50)	1(0.25)	2(0.50)	0.0	1(0.25)	0.0	14(3.50)*
72 hours								
Control I	5(1.25)	3(0.75)	0.0	0.0	0.0	0.0	0.0	3(0.75)
Control II	5(1.25)	4(1.0)	0.0	1(0.25)	0.0	0.0	0.0	5(1.25)
0.1	7(1.75)	5(1.25)	0.0	0.0	0.0	0.0	0.0	5(1.25)
0.2	9(2.25)	8(2.00)	0.0	0.0	0.0	0.0	0.0	8(2.00)
0.4	11(2.75)	9(2.25)	2(0.50)	1(0.25)	0.0	1(0.25)	0.0	13(3.25)*
0.8	12(3.00)	9(2.25)	3(0.75)	2(0.50)	3(0.75)	4(1.00)	3(0.75)	24(6.00)*

*$P < 0.05$. Values given in parentheses are percentages.
400 metaphases were scored for each concentration.

Table 2. Incidence of SCE in human lymphocytes treated with monocrotophos for 24, 48 & 72 hrs.

Treatment ug/culture	No.of Metaphases screened	24 hrs. treatment SCE/cell ± S.E.M.	48 hrs. treatment SCE/cell ± S.E.M.	72 hrs. treatmnet SCE/cell ± S.E.M.
Control I	50	5.34 ± 0.4	5.62 ± 0.5	5.90 ± 0.2
Control II	50	6.22 ± 0.9	6.40 ± 0.3	6.64 ± 0.5
0.1	50	8.42*± 0.2	8.04*± 0.4	9.20*± 0.6
0.2	50	8.96*± 0.5	9.12*± 0.8	9.94*± 0.7
0.4	50	8,92*± 0.1	9.46*± 0.6	10.32*± 0.5
0.8	50	9.12*± 0.7	10.02*± 0.3	11.50*± 0.9

*$P < 0.05$

in the presence of BrdU. The cultures were terminated by adding colchicine, and the slides were prepared according to the method described elsewhere (Moorhead et al.1960). Three day old slides were processed according to the standard method (Perry and Wolff 1974) for differential staining. The experiment was repeated twice for chromosomal aberrations and sister chromatid exchanges.

400 metaphases were scored for chromosomal aberrations and 50 metaphases for sister chromatid exchanges for each concentration and in each experiment. The aberration frequencies were similar in both the experiments. Various types of chromosomal aberrations like chromatid gaps, breaks, deletions, exchanges, dicentrics and endoreduplications were recorded. Chromatid gaps were not included in the total number of aberrations. Statistical analysis of the data was made using X^2 test for chromosomal aberrations and Student 't' test for sister chromatid exchanges.

RESULTS AND DISCUSSION

The results on the incidence of chromosomal aberrations after the treatment with 0.1, 0.2, 0.4, 0.8 ug of monocrotophos for 24, 48 and 72 hrs are given in table 1. The types of aberrations observed are chromatid breaks, gaps, deletions, fragments, exchanges, dicentrics, and endoreduplications. There was a dose dependent increase in the frequency of the gaps and breaks with a corresponding increase in the duration of exposure. A significant incidence of chromatid breaks manifest that the chemical may act mostly in the S and G_2 phase of the cell cycle. Even though very few dicentrics were observed in higher concentrations most of the aberrations recorded are of chromatid types, this reveals that the chemical may act mostly on the synthetic phase of the cell cycle than G_1 phase. Presence of deletions, exchanges and fragments at higher concentrations reveals the increased mutagenic potential of the test compound.

A significant increase in the SCEs at all concentrations and at different time intervals (Table 2) reveals that the chromosomes are more sensitive for damage caused by test compound, as SCEs is a more sensitive parameter than chromosomal aberrations. Even though the chromatid type of aberrations and SCEs are two independent parameters, the ability of the test compound to cause a significant percentage of aberrations in both the tests reveals that monocrotophos is capable of causing significant chromosomal damage at higher concentrations. There were also reports on the positive effect of this compound in mammalian systems (Vaidya and Patankar 1982) and in human lymphoid cells (Sobti et al.1982). Monocrotophos also showed positive effect in Ame's test (Moriya et al.1983). A significant increase in SCEs were found in rat tracheal epithelial and

chinese hamster ovary cells when tested with monocrotophos (Wang et al.1987). Endoreduplications were observed in BrdU cultures at 0.8 ug treatment after the 72 hours exposure. Existence of endoreduplications were not completely understood. The present study reveals that monocrotophos causes chromosome damage in human lymphocyte cultures.

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