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In Vitro Induction of Alterations in Peripheral Blood Lymphocytes by Different Doses of Diazinon

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Organophosphorus pesticides represent a major class of agricultural pesticides today. They exhibit less persistence than the organochlorine pesticides and show greater toxicity to mammals because they are cholinesterase inhibitors. The structural element common to all the organophosphates show two electrophilic sites, P and C, which permit attachment to nucleophiles. These latter can bind to either P sites, and become phosphorylated, or to C sites, were they become alkylated. Most organophosphates are chemical alkylating agents (Wild 1975) which may lead to a mutagenic potencial. Because of the extensive use of organophosphates in this country, we were prompted to study the effect of diazinon on cultures.

MATERIALS AND METHODS

Experiments were carried out on cultured human peripheral blood obtained from karyotypically normal volunteers according to standard methods. Different doses of diazinon: 5, 10, 20 and 30 μg/cc were added at the start of culture. Diazinon (Ciba-Geigy, Inquinasa SA, Spain) was first dissolved in dimethyl sulphoxide (DMSO) at an appropriate concentration. The pesticide was identified by ^{13}C and ^{1}H NMR spectroscopy and by UV and IR absorption spectrophotometry. Control cultures were made with the addition of DMSO (2.1% v/v) and without it (standard cultures). Air-dried slides were stained following G-banding tecniques. Photographs were made of all abnormal metaphase figures and karyotyping was performed whenever there appeared to be evidence of obvious chromosomal rearrangement. The criteria of Evans (1962) were used in the determination of chromatid lesions. The existence of decondensed chromosomes in fixed metaphases and the mitotic index in a total of 1000 cells were also studied.

RESULTS AND DISCUSSION

The values obtained for the mitotic index (MI) from the control cultures and the cultures with pesticide are in Table 1. The MI decreases linearly with the rise in diazinon dose, giving a

value of 5.4% in the culture containing 30 µg/cc OPP.

Table 1. Frequency of metaphases with structural chromosome aberrations, rate of chromosome condensations and mitotic index.

	Control Cultures		Cultures with µg/cc of Diazinon			
Metaphases with	Standard	DMSO	5	10	20	30
structural chromosome aberration	4.2	8.4	9.1	7.0	4.9	0
Rate of chromosome condensation (% metaphases with decondensed chromosomes)	3.0	3.6	22.8	26.0	48.5	46.8
Mitotic Index	5.8	3.4	5.4	3.1	1.5	0.9

In standard cultures it was possible to observe 4.7% of metaphases with structural aberrations and 8.3% in the cultures containing solvent. The percentage of metaphases with structural aberrations was seen to decrease with dose, giving a value of 9.1%, 7.0% and 4.9% in the cultures containing 5, 10 and 20 µg/cc of diazinon, respectively. There were no significant differences, however, between the above values and those obtained by this parameter in the control cultures.

The 30 µg/cc diazinon dose did not reveal metaphases with structural aberrations. The different types, number and percentage of structural chromosomal aberrations in the control cultures and the cultures with pesticide are in Table 2.

Table 2. Types and frequency of chromosome aberrations

	Control Cultures		Cultures with µg/cc of Diazinon			
Aberration Type	Standard	DMSO	5	10	20	30
Gaps	8.3%	53.8%	29.5%	66.7%	-	-
Breaks	16.7%	23.1%	17.6%	16.7%	75.0%	-
Deletions	-	15.4%	5.8%	-	-	-
Acentric Fragments	-	-	11.8%	-	-	-
Minute	-	-	23.5%	8.3%	-	-
Mass	-	7.7%	-	-	-	-
Not Classified	-	-	-	8.3%	-	-
Dicentric Chromosome	-	-	11.8%	-	25.0%	-
% Of Chromosome Aberrations	5.0%	12.0%	10.3%	9.0%	5.0%	0
Chromosome-Type Aberrations	-	-	11.7%	8.7%	25.5%	-
Chromatid-Type Aberrations	100%	100%	83.3%	91.3%	75.0%	-
Total of Metaphases	120	108	165	136	81	45

All the aberrations found in the control cultures are chromatid-

type. In the cultures containing diazinon the greatest frequency of chromosomal aberrations belonged to the chromatid-type, although there was a small proportion of chromosome-type aberrations. No great differences could be seen between the percentage of metaphases with abnormally condensed chromosomes (fig. 1) in the standard cultures (3.0%) and in the ones containing DMSO (3.6%). The percentage of metaphases with abnormally condensed chromosomes showed a linear increase with the dose, leading to 22.8% in the cultures containing 5 µg/cc of pesticide and 46.7% in the 30 µg/cc lot.

The first doses used in this work were seen to be highly toxic to the cells and we accordingly lowered the level in order to determine the maximum non-inhibitory dose; this was found to be 30 µg/cc diazinon per culture.

The values obtained for the MI in the control cultures are similar to those reported by Liniecki et al. (1978). The slight decrease in the MI value of the DMSO cultures compared with the standard cultures could be due to the action that this product exerts on membranes by reducing mitogenic action (Dennis 1967). When diazinon was added to the culture medium a decrease in the MI was observed which was parallel to the increase in dose. The MI tends to fall to zero in the doses greater than 30 µg/cc.

A clear relationship is apparent between MI, the structural aberration rate and chromosomal condensation, such that higher MI values are related to the higher values of metaphases with structural chromosomal aberrations and with normally condensed chromosomes. This cytotoxic effect is probably

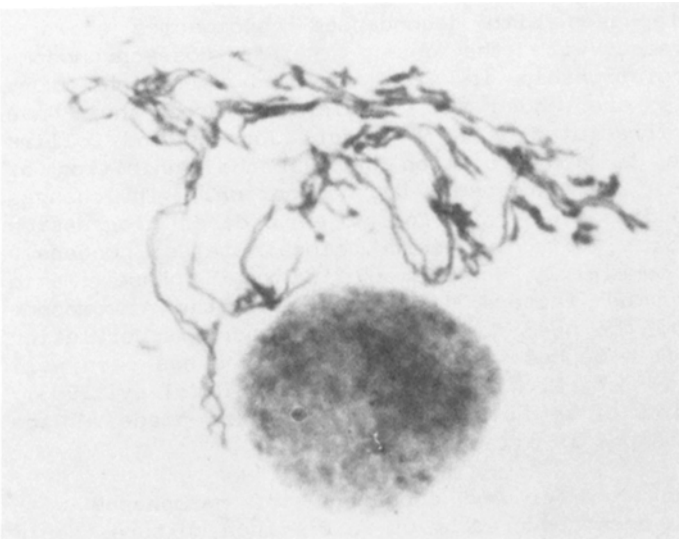

Figure 1. Metaphase with abnormaly condensed chromosomes

reversible, as evidenced by work currently being carried out in this Department. Maher and McCormick (1979), reported a direct correlation between cytotoxicity and mutation and pointed out that mutagenic and lethal lesions increase linearly with the dose. Our studies reveal that the percentage of aberrations in chromosomal structure changes conversely with the dose and does not reach the values found in the controls. The percentage decrease in structural aberrations is a direct sign of the compound's cytotoxicity in that for the highest dose, viability is more reduced than for the lower doses.

According to our findings, diazinon does not increase the percentage of structural chromosomal aberrations; this could be supported by the work of Chen et al. (1981) who described negative results on studying the increase of sister chromatid exchange (SCE) caused by this compound in V79 Chinese hamster cells, a test which is a highly sensitive indicator of the mutagenic potential of compounds.

Our results confirm those of the mutagenicity test carried out on yeast and bacteria (Dean 1972; Simmon et al. 1978; Wild 1975) diazinon, in which no mutagenic effects were observed In contrast, Tsoneba-Maneya et al. (1969) and Matsouka et al. (1979) found that diazinon brings about a high rate of chromosomal aberrations. The chromosome-type aberrations did not appear in the controls and only appeared in a small proportion in the treated cultures and are due to defects in the DNA repair mechanism (Wolff, 1982). The low frequency of chromosome-type aberrations found in this study is probably due to the low viability observed in the cultures.

The rate of metaphases with decondensed chromosomes shows a progressive increase with the dose. This dose-chromosome decondensation relationship is also observed when inhibitory DNA-repair agents are added to cellular cultures that have previously been irradiated with UV light (Johnson and Collirs 1978) and points to a correlation between the inhibition of excision DNA repair and chromosome decondensation. The changes in chromosome condensation rate follow an ordered progression along the cell cycle, with two conformational states; condensed in mitosis and completely decondensed in the S phase (Mazia 1970). Among the factors that determine the chromosome condensation rate, the need for H_1 histone hyperphosphorylation to maintain normal metaphase chromosome structure has been well documented (Gurley et al. 1974; Glotov and Nikolaev 1983). The phosphorylation of amino acid residues takes place at the sites where the histone is attached to DNA.

A possible explanation for the appearance of metaphases with decondensed chromosomes observed by us in Diazinon cultures could be that this compound can lead to an alkylation reaction by

blocking the radicals of -OH groups pertaining to serine and threonine amino acids of H_1 histones which have to undergo phosphorylation. This hypothesis is supported by the fact that the temperature-sensitive mutants t_s 85 murine carcinoma cells defective for the phosphorylation of H_1 histone may be characterized by a defective condensation of such chromosomes (Wilkinson et al. 1982).

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