Bull. Environ. Contam. Toxicol. (1987) 38:125–130

Environmental Contamination and Toxicology

Sensitivity of Human Lymphocyte Chromosome to Diazinon at Different Times During Cell Culture

D. E. Lopez and E. Carrascal

Department of Biology, Faculty of Medicine, C/ Fonseca 2, Salamanca 37007, Spain

The mutagenicity of a given compound has been classically determined by carrying out assays with different doses of such compounds maintained throughout the incubation times of the cells or tissues in question. This kind of assay must be carried out within a limited dose range, since certain compounds induce an inhibition of cell growth when acting during culture (Wild 1975; Carere et al 1981). By adding a product at short intervals at different moments of culture it is possible to observe the influence of such compounds according to the point of the Cell Cycle at which they are added (Bochnov and Yakovenko, 1978) and hence to study cellular repair merchanisms when challenged by such compounds.

In the present work a study was carried out on the influence of an organophosphorous insecticide Diazinon, by adding to standard lymphocyte cultures from human peripheral blood the maximum non-inhibitory dose of the compound (López et al 1985), 30 μg/cc at hourly pulses and at intervals of 6 hours throughout the culture period.

MATERIALS AND METHODS

Experiments were carried out on cultured peripheral blood according to standard methods. Blood samples were obtained from karyotypically normal volunteers. Diazinon, previously dissolved in dimethyl-sulphoxide (DMSO), was added for 1 hour and the cultures were then washed in HANK's solution and fresh medium was added. The dose employed was obtained from the maximum solubility of the product, decreasing the dose until the maximum non-inhibitory dose was found (30 μg/cc).

Diazinon was added to separate cultures every 6 h from culture initiation to 66 h of culture (at time 0,6,12,18,24,30,36,42,48, 54,60 and 66 h of culture) At 72 h the chromosomes were obtained. From 50 to 115 cells were investigated for each variant.

Once the chromosomal extensions had been obtained, the frequency of appearance of structural chromosomal aberrations (classified according to the classical criterion of Evans 1962), and the mitotic index (MI) were studied and the metaphases exhibited by

the condensed chromosomes were quantified. The autoradiographic criteria of Aleixandre (1981) were followed to know what cycle after lymphocyte stimulation the cell is in at the moment of exposure to the product.

RESULTS AND DISCUSSION

By studying the structural chromosomal aberrations produced by Diazinon when the pulses are conducted at different culture times it may seen (Table 1) that the percentage of such changes remains practically constant in the treatment carried out at 0,6,12 and 18 hours; after this, however, the addition of the compound induces an increase in the percentage of structural chromosomal aberrations and reaches a maximum when the pulse is performed at 36 h of culture.

Table 1. Frequency of metaphase with structural chromosome aberration, rate of chromosome condensation and mitotic index.

	%Metaphases with struct. chrom.aberr.	%Metaphases with decon. chromosomes.	Mitotic Index(%)
Control cultures:			
Standard	4.17	3.00	5.80
with DMSO	8.33	3.61	3.40
Cultures with 30 µg/cc Diazinon. Time pulses:			
0 h	10.23	8.41	5.00
6 h	10.53	12.14	2.40
12 h	13.12	6.67	0.60
18 h	11.90	7.06	0.57
24 h	15.52	6.77	0.90
30 h	12.94	0.90	1.30
36 h	26.56	14.19	0.75
42 h	3.00	10.57	1.67
48 h	3.48	3.25	1.20
54 h	13.12	13.61	0.47
60 h	9.30	2.01	1.40
66 h	3.16	0.60	3.10

In treatment later than 36 h, the cells are not very susceptible to Diazinon, as may be seen from the low percentage of structural chromosomal aberrations (5%, 4%, 8% and 3%) found when the pulses were performed at 42, 48, 60 and 66 h, respectively, with the exception of the treatment carried out at 54 h in which 15% of structural chromosomal aberrations were observed.

The values obtained for the degree of chromosomic decondensation show a parallel trend to that of the percentage of metaphases with aberrations, above all in the treatment carried out at 36 h and above, as may be seen in figure 1.

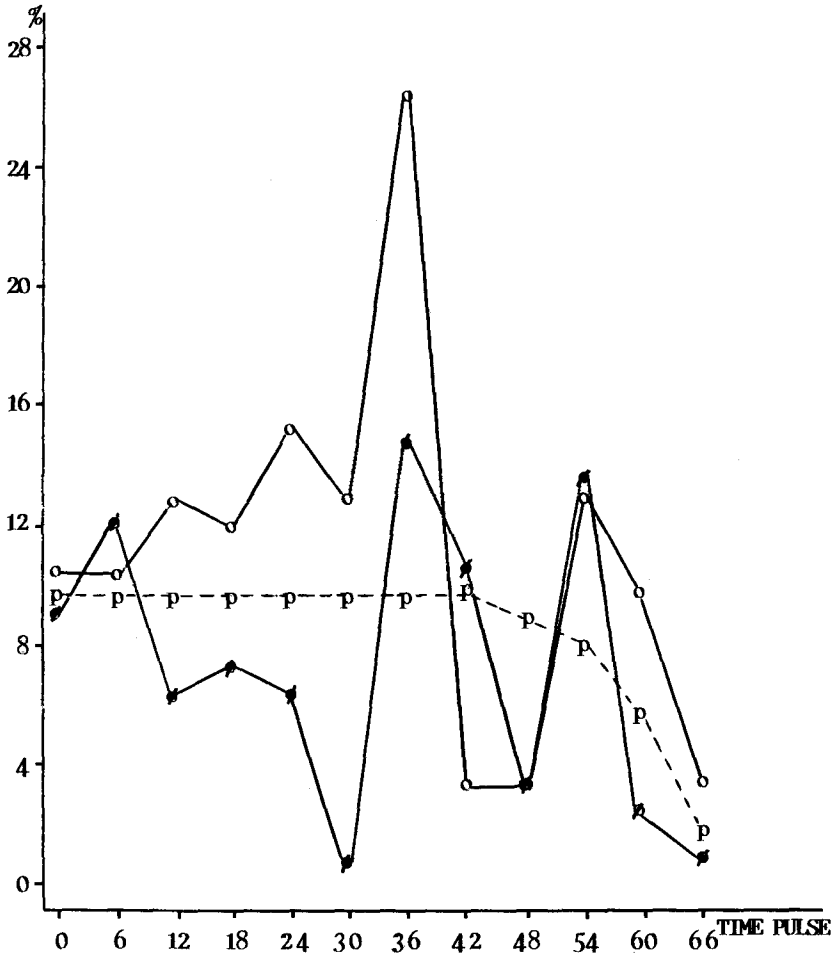

Figure 1. o-o Metaphases with structural chromosome aberrations: observed values; ø-ø Metaphases with chromosomes decondensed: observed values; p-p Expected values of metaphases with structural chromosomal aberrations and decondensed chromosomes.

The lowest degree of chromosomal decondensation is seen in the cultures in which treatment was carried out at 30, 48, 60 and 66 h and the greatest chromosomal sensitization to this decondensing action of Diazinon was found in the cultures treated at 36 and 54 h.

Upon analyzing the values obtained for the metaphase index (Table 1), no homogeneous trend could be observed; there are no great differences between the mitotic index of the cultures treated at 12 h and those treated at 60 h, ranging between 0.5 (54 h) and 1.6 (42 h). The maximum values for this parameter are

observed when the insecticide pulses were carried out at the beginning and at the end of culture, though they were always lower than the control values.

Statistical analysis of the results obtained show that there is a dependence relationship (according to the Chi squared test) between the time of application of the insecticide to the cultures and the parameters of the degree of decondensation and chromosomic aberration; these are independent of the time of application and such a relationship cannot be appreciated in the MI values. Furthermore, the three parameters studied fit a fifth order polynomial equation. The theoretical equation which predict cell behaviour **versus** the treatments carried out at different times of culture are:

$y=0,46x^5-0,21x^4-0,33x^3+0,25x^2-0,62x+0,50$
x= Time ; y= MI (%)

$y=0,57x^5-0,10x^4+0,60x^3-0,14x^2+0,95x+0,87$
x= Time ; y= % metaphases with abnormally condensed chromosomes.

$y=0,11x^5-0,15x^4+0,24x^3-0,10x^2+0,24x+0,11$
x= Time; y= % metaphases with chromosomal aberrations.

The degree of chromosomal decondensation and structural chromosomal aberration in fixed metaphases can be fitted to a single fifth order polynomial equation with a significance at 1% (p 0,01):

$y=-0,19x^5+0,25x^4+0,96x^3-0,46x^2+0,70x+0,94$
x= Time; y= % metaphases with structural chromosomal aberrations and % metaphases with fixed decondensed chromosomes.

The study of structural chromosomal aberrations shows a greater incidence of such aberrations than when the dose is maintained over 72 h of culture, in which no chromosomal aberrations can be observed (López et al 1985), showing that the cytotoxicity of the product masks the chromosomal alterations.

It may be seen that the induction of structural chromosomal aberrations is possible at different points of the Cell Cycle, though maximum values are observed when the pulses are performed when the cells are in phases S (36 and 54 h). Minimum sensitivity is noted when the cells are at the end of the S phase (42 and 66 h) and in the post-synthesis phase, G_2 (48 h), at the moment of adding the insecticide. These minima coincide with the dose obtained in treatments with different types of ethylenimide derivatives on cultures of human lymphocytes at different times (Bochnov and Yakovenko 1978).

This compound exerts a clear cytotoxic action on cells, as may be seen from the decrease observed in the mitotic index in all the assays conducted. Such action is probably reversible since when the pulses are carried out at the most distant moment from the end of the cultures (0 and 6 h) the MI values are higher. (Figure 2).

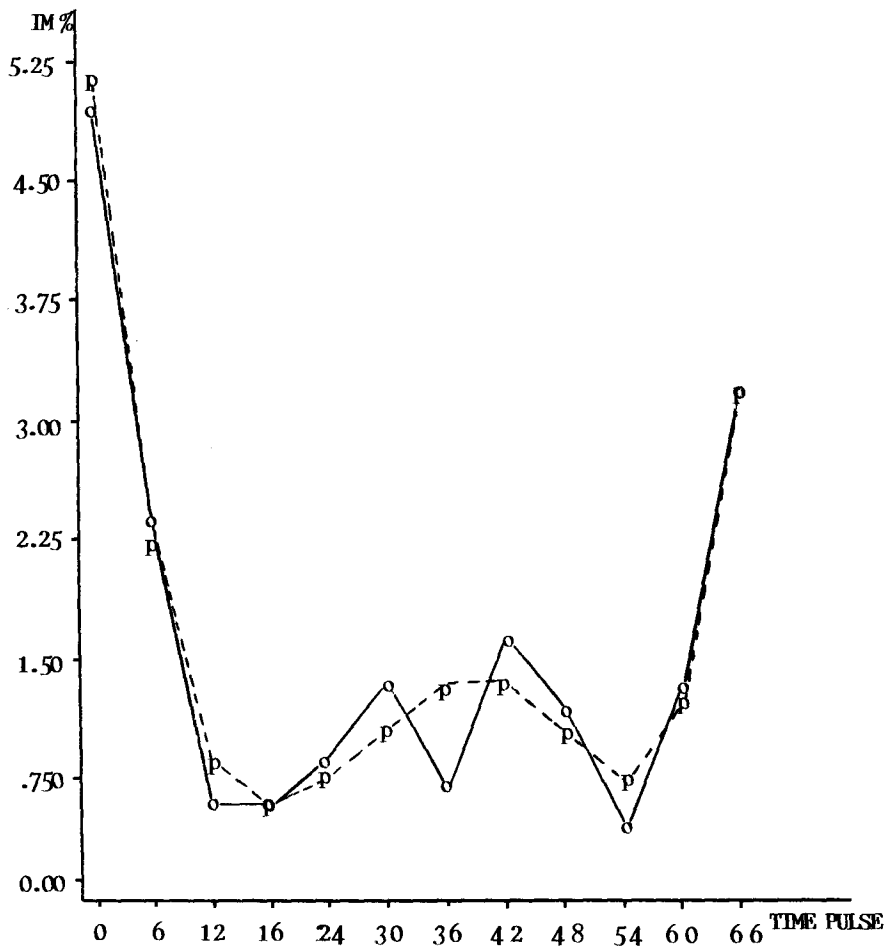

Figure 2. Mitotic index: o-o observed values; p--p Expected values

Likewise, when the treatment is carried out at 66 h, the MI scarcely differs from that of the controls, since at that moment the cells have already replicated their genetic material and there is no effect of cell division. This cytotoxic action observed in our experiments is common to all organophosphorous pesticides (Chen et al 1981) which have been reported to totally inhibit the growth of all kinds of cells at doses of 80 µg/cc by reducing cell survival (Carere and Morpurgo 1981).

The parallel trend observed between the degree of chromosomal decondensation and the degree of chromosomal aberration can be accounted for by the fact that because the chromosomes exhibit

greater decondensation they are more accessible to the action of the compound and a greater number of structural chromosomal aberrations takes place. The decondensation observed in the metaphase chromosomes can be explained in terms of the alkylating action of these organophosphorous compounds (López et al 1986) which contributes to maintaining the condensed structure of chromosomes.

REFERENCES

Aleixandre C (1981) Alteraciones cromosómicas inducidas por ^{3}HTdR en linfocitos humanos de sangre periférica. In: Universidad de Salamanca (ed). TC-290. Salamanca, Spain

Bochnov NP, Yakovenko KN (1978) Sensitivity of human limphocyte chromosomes to ethylenimine derivatives at different periods during cell culture. In: Evans HJ, Lloyd DC (ed). Mutagen induced chromosome damage in man. Edinburgh, p 290-299

Carere A, Morpurgo G (1981) Comparison of the mutagenic activity of pesticides "in vitro" in various shortterm assays. Mut Res 2:87-104

Chen HH, Sirianni SR, Hsueh JL, Huang CC (1981) Induction of sister-chromatid exchanges and cell cycle delay in cultured mammalian cells treated with eight organophosphorus pesticides. Mut Res 88:307-316

Evans HJ (1962) Chromosome aberrations induced by ionizing radiation. Int Rev Cytol 13:221-321

López DE, Aleixandre C, Merchán M, Carrascal E (1986) In vitro induction of alterations in peripheral blood lymphocytes by different doses of Diazinon. Bull Environ Contam Toxicol 37 (in press)

Wild D (1985) Mutagenicity studies on organophosphorus insecticides. Mut Res 32:113-150

Received March 2, 1986; accepted September 24, 1986.