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CYTOGENETIC STUDY OF DDT ON HUMAN LYMPHOCYTES IN VITRO

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Summary

The cytogenetic effect of DDT on human blood cultures, in vitro, was investigated. Two types of experiment were carried out: one, in which the DDT concentrations found in the culture media were similar to those found in the plasma of individuals of the Brazilian population (0.06—0.20 μg/ml); in the second experiments, doses ranging from 1 to 15 μg/ml were used.

No correlation was found between DDT doses and cells with chromosomal aberrations.

The Poisson test of comparison between means showed that at certain DDT concentrations (0.20, 4.05 and 8.72 μg/ml) the proportion of cells with structural aberrations was significantly greater than in the controls.

Introduction

Despite the countless advantages to man conferred by the use of DDT, the increasing environmental contamination by this substance has been causing great concern. Available data on its cytogenetic effects are still scarce and controversial. Hart et al. [5] found no significant increase in the incidence of chromosomal aberrations in cultures of human and rabbit blood submitted to DDT levels as high as 100 μg/ml. Yet Palmer et al. [7] reported such an increase in a cell line of *Potorous tridactylis apicalis* exposed to concentrations of 10 μg/ml and over. On the other hand, Kelly-Garvert and Legator [6] claim that DDT at 35—45 μg/ml did not produce a significant increase of chromosome aberrations in a cell line of *Cricetulus griseus*, whereas its derivative DDE induced such effects at concentrations of 30—40 μg/ml. Rabello et al. [8] sug-

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gest that DDT may cause chromatid lesions, since they found a significant increase in the frequency of cells with this type of aberration in plant labourers highly exposed to DDT. However, they found no correlation between the DDT level in the plasma and the frequencies of cells with chromosome aberrations.

The results to be presented in this paper refer to the DDT effect, in vitro, on human chromosomes in short-term lymphocyte cultures.

Material and methods

DDT extractions and analyses

The method of Dale et al. [4] was used. The extractions and dosages were performed at the Instituto Biológico of the State of S. Paulo.

Cytogenetic analysis

Two experiments were carried out, in which short-term lymphocyte cultures were submitted to DDT concentrations of 5, 10, 20 and 40 μg/ml. Heparinized blood was obtained from a normal male individual, aged 28 years, without any history of special exposure to DDT. Technical DDT * (63—77% of *p,p'*-DDT; 8—20% of *o,p'*-DDT; 3—5% of *p,p'*-DDE and 0.2—4% of *o,p'*-DDD) was used. DDT was sterilized by ultraviolet irradiation for 20 min, at a distance of about 10 cm.

In the first experiment, DDT was dissolved in acetone, then diluted in water and added to the medium at the beginning of the culture. Each culture always received 0.0001 ml acetone per ml of medium (TC Medium 199, dried, Difco Lab., supplemented with 30% bovine serum).

In the second experiment, DDT was dissolved in acetone and added directly to the culture medium. The amount of acetone added in the second experiment was twenty times greater than in the first. In both experiments, for each DDT concentration tested, five cultures with the drug and five controls were made. Control cultures received the same volume of acetone as used to dissolve DDT. The Beçak et al. [2] technique for short-term blood culture was used. A 72-h culture time was used for two reasons: (1) we were specially interested in stable chromosomal mutations; (2) any loss of chromosomal events because of a second cell division would minimize the effect we were looking for. Therefore, an eventual positive effect would be even more trustworthy. The preparations were coded and analysed in a blind test.

Cells were scored for aneuploidy, polyploidy and structural aberrations. The latter were classified as: Type A, without any detectable structural anomaly; Type B, cells with gaps or breaks involving one or both chromatids; Type C, cells with unstable (fragments, rings, dicentrics) or stable (inversions and translocations) aberrations.

Statistical analyses

To test a possible correlation between DDT dosage and frequency of cells

* *p,p'*-DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; *o,p'*-DDT, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *p,p'*-DDD, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethane; *o,p'*-DDD, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethane; *p,p'*-DDE, 1,1-dichloro-2,2-*bis*(*p*-chlorophenyl)ethylene; *o,p'*-DDE, 1,1-dichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)ethylene.

TABLE I

DDT LEVELS FOUND IN CONTROL AND EXPOSED CULTURES (μg/ml).

C.A., DDT concentration added to culture medium; C.D., DDT concentration determined by gas chromatography; tr, traces

	C.A.	C.D. p,p'-DDE	o,p'-DDT	p,p'-DDT	Total DDT
1st experiment	0	—	—	—	0
	1	—	—	—	0
	5	tr	0.04	0.16	0.20
	10	tr	0.03	0.12	0.15
	20	tr	0.03	0.11	0.14
	40	tr	0.02	0.04	0.06
2nd experiment	0	—	—	—	0
	1	0.01	0.15	0.86	1.02
	5	0.04	0.71	3.30	4.05
	10	0.06	1.53	7.13	8.72
	20	0.10	2.03	10.72	12.85
	40	0.10	2.50	13.05	15.65

with chromosomal aberrations (structural or numerical), Spearman's coefficient of rank correlation (r_s) and the exact test were calculated (Siegel [11]; Wolf [12]).

To ascertain whether the proportion of cells with structural aberrations and cells with numerical aberrations exposed to a certain DDT dose difffered from the proportions of such cells in the control, a proportional test based on the approximation of the binomial to the Poisson distribution was used. This was because the frequency of cells with structural or numerical aberrations is very small in relation to the total number of cells (Chakravarti et al. [3]).

All tests were considered at a 0.05 significance level.

TABLE II

1st EXPERIMENT. FREQUENCY OF NORMAL (TYPE A), WITH STRUCTURAL ABERRATIONS (TYPE B AND C), WITH 46 CHROMOSOMES AND ANEUPLOID CELLS IN CULTURES WITH AND WITHOUT DDT.

C.D., DDT concentrations determined in culture medium (μg/ml)

Types of cell analysed	C.D. 0	C.D. 0	C.D. 0.06	C.D. 0.14	C.D. 0.15	C.D. 0.20
A cells	91	83	86	84	85	80
B cells	7	15	14	15	14	20
C cells	2	2	—	1	1	—
Cells with structural aberrations (B + C)	9	17	14	16	15	20
Cells with 46 chromosomes	88	94	93	96	89	91
Aneuploid cells	12	6	7	4	11	9
Total number of cells analysed	100	100	100	100	100	100

TABLE III

2nd EXPERIMENT. FREQUENCY OF NORMAL (TYPE A) CELLS, CELLS WITH STRUCTURAL ABERRATIONS (TYPES B AND C), CELLS WITH 46 CHROMOSOMES, ANEUPLOID AND POLYPLOID CELLS IN CULTURES WITH AND WITHOUT DDT.

C.D., DDT concentrations determined in culture medium (μg/ml)

Types of cell analysed	C.D. 0	C.D. 1.02	C.D. 4.05	C.D. 8.72	C.D. 12.85	C.D. 15.65
A cells	144 (92.90%)	181 (90.50%)	145 (83.81%)	174 (87.00%)	180 (90.00%)	178 (89.00%)
B cells	10 (6.45%)	18 (9.00%)	27 (15.50%)	25 (12.50%)	17 (8.50%)	19 (9.50%)
C cells	1 (0.65%)	1 (0.50%)	—	1 (0.50%)	3 (1.50%)	3 (1.50%)
Cells with structural aberrations (B + C)	11 (7.09%)	19 (9.50%)	27 (15.50%)	26 (13.00%)	20 (10.00%)	22 (11.00%)
Cells with 46 chromosomes	142 (91.60%)	195 (97.50%)	155 (89.58%)	193 (96.50%)	183 (91.50%)	190 (95.00%)
Aneuploid cells	12 (7.70%)	4 (2.00%)	17 (9.80%)	7 (3.50%)	16 (8.00%)	8 (4.00%)
Polyploid cells	1 (0.60%)	1 (0.50%)	1 (0.50%)	--	1 (0.50%)	2 (1.00%)
Total number of cells with 1 numerical aberrations	13 (8.30%)	5 (2.50%)	18 (10.30%)	7 (3.50%)	17 (8.50%)	10 (5.00%)
Total number of cells analysed	155	200	173	200	200	200

Results

Concentrations of DDT added to culture media

The first observation that attracted special attention was that, as soon as DDT was added to the culture medium, it precipitated, especially in the first experiment where DDT was dissolved in acetone and then in water. Hence, samples from all culture flasks were taken and the real concentration of DDT was determined by gas chromatography. The results are shown in Table I. When DDT was diluted in acetone alone, the precipitation was smaller and proportional to the quantities added to the medium.

Cytogenetic results

The results of the cytogenetic analyses are shown in Tables II and III. The types of structural aberration found were: gaps, breaks, acentrics and translocations.

Statistical analyses

Since the frequency of type C cells was very small, B and C cells were treated as one single class, i.e. cells with structural aberrations. Aneuploid and polyploid cells were also grouped in one class, i.e. cells with numerical aberrations.

a) *Correlation between DDT concentrations in culture medium and proportion of anomalous cells.* Let Y be the variable DDT concentration; X_1 the variable proportion of cells with structural aberrations, and X_2 the variable proportion of cells with numerical aberrations; r_s is Spearman's correlation. Table IV shows the result of the correlation test. In one test (2nd experiment) the calculated r_s coincided with the critical value at the 0.05 level in the one-tailed test. All other tests were non-significant.

b) *Poisson's test of comparison between means.* Tables V and VI show the results of the comparisons between the proportion of the anomalous and normal cells in the control and exposed cultures.

In some cases ((3) in Tables V and VI) the proportion of cells with numerical aberrations in the control was greater than in the cultures with DDT.

What can be concluded from these tests is that the frequency of cells with

TABLE IV

RESULTS OF THE CORRELATION TESTS.

Y, DDT concentrations; X_1, proportion of cells with structural aberrations; X_2, proportion of cells with numerical aberrations; r_s, Spearman's coefficient

Variables	r_s	α (0.05) = 0.829 (two-tailed test) α (0.05) = 0.77 (one-tailed test)
1st experiment		
Y and X_1	0.5	Non-significant
Y and X_2	0.01	Non-significant
2nd experiment		
Y and X_1	0.77	Level of significance
Y and X_2	0.14	Non-significant

TABLE V

1st EXPERIMENT. RESULTS OBSERVED IN THE POISSON TEST. (DESCRIPTIVE LEVEL: THE LOWEST LEVEL THAT MAKES POSSIBLE THE REJECTION OF THE HYPOTHESIS.)

Classes compared		Descriptive levels of cells with structural aberrations (%)	Descriptive levels of cells with numerical aberrations (%)
Control DDT dosed	Exposed DDT dosed (μg/ml)		
0	0	8.43 [a]	11.89 [a]
0	0.06	20.24 [a]	17.96 [a]
0	0.14	11.48 [a]	3.90 [c]
0	0.15	15.37 [a]	50.00 [a]
0	0.20	3.1 [b]	33.18 [a]

[a] Descriptive levels greater than $\alpha = 0.05$ (one-tailed test). All tests are non-significant.
[b] Descriptive levels smaller than $\alpha = 0.05$ (one-tailed test). The proportion of cells with structural aberrations in this class with DDT at 0.20 μg/ml was significantly greater than the proportion of such cells in the control.
[c] Descriptive level smaller than $\alpha = 0.05$ (one-tailed test). The proportion of cells with numerical aberrations in the control was significantly greater than in the cultures with DDT (0.14 μg/ml).

TABLE VI

2nd EXPERIMENT. RESULTS OBSERVED IN THE POISSON TEST.

Compared Classes		Descriptive levels of cells with structural aberrations (%)	Descriptive levels of cells with numerical aberrations (%)
Control DDT dosed	Exposed DDT dosed (μg/ml)		
0	1.02	16.04 [a]	1.47 [c]
0	4.05	0.74 [b]	22.89 [a]
0	8.72	2.59 [b]	4.82 [c]
0	12.85	12.73 [a]	40.14 [a]
0	15.65	7.77 [a]	15.87 [a]

[a] Descriptive levels greater than $\alpha = 0.05$ (one-tailed test). All tests are non-significant.
[b] Descriptive levels smaller than $\alpha = 0.05$ (one-tailed test). The proportion of cells with structural aberrations in the cultures with 4.05 μg/ml and 8.72 μg/ml DDT are significantly greater than in the controls.
[c] Descriptive levels smaller than $\alpha = 0.05$ (one-tailed test). The proportion of cells with numerical aberrations in the cultures with 1.02 μg/ml and 8.72 μg/ml DDT are smaller than in the controls.

structural aberrations was greater in some cultures exposed to DDT than in the controls.

Discussion

The precipitation of DDT in the culture media was also mentioned by Kelly-Garvert and Legator [6]. When studying the mutagenicity of DDT and DDE in a Chinese hamster cell line, these authors found extreme variations in cellular survival among experiments in which a cytotoxic effect was evident (35—45 μg/ml). These variations were interpreted as a result of the undetermined amount of binding of DDT and DDE to the lipid moiety in calf serum (we used bovine

serum in our experiments), a slight precipitation of DDT from the medium and cell permeability. The authors did not determine the final DDT or DDE concentrations in the medium.

In our first experiment, the DDT concentrations in the culture medium were within the range of levels found in the plasma of individuals from the Brazilian population (Almeida [1]; Rabello et al. [8]; Schwartsman et al. [9].

In the second experiment, the precipitation was smaller. The lowest DDT concentration (1.02 μg/ml) was similar to the average DDT level found by Almeida [1] in the plasma of individuals occupationally exposed to the drug (0.953 μg/ml) in the State of Rio de Janeiro, Brazil. The highest concentration (15.65 μg/ml) was about 16 times larger.

Since only DDT peaks were detected in culture media by gas chromatography, we assume that the exposure of the drug to UV did not cause any modification in its chemical structure.

Statistical analysis showed no correlation between DDT concentrations and the proportion of cells with numerical or structural aberrations. This lack of correlation, however, should not be taken as a definite conclusion. In the second experiment (Table VI) the r_s calculated between DDT doses and cells with structural aberrations was equal to the critical value at $\alpha = 0.05$ in a one-tailed test. Perhaps a correlation would have been detected had we used a greater number of DDT doses and obtained the same result, because then the critical level would have had a smaller value. Nevertheless, Rabello et al. [8] found no correlation between DDT plasma levels (ranging from 0.03 to 3.25 μg/ml) and the frequency of cells with structural or numerical aberrations in a sample of 62 individuals. Eventually, a correlation different from zero might be detectable only at higher DDT concentrations.

The Poisson tests showed that, in both experiments, in some DDT doses, the exposed cultures had a smaller frequency of cells with numerical aberrations than the controls. This finding might be attributed to chance alone.

The only cases in which the proportion of cells with structural aberrations was greater in the exposed than in controls were at DDT concentrations of 0.20 (1st experiment), 4.05 and 8.72 μg/ml (2nd experiment), which were not the highest concentrations used.

Kelly-Garvert and Legator [6], when testing the cytogenetic effects of DDT and DDE in a Chinese hamster cell line, also obtained discrepant results in two experiments: in the first, a DDE level of 35 μg/ml did not produce a significant increase of cytological aberrations, whereas the same concentration in a second experiment produced a significant increase, as shown by a χ^2 test. Concentrations of DDT at 35 and 45 μg/ml failed to show a significant increase of cytological aberrations.

Our results do not agree with those of Palmer et al. [7] who obtained a significant increase of aberrations at 10 μg/ml levels and who used DMSO (dimethyl-sulphoxide) as a solvent. They are also in disaccord with those of Hart et al. [5] both in human and in rabbit blood; their sample of cells, however, was much smaller than ours (50 and 30 metaphases examined at each DDT concentration for human and rabbit cultures, respectively).

The discordance of results obtained by several authors seems to indicate that several factors may influence the results, such as different culture tech-

niques, type of cell and lineage used in the experiments and different solvents. We note that Scott and Bigger [10] reported that the lymphocytes of man and *P. tridactylis* exposed to various doses of X-rays showed different radiosensitivities.

The question of a possible cytogenetic effect of DDT remains unanswered. Experiments, in vivo and in vitro, for testing the best solvent and a greater number of observations should be encouraged so as to throw a new light on the problem.

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