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CYTOGENETIC STUDY ON INDIVIDUALS OCCUPATIONALLY EXPOSED TO DDT*

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SUMMARY

Workers from three insecticide plants in direct contact with 2,2-bis (β-chlorophenyl)-1,1,1,-trichloroethane (DDT) did not differ significantly in the frequencies of cells with chromosomal aberrations when compared with controls from the same plants but not in direct contact with the drug. The same was true when a group of workers from one plant was compared with a control group from the Instituto Butantan, with no history of occupational exposure to DDT. Yet, when the control group from one of the three plants, which showed high DDT plasmic levels, was added to the group in direct contact with the insecticide, the frequency of cells with chromatid aberrations was significantly higher, suggesting that DDT causes chromatid lesions. A positive correlation was found between DDT levels and times of exposure, but being in direct or indirect contact with DDT was not always correlated with the degree of contamination.

INTRODUCTION

Since 1940, DDT has been the world's most used pesticide both in public health and agriculture. Available data concerning its effects on human chromosomes are, however, still scarce. HART *et al.*[7] found no increase in the frequency of cells with chromosomal aberrations from human leucocyte cultures submitted *in vitro* to 1,5,10,30,50 and 100 ppm DDT. LESSA[10] found no correlation between the frequencies of cells with structural or numerical aberrations and DDT concentrations in human leucocyte cultures treated *in vitro*.

An increase in chromatid lesions has been reported in blood cultures from a group of 42 men occupationally exposed to several pesticides, DDT included, during

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Abbreviations: DDE, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene; DDT, 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane.

the spraying season as compared with short term cultures made six months before, when the same individuals had not been in contact with the pesticides for 30 days[13].

The present investigation was aimed at determining whether DDT produces chromosomal abnormalities in leucocytes from individuals submitted to different degrees of occupational exposure to DDT.

MATERIAL AND METHODS

Two samples of adult males belonging to similar socio-economical levels were studied. The first consisted of 50 workers from three insecticide plants (A,B and C) in the city of São Paulo (State of S. Paulo, Brazil): 25 of them had been directly exposed to DDT for 2 months to 10 years (mean 2 years 4 months, with an average weekly exposure of 48 h) before the experiment; the other 25 were used as controls and had not had direct contact with DDT, although working in the same plants, for periods ranging from 1 month to 19 years (mean 2 years 15 days; average weekly exposure of 48 h.). The mean age of the group directly exposed to DDT was 25 years and that of the control group was 31 years.

The second sample consisted of 8 subjects directly exposed to DDT from plant B (not included in the first sample) for at least 20 days up to 2 years (mean 11 months; 48 h per week) and of 10 labourers from the Instituto Butantan, with no history of occupational exposure to DDT. The mean age of the exposed group was 29 years and that of the control group, 34 years.

Blood samples obtained from all subjects were used for the lymphocyte cultures and for the dosage of the DDT levels in the plasma. 72-h lymphocyte cultures were prepared and harvested under similar conditions from all subjects according to BEÇAK *et al.*[2]; slides were prepared and coded by a person not concerned in the study, and 50 metaphases from each subject were studied in a blind test by one investigator. The first six cells were fully analysed to ascertain the karyotype constitution of the individual, as well as all aneuploid cells. All cells were scored for aneuploidy and chromatid and chromosomal aberrations. The types of aberration found were: gaps, breaks, acentrics, one dicentric and two rearrangements. When both chromatids of a chromosome were involved, the aberration was scored as chromosomal; when only one of the chromatids was involved, the aberration was scored as a chromatid aberration.

The extraction and analysis of the DDT plasmic levels were performed according to DALE *et al.*[5].

Two statistical tests were used: the analysis of variance to test the significance of the differences among the groups studied (both for the cytogenetic analysis and for the DDT plasmic levels), and a non-parametric test based on Spearmean's coefficient (R_S) to test the correlations between DDT plasmic levels and frequencies of chromosomal aberrations (structural and numerical) and between DDT levels and length of exposure. In all tests the level of significance considered was 0.05.

RESULTS AND DISCUSSION

Tables I and II show the results of the cytogenetic analysis and the DDT plasmic levels of the first and second samples.

66 subjects showed normal karyotypes. One subject from the experimental

group had a 45,XY,–D,–G,+t (Dq Gq) constitution, and one of the controls, phenotypically normal, presented a 47,XYY karyotype, confirmed by fluorescence microscopy. Among 3400 cells analysed, one dicentric chromosome and two rearrangements were found. The dicentric belonged to a 45,XY,–C,–C,+dic cell from one of the controls and was interpreted as resulting from the breakage of two chromosomes from group C before DNA synthesis, and loss of the acentric fragments. The cell was scored as having a chromosomal aberration. The two rearrangements showed a quadriradial configuration and were found in two 46,XY cells, one from one of the controls and the other from one of the directly exposed individuals. Both were interpreted as resulting from an interchange between chromatids (in one, between two C-chromosomes, and in the other, between two E_{16}), followed by pairing of the sister chromatids. Both cells were scored as having a chromatid aberration.

Since the blood cultures from the first sample had been prepared at three differ-

TABLE I

DDT PLASMIC LEVELS (RANGE AND AVERAGE) AND FREQUENCIES OF NORMAL AND ANOMALOUS CELLS FROM 25 SUBJECTS WORKING IN THREE PESTICIDE PLANTS (PLANTS A, B AND C), DIRECTLY EXPOSED TO DDT AND FROM 25 CONTROL SUBJECTS FROM THE SAME PLANTS INDIRECTLY EXPOSED (1ST SAMPLE)

DDT levels:	*Control group*	*Exposed group*	
Range:	*0.03–1.46 μg/ml*	*0.16–3.25 μg/ml*	
Average:	*0.38 μg/ml*	*1.03 μg/ml*	
Number of cells			$4.08 > F_{1,48}(0.05) > 4.00$
Normal	1088 (87.04%)	1034 (82.72%)	
With chromatid aberrations	116 (9.28%)	152 (12.16%)	$F = 3.83$
With chromosomal aberrations	46 (3.68%)	64 (5.12%)	$F = 1.81$
With modal number of chromosomes	1139 (91.12%)	1154 (92.32%)	
With hypermodal number of chromosomes	15 (1.28%)	10 (0.80%)	$F = 0.22$
With hypomodal number of chromosomes	96 (7.75%)	85 (6.80%)	$F = 0.84$
Total	1250	1250	

TABLE II

DDT PLASMIC LEVELS (RANGE AND AVERAGE) AND FREQUENCIES OF NORMAL AND ANOMALOUS CELLS FROM 8 SUBJECTS DIRECTLY EXPOSED TO DDT IN A PESTICIDE PLANT (PLANT B) AND FROM 10 CONTROL SUBJECTS WORKING AT THE INSTITUTO BUTANTAN (2ND SAMPLE)

DDT levels:	*Control group*	*Exposed group*	
Range:	*0.02–0.04 μg/ml*	*0.09–0.54 μg/ml*	
Average:	*0.03 μg/ml*	*0.24 μg/ml*	
Number of cells			$F_{1,16}(0.05) = 4.49$
Normal	471 (94.2%)	375 (93.75%)	
With chromatid aberrations	13 (2.20%)	14 (3.50%)	$F = 1.26$
With chromosomal aberrations	16 (3.20%)	11 (2.75%)	$F = 0.02$
With modal number of chromosomes	461 (92.20%)	273 (94.25%)	
With hypermodal number of chromosomes	4 (0.80%)	5 (1.25%)	$F = 0.37$
With hypomodal number of chromosomes	35 (7.00%)	24 (6.00%)	$F = 0.48$
Total	500	400	

ent times, analyses of variance were made for checking a possible heterogeneity. No significant differences were found.

The differences in frequencies of each type of anomalous cell were also non-significant between the groups directly and indirectly exposed to DDT (1st sample) as well as between the exposed and control groups of the 2nd sample.

The subjects from Plants A, B and C directly exposed to DDT did not differ significantly in their DDT plasmic levels. The group from Plant A, indirectly exposed to DDT, differed from the indirectly exposed group from Plants B and C, but did not differ significantly either from the group from Plant A submitted to direct exposure or the groups from Plants B and C in direct contact with the insecticide.

No significant correlation was found between the frequencies of cells with structural or numerical chromosomal aberrations and DDT levels in the plasma of the subjects investigated. Yet there was a significant positive correlation between length of exposure and DDT levels in the plasma.

The mean DDT level of the 25 directly exposed workers from the first sample (1.033 μg/ml) was similar to that found in workers from anti-malarial campaigns in Brazil, exposed to DDT for more than 6 years[1]; it was about 1.4 times greater than the levels determined by LAWS *et al.*[9] in pesticide plant workers from the USA exposed for over 5 years (0.737 μg/ml), and also by RADOMSKI *et al.*[11] in spraymen from anti-malarial programmes during 5 years in Argentina (0.709 μg/ml). The mean DDT level of the group directly exposed was 24.5 times greater than that of women from the general population of S. Paulo[14].

The mean DDT level of the 25 workers in indirect contact with the drug was 0.378 μg/ml; such a value is comparable to that determined by LAWS *et al.*[9] in pesticide plant workers submitted to medium exposure (0.358 μg/ml). The DDT plasmic level in this group, although 2.7 times smaller than that of the directly exposed group from the same plants, was 9 times greater than that of S. Paulo's general population. The directly exposed group from the second sample had a mean DDT plasmic level of 0.24 μg/ml, which is about 5.7 times greater than that of the general population.

The group from Instituto Butantan had the lowest mean DDT level of all groups studied (0.029 μg/ml); it was similar to that found in the general population of S. Paulo[12]. The observation that 2,2-bis (*p*-chlorophenyl)-1,1-dichloroethylene (DDE) was the only residue detected in the subjects of this group shows that they must have had previous contact with DDT, but their contamination is negligible at the moment.

The groups of individuals as defined according to their type of contact with DDT (direct or indirect) did not differ significantly in their frequencies of cells with chromosomal aberrations of any type.

On the other hand, the DDT determinations showed that direct or indirect contact with the insecticide is not always correlated with the degree of contamination; actually the DDT plasmic levels of the group from plant A (5 subjects) indirectly exposed did not differ significantly from those of the directly exposed groups from the three pesticide plants.

Assuming then that the criterion of classifying the individuals according to their direct or indirect contact with DDT was inadequate, we decided to add the control group from plant A, which turned out to show high DDT plasmic levels, to the directly exposed groups from Plants A, B and C (Table III). When compared with the indirectly exposed group from Plants B and C, this new class of exposed individuals proved

TABLE III

DDT PLASMIC LEVELS (RANGE AND AVERAGE) AND FREQUENCIES OF NORMAL AND ANOMALOUS CELLS OF 20 CONTROLS FROM PLANTS B AND C AND 25 SUBJECTS DIRECTLY EXPOSED FROM PLANTS A, B AND C PLUS 5 CONTROLS FROM PLANT A WITH HIGH DDT PLASMIC LEVELS

DDT levels:	*Control group (Plants B and C)*	*Exposed group (Plants A, B, C + 5 controls from A)*	
Range:	*0.03–1.46 µg/ml*	*0.16–3.25 µg/ml*	
Average:	*0.275 µg/ml*	*0.993 µg/ml*	
Number of cells			$4.08 > F_{1,43}(0.05) > 4.00$
Normal	894 (89.4%)	1228 (81.9%)	
With chromatid aberrations	88 (8.8%)	180 (12.0%)	$F = 5.22$
With chromosomal aberrations	38 (3.8%)	72 (4.8%)	$F = 0.85$
With modal number of chromosomes	906 (90.6%)	1387 (92.4%)	
With hypermodal number of chromosomes	14 (1.4%)	11 (0.73%)	$F = 1.14$
With hypomodal number of chromosomes	80 (8.0%)	69 (4.6%)	$F = 1.37$
Total	1000	1500	

to differ significantly in the frequency of cells with chromatid lesions (gaps and breaks). This result suggests that the failure in finding a significant difference in the frequency of cells with chromatid lesions in the first sample was due to the inclusion in the control group of the 5 subjects with high DDT plasmic levels.

Now, the mean DDT level of the new class of exposed individuals (0.993 µg/ml) was approximately 3.6 times greater than that of the controls from Plants B and C, whereas the mean DDT level of the exposed individuals from our second sample was 3 times greater than that of their controls. One would expect any DDT effect to be much more easily detected in the second sample. Two hypotheses could account for this not being so. First, that the significant difference found is not genuine, but results from the fact that five individuals were added to the exposed group, leaving 10 subjects of this group lacking the corresponding controls. The second hypothesis is that DDT actually causes an increase in chromatid lesions, but this effect could not be demonstrated in the second sample because it was too small. The majority of the lymphocytes when put into culture are in G_1, and DNA synthesis only begins after 24 h (ref. 3). Although chromatid lesions have been interpreted as resulting from effects occurring *in vitro*[4], it is known that many chemicals produce only chromatid type aberrations in the first and subsequent mitoses following exposure even though the cells may have been exposed to the mutagen while in the G_1 phase of the cycle[6].

Our data suggest that DDT causes chromatid aberrations, although this assertion should be considered tentative.

According to KIHLMAN[8], there are two main types of agent that produce chromosomal aberrations: (*1*) those that induce spontaneous or enzymatic DNA strand breakage and/or interfere chemically or physically with DNA synthesis, and (*2*) those that interfere with the repair mechanism. We have no evidence for deciding to which category DDT belongs; if, however, it were of the second type, this would easily account for the increase in the frequency of chromatid but not of chromosomal lesions.

The subjects from the Instituto Butantan were, among the groups studied, the most representative of the general population as to their DDT plasmic levels. They showed 2.2% of cells with chromatid lesions, a value in agreement with that found by

COURT-BROWN[4] for the general population in England. The new class of exposed individuals, with mean DDT levels about 20 times greater than the general population, showed 12% of cells with chromatid aberrations, and its control group, which had a mean DDT level 6.5 times that of the general population, presented 8% ot cells with chromatid aberrations.

Assuming that (*a*) the damage caused to the germinal cells by DDT is similar to that detected in the blood, and (*b*) there is no selection against gametes carrying chromatid aberrations, the risk for the offspring of exposed individuals could be estimated from data obtained from blood. There are no data concerning the first assumption, and the second represents an upper limit for the real situation, because a fraction of the cells carrying deletions caused by breaks is eliminated during spermatogenesis, and the majority of zygotes with chromosomal abnormalities is eliminated as spontaneous abortions early in uterine life. This leads to the conclusion that the actual risk must be much smaller than that which might be estimated from data obtained from the blood.

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