Genotoxic Monitoring of Workers at a Hazardous Waste Disposal Site in Mexico

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Chromosomal aberration and sister chromatid exchange (SCE) frequencies were determined in lymphocytes cultured from 12 high-risk individuals working at a landfill for hazardous waste disposal. Cell proliferation kinetics (CPK) was also determined. Compared with 7 control individuals, no effects were observed with respect to SCE nor on CPK. However, the workers exhibited significantly higher frequencies of chromatid and chromosomal deletions, the magnitude of which was related with exposure time. This study suggests that when high-risk exposure is suspected, determining biomarkers of genotoxic damage (e.g., chromosomal aberrations), is useful for risk assessments. — Environ Health Perspect 103(Suppl 1):111–113 (1995)

Key words: chromosomal aberrations, sister chromatid exchanges (SCE), human monitoring, hazardous waste dump-site

Introduction

The purpose of biological monitoring is to protect human health and the environment (*1*). There are numerous methods or strategies to ascertain human exposure. For example, it is possible to measure the substance(s) or its metabolites in biological fluids and/or biological alterations resulting from interactions between the reactive species and the organism. Relevant markers for genotoxic damage in biological monitoring include cytogenetic end points such as sister chromatid exchanges (SCE) and chromosomal aberrations (*2*). They are sensitive indicators of damage and have been widely used to investigate the effects of exposure on the genetic material of individuals (*3*).

Landfills for toxic materials were developed for the safe disposal of dangerous chemicals. They are built according to international recommendations to preserve the environment from the deleterious effects of hazardous wastes; nevertheless, they still represent a potential source of exposure to the workers involved in handling the residues.

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Here we report on the results of a cytogenetic study to evaluate the frequencies of SCE and chromosomal aberrations in lymphocytes cultured from a group of high-risk workers at a landfill for toxic waste. This study was done as part of a biological monitoring program performed on the same individuals to evaluate exposure (*4*). These workers had been in direct contact with toxic substances for several months without using protective equipment. They presented, when compared with control individuals, higher levels of arsenic in urine (52.24 vs 35.67 μg/g of creatinine in controls) and some of them exhibited nonspecific symptoms such as irritability and insomnia (*4*).

Materials and Methods

Twelve males employed at the dump site during 4 to 8 months and seven individuals from El Huizache, the nearby village, with similar socioeconomic and nutritional status, agreed to participate as the exposed and control individuals respectively. Each subject completed a questionnaire regarding general health condition and drinking and smoking habits. Peripheral blood samples for all the subjects were obtained early in the morning, transported to the laboratory, and processed within 24 hr.

Cell cultures, cell harvesting, slide preparation, and staining were performed as described elsewhere (*5*). Heparinized blood, 0.5 ml, was used to start cultures in 6.0 ml of RPMI 1640 culture media supplemented with L-glutamine and nonessential amino acids, 32 μM of bromodeoxyuridine (Sigma) and 0.2 ml of phytohemagglutinine (Microlab); cultures were incubated at 37°C. Lymphocytes in whole blood were cultured for 48 hr and 72 hr for the analysis of chromosomal aberrations and SCE, respectively. Cytogenetic analysis was performed in coded slides, as described earlier (*5–7*). Briefly, SCE were scored in 25 consecutive second division metaphases, all with 46 centromeres. In the same slides, cell proliferation kinetics was evaluated in 100 consecutive metaphases by counting the proportion of first, second, third, or more division cells. The replication index was calculated according to the formula $RI = (M1 + 2M2 + 3M3) / 100$. One hundred consecutive first division metaphases with 46 centromeres were analyzed for chromosomal aberrations. The aberrations were classified as chromatid-type or chromosome-type, as described (*5–7*). All the scoring was performed by the same technician to diminish scoring bias.

The frequencies of chromosomal aberrations obtained in the exposed and control groups were compared using the Mann–Whitney *U*-test at a 0.05 level of significance. A linear regression analysis was performed to study the relation between the length of exposure and the amount of scored damage (GraphPad Inplot version 4.0 by GraphPad Software). The variance was homogenized by a square root transformation of the data (*8*). The

Table 1. Sister chromatid exchanges and replication indexes observed in control and exposed individuals.

Subject/length of exposure[a]	SCE/cell	Replication index
Exposed		
17/4	5.2 ± 2.5	2.89
2/5	6.5 ± 3.3	2.38
3/5[b]	5.2 ± 2.1	2.47
5/5[b]	6.8 ± 3.7	2.57
22/6	5.2 ± 2.8	2.36
4/7[b]	5.9 ± 3.2	2.65
6/7	5.7 ± 3.1	2.36
18/7	6.0 ± 2.8	2.75
10/8	6.7 ± 2.6	2.11
13/8[b]	8.7 ± 3.7	1.75
14/8	6.1 ± 2.3	2.74
20/8	7.7 ± 3.5	2.80
Average	6.3 ± 1.1	2.48 ± 0.32
Control		
1/0[b]	7.1 ± 3.5	2.17
11/0[b]	5.3 ± 2.4	2.50
12/0	7.3 ± 5.3	2.82
15/0[b]	3.3 ± 1.8	2.47
16/0[b]	4.4 ± 2.3	2.56
21/0	9.2 ± 4.1	2.14
23/0[b]	8.7 ± 3.6	2.00
Average	6.5 ± 2.2	2.38 ± 0.28

[a]Length of exposure in months. [b]Smokers.

Table 2. Frequency and type of chromosomal aberrations.

	Aberrations/100 cells per individual				
Subject/length of exposure[a]	G′	G″	B′	B″	Total without gap
Exposed					
17/4	3	0	7	4	11
3/5[b]	3	0	5	3	8
2/5	7	0	10	3	13
22/6	10	0	8	5	13
4/7[c]	6	3	6	2	8
6/7	5	0	7	8	15
18/7	2	1	5	4	9
10/8	9	1	87	20	107
13/8[c]	5	0	28	15	43
14/8[c]	5	1	6	0	6
20/8	6	1	5	4	9
Total	69	7	184[b]	71[b]	255[b]
Controls					
1/0[c]	6	1	4	1	4
11/0[c]	3	0	2	1	3
12/0	8	0	1	2	3
15/0[c]	4	0	5	1	6
16/0[c]	3	0	1	0	1
21/0	2	0	2	1	3
23/0[c]	2	0	2	2	4
Total	28	1	17	8	24

Abbreviations: G′, chromatid gap; G″, chromosome gap; B′, chromatid aberrations; B″, chromosome-type aberrations. [a]Length of exposure in months. [b]$p<0.05$. [c]Smokers.

SCE frequencies and RI were compared using Student's t-test at a $p<0.05$.

Results

The frequency of SCE and the CPK was not different between the exposed and control groups, as shown in Table 1. However, higher frequencies of chromatid-type and chromosome-type aberrations were found in the exposed individuals (Table 2).

Due to the high frequency of aberrations found in the control group of individuals, smoking habits were taken into consideration when the results were analyzed. Four of 7 controls and 4 of 12 exposed individuals smoked between 10 and 20 cigarettes per day. When current cigarette smokers and nonsmokers were compared, no significant differences were detected in terms of the frequency of the different types of aberrations found ($p>0.05$).

A linear regression analysis was performed on the transformed data, for total aberration yield, excluding gaps, with regard to the length of exposure in months (Figure 1). Results showed that they were significantly increased with the length of exposure, taken as the number of months individuals had worked at the disposal site. The $r^2=0.37$, an ANOVA analysis of the regression, showed significance with $p<0.01$. If individual 10/8 was excluded from the analysis, $r^2=0.48$, $p<0.01$. When the same analysis was performed taking into consideration the percentage of damaged cells, $r^2=0.39$, $p<0.01$.

Discussion

A significant increased level of chromosomal damage was detected among the cultured lymphocytes of high-risk workers; this damage was found to increase with exposure time. The most frequent aberrations found were chromatid-type and chromosome-type breaks, the type of aberrations associated with occupational exposure to genotoxic chemicals (*7,9–18*).

The chemical analysis for toxic substances performed in body fluids was positive only for arsenic in urine (*4*). Blood lead, urinary mercury, cadmium in hair and blood and urinary phenol levels (biomarker for the exposure to benzene) were not detectable or were at levels without statistical significance when compared with control values (*4*). However, the low arsenic concentrations found are difficult to correlate with the type and frequencies of aberrations observed, since a higher and chronic arsenic exposure did not induce this type of damage (*5*). As arsenic is a known coclastogen (*19*), it is possible that the combination of arsenic exposure with other DNA-damaging agents, contained in the complex mixtures handled by the workers, could interact synergistically and produce the increased levels of chromosomal damage found, even if the genotoxics were at concentrations below levels of analytical detection (*1*). Therefore, when the concentration of chemical substances cannot be determined or no longer exist in body fluids due to pharmacokinetic reactions, the genotoxic biomarkers function, as in this study, as markers of exposure and of early biological effects.

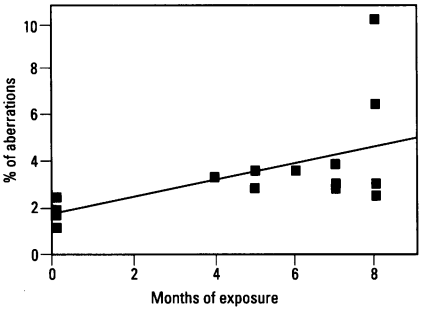

Figure 1. Linear regression curve obtained by plotting the percentage of chromosomal aberrations, without gaps, vs the length of exposure in months.

One of the workers showed 10 times the average amount of damage found in the exposed group. Even if we exclude this individual from the analysis, the difference between groups is still significant. Since the high aberration frequency in this individual could not be correlated with data from the questionnaire, physical examination, or the toxicological data, the amount of genetic damage observed could reflect differential susceptibility among individuals (*20*). The frequencies of chromosomal aberrations scored in the control individuals are higher than those found in a group of subjects living in another Mexican rural area (*5*) and also higher than those reported in other human monitoring studies (*7,9–18*). The damage found in the control population could be attributed to a sum of factors such as a small sample size, nutritional deficiencies, and to temporal exposures to substances such as pesticides (*4*).

The results of this study help to integrate the data of an early toxicological assessment (*4*) and show the relevance of analyzing several biological end points. Although the damage found could not be attributed to a particular substance, there is a clear correlation between the yield of chromosomal aberrations with the duration of exposure, which demonstrates that individuals were exposed to toxic substances.

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