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Nonrandom Distribution of Breakpoints in the Karyotypes of Workers Occupationally Exposed to Benzene

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The distribution of the breakpoints in the karyotypes of workers occupationally exposed to benzene was tested. Fifty-six workers exposed to benzene were investigated cytogenetically. A significant increase in chromosome aberrations was observed. The distribution of the breakpoints in the karyotypes of examined workers was significantly nonrandom ($p \leq 0.001$). The breakpoints accumulated mainly on chromosomes 2, 4, and 7.

Introduction

Benzene and its derivatives are used commonly in industry as solvents. Many workers are occupationally exposed to these agents (*1*). It has been shown that benzene is a myelotoxin, leading to bone-marrow depression and inducing leukemia in humans (*2–6*), as well as inducing nonhematological neoplasms in experimental animals exposed long term (*1,7*). Benzene is a clastogenic agent. An increase in chromosome abnormalities was observed both in the lymphocytes of workers occupationally exposed to benzene (*8–10*) and in the lymphocytes of animals exposed long term in experiments (*11,12*). Localization of the breakpoints in the lymphocytes of workers occupationally exposed to benzene was nonrandom (*13*).

The aim of this study was to analyze the distribution of the breakpoints in GTG-banded karyotypes of workers occupationally exposed to benzene.

Materials and Methods

Fifty-six workers employed in three different plants were investigated cytogenetically. Their work involved the use of organic solvents. The average period of exposure to benzene for each subject was 6 hr per day for 10–20 years. However, like most chemical exposures in the "real world," their exposures were not to a single agent, but to a mixture of chemicals. Personal exposure of the investigated workers, which took place at their work sites, was similar to, and included, benzene and its derivatives (toluene and xylene), butyl acetate, ethyl acetate, and butanol. The concentration of benzene was below the national occupational exposure limit set in Poland (10 ppm), and the concentrations of other chemicals were low. Neither clinical nor hematological symptoms of chronic benzene intoxication were found in the examined workers.

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Twenty persons in the control group had no occupational contact with any known chemical or physical mutagenic agents. They were matched according to sex, age, smoking habits, and alcohol consumption. The subjects in both groups were interviewed about viral infections, X-ray examinations, and drug intake during the last 3 months before cytogenetic examination.

Whole blood lymphocyte cultures were established for 50 hr of incubation in Eagle's minimal medium, supplemented with 10% fetal calf serum and stimulated with phytohemagglutinin. Colcemid was added 2 hr before havesting. Cytogenetic analysis was performed on GTG-banded (G-bands by trypsin using Giemsa) metaphases. It was not possible to analyze 100 cells in each subject. Altogether, 4000 metaphases in the groups of workers and 2000 in the controls were analyzed. Chromosome aberrations were classified according to The International System for Human Cytogenetic Nomenclature, 1985 (*14*).

Results and Discussion

In the examined group of workers occupationally exposed to benzene, structural chromosome aberrations (including gaps) were found in 2.7% $\pm$ 0.3, whereas in the control group aberrations were found in 0.9% $\pm$ 0.01 of the analyzed metaphases. Frequency of chromosomal aberrations observed in the examined groups was three times higher than in the control group. The types of aberrations observed in the presented material were mainly breaks and gaps and rarely structural chromosome rearrangements. Frequency and types of chromosomal aberrations observed in the exposed and control groups were similar to the results described by other authors (*8–17*).

Detailed band localization of the breakpoints observed in the chromosomes of exposed workers is presented in Table 1. The results suggest that distribution of the breakpoints in the karyotype of exposed workers is nonrandom. In the presented material, the breakpoints were located mainly on chromosomes numbers 2, 4, and 7 (Table 1). The number of observed break-

Table 1. Observed (O) and expected (E) numbers of the breaks and gaps and the localization of the breakpoints in the karyotype of workers exposed to benzene.[a]

Chromosome no.	Breakpoints	No. of breakpoints	No. of breaks	Gaps	No. of observed breakpoints O	E	O/E
1	p36	1					
	p31	2					
	p22	2	9	1	10	7.2	1.4
	cen	2					
	q12	1					
	q21	1					
	q31	1					
2	p25	1					
	p23	1					
	p22	1					
	p11	1					
	cen	1	9	2	11	6.8	1.6*
	q11	2					
	q21	2					
	q22	1					
	q35	1					
3	p21	4					
	p11	1					
	cen	1					
	q23	1	5	3	8	5.8	1.4
	q26	1					
4	p14	1					
	q12	3					
	q21	1	6	2	8	5.3	1.5*
	q28	2					
	q31.3	1					
5	p14	1					
	q31	1	2	1	3	5.2	0.57
	q21	1					
6	q13	2					
	q16	2	6	0	6	5.0	1.2
	q21	1					
	q23	1					
7	p14	1					
	q11	4					
	q21	1					
	q22	1	8	2	10	4.5	2.2*
	q32	3					
8	p11	2					
	q13	1	4	0	4	4.2	0.95
	q21	1					
9	cen	1					
	q12	1					
	q32	1	4	0	4	4.0	1
	q34	1					
10	q22	2	2	0	2	3.9	0.51
11	q12	2	2	0	2	3.9	0.51
12	q13	2	2	0	2	3.9	0.51
13	q14	1					
	q22	1	2	0	2	3.2	0.62
14	q21	1					
	q24	2	4	0	4	3.02	1.32
	q31	1					
15	q22	1	1	0	1	2.9	0.34
16	q22	1	1	0	1	2.8	0.35
17	q21	1	1	0	1	2.7	0.37
18	q21	1	0	1	1	2.5	0.4
19	q19	1	1	0	1	2.3	0.43
20	q12	1	1	0	1	2.3	0.43
21	0	0	0	0	0	1.6	0
22	0	0	0	0	0	1.7	0
X	q24	1	1	0	1	1.82	0.55
Y	q12	0	1	0	1	4.3	0.23

[a]Observed number of the breakpoints in each chromosome was compared with expected number using Student's *t*-test.

*Results are significant at $p < 0.001$.

points was too small to find significant statistical differences in the band distribution of these breakpoints.

Van der Berghe et al. (*18*) described the 5q anomaly observed in the lymphocytes of workers exposed to organic solvents. Mitelmann et al. (*19*) indicated the association between the exposure to solvents and specific chromosomal aberrations in neoplastic cells in acute nonlymphocytic leukemias (−5, −5q, and −7q). In the present study the observed frequency of breakpoints in chromosome 5 was less than expected, and in chromosome 7 it was more than twice as high as expected, based on relative chromosome length (Table 1). Nine out of 10 breakpoints were located on the short arm of chromosome 7. However, there was no evidence of any clonal origin of the aberration in each subject. The frequency of chromosome aberrations observed in the controls (0.9%) was similar to that described in literature (*15–17*). Apparent random distribution of the breakpoints among the chromosomes in this group may result from the small number of observed aberrations (18 only).

It is difficult to establish the localization of hot spots in the karyotypes of workers occupationally exposed to mutagenic agents for two main reasons: *a*) Workers in the "real world" are exposed to mixtures rather than to single agents and *b*) the number of chromosome aberrations observed in the groups of exposed workers is not high. To obtain a sufficient number of aberrations for the studies on band localization, the analysis should be done on extremely large groups of workers. However, it is often not possible because only relatively small numbers of employees are exposed to any given examined agent.

Conclusion

In this study, an increase in the number of chromosomal aberrations was observed in the lymphocytes of workers occupationally exposed to benzene. The distribution of the breakpoints in the analyzed karyotypes was nonrandom, with the breakpoints accumulating mainly on chromosomes 2, 4, and 7.

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