Cytogenetic Findings on Shoe Workers Exposed Long-term to Benzene

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Cytogenetic analysis of peripheral blood lymphocytes was performed to detect cytogenetical alterations in 58 shoe workers (57 male and 1 female) who had been exposed to particular mutagenic or carcinogenic agents and in 20 subjects selected from the general population as a control group. Frequencies of damaged cells, including gaps, breaks, and rearrangements (acentric fragment, deletion, translocations) were scored for both groups. The incidence of chromosomal aberrations (particularly chromatid gaps and breaks) in the study group was significantly higher than in the control group. No effects of smoking were observed and breaks alone were found to be influenced by alcohol consumption. No significant correlation was detected between the working period in the group exposed to benzene and frequency of chromosomal aberrations. Benzene content was determined to be between 0 and 28.5% in eight kinds of glues studied by fractional distillation. Hexane content ranged between 0 and 68.35% using the same method. This study indicated that the content of benzene and hexane in the glues are above normal limits. — Environ Health Perspect 104(Suppl 6):1313–1317 (1996)

Key words: shoe workers, lymphocyte cultures, chromosome aberrations, adhesive, benzene, hexane

Introduction

Benzene is an important substance widely used in industry. Most of the population occupationally exposed to these agents are exposed for a long period. Chronic benzene intoxication modulates immune responses and granulocyte enzyme systems, and leads to thrombocytopenia, leukopenia, and anemia (pancytopenia in some cases). It may trigger the formation of neoplastic diseases in some extreme cases (1–5). The frequency of neoplasia in workers occupationally exposed to benzene and its derivatives is significantly higher than in the unexposed population (6–8).

Benzene is a genotoxic agent that may affect metabolic activity (9). The metabolites that may form as a result of its metabolic activities show clastogenic, mutagenic,

and carcinogenic properties more than benzene itself (10–17). For instance, it is known that benzoquinone is one of the metabolites of benzene and that it most commonly causes DNA breaks, chromosomal damage, and sister chromatid exchanges (SCEs) (9). Some investigators indicated that numerical or structural chromosomal aberrations were increased in workers who had a long-term exposure to benzene (18–20). Moreover, they found that the chromosomal aberration ratios were increased in leukemia that occurred after exposure to benzene. Some other investigators, however, were not able to show any significant differences between the exposed and control groups (21,22). They also reported that benzene caused no health problems in exposed workers according to biochemical and hematological tests (22). In this study, we evaluated chromosomal aberrations in shoe workers who had been occupationally exposed to benzene and its derivatives.

Methods

We conducted our studies on peripheral blood lymphocytes taken from 58 shoe workers in the vicinity of Bursa, Turkey; they had been occupationally exposed to benzene for long periods of time ranging

from 5 to 50 years. Twenty healthy subjects who had not been exposed to benzene or any related physical or chemical agents, and who were living in or near Bursa, were used as a control group. Both groups were interviewed about infectious diseases, drugs, and exposure to X-rays during the 2 to 3 months before cytogenetic examination. Of those interviewed, 68% of the shoe workers and 39% of the control group were smokers; 39% of shoe workers and 5% of the control group were alcohol consumers. The study was conducted between May 1991 and July 1992. Blood samples were taken and cultured at 37°C for 72 hr in medium containing 10% TC medium 199, 20% fetal calf serum, 10 µg/ml streptomycin, 60 µg/ml penicillin, and 70% sterilized distilled water. Colchicine was added to the cultures 2 hr before the harvest. Four chromosomal preparations were made from each subject using a method described previously (23). Approximately 20 metaphases were analyzed for numerical and structural aberrations under 1000× magnification with immersion oil. A total of 435 metaphases for the control group and 1079 metaphases for the exposed group were found suitable for examination. The amounts of solvents in the glues were determined by fractional distillation; their values are presented in the results section.

Statistical analysis of chromosomal findings was performed using the Duncan test. The correlations between chromosomal findings and the working period, duration of alcohol consumption of the shoe workers, and data on smoking habits of both groups were analyzed using regression analysis and Student's t-test.

Results

The results of cytogenetic aberrations in the nonexposed group and the shoe workers are given in Tables 1 and 2. Statistical analysis (including and excluding gaps) indicated that differences in the frequencies of chromosomal damages between the exposed and nonexposed groups were significant (Table 3, Figure 1). The mean frequency of cells with total chromosomal damage was 2.6% in the control group and 22.2% in the shoe workers. Frequent gaps and breaks (particularly chromatid gaps and breaks) were determined among total chromosomal aberrations (15 and 4.3%). Gaps were the most common high abnormalities. Rearrangements such as acentric fragments, deletions, and translocations

Table 1. Percentage of cells with chromosomal damage in peripheral blood lymphocyte cultures from control subjects.

Subject	Age, years	Alcohol intake	Daily smoking, pack	No. of metaphases analyzed	Gaps	Breaks	Rearrangements	Polyploidy	Total +Gaps	Total −Gaps
1	24	−	0	20	0.00	0.00	0.00	0.00	0.00	0.00
2	31	−	0	30	0.00	0.00	0.00	0.00	0.00	0.00
3	23	−	0	20	0.00	0.00	0.00	0.00	0.00	0.00
4	26	+	1	30	0.00	0.00	0.00	0.00	0.00	0.00
5	28	−	0	20	5	0.00	0.00	0.00	5	0.00
6	31	−	0.5	20	0.00	0.00	0.00	0.00	0.00	0.00
7	23	−	0	25	0.00	0.00	0.00	0.00	0.00	0.00
8	24	−	0	20	5	0.00	0.00	0.00	5	0.00
9	28	−	0	20	0.00	0.00	0.00	0.00	0.00	0.00
10	24	−	1	20	5	0.00	0.00	0.00	5	0.00
11	27	−	0	20	0.00	0.00	0.00	0.00	0.00	0.00
12	29	−	0	25	8	0.00	0.00	0.00	8	0.00
13	29	−	0.5	20	0.00	0.00	0.00	0.00	0.00	0.00
14	23	−	0.1	20	10	5	0.00	5	20	10
15	36	−	0.33	20	5	0.00	0.00	0.00	5	0.00
16	36	−	0.75	25	4	0.00	0.00	0.00	4	0.00
17	32	−	0	20	5	0.00	0.00	5	10	5
18	31	−	0	20	0.00	0.00	0.00	0.00	0.00	0.00
19	35	−	0	20	0.00	0.00	0.00	0.00	0.00	0.00
20	29	−	0	20	5	0.00	0.00	0.00	5	0.00
Mean (SD)	28.5 (4.8)		0.21 (0.3)	21.8 (3.4)	2.6 (3.2)	0.25 (0.1)	0 (0.0)	0.5 (1.5)	3.4 (5)	0.75 (2.4)

were seen less frequently (1.4%). These abnormalities were observed in the control group. Polyploidy was the only numerical chromosomal abnormality in the shoe workers (1.8%). The frequency of polyploidy was 0.5% in the control group. The difference between polyploidy frequency of the two groups was not statistically significant (Table 3). The correlations between chromosomal data and working period, smoking, and alcohol consumption in shoe workers are shown in Table 4. No statistically significant differences were detected except for a correlation between alcohol consumption and chromosomal breaks in shoe workers.

The benzene and hexane contents of seven adhesives used in the workplace are given in Table 5. The benzene content was more than 1% in two of seven adhesives. Five adhesives contained no benzene. While five of seven adhesives contained hexane, two samples contained none. The content of hexane was more than 44% in two samples.

Discussion

Our study indicated that there is a significant increase in chromosomal aberrations in shoe workers who have been occupationally exposed long-term to benzene. The most commonly detected abnormalities were gaps and breaks, which were generally chromatid type. Rearrangements, such as acentric fragments, deletions, and translocations, were also increased in shoe workers. Acentric fragments were the most frequent rearrangements; deletions and translocations were very scarce. Although numerical abnormalities were increased slightly in shoe workers, the difference did not reach statistical significance.

Previous studies on workers who had been exposed to benzene reported that chromosomal aberrations were increased (2,18,21) and our study also seems to support those findings. Jablinicka et al. (22) however, reported that frequency of chromosomal aberrations was not significantly increased in workers exposed long-term to benzene compared with those in a control group. When we compared all exposed workers in terms of smoking and alcohol consumption, we were not able to detect any significant differences in total numerical and structural chromosomal abnormalities (Table 4, $p > 0.05$). We also could not find any significant correlation between the frequency of chromosomal damage and the length of working period, even though the frequency of chromosomal aberrations was higher in workers exposed to benzene than those in the control group. The majority of investigations could not define any connection between chromosomal aberration frequency, age, sex, and length of working period, even though the frequency of chromosomal aberrations was higher in workers exposed to benzene than those in the control groups (2,21,24). Yardley-Jones et al. (20),

however, reported that chromosomal exchanges and other chromosomal alterations were increased in older workers who had different types of neoplasia as a result of long-term exposure to benzene. Moreover, Saisiadek et al. (18) showed that there was an increase in chromosomal aberrations in leukemia that occurred in workers exposed to benzene. Numerous investigators have shown that there is a significant correlation between malignancies and chromosomal abnormalities (25–28). Chromosomal damage plays an important role in the activation of protooncogenes, and their interactions play an indirect part in the formation of malignancy (26). Although the metabolism and toxicity of benzene in the organisms are well known, the mechanisms of neoplastic cell transformation induced by benzene have not yet been elucidated.

Activation of the tyrosine kinase group of oncogenes may playing a key role in the neoplastic transformation of cells (5,29,30). Benzene may affect the tyrosine kinase groups of oncogenes by causing chromosomal damage, and malignancy may arise as a result. Although a correlation between the increase of chromosomal abnormalities, exposure time, and cancer formation in workers exposed long-term to benzene was expected, we were not able to detect any significant correlation. Chromosomal abnormalities were commonly observed in our study, but no symptom has been described

Table 2. Percentage of cells with chromosomal damage in peripheral blood lymphocyte cultures from benzene-exposed shoe workers.

Subject	Age, years	Period of exposure, years	Alcohol intake	Daily smoking, packet	No. of metaphases analyzed	Gaps	Breaks	Rearrangements	Polyploidy	Total +Gaps	Total −Gaps
1	26	16	−	1	18	11.11	11.11	0.00	11.11	33.33	22.22
2	37	29	−	2	17	17.64	0.00	0.00	0.00	17.64	0.00
3	44	35	−	0.5	17	29.41	0.00	0.00	5.88	35.29	5.88
4	24	12	+	1	17	17.64	0.00	0.00	0.00	17.64	0.00
5(F)	43	11	−	0	10	20	0.00	0.00	0.00	20	0.00
6	41	29	−	1	17	11.76	11.76	5.88	0.00	29.41	17.65
7	41	28	−	1	19	21.05	0.00	0.00	0.00	21.05	0.00
8	29	19	−	1	23	13.04	8.69	4.34	0.00	26.08	13.04
9	40	30	−	0	13	23.07	0.00	0.00	0.00	23.07	0.00
10	34	21	−	0	13	15.38	0.00	0.00	0.00	15.38	0.00
11	54	44	+	0	15	20	0.00	0.00	0.00	20	0.00
12	43	28	−	1	25	12	4	0.00	0.00	16	4
13	52	30	−	0	20	10	0.00	0.00	0.00	10	0.00
14	39	20	−	0	17	11.76	0.00	5.88	0.00	17.64	5.88
15	22	11	+	1	37	13.51	2.70	0.00	0.00	16.21	2.70
16	33	20	+	1	13	0.00	7.69	0.00	0.00	7.69	7.69
17	24	10	+	0.5	43	18.60	9.30	0.00	4.65	32.55	13.95
18	47	37	−	1	10	20	0.00	0.00	0.00	20	0.00
19	60	50	+	0	10	10	20	0.00	0.00	30	20
20	24	11	+	1	22	9.09	9.09	0.00	0.00	18.18	9.09
21	32	20	+	2	14	0.00	14.28	0.00	7.14	21.42	21.42
22	24	12	−	0	9	11.11	0.00	0.00	22.22	33.33	22.22
23	44	35	+	0	23	8.69	4.34	0.00	0.00	13.04	4.34
24	34	23	+	0	19	10.52	10.52	0.00	5.26	26.31	15.79
25	42	25	−	0.3	17	11.76	5.88	5.88	5.88	29.41	17.65
26	38	11	+	1	17	11.76	11.76	0.00	0.00	23.52	11.76
27	33	24	+	1	21	9.52	9.52	0.00	9.52	28.57	19.05
28	30	17	+	1.5	15	13.33	6.66	0.00	0.00	20	6.66
29	69	50	−	0	30	13.33	6.66	3.33	0.00	23.33	10
30	31	22	+	1.5	15	6.66	6.66	0.00	0.00	13.33	6.66
31	31	12	+	2	20	10	10	0.00	0.00	20	10
32	42	31	+	1.3	20	10	0.00	0.00	0.00	10	0.00
33	22	8	+	0	17	11.76	5.88	0.00	0.00	17.64	5.88
34	22	11	+	1	26	15.38	3.84	0.00	0.00	19.23	3.84
35	43	30	−	0	8	12.50	0.00	12.50	0.00	25	12.50
36	27	14	+	0.5	13	7.69	0.00	0.00	7.69	15.38	7.69
37	42	22	+	1.7	12	8.33	8.33	0.00	8.33	25	16.67
38	46	38	−	0.5	11	18.18	9.09	0.00	0.00	27.27	9.09
39	48	38	+	1.7	21	14.28	4.76	4.76	4.76	28.57	14.29
40	27	7	+	0	10	20	0.00	0.00	0.00	20	0.00
41	27	10	−	1	24	8.33	4.16	0.00	0.00	12.5	4.16
42	28	13	−	0	20	20	10	0.00	0.00	30	10
43	47	35	−	0.8	15	13.33	0.00	6.66	0.00	20	6.66
44	46	32	−	3	18	5.55	5.55	0.00	0.00	11.11	5.55
45	39	25	−	1	26	19.23	0.00	0.00	3.84	23.07	3.84
46	23	10	−	1	13	15.38	7.69	7.69	0.00	30.76	15.38
47	24	5	−	1	11	27.27	0.00	0.00	0.00	27.27	0.00
48	46	30	−	1	21	19.04	0.00	0.00	0.00	19.04	0.00
49	45	30	−	1.5	15	33.33	0.00	0.00	0.00	33.33	0.00
50	41	30	−	1	29	24.13	0.00	3.44	3.44	31.03	6.90
51	24	8	−	0	30	16.66	0.00	0.00	0.00	16.66	0.00
52	25	8	−	0	10	30	0.00	0.00	0.00	30	0.00
53	61	40	−	1	20	25	0.00	0.00	0.00	25	0.00
54	39	25	−	2	26	19.23	3.84	0.00	0.00	23.07	3.84
55	24	10	−	1	21	23.80	0.00	0.00	0.00	23.80	0.00
56	45	30	−	0.8	30	13.33	0.00	3.33	0.00	16.66	3.33
57	50	35	+	1.5	25	20	0.00	0.00	4	24	4
58	40	30	−	0	11	9.09	9.09	0.00	0.00	18.18	9.09
Mean	37.2 (10.9)	23.2 (11.4)		0.8 (0.7)	18.6 (7.1)	15 (6.8)	4.2 (4.8)	1.39 (3.3)	1.8 (3.9)	22.1 (6.7)	7.08 (6.84)

Table 3. Comparison of chromosomal data of shoe workers and controls.

Parameters	Means		Ft, a=0.05	Fh	p-Value
	Shoe workers, n=58	Controls, n=20			
Break	4.187	0.25	3.13	12.04	**<0.05
Gap	15.044	2.6	3.13	61.51	<0.05
Rearrangements	1.399	0	3.13	3.69	<0.05
Polyploidy	1.788	0.5	3.13	2.016	NS
Total:					
+Gaps	22.121	3.35	3.13	130.4	<0.05
−Gaps	7.075	0.75	3.13	16.255	<0.05

NS, not significant.

Table 4. Comparison of chromosomal findings with working period and alcohol intake of shoe workers and smoking habits of both groups by regression analysis and Student's t-test.
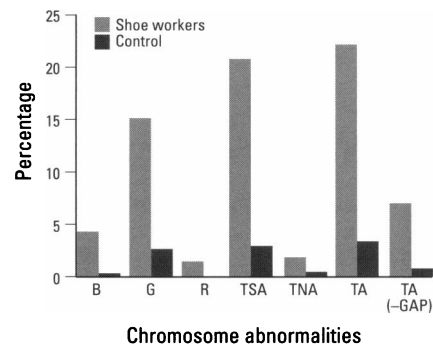
Parameters		Correlation coefficient, r	th	p-Value
I	II			
Working period	Break	0.006	0.006	NS
	Gap	0	0	NS
	Rearrangement	0.0389	0.0943	NS
	Polyploidy	0	0	NS
	Total	0	0	NS
Alcohol intake	Break	0.263	2.04	**<0.05
	Gap	−0.405	−3.33	NS
	Rearrangement	−0.1107	−0.834	NS
	Polyploidy	0.0638	0.478	NS
	Total	0	0	NS
Smoking	Break	0.149	1.1298	NS
	Gap	−0.168	−1.281	NS
	Rearrangement	−0.067	−0.502	NS
	Polyploidy	−0	−0.185	NS
	Total	0	0	NS

NS, not significant.

Figure 1. Chromosomal findings between shoe workers and control group. B, break; G, gap; R, rearrangements; TSA, total structural abnormalities; TNA, total numeric abnormalities; TA, total abnormalities.

Table 5. The content of solvents in the glues.

Samples	Percent of solvents		
	Benzene	Hexane	Others
1[a]	−	68.35	−
2	−	26.02	−
3	−	23.68	−
4[a]	28.5	16.48	−
5	−	−	26.76
6[a]	−	45	−
7[a]	20	−	30.67

[a]The amount of benzene and hexane are very high in these samples.

in the interviews related to malignancy. Instead, symptoms tended to be related to nonmalignant stomach, liver, eye, and respiratory complaints. This may suggest that the development of cancer is a multi-cause and multistep process and that the increase in chromosomal abnormalities, and probably activation of tyrosine kinase, may not be sufficient in this development. Furthermore, the formation of cancer is also closely related to genetic constitution such as deletion of DNA repair and tumor suppressor genes, and to excretion mechanisms of foreign chemicals (31–33).

We thought that the reason for the high incidence of chromosomal abnormalities in our study might be a result of high concentrations of benzene and hexane in the working environment. In Turkey, Aksoy pointed out that the maximum benzene value was between 210 to 610 ppm during working hours in workplaces in Istanbul, although the acceptable maximum allowable concentration (MAC) in the working environment is 20 ppm (34). This may be the result of a high content of benzene (more than 1%) and of hexane (more than 44%) in adhesives. Limited space in workplaces and insufficient ventilation could be other negative factors. In Turkey new regulations have been planned to limit the amount of hexane in the workplace to 55 ppm and to decrease the MAC from 20 to 1 ppm.

In conclusion, our investigation indicated that chromosomal aberrations were increased in workers exposed long-term to benzene. In Turkey, by the initiatives of Aksoy (11,14,15,34), the content of benzene in the solvents was decreased step by step. However, the present study has shown that percentages of benzene and hexane in some adhesives are still above permissible levels. Therefore, we recommend that the contents of benzene and hexane in adhesives be decreased more than 1 and 44%, respectively. MAC should be decreased from 20 ppm to none. Working conditions should be improved.

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