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Relationship Between Locations of Chromosome Breaks Induced by Vinyl Chloride Monomer and Lymphocytosis

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The distribution of vinyl chloride monomer (VCM)-induced chromosome breaks was studied in cultured lymphocytes of subjects occupationally exposed to this gas. In the examined subjects, the mean group value of chromosome aberrations is 6.5% and for sister chromatid exchange (SCE) frequencies, the mean value per cell is 7.9. These values are significantly higher than in the control population. Occupational exposure to VCM caused lymphocytosis together with disturbances of mitogenic activity in lymphocytes stimulated by phytohaemagglutinin. The results of G-banding showed that sites of chromosome breakpoints caused by VCM can be related to the lymphatic tissue disorders. © 1995 Wiley-Liss, Inc.

Key words: vinyl chloride monomer, localization of chromosome breaks, lymphocytosis, SCE, G-banding, occupational exposure

INTRODUCTION

Vinyl chloride monomer is a chemical used in the plastics industry. Today thousands of workers employed in this industry are exposed to its influence. Exposure to this gas causes multisystem disorders including acroosteolysis, thrombocytopenia, portal fibrosis, hepatic and pulmonary dysfunction, immune complex disorder, primarily angiosarcoma of the liver, lung cancer, brain tumors, and malignant melanoma [Creech and Johnson, 1974; Tabershaw and Gaffey, 1974; Waxweiler et al., 1976; Buffler et al., 1979; Doll, 1988; Ward, 1976] together with increased frequencies of SCE, chromosome aberrations, and micronuclei [Ducatman, 1975; Anderson, 1981; Sinues et al., 1991; Fučić et al., 1990a].

It has been suggested by several investigators that chromosome breakpoints caused by radiation and some chemical agents cluster on certain parts of chromosomes [Funes-Cravioto et al., 1974; Casperson et al., 1972; Holmberg and Jonasson,

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1973; Bauchinger and Gotz, 1979; Kano and Little, 1986]. This phenomenon could be connected with increasing evidences that some nonrandom chromosome changes are involved in certain cancers [Yunis and Soreng, 1984; Sandberg, 1991; Mitelman et al., 1978; Barrios et al., 1988]. According to our previous results [Fučić et al., 1990b], a nonrandom pattern of chromosomal abnormalities is also present in lymphocytes obtained from subjects occupationally exposed to VCM.

In the present study, chromosome aberrations in 20 subjects occupationally exposed to VCM were localized by G-banding and correlated with SCE frequencies, mitotic activity, and total blood counts.

MATERIALS AND METHODS

Twenty male workers aged 30–50 years from the plastics industry were chosen for cytogenetic examination. They had been employed in a polyvinyl chloride plant for 8 years, on average. Those with recent X-ray exposure and drug treatment were excluded from this study.

Exposure to VCM was measured continuously by gas chromatography. The VCM concentration in the working environment was 1 ppm, periodically reaching 300 ppm for a short period, due to technological process.

Lymphocyte cultures were initiated immediately after blood samples were collected and then cultured for 48 hr. Cultures of lymphocytes were stimulated by phytohemagglutinin (Wellcome). Eight ml of RPMI 1640 (Gipco) containing 20% calf serum was added to 0.5 ml samples of whole blood. Fixation of the cultures and preparation of the slides were carried out according to the conventional method. Complete metaphases stained by Giemsa were used for analysis. Two hundred cells per person were scored [IAEA, 1986].

Sister–chromatid exchange frequencies were measured on slides from cultures of lymphocytes to which 10 μg/ml bromodeoxyuridine was added. The cultures were harvested after 72 hr [Parry, et al., 1985].

Mitotic activity was measured on slides prepared for SCE. The first, second and third metaphase were differentiated by the level of incorporated bromodeoxyuridine. Five hundred cells per person were counted [Lamberti et al., 1983].

G-banding was performed by treatment with trypsin and staining with Giemsa. The chromosomes were identified and classified according to the ISHCN [1985]. A total of 1,200 metaphases were analyzed.

The total blood counts were analyzed automatically on Coulter Electronica 186. The presence of lymphocytosis was analyzed in 100 subjects from the same plastics industry as the rest of the examinees and in the same working environment. A control population of 100 healthy subjects, aged 30–50 years, was analyzed for blood count.

The control population for cytogenetic analysis consisted of 20 male subjects aged 30–50 years with the same smoking habits as the examinees and without recent exposure to ionizing radiation and drugs.

Frequencies of chromosome aberrations are compared as Poisson rates, and distributions of mitotic activity by Xs;2-test. Blood count data were analyzed using standard descriptive statistics. Since the Kolmogorov-Smirnov test does not show statistically significant deviation from log-normal distribution, the difference between control and experimental group was tested using the one-tailed t-test on logarithms of

TABLE I. Comparison of Frequencies of Chromosome Aberration, Sister-Chromatid Exchange Frequencies, and Mitotic Activity Between VCM Exposed and Control Subjects

		Chromosome aberration (%)						SCE		Mitotic activity (%)		
Subject	Employment (yr)	Acentric frag	Chromatic breaks	Chromosome breaks	Dicentric chromosome	Ring	tot	Per cell	Range	M1	M2	M3
1	9	0.5	5	0.5			6*	5 8	4–9	14.1	23 5	62.2
2	5	1	6	2			9*	7.4	4–13	6.8	12.5	80.2
3	4		4				4	6.7	4–10	7 2	14 4	78.3
4	4	1	1		1		3	4.4	4–6	10 5	27.8	61 5
5	3		5	1.5			6.5*	5.3	4–8	11 8	25 3	52 4
6	10		6	1			7*	6.4	4–11	15 9	21 5	52.5
7	4		6.5	1		0 5	9*	8	4–15	7.3	25.2	67 4
8	11		6				6*	8.7	4–16	6.8	39.7	53 4
9	9		2				2	5.7	4–12	13.6	40 9	45 4
10	10		7	1 5			8.5*	7.7	4–15	9 1	25 5	65
11	14	2	2		2		6*	9 5	5–17	9 8	25 3	64 8
12	12	2	4 5	1.5			8*	9.4	4–18	8.9	23 2	67.8
13	2	1	6.5	0.5			8*	8.5	4–21	10.8	16.3	72 8
14	14	0.5	6.5	0.5	0.5		8*	8.6	4–22	9 8	25.3	64 8
15	9	1	3	2			6*	9	4–21	10.5	16.9	72.5
16	10		7.5	0 5			8*	9.9	4–18	7.1	17.6	75 2
17	9	0.5	6	1			7.5*	9.6	4–14	7 2	23.7	69
18	9	1	6				7*	9.1	4–16	9 4	26.4	69
19	5		7	0.5			7 5*	8.5	4–18	9.1	34.7	63
20	8	0.5	2	0 5			3	9.3	4–21	7.6	20.3	72
Mean group values for the 20 controls		0.7	1 1	0 72			2.6	5.9	4–7	7.7	34.9	53 6

*$p < 0.01$; M1, M2, and M3, $p < 0.001$.

observed values. Statistical analysis were performed on a PC 386 computer with the STATGRAPHICS statistical package.

RESULTS

The investigated exposed subjects had a minimum of 2 years of steady daily exposure to VCM, and their histories contained no other known exposure significantly different from that of the control group.

The examined workers have higher values than the control population in both the chromosome aberration assay and SCE (Table I). The quantitatively dominant type of aberration is chromatid break, but dicentric and ring chromosomes are also present. Sister–chromatid exchange frequencies are also increased comparing to the control values. The individual range is 4–22 per cell in the examined group and 4–7 per cell in the control group.

The distribution of the values of mitotic activity in the examined population differs significantly from that in the control population (Table II). The values for the second mitosis (M2) are significantly lower than expected, while the number for the third mitosis (M3) is significantly elevated.

The G-banding technique shows that bands which correspond with chromosome breaks are located on chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14 (Fig.

TABLE II. Band Localization of Chromosome Aberrations Caused by VCM and Related Diseases

Band	Type of aberration	Related disease
1p34–32	Chromatid gap	T-ALL
1p11	Chromatid gap	MDS, AML, MPD
	Chromatid break	CHL, ML
1q21	Chromatid gap	ML, AML, ALL
	Chromatid break	
	Chromosome gap	
1q23	Deletion	ALL
1q25	Chromatid gap	MPD
1q32	Chromatid gap	ML
	Chromatid break	
2p21	Chromatid gap	random
2p23	Chromatid gap	PML, MH, T-ML
2q31	Translocation t(2;12) (q31;p12)	random
3p21	Chromatid gap	ML
3q21	Chromatid gap	AML, MDS, MPD
5q31	Chromatid gap	AML, ALL
	Chromatid break	
6q21 (14–27)	chromatid gap	ML, ALL, ATL, HCL, PLL
7p11–q11	Translocation t(7;14) (p13;p11)	ALL, ML
7p13	Deletion	ALL, AML, MPD
8q22	Chromatid gap	MPD, ML, AML-M2
9p21–22	Deletion	
9q11–q11	Chromatid gap	ALL
	Chromosome break	
9q34	Chromosome break	AML, MPD, CHL
		ALL, ML
10q11	Chromatid break	random
11q23	Chromosome break	ALL, AML, MDS
		AML-M5, AML-M4m5, ML
12p12	translation t(2;12) (q31;p12)	
13q12	Chromatid gap	MPD, MDS
14q11	Translation t(7;14) (p13–q11)	ATL, T-CRLL, T-PLL
14q24	Chromatid gap	B-CLL, ML

ALL, acute lymphoblastic leukemia (L1-L3 in FAB classification); AML, acute lyeloid leukemia (subclassified FAB M1–M7); ATL, adult T-cell leukemia/lymphoma; CLL, chronic lymphocytic leukemia; CML, chronic myeloid leukemia; HCL, hairy cell leukemia; MDS, myelodisplastic syndrome; MH, malignant histiocytosis; ML, malignant lymphoma, subclassification as B- or T-cell type; MPD, myeloproliferative disorder; PLL, prolymphocytic leukemia.

1). Banded chromosome analysis reveals that the breakpoints very often coincide with some specific cancer breakpoints, such as acute myeloid leukemia, acute lymphoblastic leukemia, and myeloproliferative disorders [Mitelman et al., 1991].

Blood test analysis show statistically significant increase in the percentage of lymphocytes in the VCM exposed examinees comparing to control. The individual range is 28–67, and the mean group value is 41.3. By contrast, the control subjects ranged from 22 to 42, with a mean group value of 30.2.

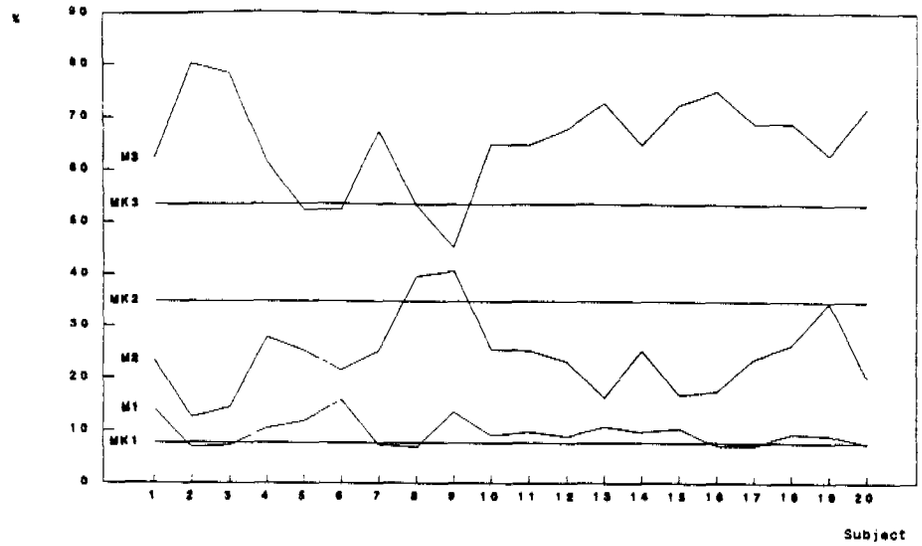

Fig. 1. Percentage of cells in the first (M1), second (M2) and third (M3) division in exposed subjects and in the control group (MK1, MK2, MK3).

DISCUSSION

After metabolic activation, VCM, a chemical used in the plastics industry, is a strong carcinogen and mutagenic agent in mammals. However, the study of the consequences of occupational exposure to VCM has been largely restricted to mortality and epidemiological studies of malignancies or severe health disorders. Too little attention has been paid to health disorders caused by exposure to low concentrations of this gas.

The metabolic products of VCM, chloroacetaldehyde and chloroethylenoxide, are known to be alkylating agents which lead to the depurination of DNA molecule [Barbin and Bartsch, 1989] and probably to an unbalanced nucleoid pool in the cell.

Another phenomenon caused by alkylation is the neighboring base effect, which is the result of specific, nucleotide sequences in the regulation of gene expression. There are adenine and thymidine-rich sequences which affect the initiation and termination of transcriptions that are dependent upon their base sequence. Modification of these or other similar sequences by alkylation could have profound effects on the cell [Briscoe and Cotter, 1985].

It is now well established that human tumors, particularly leukemias and lymphomas, are characterized by nonrandom chromosomal abnormalities such as translocations and deletions. Nonrandom chromosomal translocations are among those that define regions of the genome of oncogenic potential. At the same time, nonrandom chromosomal deletions reflect the loss of genetic information at a specific site in the genome and suggest the presence of tumor-suppressor genes.

Some neoplasias originate from exposure to various mutagenic agents from the environment. The specifically vulnerable chromosomal regions for some mutagens have already been described [Morad et al., 1973; Barrios et al., 1988; Mitelman et

al., 1978]. Additionally, elevated SCE frequencies in subjects exposed to chemical mutagens exemplify the danger of the carcinogenic action of the mutagenic agent, since sites of origin of SCE frequencies coincide with fragile sites [Glover and Stein, 1987], which can result in unequal chromosomal recombinations and rearrangements [Latt et al., 1986].

The biomonitoring results in our study confirm previous findings about the mutagenic activity of VCM. Breaks on chromosomes caused by VCM cluster on specific cancer breakpoints [Fučić et al., 1990b], which may be a predisposing factor to the induction of chromosomal rearrangements.

The breakpoints described in our study are often related to malignancies of B lymphocytes, which correlates with the findings of Ward et al. [1976], who described an increase in the circulating B lymphocytes in VCM-exposed subjects. An increased sensitivity of B lymphocytes to mutagens was also found in a population of gasoline pump mechanics [Hogstedt et al., 1991].

Blood test analyses confirm that a population occupationally exposed to VCM exhibit disorders of the lymphopoietic system; of the 100 subjects in this study, 75% exhibited an increase in the percentage of lymphocytes together with disturbances of mitotic activity. It is important to emphasize that none of the subjects suffered from any chronic disease or expressed symptoms typical of VCM disease. Thus, lymphocytosis might be hidden for a long period of latency without visible health disorders.

The epidemiologic study of Smulevich et al. [1988] revealed an elevated number of subjects suffering from leukemias and lymphomas after occupational exposure to VCM. An increase in lymphomas and leukemias is especially present among female workers, who are usually exposed to lower concentrations of VCM. This result is partly proved in experiments [Kurliandsky et al., 1981; Smulevich et al., 1988].

In conclusion, continuous biomonitoring of workers in the plastics industry is of extreme importance since the results indicate that lymphomas and leukemias can be expected even with the use of modern VCM technologies in which the concentration of VCM in the working environment is 1 ppm.

REFERENCES

Anderson D, Richardson CR, Purchase IFH, Evans HJ, Oriordan ML (1981): Chromosomal analysis in vinyl chloride exposed workers: Comparison of the standard technique with the sister chromatid exchange technique. Mutat Res 83:137–144.

Barbin A, Bartsch H (1989): Nucleophilic selectivity as a determinant of carcinogenic potency (TD50) in rodents a comparison of mono and bifunctional alkylating agents and vinyl chloride metabolites. Mutat Res 215:95–106.

Barrios L, Miro R, Caballin MR, Fuster C, Guedea F, Subias A, Egozcue J (1988): Cytogenetic effects of radiotherapy; breakpoint distribution in induced chromosome aberrations. Cancer Genet Cytogenet 70:41–46.

Bauchinger M, Gotz G (1979): Distribution of radiation induced lesions in human chromosomes and dose-effects relation analyzed with G-banding. Radiat Environ Biphys 16:335–366.

Briscoe W, Cotter L-E (1985): DNA sequence has as effect on the extent and kind of alkylation of DNA by a potent carcinogen Chem Biol Interact 56:321–331.

Buffler PA, Wood S, Eifler C, Suarez L, Kilian DJ (1979): Mortality experience of workers in vinyl-chloride monomer production plant. J Occup Med 21.191–202.

Casperson T, Haglund V, Lindell B, Cech L (1972): Radiation induced non-random chromosome breakage. Exp Cell Res 75:541–543.

Creech JL, Johnson MN (1974): Angiosarcoma of liver in the manufacture of polyvinyl workers. J Occup Med 16:150–151.

Doll R (1988): Effects of exposure to vinyl chloride. Scand J Work Environ Health 14:461–478.

Ducatman A, Hirschhorn K, Selikoff IJ (1975): Vinylchloride exposure and human chromosome aberrations. Mutat Res 31:163–168.

Fučić A, Horvat D, Dimitrović B (1990a): Mutagenicity of vinyl chloride in man: Comparison of chromosome aberrations with micronucleus and sister–chromatid exchange frequencies. Mutat Res 242:265–270.

Fučić A, Horvat D, Dimitrović B (1990b): Localization of breaks induced by vinyl chloride in the human chromosomes of lymphocytes. Mutat Res 243:95–99.

Funes-Cravioto F, Yakovienko KN, Kuleshov NP, Zhukov VS (1974): Localization of chemically induced breaks in chromosomes of human lymphocytes. Mutat Res 23:87–105.

Glover TW, Stein CK (1987): Induction of sister chromatid exchanges at common fragile sites. Am J Hum Genet 41:882–890.

Hogstedt B, Holmen A, Karlsson A., Raihce G, Nilluis K, Vestlund K (1991): Gasoline pump mechanics and increased frequencies and sites of micronuclei in lymphocytes stimulated by pokeweed mitogen. Mutat Res 263:51–55.

Holmberg H, Jonasson J (1973): Preferential location of x-ray induced chromosome breakage in the R-bands of human chromosomes. Hereditas 74:57–68.

International Atomic Energy Agency (1968): Biological dosimetry. Chromosome aberration analysis for dose assessment. Technical report series No 260. Vienna: IAEA, pp 59–63.

International System for Human Cytogenetic Nomenclature (1985): Handen DG, Kungen HP (eds). Basel, S Karger.

Kano Y, Little JB (1986): Site-specific chromosomal rearrangement induced in human diploid cells by x-radiation. Cytogenet Cell Genet 41:22–29.

Kurliandsky BA, Stoubur NN, Turusov VS (1981): Towards the question of hygienic reclamentation of vinyl chloride. Gigiena Sanit 3:74–86.

Lamberti L, Ponzetto PB, Ardito G (1983): Cell kinetics and sister chromatid exchange frequency in human lymphocytes. Mutat Res 120:193–199.

Latt SA, Shiloh Y, Sacai K, Brodeur G, Donlon T, Korf B, Shipley J, Bruns G, Heartlean M, Kanda N, Kohl N, Alt F, Seeger R (1986): Novel DNA rearrangement phenomena associated with DNA amplification in human neuroblastomas and neuroblastoma cell lines. In "Genetic Toxicology of Environmental Chemicals Data: Basic Principles and Mechanisms of Action." New York: Alan R Liss, pp 691–612.

Mitelman F, Brandt L, Nilsson PG (1978): Relation among occupational exposure to potential mutagenic/carcinogenic agents, clinical findings, and bone marrow chromosomes in acute non-lymphocytic leukemia. Blood 52:1229–1237.

Mitelman F, Kaneko Y, Trent J (1991): Report of the committee on chromosome changes in neoplasia. Cytogenet Cell Genet 58:1053–1079.

Morad M, Jonasson J, Lindsten J (1973): Distribution of mitomycin C induced breaks on human chromosomes. Hereditas 74:232–282.

Parry EM, Sharp DC, Parry JM (1985): The observation of mitotic division aberration in mammalian cells exposed to chemical and radiation treatments. Mutat Res 150:169–181.

Sandberg AA (1991): Chromosome abnormalities in human cancer and leukemia. Mutat Res 247:231–240.

Sinues B, Sanz A, Bernal ML, Tres A, Alcala A, Lanuza J, Caballos C, Saenz MA (1991): Sister chromatid exchanges, proliferative rate index and micronuclei in biomonitoring of internal exposure to vinyl chloride monomer in plastic industry workers. Toxicol Appl Pharmacol 108:37–45.

Smulevich UB, Fedotova IV, Filatova VS (1988): Increasing evidence of the rise of cancer in workers exposed to vinylchloride. Br J Ind Med 45:93–97.

Tabershaw IR, Gaffey WR (1974): Mortality study of workers in the manufacture of vinyl chloride and its polymers. J Occup Med 16:509–518.

Ward AM, Udnoon S, Watkins J, Walker AE, Darke CS (1976): Immunological mechanisms in the pathogenesis of vinyl chloride disease. BMJ 1:936–938.

Waxweiler RJ, Stringer W, Wagner JK, Jones J, Falk H, Carter C (1976): Neoplastic risk among workers exposed to vinyl chloride. Ann NY Acad Sci 211:39–42.

Yunis JJ, Soreng AL (1984): Constitutive fragile sites and cancer. Science 226:1199–1204.