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Mutagenicity of vinyl chloride in man: comparison of chromosome aberrations with micronucleus and sister-chromatid exchange frequencies

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Summary

The mutagenic effects of vinyl chloride monomer in man were studied in the lymphocyte culture with 3 methods: the chromosome aberration assay, the micronucleus assay and the sister-chromatid exchange method. Compared with control, values obtained by these tests are increased in workers occupationally exposed to vinyl chloride. In relation to non-smokers, smokers exposed to vinyl chloride show significant increases in sister-chromatid exchange frequencies. The problem of correlating the results of the chromosome aberration assay with micronucleus and sister-chromatid exchange frequencies is discussed.

Vinyl chloride monomer (VCM) is a well-known carcinogenic and mutagenic substance (Anderson et al., 1980, 1981; Purchase et al., 1976, 1978; Hansteen et al., 1978; Green and Hathway, 1978; Maltoni and Lefemine, 1975; Ducatman et al., 1975; Funes-Cravioto et al., 1975). During the past 20 years, the accumulated knowledge about hazards for people employed in the plastic industry has made it necessary to develop a technology with closed systems which even use robots. In the most developed countries these workers now run much the same risk as the entire population of coming into contact with VCM through polluted air, food or water. The mutagenic activity of VCM is unfortunately still a matter of extreme interest, because there are a great number of factories with old technology that provide no real protection for employees.

Vinyl chloride monomer is a substance that requires activation by metabolic enzymes in the liver (Bartsch and Montesano, 1975). Its metabolites, chlorethyleneoxide and chloracetaldehyde, as alkylating agents, react with amino acids and DNA (Green and Hathway, 1978; Osterman-Golkar et al., 1977).

In order to show and compare the consequences of the action of VCM on lymphocyte chromosomes in workers exposed to VCM in our study we used three methods: the chromosome aberration assay, the micronucleus assay and the sister-chromatid exchange method. The influence of smoking on the mutagenic activity of VCM was also investigated.

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TABLE 1

COMPARISON OF FREQUENCIES OF CHROMOSOME ABERRATIONS, MICRONUCLEI AND SISTER-CHROMATID EXCHANGES BETWEEN EXPOSED AND CONTROL SUBJECTS

Subject	Employment (years)	Cigarettes/day	Micronuclei (%)						Chromosome aberrations (%)					SCE	
			Total	0	1	2	3	4	Total	Chromatid breaks	Chromosome breaks	Dicentric chrom.	Acentric frag.	Per cell	Range
1	12	20	2.1	97.9	2.1	0	0	0	4	2	1	0.5	0.5	9.1	5–17
2	10	0	3.6	96.4	3.6	0	0	0	5.5	2.5	1.5	0.5	1	9	4–14
3	15	30	3.7	96.3	2.8	0.9	0	0	9	6	1.5	0	1.5	11	5–23
4	15	0	4.9	95.1	4.9	0	0	0	8.5	5.5	1.5	0	1.5	7.6	4–15
5	12	20	6.4	93.6	5.6	0.7	0	0	11.2	5	2	1.4	2.8	11.1	5–23
6	12	0	9.4	90.6	7.6	1	0.7	0	6.2	2.8	2.8	0	0.6	8.5	5–13
7	25	0	10.3	89.7	10.3	0	0	0	10.8	8.1	1.2	0	1.5	6.4	4–11
8	16	20	10.5	89.5	10.5	0	0	0	10	5.5	2	0	2.5	8.1	4–14
9	9	20	9.9	89.1	9.17	0.8	0	0	10.5	6	2.5	0	2	12.3	4–26
10	15	20	11.2	88.6	9	2.2	0	0	8	6	0.5	0.5	1	13.1	4–24
11	3	0	11.5	88.7	9.6	0.9	0	0	4	1.5	1	0.5	1	8.6	4–11
12	15	20	12.3	87.7	5.4	2.7	4.1	0	8.5	7	1	0.5	0	11.5	5–27
13	25	15	14.6	84.4	14.6	0	0	0	9	6	1	0.5	1.5	10	4–20
14	15	0	15	85	14	1	0	0	5.5	2	1.5	0.5	1.5	6.3	4–10
15	22	0	16.2	83.8	16.2	0	0	0	13	3.5	3	1.5	5	6.3	4–13
16	15	0	16.3	83.7	13.6	2.7	0	0	12	8	2	0.5	1.5	8.9	4–16
17	15	30	20.9	79.1	16.8	1.5	1.1	1.5	8.5	6.5	1	0.5	0.5	10.7	4–14
18	22	0	23	77	12	8	3	0	9.5	7	1.5	0.5	0.5	8	4–15
19	14	15	26.9	73.1	20.2	6.7	0	0	10.5	8.5	1.5	0	0.5	9.3	4–14
Mean group values			12.1 ±6.5	87.8 ±6.5	9.45 ±5.2	1.5 ±2.1	0.4 ±1.06	0.07 ±0.3	8.5 ±2.6	5.2 ±2.1	1.5 ±0.6	0.4 ±0.4	1.4 ±1.1	9.2 ±1.9	4–27
Mean group values for the 20 controls			6.6 ±2.3	98.4 ±2.8	5.8 ±1.7	0.7 ±0.9	0.05 ±0.2	0	1.1 ±0.3	0.7 ±0.4	0.2 ±0.2	0	0.15 ±0.2	5.9 ±1.1	0– 7

The frequencies of abnormal cells in the group exposed to VCM are significantly greater than in controls ($P < 0.001$).

Material and methods

Nineteen workers from the plastic industry were chosen for cytogenetic examination. As a control group we chose 20 male subjects from the general population, 40–50 years old. The examined workers had been employed in the polyvinyl chloride plant for 15 years on average. Those with recent X-ray exposure and drug treatment were excluded from the study. Exact measurements of exposure to VCM were performed continuously. The VCM concentration in the working environment was 50 ppm. Due to the technological process the concentration could periodically reach 2000 ppm for a short period of time.

The chromosome aberration assay was carried out on cultures of phytohemagglutinin-stimulated blood lymphocytes. Into 0.5-ml samples of whole blood 8 ml of F-10 medium (Gibco) containing 20% of calf serum was added. Lymphocytes were incubated at 37°C for 48 h. After 45 h colchicine was added. Fixation of the cultures and preparation of slides were carried out according to conventional methods (IAEA, 1986). Two hundred well-spread and complete metaphases were analyzed for every person and results are presented as percentages.

For micronucleus preparations cytochalasin B at a final concentration of 3 μg/ml was added after 44 h to the lymphocyte cultures according to the method of Fenech and Morley (1985). Micronucleus slides were made by fixation in methanol:acetic acid (3:1) without hypotonic treatment. In the micronucleus test 1000 binucleated cells per person were analyzed. Results are presented as the distribution of the percentage of cells with 1, 2, 3 and more micronuclei per cell.

Bromodeoxyuridine in a concentration of 10 μg/ml was added to the culture medium for sister-chromatid exchange preparations. The cultures were harvested at 72 h. The sister-chromatid exchanges were compared with the percentage of chromosomal breakage and the micronucleus frequency from the same blood sample. Smoking habits of the individuals were examined.

The results are statistically compared by Student's *t*-test (Pavlić, 1970).

Results

We examined 19 workers from the plastic industry and 20 control individuals from the general population. The individual and mean group results are presented in Tables 1 and 2. The values of chromosome aberrations, micronuclei and sister-chromatid exchange frequencies in workers exposed to VCM show statistically significant increases compared with the control group ($P < 0.001$). The mean group value for micronuclei is 12.2% with a range of 2.1–26.9%. With increasing numbers of micronuclei per binucleated cell the number of cells with more than one micronucleus increases.

The mean group value for chromosome aberrations is 8.5%. Chromatid breaks are the predominant type of aberration and they represent 61.2% of all breaks. Chromosome breaks, dicentric chro-

TABLE 2

VINYL CHLORIDE EXPOSURE AND SISTER-CHROMATID EXCHANGE FREQUENCIES IN SMOKING AND NON-SMOKING SUBJECTS

Subject	Cigarettes/day	Employment (years)	SCE/cell	Range
Smokers				
1	20	12	9.1	5–17
2	30	15	11	5–23
3	20	12	11.1	5–23
4	20	16	8.1	4–14
5	20	9	12.3	4–26
6	20	15	13.1	4–24
7	20	15	11.5	5–27
8	15	25	10	4–20
9	30	15	10.7	4–14
10	15	15	9.3	4–14
Mean for smokers			10.6 ± 1.4	4–27
Non-smokers				
1	0	10	9	4–14
2	0	1.5	7.6	4–15
3	0	12	8.5	5–13
4	0	25	6.4	4–11
5	0	3	8.6	4–14
6	0	15	6.3	4–10
7	0	22	6.3	4–13
8	0	15	8.9	4–16
9	0	22	8	4–15
Mean for non-smokers			7.8 ± 1.07	4–16

mosomes and acentric fragments are also present. The results of the micronucleus and chromosome aberration assays are comparable. Increasing numbers of micronuclei per binucleated cell and increased numbers of cells with more than one micronucleus are followed by a higher percentage of chromosome aberrations or more severe chromosomal damages.

Sister-chromatid exchange frequencies are also increased, with a mean group value of 9.2 per cell. The individual range of sister-chromatid exchange frequencies is 4–27 per cell. Table 2 shows the influence of smoking on the results of the sister-chromatid exchange assay compared with non-smoking subjects.

Discussion

During the last few decades it has been shown that VCM, as a mutagenic and carcinogenic substance, causes liver damages, acro-osteolysis, brain, lung and skin cancer in workers chronically exposed to this gas (Bartsch and Montesano, 1975; Smulevich et al., 1988).

There is a strong supposition that the modification of the DNA structure by VCM and the observed mutagenic properties are due to a process of depurination (Green and Hathway, 1978). The gap so produced might be filled by various bases resulting in 'mispairing' during DNA replication and there is a high accordance between the severe damaging effect of DNA depurination and mutagenicity (Roberts, 1975; Loveless, 1966; Hathway, 1977).

In our study we examined 19 workers from a polyvinyl plant with an average duration of employment of 15 years. We used a battery of 3 tests (chromosome aberration, micronucleus and sister-chromatid exchange assay) to analyze the consequences of the action of this dangerous gas on lymphocyte chromosomes.

The subjects in our study showed an increased percentage of chromosome aberrations, micronuclei and increased values of sister-chromatid exchanges. Compared with the control all results were statistically highly significant ($P < 0.001$). These results may be due to the relatively long period of employment in this plant and the continuous exposure since it is known that the formation of the 3 major urinary metabolites of VCM in the organism involves glutathione utilization and this process seriously depletes the glutathione pool, despite the compensating mechanism. It is proposed that the fundamental role of glutathione in the body may be to protect the tissue against electrophilic attack by drug metabolites and other alkylating agents. In that way chronic exposure to a high concentration of VCM lowers the body's ability to protect itself against attack by reactive metabolites (Green and Hathway, 1977).

The results of the micronucleus assay show that the mean group value is 12% with an individual range of 2.1–26.9%. The appearance of more than one micronucleus per binucleated cell is related with the results of chromosome aberrations. If the number of cells with more than one micronucleus increases, the percentage of chromosome aberrations increases simultaneously or chromosome damages are more severe. Although the results of the micronucleus and chromosome aberration assays did correlate it was evident that the values of chromosome aberrations ranged between 3% and 13% while at the same time the values of the micronucleus assay ranged between 2.1% and 26.9%. From this observation we can suppose that micronuclei could be a result not only of lost genetic material but also of some yet unknown mechanism which affects other cell processes such as the spindle action.

The distribution of types of chromosomal aberrations shows that 61% are chromatid breaks, which is characteristic for the action of most chemical agents. At the same time, chromosome breaks, acentric fragments and dicentric chromosomes are also present in lower percentages.

As the third method we used the sister-chromatid exchange assay. The mean group value of sister-chromatid exchange frequencies per cell was 9.2. The mean individual values ranged between 6.3 and 13.1 per cell. It is important to mention that apart from objective differences in exposure depending on the working microenvironment there exist various environmental, biological and physiological influences which affect the frequencies of sister-chromatid exchanges in human peripheral lymphocytes (Tucker et al., 1988). On the other hand, the molecular mechanism of sister-chromatid exchange induction is not clear. Our current

knowledge suggests that the mechanisms of formation of sister-chromatid exchanges and aberrations are different (Wolf, 1982), and hence, sister-chromatid exchange frequencies and chromosome aberrations cannot be correlated. The conclusion from our results is that sister-chromatid exchanges and chromosome aberrations cannot be used to predict one from the other, moreover such findings are in agreement with some other authors (Galloway et al., 1986; Hansteen et al., 1978).

Comparing the results of sister-chromatid exchange frequencies and the micronucleus assay, it could be observed that values differ. This phenomenon has already been described by some authors (Madle et al., 1986; Lin-Lee et al., 1984) and it is explained by different mechanisms in the formation of genetic alterations.

The adverse health effects of smoking have been documented in hundreds of studies during the last decades. The exact mechanism by which cigarette smoking leads to the formation of cancer is still unknown. The variety of biologically active compounds inhaled during smoking obviously provides an inductive agent for both initiating and promoting steps of malignant growth. It is also interesting that VCM is present in the cigarette smoke (Antweiler, 1976). The present data show increased sister-chromatid exchange frequencies in lymphocytes of smoking subjects compared with non-smokers. The mean group and individual values of sister-chromatid exchanges are lower in non-smokers than in smokers. Duration of employment has no influence on the results.

Our data confirm the findings of previous studies that humans professionally exposed to high concentrations of VCM during several years of employment demonstrate increased values of chromosome aberrations, increased numbers of micronuclei and high frequencies of sister-chromatid exchanges. Sister-chromatid exchanges per cell are even more elevated in subjects who smoke. The positive correlation between the results of the micronucleus and the chromosome aberration assays documents that they are good indicators of exposure to chemical agents and that the results of the micronucleus assay, which is a cheaper and simpler method, can be used for screening purposes.

It is our duty to use those data to improve the working conditions of people employed in the plastic industry, since cytogenetic monitoring studies have shown that no biologically significant increases in the frequencies of chromosomal aberrations are found in workers in plants where modern technology is used compared with control populations.

References

Anderson, D., C.R. Richardson, T.M. Weight, I.F.H. Purchase and W.G.F. Adams (1980) Chromosomal analysis in vinyl chloride exposed workers, results from analysis 18 and 42 months after an initial sampling, Mutation Res., 79, 151–162.

Anderson, D., C.R. Richardson, I.F.H. Purchase, H.J. Evans and M.L. O'Riordan (1981) Chromosomal analysis in vinyl chloride exposed workers: comparison of the standard technique with the sister-chromatid exchange technique, Mutation Res., 82, 137–144.

Antweiler, H. (1976) Studies on the metabolism of vinyl chloride, Environ. Health Perspect., 17, 217.

Bartsch, H., and R. Montesano (1975) Mutagenic and carcinogenic effects of vinyl chloride, Mutation Res., 32, 93–114.

Ducatman, A., K. Hirschorn and I.J. Selikoff (1975) Vinyl chloride exposure and human chromosome aberration, Mutation Res., 31, 163–168.

Fenech, M., and A.A. Morley (1985) Solution to the kinetic problem in micronucleus assay, Cytobios, 43, 233–246.

Funes-Cravioto, F., B. Lambert, J. Lindsten, L. Ehrenberg, A.T. Natarajan and S. Osterman-Golkar (1975) Chromosome aberrations in workers exposed to vinyl chloride, Lancet, i, 459.

Galloway, S.M., P.K. Berry, W.W. Nichols, S. Wolman, K.A. Soper, P.D. Stolley and P. Archer (1986) Chromosome aberrations in individuals occupationally exposed to ethylene oxide and in a large control population, Mutation Res., 170, 55–79.

Green, T., and D.E. Hathway (1975) The biological fate in rats of vinyl chloride in relation to its oncogenicity, Chem.-Biol. Interact., 11, 545–562.

Green, T., and D.E. Hathway (1978) Interactions of vinyl chloride with rat liver DNA in vivo, Chem.-Biol. Interact., 22, 211–224.

Hansteen, I.L., L. Hillestad, L. Thiss-Evensen and S.S. Heldaas (1978) Effects of vinylchloride in man, a cytogenetic follow up study, Mutation Res., 51, 271–278.

Hathway, D.E. (1977) Comparative mammalian metabolism of vinyl chloride and vinylidene chloride in relation to oncogenic potential, Environ. Health Perspect., 21, 55–59.

IAEA (1986) Biological dosimetry. Chromosome aberration analysis for dose assessment, Technical Reports Series No. 260, International Atomic Energy Agency, Vienna, pp. 59–63.

Lin-Lee, V.W., J.A. Heddle, C.F. Arlett and B. Broughton (1984) Genetic effects of specific DNA lesions in mammalian cells, Mutation Res., 127, 139–147.

Loveless, A. (1966) Genetic and Allied Effects of Alkylating Agents, Butterworths, London.

Madle, E., A. Korte and B. Beech (1986) Species differences in mutagenicity testing. II. Sister-chromatid exchange and micronucleus induction in rats, mice and Chinese hamsters treated with cyclophosphamide, Mutagenesis, 1, 419–422.

Maltoni, C., and G. Lefemine (1975) Carcinogenicity bioassays of vinyl chloride: current results, Ann. N.Y. Acad. Sci., 246, 195–218.

Osterman-Golkar, S., D. Hultmark, D. Segerbach, C.J. Calleman, R. Göthe, L. Ehrenberg and C.A. Wachtmeister (1977) Alkylation of DNA and proteins in mice exposed to vinyl chloride, Biochem. Biophys. Res. Commun., 76, 259–266.

Pavlić, I. (1970) Statistička teorija i primjena, Tehnička Knjiga, Zagreb, pp. 233–237.

Purchase, I.F.H., C. Richardson and D. Anderson (1976) Chromosomal effects in peripheral lymphocytes, Proc. R. Soc. Med., 69, 290–291.

Purchase, I.F.H., C.R. Richardson, D. Anderson, G.M. Paddle and W.G.F. Adams (1978) Chromosomal analysis in vinyl chloride exposed workers, Mutation Res., 57, 325–334.

Roberts, J.J. (1975) Nucleic acid modifications and cancer, in: E.J. Ambrose and F.J.C. Roe (Eds.), Biology of Cancer, Halstead, Chichester.

Smulevich, V.B., I.V. Fedotova and V.S. Filatova (1988) Increasing evidence of the rise of cancer in workers exposed to vinyl chloride, Br. J. Ind. Med., 45, 93–97.

Tucker, J.D., L.K. Ashworth, G.R. Johnston, N.A. Allen and A.V. Carrano (1988) Variation in the human lymphocyte sister-chromatid exchange frequency: results of a long-term longitudinal study, Mutation Res., 204, 435–444.

Wolff, S. (1982) Chromosome aberrations, sister-chromatid exchanges and the lesions that produce them, in: S. Wolf (Ed.), Sister Chromatid Exchange, Wiley, New York, pp. 41–57.