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EFFECTS OF VINYL CHLORIDE IN MAN
A CYTOGENETIC FOLLOW-UP STUDY

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Summary

Cytogenetic studies were performed on 39 workers from a PVC plant in 1974. 16 healthy men without any connection with the plant were chosen as controls. The cytogenetic study was repeated for 37 of the 39 workers 2—2.5 years later. During this time interval the workers had only had a minimal exposure to VCM. This repeated study was performed with 32 matched controls from the office employees in the factory.

Breaks, gaps and stable rearrangements were scored in 100 metaphases per person from 48-h lymphocyte cultures.

The mean chromosome-breakage frequency for the workers (3.41%) was significantly higher than for the controls (1.79%) in the first investigation. In the repeated study no difference was found in mean chromosome-breakage frequency between the workers and their matched controls. Neither was there any difference between these breakage frequencies and the breakage frequency for the previous control group. These results might indicate a relationship between the reduction in exposure to VCM and the normalized chromosome-breakage frequency. Sister-chromatid exchanges were studied for 16 workers with matched controls in the repeated study. A mean of 7.6 SCEs per cell was found for both workers and controls.

Bone-marrow samples from 4 workers were studied in the first investigation. The mean chromosome-breakage frequency was higher in the bone marrows (4.2%) than that reported for normal bone marrows (0.2, 0.4, 1.7%), and higher than for the corresponding lymphocyte cultures.

Abbreviations: BrdU, 5-bromodeoxyuridine; SCE, sister-chromatid exchange; VCM, vinyl chloride monomer.

Introduction

Reports on the clastogenic effect of vinyl chloride monomer (VCM) have been published for workers in PVC plants [1—3,5,13,17]. The PVC workers had been exposed to VCM for years. Yoder et al. [19] found a high chromosome-breakage frequency in agricultural workers during extensive occupational exposure to pesticides, but the chromosome-breakage frequency was back to normal after some months without exposure. We therefore repeated the cytogenetic study of the PVC workers 2—2.5 years after the first examination to see if we could find the same effect. During this time the workers had only minimal exposure to VCM.

The results for both the first and second cytogenetic study are presented in this report.

Methods

Cytogenetic studies were performed on 39 workers from a PVC plant in 1974. 14 of these were chosen at random. 13 were chosen because they had been heavily exposed to VCM for years. Moreover, chromosome analyses were performed in connection with clinical re-examination on 12 workers whose first health screening revealed abnormalities. At the re-examination all the laboratory tests proved normal.

16 healthy men, who had no connection with the PVC plant, were chosen as controls. Matching for age between the workers and the controls was attempted. but was not entirely successful.

The cytogenetic study was repeated for 37 of the 39 workers 2—2.5 years later. This repeated study was performed with 32 matched controls from the office employees at the factory. They were matched for sex, age, ethnic background and time of employment. In addition, 6 of the previous control group were re-examined.

A medical history, including viral infection, radiation exposure and drug intake, was obtained for all participants in both studies.

Exact measurements of the exposure in the PVC plant were provided from 1974. The estimated values for the previous years indicate that the exposure had been considerable (Table 1).

Immediately after the blood samples were collected, lymphocyte cultures were initiated and cultured for 48—53 h according to conventional methods in both the initial and the repeated study. Whenever possible, 100 metaphases were studied for each individual. 20 metaphases, including suspect rearrangements, were karyotyped. Another 5 were analysed under the microscope. The remaining metaphases were counted and scored for breaks and gaps. The system of Hirschorn and Cohen [6], which considers all breaking events in the cells, was applied to these data, and the results are reported as breakage frequency per 100 cells. In the repeated study only cells with suspect rearrangements were karyotyped.

Chromosome studies were done on bone-marrow samples from 4 workers in the initial study.

The sister-chromatid exchange (SCE) technique [11] was used on 16

TABLE 1

AIR CONCENTRATION OF VCM IN THE PVC PLANT

Estimates are based upon the level of production, and the number and types of autoclave used in the respecuve time periods.

Time periods		Air conc. of VCM in ppm
1950—1954		2000
1955—1959		1000
1960—1967		500
1968—1972		100
1973—1974		80
1974	Measured	25
1975—		1

workers and their matched controls in the repeated study only. BrdU (2 μg/ml) was added to the cultures 24 h after initiation. The cultures were harvested at 72 h. The 33258 Hoechst—Light—Giemsa method described by Goto et al. [4] was used for staining with a high-voltage mercury lamp (Zeiss HBO 200 W/4) as light source. The SCEs were scored in 30 cells from each individual, and the values compared with the breakage frequency found in 48-h cultures from the same blood sample.

Results

The results for the control groups in 1974 and 1977 are presented in Table 2 together with the results for the workers chosen at random (Group I), for the workers examined on clinical grounds (Group II), and for the heavily exposed workers (Group III), with their matched controls. The mean breakage frequency for all the 39 workers was 3.7% in the initial study. After thorough examination of all the subjects, different factors (as radiation and drugs) which may influence the breakage frequency were found for one worker in Group I, for one in Group II, and for two workers in Group III. These workers were omitted. The mean breakage frequency for the remaining 35 workers was then 3.41% compared with the controls with 1.79% (Table 2). The mean breakage frequency for the heavily exposed workers, 3.97% (Table 2), was slightly higher than for the rest of the workers with 3.15% (Table 2).

The results were analysed statistically by the Wilcoxon two-sample ranking test [7]. All tests for workers versus controls (Table 3) showed that workers scored significantly higher on a 2.5% significance level in the initial study.

In the repeated cytogenetic study 2.5 years later, no significant difference was found between chromosome breakage frequency for the workers and their matched controls (Table 4). The chromosome-breakage frequencies for each of the three groups of workers were back to normal. No significant difference was found between the control group in 1974 and the matched controls either (Table 4).

A closer study of the individual values revealed that the worker omitted from Group I had a high breakage frequency at both examinations. He was found to have carcinoma labii. The rest of the randomly chosen workers with

TABLE 2

RESULTS OF THE INITIAL (1974) AND REPEATED (1977) CYTOGENETIC STUDY OF THE PVC WORKERS AND THE TWO CONTROL GROUPS

Groups	Age 1974	Employment in years 1974	Breakage frequency per 100 cells		
			1974	1977	
				Workers	Matched controls
	$\bar{X}$ (range)	$\bar{X}$ (range)	$\bar{X}$ (SD)	$\bar{X}$ (SD)	$\bar{X}$ (SD)
Controls	42.8 (27–66)		1.79 (1.87)		
Repeated control (6 workers)					2.33
Workers					
Group I	44.9 (23–66)	10.8 (1–18.5)	3.72 (2.12)	1.58 (1.11)	1.25 (1.09)
Group II	51.1 (26–67)	13.7 (4–21)	2.48 (1.25)	1.40 (1.43)	1.60 (1.28)
Group III	51.7 (39–63)	16.5 (10.5–24)	3.97 (1.61)	1.82 (1.85)	1.91 (1.16)

Group I, workers chosen at random. Group II, workers examined on clinical grounds. Group III, workers heavily exposed.

TABLE 3

STATISTICAL EVALUATION WITH WILCOXON TWO-SAMPLE RANKING TEST

The probability percentage reflects the extent to which the two samples compared are statistically identical.

Samples tested 1974 for total breakage frequency	Probability percentage
Heavily exposed workers versus controls	0.33
The rest of the workers versus controls	2.1
All workers versus controls	0.45

TABLE 4

STATISTICAL EVALUATION WITH WILCOXON TWO-SAMPLE RANKING TEST

The probability percentage reflects the extent to which the two samples compared are statistically identical.

Samples tested 1977 for total breakage frequency		Probability percentage
Workers at random versus controls	Group I	20
Workers — clinical versus controls	Group II	64
Workers heavily exposed versus controls	Group III	61
All workers versus controls		43
Controls 1977 versus controls 1974		41

TABLE 5

SCE AND TOTAL BREAKAGE FREQUENCY IN 16 PVC WORKERS AND THEIR MATCHED CONTROLS

No.	Workers			Matched controls		
	Breakage frequency 48-h culture	SCE per cell 72-h culture	(range)	Breakage frequency 48-h culture	SCE per cell 72-h culture	(range)
18	6	10.5	(2—17)	4	7.2	(2—13)
7	5	6.8	(2—14)	2	5.8	(3—10)
16	4.5	6.4	(3—17)	3	11.8	(5—21)
14	4	6.1	(2—11)	3	5.2	(2—10)
11	4	8.2	(4—16)	2	7.6	(1—15)
23	3	9.3	(3—19)	1	6.3	(3—11)
25	3	7.9	(4—22)	3	5.8	(2—9)
26	3	6.8	(2—13)	1	5.9	(1—15)
36	3	6.7	(4—13)	3	6.5	(3—11)
37	3	4.7	(2—11)	0	7.6	(3—14)
3	2.5	7.3	(2—17)	4	5.6	(0—11)
27	1.5	5.8	(1—16)	3	11.5	(5—28)
30	1	6.6	(3—12)	2	7.4	(1—18)
39	0	9.9	(4—21)	0	8.1	(2—16)
15	0	9.8	(3—21)	4	11.1	(3—24)
31	0	9.5	(4—20)	0	8.8	(3—19)
$\bar{X}$	2.6	7.6	(1—21)	2.3	7.5	(0—24)

high breakage frequencies in 1974 had lower values in 1977. None of the workers chosen on clinical grounds had higher breakage frequencies than the highest control value in either investigation. The two heavily exposed workers, who were omitted from the statistical evaluation in 1974 because of drug intake, had lower breakage frequencies in 1977 despite their using the same drugs. The rest of the heavily exposed workers also had lower breakage frequencies in 1977, except one. He died of lung cancer four months after the last blood sample was taken.

There was no difference in the mean number of SCEs per cell between the workers and their matched controls 2.5 years after exposure (Table 5), just as there was no difference in breakage frequency. By comparing the chromosome-breakage frequency and SCEs for each individual, we found a high breakage frequency with a high number of SCEs (Table 5, No. 18) and a low number of SCEs (Table 5, No. 7). We also found low breakage frequencies with both high and low numbers of SCEs (Table 5, last cases).

Bone-marrow samples from 4 workers were studied in the first investigation. A total of 288 cells was examined. The mean breakage frequency was 4.2% with range 0—7.

Congenital chromosomal abnormalities were found in 2 of the workers studied. One had 46,XY, with rcp (5; 12) (5qter → 5q34::12qter → 12q15) and rcp (15; 19) (15qter → 15q22::19) in all cells studied. The other had a normal karyotype in 2/3 of the cells studied, and a deleted Y chromosome in the remaining cells.

Discussion

The chromosome-breakage frequency for the workers in 1974 was significantly higher than for the controls. It was then concluded that VCM might be the cause of this increased frequency. As the control group was chosen from premises outside the factory, one could not completely exclude that other agents than VCM could be the cause of the increase. When the cytogenetic study was repeated 2—2.5 years later, a control group was therefore selected from the office employees at the factory.

The repeated study demonstrated that the chromosome-breakage frequency was back to normal after a significant reduction of exposure of the workers for two years, the same effect as Yoder et al. [19] found for pesticides. There was no significant difference between the chromosome-breakage frequency in the first and second control groups (Table 4). These two observations provide evidence for VCM as the real cause of the chromosome damage found in the first investigation, as also demonstrated by others [1—3,13,17].

In the first part of the study the heavily exposed workers were more significantly different from the controls than were the rest of the workers, but a dose—response relationship could not be established. Low as well as high breakage frequencies were found in heavily exposed workers. Workers with high breakage frequencies during chronic exposure may be at risk and will be followed with special attention.

At present little is known about the significance of the chromosomal aberrations incurred by workers exposed to VCM or other chemicals. In the present

work no relationship between chromosomal aberrations and VCM disease could be traced, except perhaps for one of the heavily exposed workers. This worker, however, was omitted from the statistical evaluation, as only follow-up examinations can provide the answer to the likelihood of a VCM disease. His chromosome-breakage frequency was back to normal, too, after his removal from exposure. The chromosome-breakage frequency was consistently high in two workers. Cancer was discovered in these two workers. The histological type of the cancers has not been confirmed to have any connection with VCM exposure.

A salient point in chromosome studies is whether the findings in peripheral blood can also be detected in the tissues and in the germinative apparatus. In this study, 4 bone-marrow samples were subjected to chromosome analysis. A higher total breakage frequency was found in the bone-marrow samples compared with the lymphocyte cultures from the two workers who were adequately studied: 4% versus 2% for one, and 7% versus 2.5% for the other. The quality of the bone-marrow preparations was rather poor in the two other bone marrows. The mean breakage frequency for the marrows was 4.2%. Unfortunately, control samples were not available, but reports on haematologically normal bone marrow in the literature [8,9,14] give mean frequencies of aberrations of 0.4, 1.7 and 0.2% respectively. No conclusions can be drawn from studies of the 4 bone-marrow samples. At present it can be noted, however, that the mean breakage frequency was higher in the bone marrows from the PVC workers than that reported for normal bone marrows.

Breaks and gaps were the predominant aberrations in bone marrow as well as in the lymphocyte cultures.

Many papers have recently reported increases in SCEs after acute exposure to different chemicals in cell-culture systems [12,13], in animals [16], and in man [12,18]. In the present study the same mean value was found for workers and their matched controls, which establishes a normal mean incidence of SCEs per cell of 7.6. This is in agreement with Morgan and Crossen's [10] finding of 7.9 SCEs per cell in human lymphocyte cultures.

A normal incidence of SCEs is what we would expect when the chromosome-breakage frequency is within normal variation for both workers and controls. It does not necessarily follow, however, that we would have found a higher number of SCEs in the workers in 1974 during chronic exposure, as in the present investigation we found both high and low numbers of SCEs with the highest breakage frequencies (Table 5). The question about a connection between chromosome damage scored as chromosome-breakage frequency and as number of SCEs per cell will therefore have to be investigated further before we can evaluate the use of SCE in the study of chronic exposure.

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References

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

2 Fleig, I., and A.M. Thiess, External chromosome studies undertaken on persons and animals with VC illness, Abstract of 2nd Int. Conf. Environ. Mutagens, 1977, p. 219; Mutation Res., 53 (1978) 187.
3 Funes-Cravioto, F., B. Lambert, J. Linsten, L. Ehrenberg, A.T. Natarajan and S. Osterman-Golkar, Chromosome aberrations in workers exposed to vinyl chloride, Lancet, I (1975) 459.
4 Goto, K., T. Akematsu, H. Shimazu and T. Sugiyama, Simple differential Giemsa staining of sister chromatids after treatment with photosensitive dyes and exposure to light and the mechanism of staining, Chromosoma, 53 (1975) 223—230.
5 Hansteen, I.L., L. Hillestad and E. Thiis-Evensen, Chromosome studies in workers exposed to vinyl chloride, Abstract of Vth Annual Meeting of EEMS, (1975) 63; Mutation Res., 38 (1976) 112.
6 Hirschorn, K., and M.M. Cohen, Drug-induced chromosomal aberrations, Ann. N.Y. Acad. Sci., 151 (1968) 955—987.
7 Hodges Jr., J.L., and E.L. Lehmann, Grundbegreper i sandsynlighetsregning og statistikk, 2nd edn., Nyt Nordisk Forlag, Arnold Busck, Copenhagen, 1972.
8 Knuutila, S., O. Simell, P. Lipponen and I. Saarinen, Bone-marrow chromosomes in healthy subjects, Hereditas, 82 (1976) 29—36.
9 Krogh Jensen, M., J. Eriksen and B.W. Djernes, Cytogenetic studies in myelomatosis, Scand. J. Haematol., 14 (1975) 201—209.
10 Morgan, W.F., and P.E. Crossen, The incidence of sister chromatid exchanges in cultured human lymphocytes, Mutation Res., 42 (1977) 305—312.
11 Perry, P., and S. Wolff, New Giemsa method for the differential staining of sister chromatids, Nature (London), 251 (1974) 156—158.
12 Perry, P., and H.J. Evans, Cytological detection of mutagen—carcinogen exposure by sister chromatid exchange, Nature (London), 258 (1975) 121—125.
13 Purchase, I.F.H., C.R. Richardson and D. Anderson, Chromosomal and dominant lethal effects of vinyl chloride, Lancet, II (1975) 410—411.
14 Secker Walker, L.M., The chromosomes of bone-marrow cells of hematologically normal men and women, Brit. J. Haematol., 21 (1971) 455—461.
15 Solomon, E., and M. Bobrow, Sister chromatid exchanges — a sensitive assay of agents damaging human chromosomes, Mutation Res., 30 (1975) 273—278.
16 Sterka, D.G., and S. Wolff, Sister chromatid exchange as an assay for genetic damage induced by mutagen—carcinogens, 1. In vivo test for compounds requiring metabolic activation, Mutation Res., 41 (1976) 333—342.
17 Szentesi, I., E. Hornyák, G. Ungváry, A. Czeizel, Z. Bognár and M. Timar, High rate of chromosomal aberrations in PVC workers, Mutation Res., 37 (1976) 313—316.
18 Waksvik, H., A. Brøgger and J. Stene, Psoralen/UVA treatment and chromosomes. 1. Aberrations and sister chromatid exchange in human lymphocytes in vitro and synergism with caffeine, Hum. Genet., 38 (1977) 195—207.
19 Yoder, J., M. Watson and W.W. Benson, Lymphocyte chromosome analysis of agricultural workers during extensive occupational exposure to pesticides, Mutation Res., 21 (1973) 335—340.