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Localization of breaks induced by vinyl chloride in the human chromosomes of lymphocytes

Aleksandra Fucic, Durda Horvat and Boris Dimitrovic

Institute for Medical Research and Medical Health, Mose Pijade 158, Zagreb (Yugoslavia)

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Summary

A group of 67 workers occupationally exposed to VCM was examined for the presence and distribution of breaks along the chromosomal length. Breaks induced by VCM are not randomly distributed as had been expected in a normal population. According to our results there exist highly sensitive and highly resistant locations along the chromosomes to the actions of VCM. The link between the highly sensitive segments of chromosomes, fragile sites and the activation of oncogenes is discussed.

Effects on health of vinyl chloride monomer (VCM) (Petty et al., 1930) were reported for the first time in 1930. Since then there have been numerous reports which associated exposure to VCM with lung cancer, brain tumors, cancer in the digestive system, primarily angiosarcoma of the liver and malignant melanoma (Creech and Johnson, 1974; Monson et al., 1974; Takershaw and Gaffey, 1974; Waxweiler et al., 1976; Buffler et al., 1979; Storetvedt et al., 1984). Nowadays the link between carcinogenesis and chromosome changes is a well-known and accepted fact. Vinyl chloride monomer is used in the plastics industry. Not only are the thousands of workers employed in this industry all over the world affected by being exposed to its influence, but also the entire population as a result of numberless plastic products which contain some percentage of the chemically uncoupled monomer in everyday life. In past years there have been numerous reports from many countries which demonstrated a significant excess of chromosomal aberrations among workers exposed to VCM (Ducatman et al., 1975; Funes-Cravioto et al., 1974; Anderson et al., 1981). Experiments have shown that VCM metabolites induce mutations in microbial systems and in mammalian cells (Bartsch and Montesano, 1975; Rannug et al., 1974). There are rapidly accumulating data which suggest that some non-random chromosomal changes involved in certain cancers have break-points that coincide with chromosomal fragile sites (DeBraekeleer, 1985; LeBeau and Rowley, 1984; Yunis and Soreng, 1984). According to some recent analyses (DeBraekeleer, 1985; Heim

Correspondence: Dr. Boris Dimitrovic, 'Vinilplastika', Medical Department, Lole Ribara 12, Zadar (Yugoslavia).

and Mitelman, 1987) of the distribution of chromosomal breaks, the majority of the bands which contain fragile sites are involved in structural aberrations in cancer and some of them are associated with specific chromosomal structure changes in specific types of cancer. The aim of our study was to show that chromosomal breaks in workers exposed to VCM are not randomly distributed along the chromosomal length. Such data can be used in evaluating the link between a mutagenic substance and, depending on the fragment of chromosome which is affected, the type of malignant transformations which could appear in the organism.

Material and methods

For this study 67 workers in the plastic industry were chosen for cytogenetic examination. The workers examined had been employed in the polyvinyl chloride plant for 15 years on average. Exact measurements of the exposure to VCM were performed continuously. The VCM concentration in the working environment was 5 ppm. Due to the technological process the concentration periodically reached 2000 ppm for short periods of time.

Lymphocyte cultures were initiated immediately after blood samples were collected and cultured for 48 h. 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 analyses. 2000 cells per person were scored for chromatid and bichromatid chromosomal breaks.

To estimate the number and locations of breaks each chromosome was divided into 5 (submetacentric chromosome) and 6 segments (metacentric chromosome). This scheme of division of the human metaphase chromosomes into segments was taken from the study of Funes-Cravioto et al. (1974) (Fig. 1). The breaks within A1, A2, A3, B, C and D chromosome groups were taken into account. The distribution of breaks was based on the study of 14 400 analyzed cells.

The distribution of the units of length of the metaphase chromosomes was based on the reports from the Denver Conference (1963). For statistical analyses of observed and expected frequencies the 'chi-square' criterion was used (Pavlic, 1970).

Results

In our study we analyzed the distribution of chromatid and bichromatid chromosome breaks in the population of workers employed in the polyvinyl chloride plant. During their work the workers were exposed to concentrations of VCM varying from 5 to 2000 ppm. In 14 400 complete metaphases we counted 626 breaks in chromosomes A1, A2, A3, and groups of chromosomes B, C, and D. The results of our study revealed (Table 1) that the distribution of breaks along the chromosome arms in the lymphocytes of the population exposed to VCM was not identical to the expected random distribution of breaks in the lymphocytes of an unexposed normal population. The comparison of the distribution of breaks within the groups and segments of chromosomes with the distribution expected on the basis of the relative length of metaphase chromosomes showed statistically significant values on the 1st segment of chromosome A1 ($P<0.001$), 1st ($P<0.05$), 5th and 6th ($P<0.001$) segment of chromosome A2, 1st

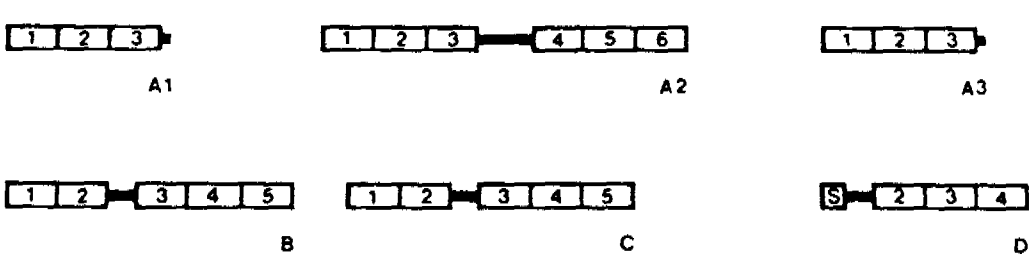

Fig. 1. Scheme of the division of the human metaphase chromosomes into segments.

TABLE I

LOCALIZATION AND FREQUENCY OF OBSERVED BREAKS ALONG THE LENGTH OF INDIVIDUAL CHROMOSOMES OR GROUPS OF CHROMOSOMES INDUCED BY VCM COMPARED TO THE EXPECTED VALUES IN A NORMAL POPULATION

Group of chromosomes	Chromosome segment	Observed values	Expected values	Probability of breakage
A1	1	56	21.9	$<<0.001$
	2	55	21.9	$<<0.001$
	3	31	21.9	0.10
A2	1	1	8.1	<0.05
	2	5	8.1	0.30
	3	6	8.1	0.80
	4	8	12.2	0.20
	5	28	12.2	$<<0.001$
	6	45	12.2	$<<0.001$
A3	1	7	17.4	<0.05
	2	17	17.4	0.90
	3	5	17.4	<0.01
B	1	3	13.2	<0.01
	2	5	13.2	<0.05
	3	32	21.7	<0.05
	4	48	21.7	$<<0.001$
	5	37	21.7	<0.01
C	1	13	45.8	$<<0.001$
	2	21	45.8	$<<0.001$
	3	42	60.9	<0.05
	4	83	60.9	<0.01
	5	37	60.9	<0.01
D	1	0	12.7	$<<0.001$
	2	13	22.2	0.10
	3	20	22.2	0.70
	4	8	22.2	<0.01
		626.0	626.0	

($P<0.05$) and 3rd segment of chromosome A3, on all segments of the chromosomes of groups B and C and on 1st and 4th segment of the chromosomes from group D.

A statistical larger number of breaks compared wiht that expected was oberved on the 1st and 2nd arm of chromosome A1, the 5th and 6th segment of chromosome A2, the 3rd, 4th and 5th segment of chromosomes in group B and the 3rd, 4th and 5th segment in the chromosomes from group C and a statistically significant smaller number of observed breaks on the 1st segment of chromosome A2, the 1st and 3rd segment of chromosome A3, the 1st and 2nd segment of chromosomes from group B, the 1st and 2nd segment of chromosomes from group C and the 1st and 4th segment of chromosomes from group D.

Discussion

The evidence for random distribution of spontaneous breaks in human lymphocytes is well

known from an extensive study by Funes-Cravioto et al. (1975) who analyzed spontaneous breaks in the lymphocytes of newborns. Newborns were chosen because they represent a population which can give us much more reliable data than a population of adults exposed to numberless chemical substances during their lifetime. The results of studies which consider the question of the distribution of breaks along the chromosomal length provoked by the influence of different chemical substances are opposed to these findings. Such studies show that the distribution of breaks is not random and that some parts of chromosomes are more or less affected (Funes-Cravioto et al., 1975; Hampel et al., 1966; Glover et al., 1984). In earlier papers such locations were called 'hot and cold spots' but nowadays they are connected with fragile sites and even non-random changes in certain cancers (DeBraekeleer, 1985; LeBeau and Rowley, 1984; Yunis, 1983).

In our study we examined a group of 67 workers occupationally exposed to VCM for the presence of chromosomal aberrations and their distribution along the chromosomal length. This investigation showed that the breaks induced by VCM were located in a non-random fashion i.e. there were groups of chromosomes with a disproportionally high frequency of breaks as well as groups of chromosomes where damage was below the expected values. The highest frequency of breaks compared to that expected in the normal unexposed population was located on the 1st and 2nd segment of chromosome A1, the 5th and 6th segment of chromosome A2, the 5th segment of chromosomes from group B and the 4th and 5th segment of chromosomes from group C. Locations with lower frequencies than expected were the 1st and 2nd segment of the chromosomes from group C and the 1st segment of the chromosomes from group D.

The analyses of the locations of breaks along the chromosomal length allowed us to judge the mechanism of action of different chemical substances depending on the chemical organization of each chromosome. According to our results there exist locations highly sensitive and highly resistant to the actions of VCM along the chromosomes (Fig. 2).

Some banding methods indicate that fragile sites are points on chromosomes which tend to break non-randomly when exposed to specific chemical agents. Their expression is mediated by perturbations of the nucleotide pool (Sutherland and Hecht, 1984). If we correlate this definition of fragile sites with the mechanism of action of VCM metabolites on DNA, we can see that the DNA is depurinated by reaction with this mutagenic substance (Roberts, 1975). The unbalanced pool of nucleotides is a condition which may lead to the expression of specific fragile sites connected with disturbances in the concentrations of purines. On the other hand, fragile sites are frequently located at breakpoints of chromosomal rearrangements found in tumor karyotypes (LeBeau and Rowley, 1986; Hecht and Glover, 1984; Yunis and Soreng, 1984; DeBraekeleer et al., 1985; Hecht and Hecht, 1986; Glover et al., 1986). From such a point of view there is a correlation between specific fragile sites and cancer breakpoints and there is also a possibility that the expression of fragile sites is a predisposing factor in the occurrence of cancer in some people.

The distribution of breaks in the chromosomes induced by VCM shows us that the most sensitive parts of the chromosomes are the terminal segments of chromosome A1, chromosome A2 and the terminal segments of chromosomes from

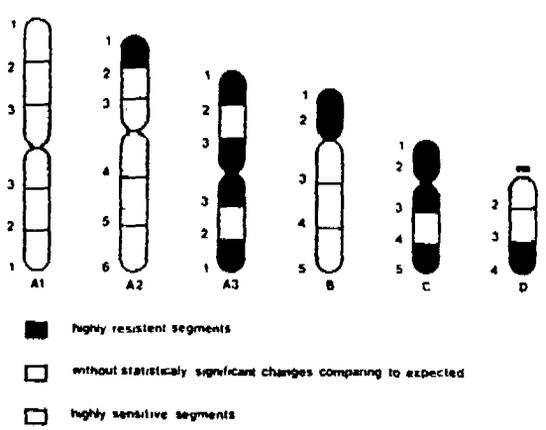

Fig. 2. Scheme of the distribution of breaks along chromosomal length.

groups B and C. For some types of cancer maps already exist with cancer-specific breakpoints and fragile sites (Heim and Mitelman, 1987; Yunis and Soreng, 1984). The carcinogenic action of VCM is described in numerous studies (Creech and Johnson, 1974; Monson et al., 1974; Takershaw et al., 1974). If non-randomly distributed breaks along chromosomes induced by VCM represent a specific family of fragile sites induced by an unbalanced pool of purines and if we link these regions with regions which carry genes related to cancer (oncogenes) or if they are predisposing factors for chromosomal rearrangements it may be possible to find confirmation of occupationally connected malignant transformations at DNA level for different chemical substances.

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