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The persistence of sister-chromatid exchange frequencies in men occupationally exposed to vinyl chloride monomer

Aleksandra Fučić [1], Verica Garaj-Vrhovac [1], Boris Dimitrović [2] and Mladen Škara [2]

[1] *Institute for Medical Research and Occupational Health, University of Zagreb and* [2] *'Vinilplastika' Medical Department, Zadar, Croatia (Yugoslavia)*

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Summary

The persistence of sister-chromatid exchange frequencies in a population occupationally exposed to the well known chemical mutagen vinyl chloride monomer was studied. It was shown that increased values of sister-chromatid exchange frequencies were still present in the lymphocytes of workers who had not been exposed for 8–120 days and retired persons for 5–10 years after exposure. The possible ability of vinyl chloride monomer alkylating metabolites to cause long-lasting damage of the DNA molecule is discussed.

Vinyl chloride monomer (VCM) is a well known carcinogen and mutagen in humans (Anderson et al., 1980; Purchase et al., 1976; Creech and Johnson, 1974; Fučić et al., 1990). After metabolic activation by cytochrome P-450-dependent monooxygenases VCM is transformed into alkylating intermediates which express mutagenic and carcinogenic activity (Bartsch and Montesano, 1975; Zajdela et al., 1980; Barbin and Bartsch, 1989). These intermediates are potent inducers of increased sister-chromatid exchange frequencies (Perry and Evans, 1975; Fučić et al., 1990; Uzych, 1988; Simes et al., 1991).

Sister-chromatid exchange is a selective and sensitive method for detecting the mutagenic activity of chemicals (Natarajan and Mullenders, 1987) although it has been shown that sister-chromatid exchange frequencies are decreased 4–16 weeks after the end of exposure (Stetka and Wolff, 1976; Uzych, 1988).

The aim of our study was to draw attention to the persistence of changes in the DNA molecule which lead to expression of increased sister-chromatid exchange frequencies after occupational exposure to high concentrations of VCM.

Material and methods

Fifteen plastics industry workers were chosen for the study of sister-chromatid exchange frequencies. As a control population we chose 10 male subjects from the general population 40–50

Correspondence: Dr. A. Fučić, Institute for Medical Research and Occupational Health, University of Zagreb, Ksaverska c. 2, Zagreb, Croatia (Yugoslavia).

years old. In the experimental group the examined workers had been employed in a polyvinyl chloride plant for 1.5–35 years. Those with recent X-ray exposure and drug treatment were excluded from the study. The VCM concentration in the working environment was 2000 ppm. Due to the technological process the concentration could occasionally reach 2000 ppm for a very short period of time.

Sister-chromatid exchange frequencies were measured during and 8, 30 and 120 days after exposure. Retired workers were examined 5–10 years after exposure.

Sister-chromatid exchange frequencies were measured on slides from cultures of blood lymphocytes stimulated with phytohemagglutinin. To 0.5-ml samples of whole blood 8 ml of F-10 medium (Gibco) containing 20% calf serum and 10 μg/ml bromodeoxyuridine was added. The cultures were harvested after 72 h. Smoking habits of the individuals were taken into account. For statistical analysis the *t*-test was used (Pavlić, 1970).

Results

Sister-chromatid exchange frequencies in 15 plastics industry workers and 10 control individuals from the general population are shown in Table 1. The periods during which workers had not been exposed were 8, 30 and 120 days. Retired persons had not been exposed for periods of 5–10 years. Two retired persons were examined twice with a 2-year interval. There was no statistically significant difference between sister-chromatid exchange frequencies in workers during exposure and 8, 30 and 120 days after exposure. The range of sister-chromatid exchange frequen-

TABLE 1

COMPARISON OF SISTER-CHROMATID EXCHANGE FREQUENCIES IN SUBJECTS DURING AND AFTER EXPOSURE TO VCM

Subject No.	Employment (years)	Cigarettes/ day	SCE frequencies				
			During exposure		After exposure		
			per cell	range	period	per cell	range
Employed							
1	16	0	9.8	6–15	8 days	11.5	7–17
2	12	20	10.7	4–17	8 days	9.1	5–16
3	1.5	0	7.6	4–15	30 days	8.0	4–14
4	12	20	9.7	4–18	30 days	11.1	6–23
5	3	0	8.6	5–14	30 days	8.6	5–15
6	12	0	8.5	6–12	30 days	9.8	4–13
7	2	25	8.5	4–14	30 days	8.5	4–14
8	10	0	9	4–14	30 days	8.3	4–14
9	10	20	9.7	4–19	30 days	9.7	4–18
10	9	20	12.3	5–26	30 days	10.4	4–19
11	15	30	12.7	8–16	30 days	11	5–23
12	20	30	11.1	6–19	120 days	10.9	6–18
Retired							
1	17	15			8 years	10.4	6–20
					10 years	8.6	4–13
2	35	2			5 years	9.5	5–19
					7 years	10.1	5–18
3	7	0			9 years	7.5	5–13
						5.9	0– 7
Control subjects (mean value, n = 10)							

For all calculations 50 cells per person were counted.

cies during exposure was 4–26, and after exposure it was 4–23. Even 10 years after chronic exposure to high concentrations of VCM retired persons showed significantly increased values of sister-chromatid exchange frequencies ranging from 4 to 20. The value of sister-chromatid exchange frequencies for control individuals was 5.9, with a range of 3–7.

Discussion

Sister-chromatid exchange frequencies provide useful information about exposure to chemical mutagens. Although the mechanism of sister-chromatid exchange induction is still a matter for speculation this method is very sensitive and very frequently used in biomonitoring of people occupationally exposed to a wide range of chemicals. It is generally believed that in contrast to severe morphological changes in chromosomes which may persist for a long time, sister-chromatid exchange frequencies in lymphocytes may maintain a high level for 4–16 weeks after exposure (Stetka and Wolff, 1976; Uzych, 1988). The transient nature of increased sister-chromatid exchange frequencies raises the question of the mode of action of the substances that cause them. The question is whether the decrease in sister-chromatid exchange frequencies is due to the disappearance of the chemical agent or its active metabolite from the organism or to the repair process.

Sister-chromatid exchange is a result of a mechanism independent from that which causes chromosome breakage (Wolff and Bodycote, 1975; Lin and Wertelecki, 1982). However, fragile sites are hot spots for DNA recombination as measured by sister-chromatid exchange (Glover and Stein, 1987). This is also confirmed by the observation that chemicals that do not induce gross chromosome aberrations significantly increase sister-chromatid exchange frequencies (Latte, 1974; Perry and Evans, 1975).

Vinyl chloride monomer is a mutagen which increases the frequencies of sister-chromatid exchange in people exposed to high concentrations of it. In contrast to the general opinion today we were able to show increased values of sister-chromatid exchange in humans exposed to high concentrations of VCM as long as 10 years after the exposure. Our findings, which demonstrate that there was no significant decrease in sister-chromatid exchange levels from 8 days to 10 years after exposure, present a new aspect of the mechanism of sister-chromatid exchange and the action of VCM. The primary adducts isolated from DNA after exposure to VCM were 3,N^4-ethenodeoxycytosine and 7-(2-oxoethyl)guanine along with smaller quantities of 1,N^6-ethenodeoxyadenine (Green and Hathway, 1978; Barbin et al., 1981). The aromatic character of the suspected persistent monoadduct ethenodeoxycytosine ensures its easy formation and stability. The presence of such etheno derivates in synthetic nucleic acids has been associated with misincorporation of bases during DNA synthesis (Barbin et al., 1981; Hall et al., 1981). The carcinogenic potency of VCM may be related to the persistence of such potentially miscoding lesions. It is suggested that the mode of action of chemicals such as VCM is a multistep mechanism which also includes the formation of interstrand cross-links with either its complementary base or an adjacent base on the complementary strand (Conner and Cheng, 1983). Such cross-links may present yet unknown highly persistent sister-chromatid exchange-inducing lesions. The levels of sister-chromatid exchange even 120 days after exposure to VCM were not decreased regardless of smoking habit. The most interesting subjects were retired persons who, even 10 years after exposure, still carried high levels of sister-chromatid exchanges. The range of their sister-chromatid exchange frequencies did not differ statistically significantly from that in still working subjects.

Consequently it may be assumed that even bone marrow stem cells are affected and that these lesions are not being repaired during the S-phase of the mitotic cycle as described in some hypotheses on the origin of sister-chromatid exchange (Schofeld and Lajtha, 1973).

Further investigation is necessary to prove the persistence of alkylated sites in the DNA molecule caused by VCM and detectable by the sister-chromatid exchange method after long exposure to high concentrations of it. At the same time it is

necessary to consider this population as having permanent damage to the genetic pool.

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