

Increase of sister chromatid exchange and unscheduled synthesis of deoxyribonucleic acid by acrylonitrile in human lymphocytes in vitro

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PEROCCO P, PANE G, BOLOGNESI S, ZANNOTTI M. Increase of sister chromatid exchange and unscheduled synthesis of deoxyribonucleic acid by acrylonitrile in human lymphocytes in vitro. *Scand j work environ health* 8 (1982) 290–293. The investigation has been carried out on cultures grown in the presence of 5×10^{-1} – 5×10^{-5} M acrylonitrile with or without a rat liver metabolizing system (S-9 mix). The results obtained showed that acrylonitrile was toxic starting from the 5×10^{-3} M concentration, caused a significant increase in the sister chromatid exchange (SCE) frequency in comparison with the controls ($p \leq 0.001$) when the concentration was 5×10^{-4} M, and elicited reparative deoxyribonucleic acid (DNA) synthesis, determined by tritiated thymidine uptake, particularly when the concentration was 5×10^{-1} M. These effects were observed in lymphocytes of different donors and after drug activation by the S-9 mix metabolizing system.

Key terms: chemical mutagenicity, deoxyribonucleic acid repair, plastics industry monomers.

Some monomers utilized in the plastics industry show mutagenic and carcinogenic properties either towards bacterial cells and experimental animals or directly towards man. These properties are well known for the halogenated vinyl monomers and for other chemically related molecules. However, limited experimental data are available for many of these substances despite their wide industrial use.

Acrylonitrile ($\text{CH}_2 = \text{CHCN}$, vinyl cyanide, cyano-ethylene, propenenitrile) belongs to this class of compounds. This monomer is widely used as an intermediate in the manufacture of synthetic fibers, plastics, and rubbers and as a grain fumigant, and it has been shown to have mutagenic power in assay systems utiliz-

ing bacterial cells (7, 17) and carcinogenic activity in animals (6, 15, 16). Furthermore, acrylonitrile is toxic and a suspected carcinogen to man (4), even if negative results have been obtained concerning its genotoxic action in cytogenetic studies on mice and rats (11) and in epidemiologic investigations carried out with exposed workers (14).

In this study additional data are reported on the damaging actions of this substance on deoxyribonucleic acid (DNA) and its mutagenic effect on human lymphocytes cultured in vitro in the presence (treated) or absence (controls) of three scalar doses of acrylonitrile.

The parameters studied were (i) the scheduled DNA synthesis (replicative synthesis), (ii) the unscheduled DNA synthesis (reparative synthesis) in cultures grown in the presence of hydroxyurea, and (iii) the frequency of sister chromatid exchanges.

Scheduled and unscheduled DNA syntheses were measured through the tritiated

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thymidine uptake by lymphocytes, and all the experiments were carried out with or without metabolic activation of the drug.

Materials and methods

Lymphocyte cultures

The experimental conditions have been previously described (9, 12). Briefly, 50–60 ml of blood from healthy adult donors was collected, and lymphocytes were separated according to Böyum (1). Lymphocytes, 2×10^5 /well of microtest culture plate, were cultured in a volume of 0.2 ml of Roswell Park Memorial Institute (RPMI) 1640 medium, supplemented with 2 mM glutamine and containing 0.25 μ Ci (9,250 Bq) of tritiated thymidine (^{3} H-TdR) (The Radiochemical Centre, Amersham, England).

Cultures were grown for 4 h at 37 °C in humidified atmosphere containing 5 % carbon dioxide in the absence (controls) or presence of acrylonitrile in concentrations of 5×10^{-1} to 5×10^{-3} M (99 % purity; Merck-Schuchardt, Darmstadt, Federal Republic of Germany).

Toxic effects of the drug were measured by the ^{3} H-TdR uptake for the DNA scheduled synthesis.

To study the action of acrylonitrile as a DNA-damaging agent, the ^{3} H-TdR uptake for the unscheduled DNA synthesis was measured by the addition of 10 mM hydroxyurea (Sigma, St Louis, MO, USA) to the cultures to block the DNA replicative synthesis (3, 5).

The metabolic activation of the drug was obtained through a phenobarbital-induced rat liver metabolizing system (S-9 mix) as previously described (9).

At the end of the culture periods, ^{3} H-TdR uptake to determine the scheduled or the unscheduled DNA syntheses, in sextuplicate wells for each experimental combination, was measured by a liquid scintillation spectrometer as previously described (12), and it was exposed in disintegrations per minute $\pm$ standard error.

Sister chromatid exchange frequency

Whole blood from healthy adult donors was cultured at 37 °C in RPMI 1640 medium supplemented with 10 % human pooled sera in the presence of phytohe-

moagglutinin (Difco, Detroit, MI, USA) and bromodeoxyuridine (BUdR) (Sigma) in concentrations of 1 and 10 μ g/ml, respectively.

Acrylonitrile, or only medium to the controls, was added to the cultures for 1 h at 48 h of incubation at concentrations ranging from 5×10^{-3} to 5×10^{-5} M in the presence or absence of S-9 mix. Thereafter, the culture tubes were centrifuged, the supernatants were discarded, and the cells were suspended in fresh BUdR-containing medium.

Cultures, carried out under dim light, were stopped at 72 h and colcemid (0.2 μ g/ml) (CIBA, Basel, Switzerland) was added for the last 2 h of culture.

After hypotonic treatment with 0.075 M potassium chloride, air-dried preparations of chromosomes were obtained by the standard procedure. The staining technique used was essentially according to Schneider et al (13) and the fluorescence plus Giemsa method of Perry & Wolff (10); the slides were stained with Hoechst 33258 (15 μ g/ml) in 50 mM phosphate buffer (pH 6.8) for 15 min, rinsed, mounted in 50 mM citric acid-100 mM phosphate buffer (pH 7), and exposed to a 100-W visible-light lamp from a distance of 6 cm for 2 h. The slides were rinsed and stained with 3 % Giemsa in 50 mM phosphate buffer (pH 6.8) for 15 min. For each experimental combination 20 metaphases with ≥ 44 chromosomes were scored for sister chromatid exchanges. The differences in the means of the treated and control groups were analyzed by Student's t-test (2 p).

Results and discussion

Fig 1 reports the values for the ^{3} H-TdR uptake in the cultures of lymphocytes from two different donors treated with three doses of acrylonitrile, plus untreated controls, for 4 h in the presence or absence of 10 mM hydroxyurea and S-9 mix. It appears that acrylonitrile is toxic at high doses, ie, 5×10^{-1} and 5×10^{-2} M, in cultures grown without metabolic activation, as shown by the low ^{3} H-TdR uptake in comparison with that of the controls (dotted line in figure). On the contrary, the ^{3} H-TdR uptake was significantly higher than that of the controls, particularly in 5×10^{-1} M acrylonitrile-treated cultures grown in the presence of S-9 mix.

If the presence of hydroxyurea (which inhibits scheduled DNA synthesis) is taken into account, we can argue that these high values of thymidine uptake are due to

unscheduled DNA synthesis after metabolic activation of the drug to a DNA-damaging form.

These toxic and mutagenic actions of acrylonitrile are confirmed by the sister chromatid exchange assay reported in table 1. In fact, a small but statistically significant increase ($p \leq 0.001$) of sister chromatid exchange frequency in comparison with that of the controls has been obtained for human lymphocytes from two different donors after treatment with 5×10^{-4} M acrylonitrile for 1 h with metabolic activation.

The sister chromatid exchange frequency following treatment with 5×10^{-3} M acrylonitrile could not be determined because only few cells with pycnotic nuclei and without mitotic figures were observable.

The frequency of sister chromatid exchange obtained with 5×10^{-5} M acrylonitrile was not significantly different from that of the controls, either with or without the metabolic activation of the drug.

These results are in agreement with those reported in the literature. In fact, the DNA-damaging and mutagenic activities of acrylonitrile towards human lymphocytes confirm the toxic, teratogenic, and carcinogenic power observed for this substance in studies carried out on rodents (6, 15, 16) and its mutagenic effects towards mammalian and bacterial cells cultured in vitro (7, 8, 17).

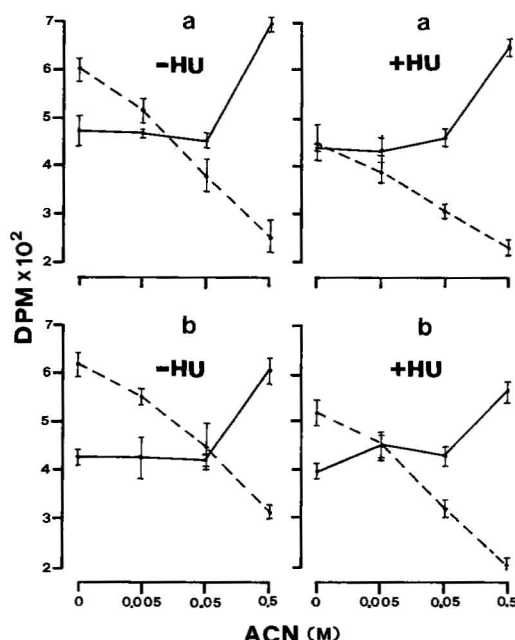


Fig 1. Tritiated thymidine uptake by human lymphocytes from two different donors (a, b). The lymphocytes were cultured in vitro for 4 h in the presence or absence (controls) of acrylonitrile (ACN) with (—) or without (---) S-9 mix metabolizing system and 10 mM hydroxyurea ($\pm$ HU). Data, in disintegrations per minute, are the means of sextuplicate samples $\pm$ standard error.

Table 1. Sister chromatid exchange (SCE) frequency in human lymphocytes from two different donors (a, b). The lymphocytes were cultured in vitro for 1 h in the presence or absence (controls) of acrylonitrile with or without the S-9 mix metabolizing system. Significance has been calculated by the Student's t-test.

Acrylonitrile doses (M)	- S-9 mix			+ S-9 mix		
	SCE/cell ^a	t ^b	p-value	SCE/cell ^a	t ^b	p-value
Donor a						
5×10^{-5}	8.15 ± 0.44	0.6	NS ^c	8.30 ± 0.61	0.4	NS
5×10^{-4}	8.75 ± 0.54	0.3	NS	11.10 ± 0.53	3.8	$p < 0.001$
5×10^{-3}	— ^d	—	—	—	—	—
Control	8.55 ± 0.38			7.95 ± 0.62		
Donor b						
5×10^{-5}	7.40 ± 0.50	0.1	NS	8.90 ± 0.55	1.4	NS
5×10^{-4}	8.50 ± 0.55	1.3	NS	10.80 ± 0.69	3.5	$p = 0.001$
5×10^{-3}	—	—	—	—	—	—
Control	7.50 ± 0.46			7.85 ± 0.44		

^a Mean of 20 metaphases scored $\pm$ standard error.

^b Treated/control groups.

^c Not statistically significant.

^d No visible metaphases.

Our results also confirm that acrylonitrile needs metabolic activation by S-9 mix to exert its highest mutagenic power, as reported in previous studies utilizing the Ames *Salmonella*/microsome test (7) and in investigations carried out in vitro on the metabolic pathways of acrylonitrile (2). However, this substance was also found to be a directly acting mutagen for some strains of *Escherichia coli*, and it has been suggested that this action could be due to a peculiar sensitivity of these bacteria to mutagenic chemicals (17).

The toxic and mutagenic actions of acrylonitrile were observable only at very high doses, which varied in the two experimental conditions. This discrepancy might be ascribed to genetic damage induced by different mechanisms and/or sensitivity of the tests.

The disagreement between the significance of the sister chromatid exchange frequency observed in this study and the lack of chromosome aberrations in lymphocytes of acrylonitrile-exposed workers (14) is probably due to differences in experimental conditions and acrylonitrile doses (< 20 ppm for the exposed workers).

As far as we know, no data are available in the literature on the mutagenic action exerted by this chemical directly on human cells. On the other hand our results were obtained with concentrations which are not comparable with those of workplaces. However, if the massive production and use of acrylonitrile are taken into account, it cannot be excluded that genotoxic effects could be determined in man by a prolonged and continuous exposure to this substance.

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