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Influence of cysteine on mechlorethamine-induced chromosomal aberrations in cultured human lymphocytes

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Abstract. The aim of the present paper was to find out by in vitro chromosomal aberration test using human lymphocytes whether cysteine has anticlastogenic properties towards a well-known mutagen – mechlorethamine. The lymphocytes tested were obtained from three healthy donors. Two doses of cysteine (1.0 and 2.0 μg/ml) and three doses of mechlorethamine (0.1, 0.2 and 0.3 μg m^{-1}) were tested. It was found that cysteine had anticlastogenic properties and that it reduced the number of metaphases with chromosomal aberrations induced by mechlorethamine.

Key words: anticlastogens, chromosomal aberrations, cysteine, in vitro, mutagenicity testing, mechlorethamine.

Many experiments have been carried out to decrease the risk of mutagenic and carcinogenic compounds. Their aim was to find compounds which could counteract with mutagenicity of chemical substances. Groups of antimutagenic and anticarcinogenic compounds have already been identified (INOUE et al. 1985, GOTOH et al. 1988), for example thiol compounds, natural (e.g. reduced glutathione) or synthetic (e.g. N-acetylcysteine). It is known that thiols possess antioxidant and nucleophilic properties and can stimulate various cytosolic enzyme activities, as well as enzymes involved in DNA repair (De FLORA, RAMEL 1988). It was found that thiols, such as cysteine, cystamine, glutathione, significantly reduced the number of mutagen-induced SCEs (INOUE et al. 1985).

The goal of the present study was to find out by in vitro chromosomal aberration test carried out on human lymphocytes whether cysteine has anticlastogenic properties towards a well-known mutagen – mechlorethamine.

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Material and methods

Lymphocytes obtained from peripheral blood of three (A, B, C) healthy male donors (aged 19-23) were cultured for 72 h at 37°C on the Eagle medium (80%) with calf serum (20%), antibiotics (penicillin – 100 units ml^{-1}, streptomycin – 0.2 mg m^{-1}) and a mitogenic substance LF-7 (Biomed – Cracow, Poland). The tested compounds – mechlorethamine (2-chloro-N-[2-chloroethyl]-N-methylethanamine) and cysteine – were added to the medium during the last 24 h. Concentrations of clastogen defined by preliminary tests were 0.1, 0.2, 0.3 μg m^{-1}. Cysteine doses were 1.0 and 2.0 μg m^{-1}. Chromosomal preparations were obtained by a routine method after a hypotonic shock caused by 0.075 M KCl and fixation in the mixture of methanol and acetic acid (3:1). Following the routine Giemsa staining the number and type of aberrations were analysed. The data were statistically treated by Chi-square test.

Results

The mutagen mechlorethamine caused numerous chromosomal aberrations in human lymphocytes cultured in vitro (Table 1), namely, chromatid exchanges, breaks and gaps. Many metaphases were observed to have several aberrations. The rate of aberrant metaphases was high – on the average, 40% for 0.1 μg m^{-1}, 50% for 0.2 μg m^{-1} and 62% for 0.3 μg m^{-1}. Addition of cysteine to lymphocyte cultures together with mechlorethamine decreased the number of aberrant metaphases (Table 1). A pronounced protective effect of cysteine was observed in the lymphocyte cultures from donor B, which showed a statistically significant decrease in the number of aberrant metaphases in all experimental variants (Table 1). A decrease in the number of aberrations per cell was observed in the lymphocyte cultures from donor B and in some variants from donors A and C (Table 1). A slight increase in the number of aberrations per cell was found in some cultures from donors A and C which was due to numerous lesions in single metaphases.

Discussion

Cysteine belongs to thiol compounds which exhibited antimutagenic effect towards many chemical compounds in many tests (INOUE et al. 1985, HAYATSU et al. 1988).

Table 1. Results of antimutagenic activity of cysteine testing by in vitro chromosomal aberration test (human lymphocytes)

Donor	Compounds tested (μg ml^{-1})		Number of analysed cells	Number of aberrant cells	Number of aberrations per cell
	ME	CYS			
A	0	0	100	1.0	0.01
	0	1.0	100	4.0	0.04
	0	2.0	150	4.1	0.04
	0.1	0	175	34.8	0.53
	0.1	1.0	117	28.2	0.51
	0.1	2.0	101	26.8	0.57
	0.3	0	102	62.7	1.18
	0.3	1.0	100	61.0	1.32
	0.3	2.0	59	40.7*	0.90
B	0	0	100	0	0
	0	1.0	100	3.0	0.03
	0	2.0	100	3.0	0.03
	0.1	0	116	35.3	0.50
	0.1	1.0	100	15.0*	0.15
	0.1	2.0	101	9.9*	0.13
	0.3	0	71	60.6	1.11
	0.3	1.0	59	40.7*	0.90
	0.3	2.0	53	26.4*	0.47
C	0	0	100	4.0	0.04
	0	1.0	100	6.0	0.06
	0	2.0	100	4.0	0.04
	0.1	0	100	50.0	0.94
	0.1	1.0	100	59.0	1.09
	0.1	2.0	100	45.0	0.82
	0.2	0	100	63.0	1.27
	0.2	1.0	84	50.0	0.82
	0.2	2.0	100	54.0	0.76

ME – mechlorethamine, CYS – cysteine, * $P < 0.05$

It was shown, for example, that cysteine inhibited mutagenicity of 2-acetoxyacetylaminofluorene and glutathione and that of benzo(a)pyrene in in vitro cultures of mammalian cells (HAYATSU et al. 1988). It was also found that cysteine and cystamine reduced the number of SCE induced in vitro in human lymphocytes by nitrosocimetidine (INOUE et al. 1985). Cysteine reduced also the frequency of SCE induced by hydroxylamine, hydrazine and isoniazid in the V-79 cell line of the Chinese hamster; it was also active in the bone marrow cells of the Chinese hamster (SPEIT et al. 1980). In the present experiments it was observed that cysteine decreased the number of aberrations and that of aberrant metaphases induced by mechlorethamine in in vitro cultured human lymphocytes. However, lymphocytes from different donors had different re-

actions. Variation in individual sensitivity to a genotoxic effect of different chemicals was reported by several investigators, with a special emphasis on those compounds which interact with DNA (OBE, BEEK 1984). Inter-individual variation is related to a variety of factors such as age, metabolism, intrinsic repair competence, different susceptibility of lymphocyte populations. Besides variation among individuals in their response to a single tested agent, they can also show considerable variation in their response to different agents (CROSSEN 1982). So, individual susceptibility to a mutagen tested can be also different from the susceptibility to an antimutagen studied. In the present paper only three individuals were tested. Different reactions of lymphocytes were most clearly visible when cysteine effects were compared in the lymphocytes from donors A and B in the dose group of 0.1 $\mu g\ m^{-1}$ and 0.3 $\mu g\ m^{-1}$. Anticlastogenic activity of cysteine was the highest in the lymphocytes from donor B, and the level of mutations was the lowest in the control lymphocytes from this donor.

The highest lymphocyte susceptibility to the mutagen tested was observed in donor C, whereas cysteine activity in this donor was low. Different response of lymphocytes to mutagen and antimutagen could be also related to different kinetics of lymphocyte proliferation in unsynchronized lymphocyte cultures. Data from other studies show that there can be considerable variation among individuals in their proliferative response in the presence of the compounds tested (CROSSEN 1982). In the present paper mechlorethamine and cysteine action on cell sub-populations with different velocity of proliferation was not controlled by the use of bromodeoxyuridine labeling.

The mechanism of antimutagenic effect of thiol compounds including cysteine has not been well understood so far. It is supposed that they can act as potential nucleophilic compounds which compete with DNA in chemical reactions with mutagenic electrophiles (De FLORA, RAMEL 1988). These compounds can also induce changes in promutagen metabolism, thus enabling them to transform into a mutagenic form (De FLORA, RAMEL 1988, HAYATSU et al. 1988).

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