MODIFICATION OF THE CYTOGENETIC EFFECT OF CYTOSINE ARABINOSIDE BY CYSTEINE IN HUMAN PERIPHERAL BLOOD LYMPHOCYTE CULTURES

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The modifying action of L-cysteine on chromosomal aberrations induced by the pyrimidine base analog cytosine arabinoside in cultures of human lymphocytes was studied. The effect of L-cysteine is manifested more clearly if there is an increased level of induced aberrations. The manifestation of the effect differs at different stages of the cell cycle. In cultures fixed in the late stages no modifying effect of L-cysteine is seen, because the mutagen acts on cells entering on the second mitotic division, whereas treatment with L-cysteine was carried out before their first division.

In investigations to study the action of cell compounds on chromosomal aberrations induced by chemical mutagens in cultures of human leukocytes, alkylating compounds (myleran [5], trenimon [6], and thio-TEPA [1]) and the chelating compound 8-hydroxyquinoline sulfate [4] have been used as mutagens. The effect of the protector was found to depend on the time of its administration and on the times of fixation of the cultures, and it is manifested more clearly with an increase in the level of aberrations induced by the mutagen.

TABLE 1. Relationship between Modifying Effect of Cysteine and Time of Its Administration

Preparation added	Time (in h) of addition of preparation	Aberrant cells			Chromosomal aberrations		
		number	%	P	number	for 100 cells	P
CA	57	47	47,0	—	58	58,0	—
+C	0	38	38,0	>0,05	38	38,0	<0,01
+C	4	38	38,0	>0,05	40	40,0	<0,05
+C	8	37	37,0	>0,05	39	39,0	<0,01
+C	12	37	37,0	>0,05	44	44,0	<0,05
+C	16	18	36,0	>0,05	19	38,0	<0,05
+C	20	35	35,0	>0,05	39	39,0	<0,01
+C	24	32	32,0	<0,05	39	39,0	<0,01
+C	28	24	24,0	<0,001	25	25,0	<0,001
+C	32	29	32,9	<0,05	37	40,6	<0,05
+C	36	25	31,6	<0,05	27	34,2	<0,01
+C	40	39	39,0	>0,05	46	46,0	>0,05
+C	44	18	36,0	>0,05	20	40,0	<0,01
+C	48	38	38,0	>0,05	46	46,0	>0,05
+C	52	44	44,0	>0,05	53	53,0	>0,05
+C	56	42	42,0	>0,05	55	55,0	>0,05

Legend here and in Tables 2 and 3: C) cysteine; CA) cytosine arabinoside.

The object of the present investigation was to study the effects of time and dosage on the action of cysteine and chromosomal aberrations induced by cytosine arabinoside in cultures of human peripheral blood lymphocytes.

EXPERIMENTAL METHOD

Blood of a clinically healthy donor aged 25 years was cultivated by the method described previously [1]. Cytosine arabinoside, added in all experiments 3 h before fixation (the G_2 stage), was used as the mutagen.

In the experiments of series I L-cysteine was added to the cultures between hours 0 and 56 of cultivation at intervals of 4 h. Fixation was carried out after 60 h. In experiments of series II and III cysteine was added at the 28th hour of cultivation and cytosine arabinoside at the 55th hour.

Colchichine (10^{-5} M) was added to all cultures 2.5 h before fixation. From 100 to 150 metaphase plates were analyzed in each case, and the experiments

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TABLE 2. Modifying Effect of Cysteine with Different Concentrations of Cytosine Arabinoside

Preparation added	Times of fixation	Aberrant cells			Chromosomal aberrations		
		number	%	P	number	for 100 cells	P
CA	58	27	22,6		36	36,0	
C + CA		15	10,0	<0,01	16	10,7	<0,001
CA	62	14	14,0		16	16,0	
C + CA		24	24,0	>0,05	23	23,0	>0,05
CA	66	19	12,9		37	25,2	
C + CA		20	13,3	>0,05	28	18,7	>0,05
CA	70	30	20,0		40	26,7	
C + CA							>0,05
CA	74	31	20,7	>0,05	38	25,3	
C + CA		33	22,0	>0,05	39	26,0	>0,05
CA	78	32	21,3		46	30,7	
C + CA		21	14,0	>0,05	32	21,3	>0,05
CA	82	24	24,0		25	25,0	
C + CA		21	21,0	>0,05	30	30,0	>0,05

TABLE 3. Modifying Effect of Cysteine with Differential Concentrations of Cytosine Arabinoside

Preparation added	Aberrant cells			chromosomal aberrations		
	number	%	P	number	for 100 cells	P
CA ($2{,}25 \cdot 10^{-6}$ mole)	9	9,0	>0,05	9	9,0	
C + CA ($2{,}25 \cdot 10^{-6}$ mole)	7	7,0		7	7,0	>0,05
CA ($4{,}6 \cdot 10^{-6}$ mole)	13	14,0	>0,05	13	14,0	>0,05
C + CA ($4{,}6 \cdot 10^{-6}$ mole)	12	12,0		12	12,0	
CA ($0{,}9 \cdot 10^{-5}$ mole)	22	24,7		28	31,5	
C + CA ($0{,}9 \cdot 10^{-5}$ mole)	17	17,0	>0,05	19	19,0	<0,05
CA ($1{,}8 \cdot 10^{-5}$ mole)	40	40,0		46	46,0	
C + CA ($1{,}8 \cdot 10^{-5}$ mole)	16	17,8	<0,001	16	17,8	<0,001
CA ($3{.}6 \cdot 10^{-5}$ mole)	30	45,5	<0,01	32	48,5	<0,001
C + CA ($3{,}6 \cdot 10^{-5}$ mole)	18	21,2		18	21,2	

were repeated twice. The following chromosomal aberrations were considered: single and paired fragments and chromatid and chromosomal exchanges.

EXPERIMENTAL RESULTS

In the experiments of series I cysteine had no modifying effect whatsoever when added at the late stages of cultivation, and the greatest decrease in the number of aberrant cells was found when cysteine was added from the 24th to the 36th hour of cultivation (Table 1). Cytosine arabinoside was added in a dose of 1.8×10^{-5} mole and cysteine in a dose of 10^{-3} mole.

In the experiments of series II the way in which the effect of the protector depended on the time of fixation of the cultures was studied. The results of this series show that a modifying effect of cysteine was observed only at the 58th hour of cultivation (Table 2). The doses were the same as in series I.

In the experiments of series III (Table 3) to study the effect of cysteine with different concentrations of cytosine arabinoside, the results of the first two series were taken into account. The results of this series show that the effect of cysteine in a dose of 10^{-3} mole was demonstrated more clearly with an increase in the level of aberrations induced by cytosine arabinoside, the same effect as was also found with aberrations induced by radiation [2].

These experiments showed that the manifestation of the modifying effect of cysteine does not depend on its simultaneous administration with cytosine arabinoside.

The maximal effect was found when cysteine was added from the 24th to the 36th hour of cultivation, in agreement with the results of an earlier study of the action of cysteine on cells treated with thioTEPA. The results suggest the existence of a protective effect of cysteine which is independent of its interaction with cytosine arabinoside.

The effect of cysteine was manifested when the mutagen was added after 55-57 h of cultivation. If the mutagen was added later than this, no action of the protector was manifested. This was probably because the cells had commenced the second mitotic division. Similar weakening of the action of the protector has also been postulated for chromosomal aberrations induced by radiation [3].

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