CHROMOSOMAL ABERRATIONS IN EMBRYONIC LIVER AND BONE MARROW CELLS OF A/He AND C57BL/6 MICE INDUCED BY THIOTEPA

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Chromosomal aberrations in the first mitosis of embryonic liver and bone marrow cells were studied in A/He and C57Bl/6 mice after injection of the alkylating agent thioTEPA in the g_1-S period of the cell cycle. Chromosomes of A/He mice were shown to be more sensitive to the mutagenic action of the compound.

KEY WORDS: interlinear differences; chromosomal aberrations; thiotepa.

The object of this investigation was to study the sensitivity of chromosomes of embryonic liver and bone marrow cells of two inbred lines of mice to the mutagenic action of the alkylating agent thioTEPA. The choice of lines of mice with different sensitivity is of considerable interest for the analysis of the nature and genetic control of chromosomal mutability.

EXPERIMENTAL METHOD

To obtain metaphase plates in embryonic liver cells pregnant females aged 2-3 months were killed 13-14 days after discovery of a vaginal plug (control group). Animals of the experimental group received an injection of the thioTEPA in Hanks's solution in a dose of 2.5 mg/kg intraperitoneally 12 h before sacrifice. The dose of the compound and the time of fixation were chosen after preliminary experiments which showed that the greatest number of chromosomal aberrations is observed 10-12 h after injection of the mutagen. In material fixed in this way many of the embryonic liver cells are in the g_1-S phase of the cell cycle at the time of contact with the mutagen [2]. Metaphase plates of embryonic liver cells were obtained from two embryos taken from the same mother (one sample) by the method described in [4, 8-10].

Bone marrow cells of male mice aged 2-3 months were studied. ThioTEPA in Hanks' solution was injected intraperitoneally. The dose of the compound (5 mg/kg) and the time of fixation (24 h after injection of the mutagen) were chosen on the basis of data in [6]. Bone marrow preparations were obtained by the standard method [4] with certain modifications: a 0.05% solution of colchicine in a volume of 0.025 ml/g body weight was injected into the mice 1.5 h before sacrifice.

To select metaphase plates suitable for analysis the standard criteria were used [1].

Metaphase plates with 38-41 chromosomes were analyzed. The following structural aberrations were taken into account: single and paired fragments, rings, and exchanges. Chromatid and isochromatid deficiencies were considered separately. Cells containing 10 breaks or more were classed as "multiple aberrations." The significance of differences in the percentage of damaged cells between mice of different lines of intact animals was determined by the φ method [5], and in animals after treatment with thioTEPA, by Student's t-test.

EXPERIMENTAL RESULTS

The results of determination of the percentage of cells with chromosomal aberrations and deficiencies in the embryonic liver of intact mice and mice receiving thioTEPA are given in Table 1. The results for the percentage of cells with structural aberrations and achromatin deficiencies were pooled and included in the column

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TABLE 1. Frequency and Types of Chromosomal Aberrations in Mouse Embryonic Liver Cells 12 h after Administration of ThioTEPA in a dose of 2.5 mg/kg

Experimental conditions	Line of mice	Number of specimens	Number of cells studied	Number of chromosomal aberrations of different types				Percent of cells damaged (M ± m)	Percent of cells with structural aberrations (M ± m)	Percent of cells with multiple aberrations ***
				single fragments	paired fragments	rings*	exchanges**			
~~Thiotepa~~	A/He	10	1000	163	54	35	68	56,5±8,9	27,3±6,1	1,7
	C57BL/6	10	1000	52	23	8	18	28,7±9,0	8,6±3,7	0,2
Control	A/He	10	1000	3	3	1	—	3,2	0,7	
	C57BL/6	10	1000	2	—	—	—	1,0	0,2	—

Here and in Table 2: *) acentric and centric rings with accompanying fragments; **) exchanges with accompanying fragments; ***) of the number of cells with structural aberrations.

TABLE 2. Frequency and Types of Chromosomal Aberrations in Bone Marrow of Mice 24 h after Treatment with ThioTEPA in Dose of 5 mg/kg

Experimental conditions	Line of mice	Number of animals	Number of cells studied	Number of chromosomal aberrations of different types				Percent of cells damaged (M ± m)	Percent of cells with structural aberrations (M ± m)	Percent of cells with multiple aberrations ***
				single fragments	paired fragments	rings*	exchanges**			
Thiotepa	A/He	13	1270	571	51	100	137	53,8±11,6	41,5±9,9	8,8
	C57BL/6	9	850	264	18	42	102	46,2±9,9	31,8±8,6	1,4
Control	A/He	11	1020	8	—	—	—	2,1	0,8	—
	C57BL/6	10	1000	5	—	—	—	1,8	0,5	—

of the table headed "Percent of damaged cells," for electron-microscopic investigations have shown that it is not always possible to distinguish deficiencies exactly from true breakages when chromosomal aberrations are studied in the light microscope. Often deficiencies visible in the light microscope are found in electron micrographs to be breakages, and vice versa [7].

It will be clear from Table 1 that the embryonic liver of intact A/He animals contained more cells with injuries (with structural aberrations and achromatin deficiencies) and more cells with structural changes only than in C57BL/6 mice. The differences are statistically significant ($P<0.01$ and $P<0.05$ respectively).

After administration of thioTEPA the number of cells with chromosomal aberrations and deficiencies in A/He and C57BL/6 mice reached 56.5 and 28.7% respectively. Interlinear differences for this index are statistically significant ($P<0.01$). Differences between mice of the two lines for the number of cells with structural aberrations only also are significant ($P<0.001$). The spectrum of chromosomal aberrations is shown in Table 1, from which it can be seen that one type of injury frequently found is a single fragment. In mice of both lines treated with thioTEPA cells with multiple injuries to the chromosomes appeared: in A/He mice this class of cells accounted for 1.7%, but in C57BL/6 mice for only 0.2%. ThioTEPA also induced the formation of achromatin deficiencies with a frequency of 0.85 deficiency per cell in A/He and 0.35 in C57BL/6 mice. In the control, 0.027 and 0.008 deficiency per cell was observed in the mice of these lines respectively.

The results of determination of the number of cells with chromosomal aberrations in the bone marrow of the table headed "Percent of damaged cells," for electron microscopic investigations have shown that it is - tact animals differences in the level of chromosomal aberrations were not statistically significant. ThioTEPA induced more damaged cells and cells with structural changes only in A/He mice than in C57BL/6 mice. Differences in the percentage of cells with only structural changes are statistically significant ($P<0.01$). The spectrum of chromosomal aberrations in the bone marrow of the mice of these lines, just as in the embryonic liver, was about the same after treatment with thioTEPA. After exposure to the mutagen, cells with multiple aberrations also appeared in the bone marrow: in A/He 8.8% of them were induced, but in C56BL/6 mice only one-sixth as many (1.4%). Just as in the embryonic liver, thioTEPA induced the formation of achromatin deficiencies in the bone marrow of both lines of mice.

Comparison of the results of the present investigation with those obtained previously shows that the chromosomes of A/He mice are characteristically more sensitive to thiotepa than those of C57BL/6 mice, whether the cells treated are in the g_0 phase of the cell cycle (the liver of adult animals) [3] or actively proliferating embryonic liver and bone marrow cells are treated in the g_1-S phase of the cell cycle. Calculation of the percentage of cells with damaged chromosomes (structural aberrations and deficiencies) per unit dose of the compound showed that embryonic liver cells are rather more sensitive to the mutagenic action of thioTEPA than bone marrow cells of adult animals. For instance, per milligram of thioTEPA, 22.6 and 11.5% of damaged cells were observed in the embryonic liver and 10.7 and 9.2% in bone marrow of A/He and C57BL/6 mice respectively.

A/He mice differ from C57BL/6 mice not only in the greater number of aberrant cells after thioTEPA treatment, but also in the fact that more cells with multiple chromosomal injuries are induced both in the embryonic liver and in the bone marrow of mice of this line. In the writers' view, the existence of a greater number of these cells among the total number of aberrations in A/He mice reflects the greater severity of the damage and, consequently, the higher sensitivity of mice of this line to the mutagen.

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BLOOD SERUM AS A FACTOR IN CHROMATIN CONDENSATION IN TRISOMY-21 (DOWN'S SYNDROME)

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The effect on the blood serum of patients with Down's syndrome and of healthy persons and also separate fractions of sera on structural parameters of model nucleohistone systems (DNP-systems) was studied. Unfractionated patients' sera were found to have a condensing effect on DNP-systems, unlike healthy human sera. Analysis of the action of the individual serum fractions showed that different degrees of condensation can be attributed to the influence of high-molecular weight, undialyzable, thermolabile components, the action of which disappears after gel-filtration of the serum proteins. The problem of possible humoral control over the structural organization of chromatin in vivo is discussed in the light of data showing similarity between blood serum proteins and certain nonhistone proteins of chromatin.

KEY WORDS: chromatin; condensation, serum.

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