Mutagenic Activity of Potentiated Antibodies to Erythropoietin

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Single and repeated administration of ultralow doses of antibodies to erythropoietin did not increase the count of aberrant metaphases in bone marrow cells of BALB/c mice and were not genotoxic for *Drosophila melanogaster* wing cells in the test of somatic mosaicism.

Key Words: *antibodies to erythropoietin; mutagenicity*

Various preparations of erythropoietin are used in clinical practice for the correction of anemia [5,7]. These preparations stimulate the erythroid hemopoietic stem, but cause side effects [6]. The search for new homeopathic preparations containing active substances in ultralow doses and not producing side effects during long-term treatment is an urgent problem [8,9].

Here we evaluated the cytogenetic effect of a new homeopathic preparation containing antibodies to erythropoietin (ABE), synthesized at the "Materia Medica Holding" Research-and-Production Company, and studied as a hemostimulator at the Institute of Pharmacology.

MATERIALS AND METHODS

The effects of single or repeated treatment with the homeopathic preparation were studied by counting chromosome aberrations in bone marrow cells from 47 BALB/c mice weighing 18-22 g. The animals were obtained from the Laboratory of Biological Models (Institute of Pharmacology) and kept according to the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986) and order No. 1179 of the Russian Ministry of Health (October 10, 1983).

In series I the preparation of ABE (a mixture of homeopathic dilutions C12+C30+C200) was administered intragastrically in a single dose of 0.5 ml/20 g to male mice. In series II ABE were administered intragastrically in a daily dose of 0.2 ml/20 g for 5 days to male and female mice. Bone marrow cells were fixed 24 h after the last treatment. Some mice received potentiated water (0.25 ml/10 g, C12+C30+C200, negative control). Other animals (positive control) received intraperitoneal injections of cis-platinum(II)diamine dichloride with cytogenetic activity in a single dose of 17 mg/kg (LD_{50}). These mice were compared with animals intraperitoneally injected with the solvent (0.9% NaCl). For accumulation of metaphases the mice received 0.025% colchicine (0.01 ml/g intraperitoneally) 1.5 h before the end of 24-h exposition. Bone marrow cells were obtained by the method of Ford with modifications [3] and stained with azure II and eosin for 30 min. We analyzed 50-100 metaphases from each animal. Undestroyed cells having a round shape, characterized by a wide variation of chromosomes and modular number of 40, and not containing overlaps were assayed. The ratio of aberrant metaphases and chromosome aberrations were estimated in bone marrow cells. Structural changes in chromosomes included single and paired fragments and exchanges.

The results were analyzed by Student's *t* test.

The method of somatic mosaicism is based on counting of mosaic spots in *Drosophila melanogaster* wings, whose formation is associated with complex changes in the genotype. Virgin mwh/mwh females (n=10) and flr^3/TM3 males (n=5) were kept together in tubes with the standard nutrient medium. After 60-62 h parent flies were placed into another tubes with a freshly prepared nutrient medium. ABE were added to the initial tubes (500 µl per 2 ml medium). Flies were examined 9-10 days after the start of experiments. The flies with mwh+/+flr^3 genotype were isolated and fixed in 70% ethanol. Microscopic preparations of wings were obtained using Fore's fluid. Both wing surfaces presented by cell monolayers were examined under a microscope (×400). Mutant spots were divided into the following classes: single spots

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TABLE 1. Effect of ABE on the Incidence of Chromosome Aberrations in Bone Marrow Cells from BALB/c Mice (*M*±*m*)

Parameter	Intact (*n*=5)			Platin (*n*=7)	ABE (*n*=5)
	Control	H_2O (*n*=6)	0.9% NaCl		
Count of studied cells	500	600	500	700	500
Aberrations, %	2.40±1.53	1.17±0.60	2.40±1.47	27.00±2.13*	0.6±0.4+
including:					
fragments					
single	2.40±1.53	1.17±0.60	2.40±1.47	23.00±2.08*	0.6±0.4+
paired	0±0	0±0	0±0	0.14±0.14	0±0
exchanges	0±0	0±0	0±0	3.86±0.83*	0±0+
Percent of aberrant metaphases	1.20±0.38	1.17±0.60	2.40±1.47	19.00±1.43	0.6±0.4+

Note. Significant differences: *compared to the control (0.9% NaCl); +compared to platin.

consisting of 1-2 cells (mwh or flr), large single spots consisting of 3 and more cells (mwh or flr), and double spots of mwh and flr cells localized close to each other. The incidence of each type of mutant wing spots was determined [2].

The results were analyzed by χ^2 test [4].

RESULTS

The percentage of damaged cells in mice receiving ABE in a dose of 0.5 ml/20 g during 24-h exposure corresponded to structural changes in animals receiving the solvent or potentiated water (negative control) and was much lower than in animals receiving the cytostatic (Table 1).

In series II, chromosome aberrations in males 24 h after treatment were found in 1.40±0.75% cells (per 500 metaphases). These aberrations were presented by single fragments, which corresponded to the control level. In 5 females the ratio of damaged cells in 500 metaphases was 1.00±0.45% 24 h after ABE administration. Chromosome aberrations included only single fragments.

Therefore, single and repeated intragastric administration of ABE did not increase the count of aberrant metaphases in mouse bone marrow cells.

ABE markedly decreased the incidence of mutant spots (Table 2). After treatment with this preparation we found only individual cells with 2 or 3 villi that were localized in various cells of the wing surface. The differences between experimental, control, and intact flies were statistically significant (Table 2). Thus, the preparation of ABE was not genotoxic for *D. melanogaster*.

Our results show that single and repeated intragastric administration of ABE did not increase the incidence of cytogenetic abnormalities in bone marrow cells from BALB/c mice. The preparation posseses no genotoxic activity and decreases the number of spontaneous mutations and recombinations in *D. melanogaster* wing.

TABLE 2. Somatic Mosaicism in *Drosophila melanogaster* Wings (*n*=100) Evaluated Using mwh and flr Markers

Parameter	Intact	Control	ABE
Spots	48	43	19
individual (mwh/flr)	48/0	41/0	19/0
large (mwh/flr)	0/0	1/0	0/0
double (mwh and flr)	0	1	0
Incidence of induction, $\times 10^{-5}$	1.58	1.42	0.63
χ^2	9.29	12.56	—

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