Cytogenetic Effects of Antibodies to γ-Interferon in Ultralow Doses

O. L. Voronova, O. P. Rogozina, A. V. Martyushev*, S. A. Sergeeva*, O. I. Epstein*, A.M. Dygai and E.D. Goldberg.

Single and course administration of ultralow doses of antibodies γ-interferon did not increase the incidence of cytogenetic abnormalities in bone marrow cells from BALB/c mice and produced no genotoxic effect on *Drosophila melanogaster* wing cells in the test of somatic mosaicism.

Key Words: *ultralow doses; antibodies to human γ-interferon; mutagenicity*

Long-term treatment with homeopathic preparations containing active substances in ultralow doses does not cause side affects [5,6]. It remains unclear whether these preparations possess antimutagenic activity. Here we evaluated the cytogenetic effect of a new homeopathic preparation containing antibodies to γ-interferon. Experiments were performed using routine tests recommended by the Russian Pharmacological Committee for studying mutagenic properties of pharmacological substances: evaluation of chromosome aberrations in mouse bone marrow cells (BMC) and gene mutations in *Drosophila melanogaster* [1].

MATERIALS AND METHODS

The effects of single or course treatment with the homeopathic preparation on the incidence of chromosome aberrations were studied in BMC from 34 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 antibodies to human γ-interferon (AB-IFN, a mixture of homeopathic dilutions C12+C30+C200) were administered to male mice intragastrically in a single dose of 0.5 ml/20 g. In series II AB-IFN were administered intragastrically in a daily dose of 0.2 ml/20 g to male and female mice for 5 days. BMC were fixed 24 h after the last treatment. Control mice received potentiated distilled water (0.25 ml/10 g, C12+C30+C200, PDW). Some animals (positive control) received intraperitoneal injections of cis-platinum(II)diamine dichloride, preparation exhibiting high 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 exposure. BMC were obtained by the method of Ford with some modifications [3] and stained with azure II and eosin for 30 min.

We analyzed 50-100 metaphases from each animal. Analysis was performed on undestroyed round cells with well spread chromosomes without overlaps and modulal number (40). The ratio of damaged BMC and chromosome aberrations were estimated (per 100 cells). Single and paired fragments and exchanges were counted. The significance of differences was analyzed using Student's *t* test.

For evaluation of somatic mosaicism virgin mwh/mwh females (n=10) and flr^3/TM3 males (n=5) were kept together in tubes with standard nutrient medium. After 60-62 h parent flies were removed and AB-IFN were added in a dose of 500 μl per 2 ml medium. Young flies were examined on days 9-10 of the experiments. Flies with mwh+/+flr^3 genotype were isolated and fixed in 70% ethanol. Microscopic preparations of wings were treated with Fore fluid. Both wing surfaces presented by cell monolayers were examined under a microscope (×400). Mutant spots were divided into the following classes: single spots 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 cell clones localized close to each other.

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences; "Materia Medica Holding" Research-and-Production Company, Moscow

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TABLE 1. Effect of AB-IFN on the Incidence of Chromosome Aberrations in BMC from BALB/c Mice ($M \pm m$)

Parameter	Intact (n=500)	Cytostatic		Homeopathic preparation	
		control (physiological saline, n=500)	platin (n=500)	PDW (n=600)	AB-IFN (n=400)
Number of aberrations, %	2.4±1.5	2.40±1.47	24.4±1.5*	1.40±0.68	1.00±0.41$^+$
including:					
fragments single	2.4±1.5	2.40±1.47	21.20±2.22*	1.40±0.68	1.00±0.41$^+$
paired	0±0	0±0	0.2±0.2	0±0	0±0
exchanges	0±0	0±0	3.00±0.89*	0±0	0±0$^+$
Ratio of damaged cells, %	1.20±0.38	2.40±1.47	19.00±2.05*	1.17±0.60	1.00±0.41$^+$

Note. Significant differences: *compared to the control; $^+$compared to platin; n, count of studied cells

The incidence of each type of mutant wing spots was determined [2]. The results were analyzed by χ^2 test [4].

RESULTS

Platin markedly increased the count of cells with chromosome aberrations, especially with single fragments (Table 1). The number of chromatid exchanges increased significantly. Therefore, these animals were characterized by the highest incidence of chromosome aberrations.

In mice receiving AB-IFN changes in chromosomes were presented by single fragments. The number of aberrant metaphases in these mice did not differ from that in control and intact animals. The number of damaged cells, chromosome aberrations, and single fragments and exchanges in mice receiving AB-IFN were much lower than in animals treated with the cytostatic (Table 1).

Tweenty-four hours after repeated treatment with AB-IFN chromosome aberrations were found 1.00± 0.58% bone marrow cells from males (per 400 metaphases examined). These aberrations were presented by single fragments, which corresponded to the control level. In females the ratio of damaged cells in 400 metaphases was 1.50±0.58%. Chromosome aberrations were presented by single fragments.

Therefore, single and course intragastric administration of AB-IFN did not increase the ratio of BMC with aberrant metaphases in mice.

AB-IFN markedly decreased the incidence of mutant spots compared to control and intact flies (Table 2). Thus, AB-IFN were not genotoxic for *D. melanogaster*.

TABLE 2. Somatic Mosaicism in *Drosophila melanogaster* Wings (per 100 Wings) Evaluated using mwh and flr Markers

Parameter	Intact	PDW	AB-IFN
Spots	48	43	23
individual	48/0	41/0	20/0
large	0/0	1/0	3/0
double	0	1	0
Incidence of induction, $\times 10^{-5}$	1.58	1.42	0.72
χ^2	8.81	6.06	—

Note. χ^2 was calculated for intact flies and flies grown on medium with PDW in comparison with flies grown in the presence of AB-IFN.

Our results suggest that single and repeated intragastric administration of AB-IFN did not increase the incidence of cytogenetic abnormalities in BMC of BALB/c mice. The preparation possesses no genotoxic activity and decreased the number of spontaneous mutations and recombinations in *D. melanogaster* wings.

REFERENCES

1. A. D. Durnev, Yu. A. Revazova, O. L. Verstakova, *et al.*, *Manual on Experimental (Preclinical) Studies of New Pharmacological Preparations* [in Russian], Moscow (2000), pp. 47-60.
2. L. P. Zakharenko and I. K. Zakharov, *Genetika*, **32**, No. 6, 755-758 (1996).
3. V. N. Orlov, G. A. Chudinovskaya, and E. P. Kryukova, *Chromosome Sets in Mammals* [in Russian], Moscow (1976).
4. H. Frei and F. E. Wurgler, *Mutat. Res.*, **203**, 297-308 (1988).
5. B. Kaplan, *Prof. Care Mother Child*, **4**, No. 5 (1994).
6. B. Poitevin, *Bull. WHO*, **77**, No. 2, 160-166 (1999).