Preclinical Study of Proproten-100 for Mutagenicity

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, Suppl. 1, pp. 187-188, September, 2009
Original article submitted August 1, 2008

Experiments on CBA/CaLac mice showed that single and course administration of proproten-100 did not increase the percent of abnormal metaphases in red bone marrow cells and produced no genotoxic effects in *Drosophila melanogaster* wing cells in the test of somatic mosaicism.

Key Words: *ultralow doses; proproten-100; mutagenicity; preclinical study*

The studies of ultralow doses of antibodies to neurospecific S-100 protein led us to a creation of a new preparation for the treatment of abstinent syndrome, proproten-100 [1,5]. This preparation is now approved for clinical application by Federal Agency for Health Care and Social Development [6].

Here we studied the cytogenetic effects of proproten-100 in two tests obligatory at the first stage of preclinical study of drugs and recommended by Pharmacological Committee of the Russian Federation for evaluation of mutagenic properties of pharmacological drugs [2]: counting of chromosome aberration (CA) in mouse red bone marrow cells (RBM) and gene mutations in the somatic mosaicism test system on *Drosophila melanogaster* wing cells.

MATERIALS AND METHODS

The effect of single and course treatment with proproten-100 was studied on 39 CBA/CaLac mice weighing 18-22 g (Rassvet nursery) maintained in accordance with European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (Strasburg, 1986) and Order of Ministry of Health of USSR No. 1179, October 10, 1983).

In series I, proproten-100 (antibodies to S-100 protein in a homeopathic dilution C_{1000}) was administered intragastrically to male mice in a single dose of 0.5 ml per 20 g body weight. In series II, the preparation was administered intragastrically to male and female mice in a dose of 0.5 ml per 20 g body weight daily for 5 days. RBM cells were fixed 24 h after the last dose. The mice comprising the reference groups received distilled water (negative control) in a volume of 0.25 ml per 10 g body weight. Cyclophosphamide (single dose of 20 mg/kg, $^1/_{10}$ LD_{50}) exhibiting cytogenetic activity was administered intraperitoneally as the positive control. For accumulation of metaphases, colchicine (0.025%, 0.01 ml per 1 g body weight) was injected intraperitoneally 1.5 h before the end of exposure (24 h). RBM cell preparations were prepared by the modified Ford method [4] and stained with azure II and eosin for 30 min.

In all groups, 50-100 metaphases from each animal were analyzed. Round unbroken cells with good chromosome spreading without overlapping and with module number (40) were selected for the analysis. The number of damaged cells and the number of CA per 100 RBM cells were determined. Among structural abnormalities, single fragments and exchanges were recorded. During statistical processing, the reliability of differences was evaluated using Student's *t* test.

For the analysis of somatic mosaicism, 10 virgin female drosophila flies (mwh/mwh) were placed together with flr^3/TMZ males in tubes containing standard nutrient medium. After 60-62 h the parents were separated and proproten-100 was added to the tubes in a volume of 500 µl per 2 ml medium. The offspring flies were examined starting from days 9-10 of the experiment, the mice with genotype mwh+/+flr^3 were selected and fixed in ethyl alcohol. Microscopic

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0007-4888/09/1483-0547

TABLE 1. Effect of Proproten-100 on the Incidence of CA in RBM Cells of Male CBA/CaLac Mice (*M*±*m*)

Parameter	Control (*n*=550)	Cyclophosphamide (*n*=580)	Proproten-100 (*n*=400)
Number of CA, %	1.29±0.57	35.57±6.72*	0.75±0.48**
including:			
single fragments	1.29±0.57	32.07±5.8*	0.75±0.48**
exchanges	0	3.50±1.34	0
Cells with multiple aberrations	0	1.00±0.52	0
Percent of aberrant cells	1.29±0.57	21.33±4.10*	0.75±0.48**

Note. $p<0.05$ compared to: *control, **cyclophosphamide.

preparations of the wings of these flies were prepared in the Fore fluid. Both sides of the wing consisting of cell monolayers were examined under a microscope at ×400. Mutant spots were classified as follows: spots containing 1-2 cells (mwh or flr), large solitary spots consisting of 3 and more cells (mwh or flr), and double spots consisting of neighboring mwh и flr cell clones. The incidence of each type of spots was analyzed separately [3].

During statistical processing, the reliability of differences was evaluated using χ^2 test [7].

RESULTS

Under the effect of cyclophosphamide, the number of cells with CA in 580 analyzed metaphase plates considerably increased compared to the control (Table 1). The total number of CA increased to 35.57±6.72%, primarily due to single fragments and exchanges (Table 1).

In mice receiving proproten-100, CA were less incident and were primarily presented by single fragments. The number of abnormal metaphases remained at the control level. The number of CA (single fragments and exhanges) was considerably lower than during cyclophosphamide treatment (Table 1).

In male mice receiving course treatment with proproten-100, the incidence of CA after 24 h was 0.75±0.48% (in 500 metaphases) and they were presented by single fragments, which corresponded to the control level. In female mice receiving proproten-100, the corresponding parameter was 1.50±0.50% (in 350 metaphases). CA were presented by single fragments.

Thus, single and course intragastric administration of proproten-100 did not increase the number of abnormal metaphases in mouse RBM cells.

In the test of somatic mosaicism, the number of mutant spots decreased under the effect of proproten-100 to 1 per wing (*vs*. 1.9 in the control), which attested to the absence of genotoxicity of proproten-100 for drosophila (Table 2).

TABLE 2. Somatic Mosaicism (mwh/flr Markers) on the Wing of *Drosophila melanogaster* (per 100 Cells)

Parameter	Control	Proproten-100
Mutant spots	57	30
including:		
single	35	21
large	19	7
double	3	2
Incidence of induction, 10^{-5}	1.9	1.0
χ^2	8.39	—

These findings suggest that single and course intragastric administration of proproten-100 does not increase the incidence of cytogenetic disturbances in RBM cells of CBA/CaLac mice. The preparation exhibits no genetic toxicity and even decreases spontaneous level of mutations and recombinations on the wing of *Drosophila melanogaster*.

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