PHARMACOLOGY AND TOXICOLOGY

Specific Activities of Poetam Preparation (Superlow-Doses of Antibodies to Erythropoietin) and Recombinant Erythropoietin

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We compared the capacity of superlow-dose of antibodies to erythropoietin (Poetam) and recombinant erythropoietin (Recormon) to stimulate the recovery of adriamycin-suppressed erythropoiesis in mice. Both preparations exhibited high erythron activation capacity and considerably increased the content of erythrocytes and reticulocytes in the peripheral blood and content of erythrokaryocytes and erythroid precursors in the hemopoietic tissue of experimental animals. The effect of Recormon manifested immediately after injection, while the effect of Poetam was somewhat delayed, but more lasting (due to activation of host erythropoietin system).

Key Words: *myelosuppression; erythropoietin; superlow doses of antibodies; erythropoiesis*

Anemias caused by hemopoiesis insufficiency remain a pressing problem of modern hematology. The method based on the use of recombinant erythropoietin (EPO, natural hemopoiesis regulator) are widely used in the treatment of these diseases [5,7]. However, these drugs, active stimulators of the erythroid hemopoietic stem, are not free from side effects, such as hypertension, hemostasis disorders, serum iron deficiency, *etc.* [6,8,9]. Poetam, an original preparation containing superlow doses of antibodies to EPO, has an undeniable advantage: it can be used for a long time, because it causes no side effects. We previously studied specific activity of Poetam. The drug activated bone marrow erythropoiesis, suppressed mainly because of increased concentrations of humoral factors in the peripheral blood serum. The results indicated that the drug effect was aimed mainly towards stimulation of the distant component of erythropoiesis neurohumoral regulation [2].

We compared the specific activities and mechanisms of action of superlow-dose of antibodies to EPO (Poetam) and recombinant human EPO (Recormon).

MATERIALS AND METHODS

The study was carried out on 2-month-old male CBA/CaLac mice (n=315). Conventional certified 1st category mice from Breeding Center of Institute of Pharmacology were divided into 3 groups: 2 experimental and control. Cytostatic myelosuppres-

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sion was induced by a single intraperitoneal injection of adriamycin in the maximum tolerable dose (MTD; 6 mg/kg according to probit analysis). Group 1 animals received Poetam (superlow-dose preparation of antibodies to EPO, Materia Medica Holding). The preparation was administered intragastrically through a tube, 0.2 ml/mouse daily, starting from day 5 before adriamycin injection until day 10 after the cytostatic treatment. Group 2 mice were injected with recombinant human EPO (epoetin-β) Recormon (Hoffmann-La Roche Ltd.) in a dose of 20 U/mouse subcutaneously according to the same protocol. Controls received distilled water orally (0.2 ml/mouse) for 15 days and an injection of adriamycin. Basal values were evaluated in intact mice.

The animals were sacrificed by cervical dislocation or decapitation under ether narcosis on days 3-10, 12, and 15 after injection of the cytostatic. The peripheral components of the erythron system (hemoglobin content, erythrocyte count, hematocrit, mean corpuscular concentration of hemoglobin) were evaluated in mice on an ABACUS automated hematological analyzer (Diatron) in the veterinarian mode. Reticulocyte count in the peripheral blood and parameters of bone marrow hemopoiesis (total count of myelokaryocytes, myelogram) were evaluated routinely [3]. The content of committed erythropoiesis precursor cells (CFU-E) in the bone marrow was studied *in vitro* by cloning nonadhesive myelokaryocytes in semisolid culture medium [1]. Serum EPO was measured by enzyme immunoassay using Biomerica kit according to manufacturer's instruction. The intensity of staining was evaluated on an Uniplan AIFR-01 counter (PI-KONT Firm).

The data were processed statistically using Student's t and Mann—Whitney tests.

RESULTS

Treatment with the test preparations accelerated recovery of erythropoiesis in mice suppressed by adriamycin in MTD. Both test drugs increased the counts of erythrokaryocytes in hemopoietic tissue in comparison with control animals receiving the solvent on days 4-6, 8-10, 12 (Recormon) and on days 6, 9, and 12 (Poetam). Recormon caused a more pronounced increase in content of bone marrow erythroid stem cells (up to 359.6% of control on day 5) during the initial period of the experiment, while later (days 12, 15) the superlow-dose antibodies to EPO were more effective. On day 15, the content of erythroid nucleated cells in the hemopoietic tissue of mice treated with Poetam was significantly higher than in animals treated with Recormon (Fig. 1).

Both drugs modified the colony-forming capacity of the regenerating bone marrow. The growth of erythroid colonies in methylcellulose medium increased on days 4-10 and 15 after Recormon treatment (up to 300% on day 4) and on days 4, 10, 12, and 15 after Poetam treatment (up to 218.11% on day 15). Comparative analysis of this parameter in two experimental groups revealed higher level of erythroid colony formation in mice treated with recombinant EPO on days 3-8 of the experiment. At later terms Poetam treatment was associated with

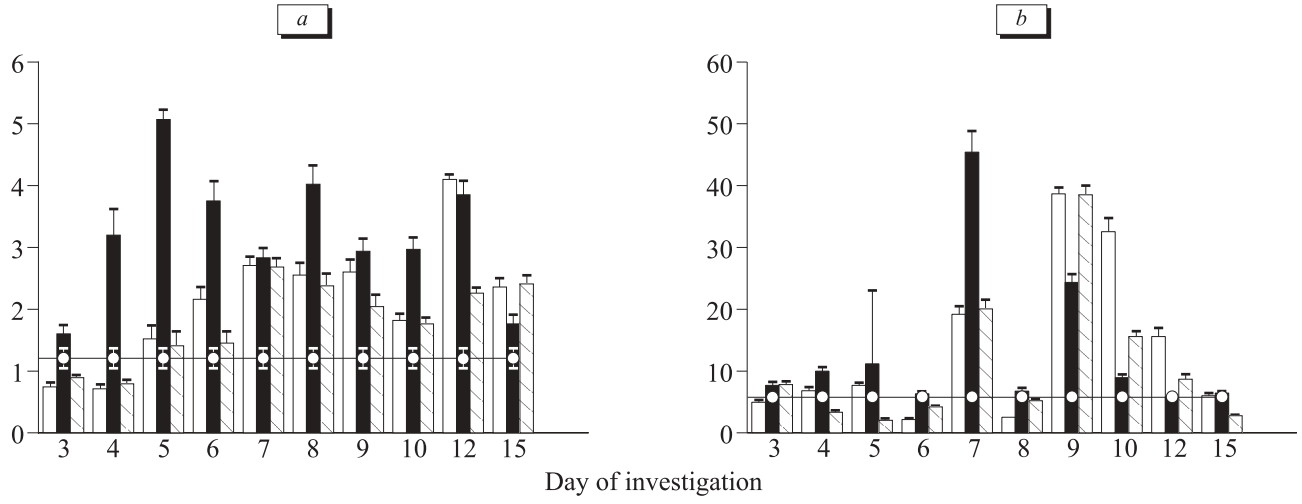

Fig. 1. Content of erythrokaryocytes (*10^6/femur; *a*) and erythroid precursor cells (per 10^5 nonadhesive myelokaryocytes; *b*) in the bone marrow of CBA/CaLac mice treated with adriamycin (6 mg/kg) and Poetam (light bars), adriamycin and Recormon (dark bars), or adriamycin and distilled water (cross-hatched bars). Here and in Fig. 2: horizontal line shows the basal level. Confidence intervals at p=0.05.

a statistically significant increase in the content of erythropoiesis precursors in the bone marrow on days 9, 10, and 12 of the experiment (Fig. 1).

The more pronounced (compared to control mice) increase in blood reticulocyte count throughout the observation period reflected activation of erythropoiesis during treatment with the stimulants. Reticulocytosis was paralleled by an increase in erythrocyte count, hematocrit, and hemoglobin content. More pronounced changes in these parameters were observed in animals treated with Recormon, except day 15, when reticulocyte count was significantly higher in animals of experimental group 1 (Fig. 2). Comparison of the qualitative characteristics of mature red blood cells showed significant differences between the experimental groups. The mean corpuscular concentration of hemoglobin decreased in animals treated with Recormon in comparison with the controls, while Poetam treatment significantly increased erythrocyte saturation with hemoglobin on days 4, 6, 8, 9, and 12 of the experiment (Fig. 2).

The study of the erythron regulatory component during the experiment showed that EPO production increased on days 4-5 after injection of adriamycin in control mice: the peak value (168% of the initial level) was observed on day 6 of the experiment. This was followed by a reduction of blood hemopoietin level, after which a pronounced increase was observed on days 10-12 (on day 10 hemopoietin content 45-fold surpassed the mean basal level; Table 1). The detected increase in EPO content seems to be a reaction of the erythropoietin-producing system to anemia developing after adriamycin injection increasing the production of erythroid precursor cells in the bone marrow on days 7 and 9-10 of the experiment.

Blood EPO level in mice treated with Recormon significantly increased throughout the experiment. The maximum values (458 and 311 U/ml) were recorded on days 3 and 4 after injection of the cytostatic, respectively. The decrease in EPO content was recorded only on day 15. Analysis of the time course of this parameter indicates that high

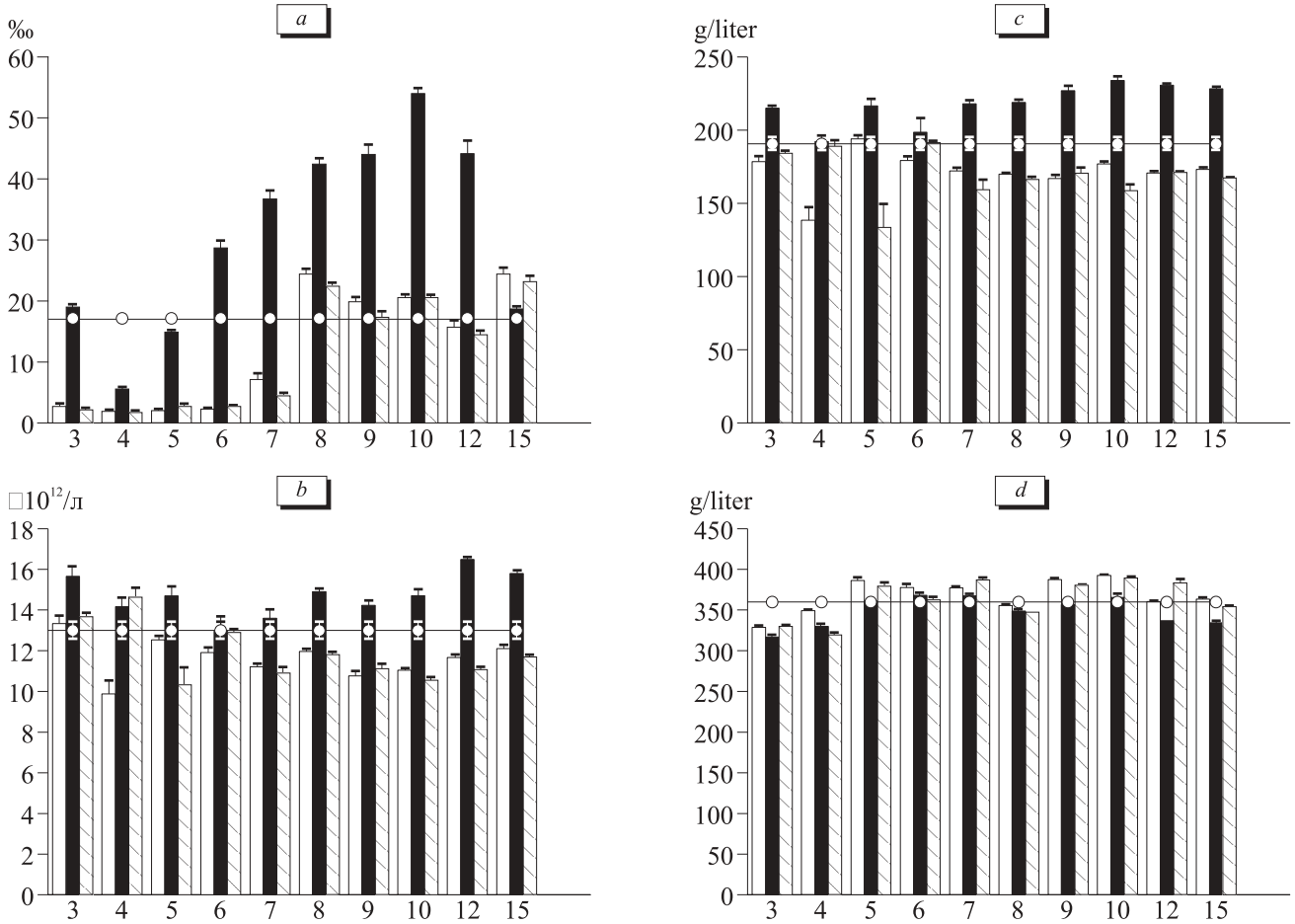

Fig. 2. Content of reticulocytes (*a*), erythrocytes (*b*), hemoglobin (*c*), and mean corpuscular concentration of hemoglobin (*d*) in the peripheral blood of CBA/CaLac mice injected with adriamycin (6 mg/kg) and Poetam (light bars), adriamycin and Recormon (dark bars), or adriamycin and distilled water (cross-hatched bars).

TABLE 1. Serum Content of EPO in CBA/CaLac Mice Injected with Adriamycin (6 mg/kg) and Poetam (Group 1), Adriamycin and Recormon (Group 2), or Adriamycin and Distilled Water (Control): Enzyme Immunoassay Values (U/ml; $X \pm m$)

Day of investigation; group		EPO
Before injection		5.79±0.32
3	control	4.33±0.27*
	1	7.36±0.40*+
	2	457.78±3.91*+x
4	control	8.79±0.42*
1		9.24±0.69*
	2	310.79±12.69*+x
5	control	8.43±0.52*
	1	8.65±0.84*
	2	124.71±1.52*+x
6	control	9.73±1.02*
	1	7.66±0.82
	2	103.78±1.58*+x
7	control	4.74±0.35*
	1	4.47±0.36*
	2	230.90±0.22*+x
8	control	4.64±0.24*
	1	5.71±0.34+
	2	59.39±0.61*+x
9	control	5.72±0.66
	1	4.35±0.33*
	2	72.37±1.60*+x
10	control	258.72±30.61*
	1	468.44±3.45*+
	2	106.52±2.19*+x
12	control	42.53±8.69*
	1	40.47±6.21*
	2	136.97±0.00*+x
15	control	3.53±0.23*
	1	3.66±0.65*
	2	2.86±0.13*+

Note. $p<0.05$ compared to *initial level, +control, xexperimental group 1.

level of EPO in the blood of these animals was a result of treatment with recombinant EPO (Recormon) in high doses. The level of EPO decreased when the treatment was discontinued.

Treatment with superlow-dose preparation of antibodies to EPO before and after adriamycin injection led to a certain increase in the serum level of endogenous EPO starting from the earliest periods of the study. However, a manifest increase in this parameter was observed on days 10-12. Increased content of erythroid precursors and erythrokaryocytes in hemopoietic tissue on days 10-12 and 12, respectively, in comparison with the parameters in controls receiving the solvent, seemed to be a result of this phenomenon.

Hence, recombinant EPO and superlow-dose preparation of antibodies to EPO exhibited pronounced erythropoiesis-stimulating effects. Treatment with these drugs caused a pronounced increase in the content of erythrokaryocytes in the hemopoietic tissue and erythrocytes and reticulocytes in the peripheral blood of experimental animals during postcytostatic recovery. Poetam treatment under conditions of cytostatic myelosuppression led to production of qualitatively more valuable (in comparison with that after Recormon) erythrocytes with a higher corpuscular concentration of hemoglobin.

Specific activity of Recormon manifests directly after its injection and is short-lasting, while the effect of Poetam is delayed and long-lasting (due to mobilization of humoral systems of erythropoiesis regulation, which takes some time). The stimulatory effect of these drugs on erythropoiesis precursor cells can be attributed to their effect on EPO production. The maximum increase in the content of endogenous EPO in the blood of mice treated with Poetam was observed during the same period (on day 10 after injection of the cytostatic) as in animals receiving the solvent before and after adriamycin, but the value was 2-fold higher in the Poetam group in comparison with the controls. This fact attests to potentiating effect of Poetam on the production of renal EPO under conditions of hypoplastic anemia.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
2. V. V. Zhdanov, E. V. Simanina, Yu. L. Dugina, *et al.*, *Byull. Eksp. Biol. Med.*, Suppl., 85-87 (2003).
3. V. V. Men'shikov, Ed., *Laboratory Methods of Investigation in Clinical Practice: Handbook* [in Russian], Moscow (1987).
4. J. W. Fisher, *Exp. Biol. Med.* (Maywood), **228**, No. 1, 1-14 (2003).
5. R. G. Geissler, P. Schulte, and A. Ganser, *Int. J. Hematol.*, **65**, No. 4, 339-354 (1997).
6. K. Jabs and W. E. Harmon, *Adv. Ren. Replace Ther.*, **3**, No. 1, 24-36 (1996).
7. W. Jelkmann and E. Metzen, *Ann. Anat.*, **178**, No. 5, 391-403 (1996).
8. G. Singbart, *Clin. Investig.*, **72**, Suppl. 6, S36-S43 (1994).
9. B. Sowade, O. Sowade, K. Mocks, *et al.*, *Int. J. Mol. Med.*, **1**, No. 2, 303-314 (1998).