A Comparative Study of Stimulation of Erythropoiesis during Renal Anemia with the Preparation of Antibodies against Erythropoietin in Ultralow Doses and Recormon

O. I. Epshtein*, A. M. Dygai, V. V. Zhdanov, S. A. Sergeeva*, G. N. Zyuz'kov, E. V. Udut, T. Yu. Khrichkova, E. V. Simanina, L. A. Gur'yantseva, L. A. Stavrova, and E. D. Gol'dberg

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We compared the stimulatory effects of a preparation of antibodies against erythropoietin in ultralow doses (Poetam) and recombinant erythropoietin (Recormon) on erythropoiesis that was suppressed by carboplatin. Both drugs possessed high erythropoiesis-stimulating activity, which was manifested in an increase in the number of erythrokaryocytes and erythroid precursors in hemopoietic tissue and count of erythrokaryocytes and reticulocytes in the peripheral blood during postcytostatic recovery. Recormon produced a rapid and short-term stimulatory effect on the erythron. The effect of Poetam developed slowly, but persisted for a longer time.

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

The number of diseases accompanied by anemia syndrome progressively increases. Hence, the search for new methods of correction of this disorder is an urgent problem. Erythropoiesis-stimulating treatment with recombinant forms of a natural hemopoietic regulator (erythropoietin, EP) is most effective under these conditions [5,7]. However, these drugs cause several side effects, including hemostatic disorders, hypertension, and serum iron deficiency [6,8,9]. Original preparation Poetam, which contains antibodies against EP in ultralow doses, has several advantages. No side effects were observed even after long-term treatment with this drug. Our previous studies revealed a potent erythropoiesis-stimulating effect of Poetam on the model of adriamycin-induced myelosuppression. This effect was related to an increase in plasma concentration of humoral factors, and, therefore, stimulation of distant neurohormonal mechanisms of erythropoiesis regulation [2]. The model of myelosuppression induced by carboplatin is of considerable methodological interest. It allows us to evaluate the mechanisms of hemopoiesis regulation and to study the effect of hemopoietic stimulators, since nephrotoxicity of this cytostatic contributes to the pathogenesis of myelosuppression [4].

Here we evaluated specific activity and mechanism underlying the action of Poetam, a preparation containing antibodies to EP in ultralow doses, during carboplatin-induced myelosuppression. Recombinant human EP, Recormon, served as the reference drug.

MATERIALS AND METHODS

Experiments were performed on 465 CBA/CaLac mice (class I conventional strain) aging 2 months and obtained from the nursery of the Institute of

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

Pharmacology (Tomsk Research Center). The mice were divided into 3 groups. Myelosuppression was modeled by single intraperitoneal injection of carboplatin in the maximum tolerated dose (MTD 100 mg/kg, probit analysis). Poetam (Materia Medica Holding) was administered to group 1 animals daily through a probe over 5 days before and 10 days after cytostatic treatment in a volume of 0.2 ml. The preparation of recombinant human EP (epoetin-β), Recormon (20 U, Hoffman-La Roche Ltd.), was injected subcutaneously to group 2 mice in the same periods of the study. Control animals were treated with carboplatin and daily received distilled water (0.2 ml perorally) for 15 days. Baseline parameters were measured in intact mice.

The animals were euthanized by cervical dislocation or decapitation under ether anesthesia on days 3-10, 12, and 15. The function of the peripheral erythron (hemoglobin level, erythrocyte count, hematocrit, and average corpuscular concentration of hemoglobin) in treated and control mice was studied on an ABACUS automatic blood analyzer (Diatron) under veterinary conditions. The count of peripheral blood reticulocytes and parameters of bone marrow hemopoiesis (total number of bone marrow myelokaryocytes and myelogram) were estimated by standard methods [3]. The number of committed precursor cells for erythropoiesis in the bone marrow was measured by *in vitro* cloning of nonadherent myelokaryocytes in a semisolid medium [1]. Serum EP level was measured by enzyme immunoassay (ELISA) using Biomerica kits according to the manufacturer's recommendations. The intensity of staining in samples was estimated on an Uniplan AIFR-01 counter (PIKON).

The results were analyzed by the method of variation statistics (Student's *t* test). The significance of differences between samples with the non-Gaussian distribution of variants was evaluated by nonparametric Wilcoxon test and Mann—Whitney test.

RESULTS

Administration of the test drugs significantly modulated the recovery of erythropoiesis, which was suppressed by carboplatin. The number of peripheral blood reticulocytes in treated mice increased more significantly than in the cytostatic control. In all periods of the study this parameter was increased in animals receiving Recormon (up to 1003.8% of the control, day 5) and antibodies against EP in ultralow doses (up to 392.0% of the control, day 12). In nearly all periods of the study, increased migration of reticulocytes into the blood was accompanied by an increase in the number of erythrocytes, hematocrit, and hemoglobin concentration in animals receiving Recormon and Poetam (Fig. 1). Changes in these parameters were most pronounced in animals receiving Recormon. However, in some periods the number of peripheral blood reticulocytes, hematocrit, and hemoglobin concentration significantly decreased in mice receiving Recormon (days 9, 10, and 12) and Poetam (day 9). The observed changes were most pronounced in animals of the Recormon group.

TABLE 1. Serum EP Level in CAB/CaLac Mice after Single Administration of Carboplatin and Treatment with the Solvent (1), Recormon (2), or Poetam (3). EIA (U/ml, $X \pm m$)

Period, days		EP
Before administration		1.24±0.05
3	1	0.21±0.02*
	2	86.24±0.52*+
	3	0.74±0.11*+x
4	1	1.35±0.08
	2	50.66*+
	3	1.31±0.02x
5	1	1.35±0.22*
	2	145.42*+
	3	5.47±0.06+x
6	1	2.42±0.21*
	2	68.83±1.17*+
	3	3.60±0.12*+x
7	1	2.26±0.17*
	2	54.86±0.53*+
	3	3.73±0.08*+x
8	1	4.33±0.11*
	2	0.85*+
	3	2.21±0.21*+x
9	1	2.81±0.48*
	2	62.08±1.15*+
	3	14.89±0.63*+x
10	1	33.39±1.15*
	2	127.69±2.74*+
	3	75.93±0.53*+x
12	1	35.82±0.23*
	2	75.77±1.25*+
	3	30.91±0.59*+x
15	1	15.34±0.45*
	2	2.76±0.20*+
	3	3.87±0.11*+x

Note. $p<0.05$: *compared to the pretreatment level; +compared to the solvent; xcompared to Recormon.

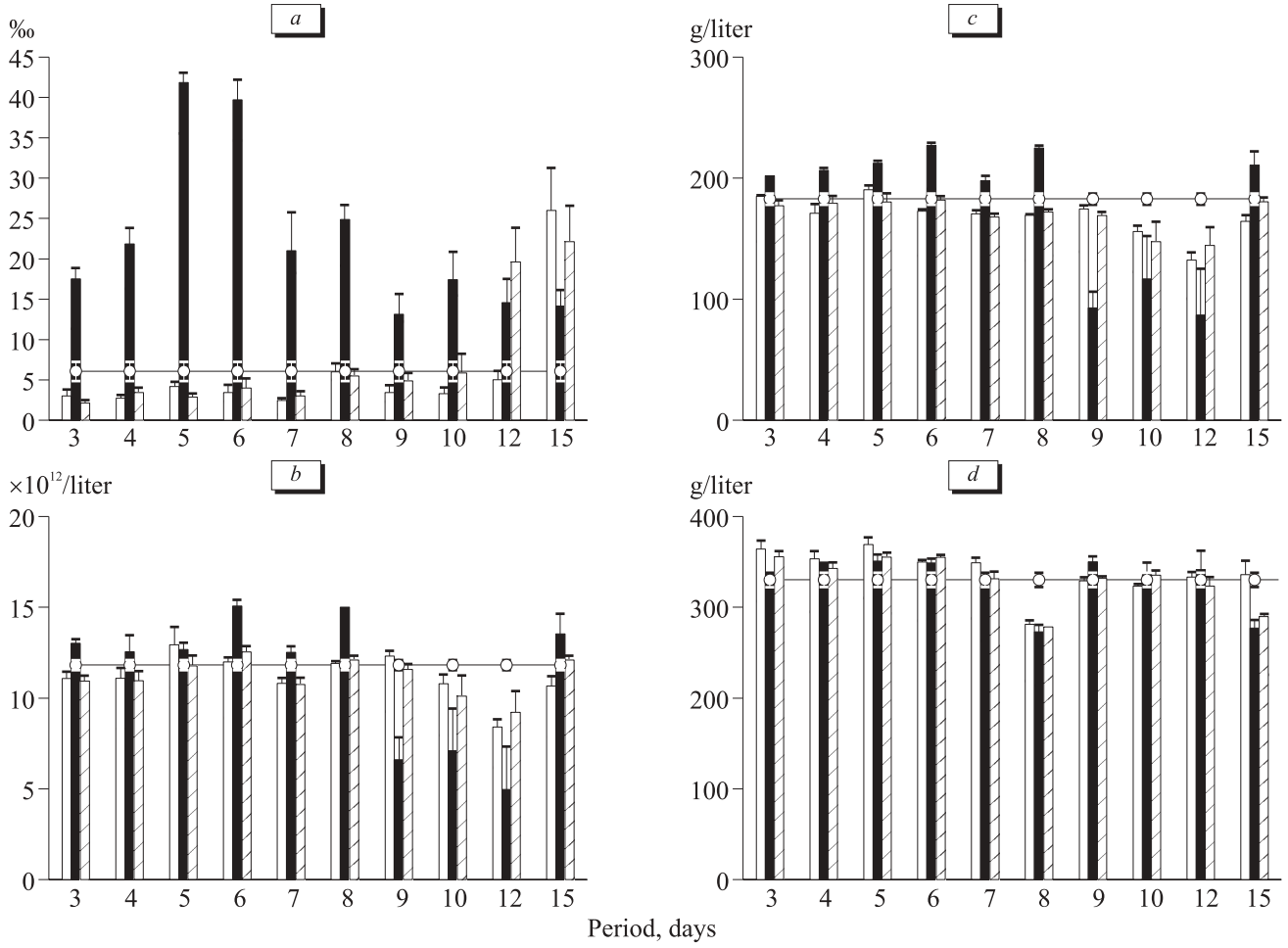

Fig. 1. Number of reticulocytes (*a*) and erythrocytes (*b*), hemoglobin level (*c*), and average corpuscular concentration of hemoglobin (*d*) in the peripheral blood of CBA/CaLac mice receiving the solvent (light bars), Recormon (dark bars), or Poetam (shaded bars) after single administration of carboplatin in a dose of 100 mg/kg. Here and in Fig. 2: solid horizontal line, baseline level. Confidence intervals at p=0.05.

Peripheral blood parameters reflected changes in bone marrow erythropoiesis. We revealed an increase in the number of erythrokaryocytes in hemopoietic tissue of mice receiving Recormon (days 3-5, 7, and 12) and Poetam (days 4, 5, and 12) compared to control animals of the solvent group. In the early period (days 3-5) parameters of bone marrow erythropoiesis increased most significantly in Recormon-receiving mice (up to 700% of the control, day 5). However, Poetam was more potent than Recormon during the late period (days 6, 8, and 15). On day 15, the number of nucleated erythroid cells in the bone marrow of Poetam-treated mice was 74.74% higher than in animals of the Recormon group. During this period the number of erythrocytes in Recormon-receiving mice decreased to 59.2% of the control. These changes and decrease in quantitative parameters of the erythron on days 9-12 were probably associated with the scheme of drug treatment. Experimental animals received the cytostatic after stimulation of the erythroid hemopoietic stem with Recormon, which resulted in an increase in the damaging effect of carboplatin on hemopoiesis (Fig. 2).

The test drugs modulated colony-forming activity of the regenerating bone marrow. The course of treatment with hemopoietic stimulators increased the yield of erythroid colonies in the methylcellulose medium. This effect was observed in animals receiving Recormon (days 3, 7, and 8; up to 248% of the control on day 8) or treated perorally with Poetam (days 4, 8, and 15; up to 338.3% of the control on day 15). However, administration of Recormon during the late period was accompanied by a decrease in this parameter compared to the control (up to 53.33% on day 15, Fig. 2).

Hemopoiesis is regulated by local and distant mechanisms. Humoral hemopoietic factors play an important role in the response of hemopoietic tissue. We revealed that the test drugs produce a strong

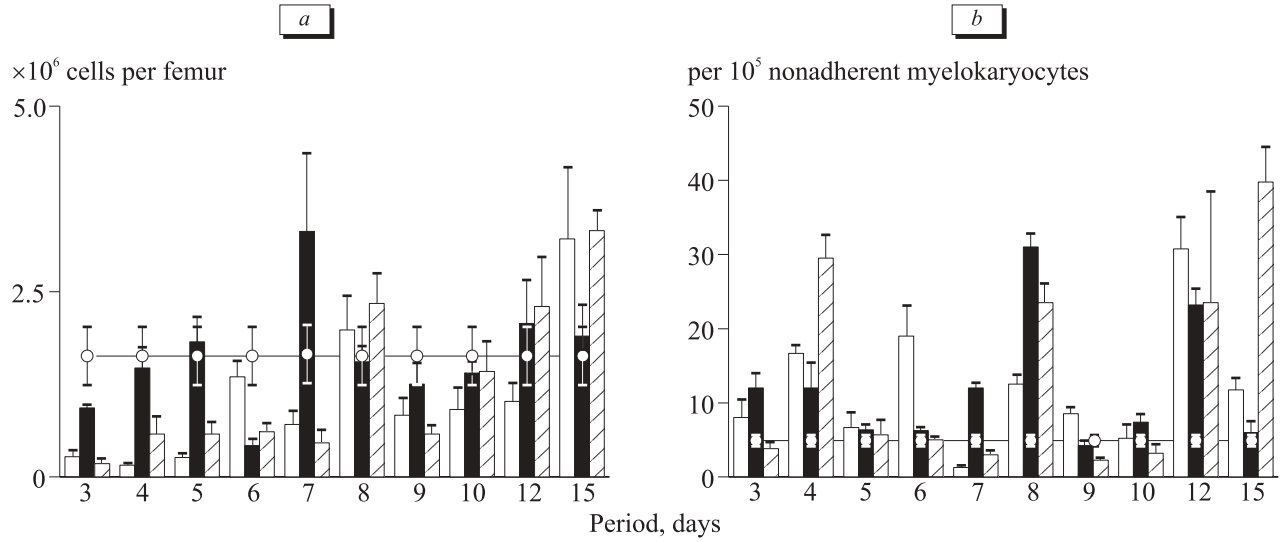

Fig. 2. Number of erythrokaryocytes (*a*) and erythroid precursors (*b*) in the bone marrow of CBA/CaLac mice receiving the solvent (light bars), Recormon (dark bars), or Poetam (shaded bars) after single administration of carboplatin in a dose of 100 mg/kg.

effect on serum EP level. EP production in the kidneys was suppressed on day 3 after injection of carboplatin in MTD. However, administration of carboplatin after treatment with hemopoietic stimulators was followed by a significant increase in EP concentration. This parameter increased most significantly in mice receiving daily injections of Recormon (up to 10,771.85% of the control on day 5), which was related to the detection of an exogenous substance in blood plasma. However, serum EP level in treated mice significantly decreased compared to the cytostatic control on day 15 (cessation of treatment with hemopoietic stimulators). It was particularly pronounced in Recormon-receiving animals (day 15, Table 1). These changes were probably related to the development of withdrawal syndrome, which resulted from the cessation of treatment with EP preparations.

Our results indicate that the test drugs exhibit high erythropoiesis-stimulating activity on the model of carboplatin-induced myelosuppression. Under these conditions, the specific effect of Recormon developed rapidly, but was short-lasting and impaired deep reserve of erythropoiesis, which manifested in exhaustion of the pool of committed precursor cells. Poetam produced a delayed effect not accompanied by exhaustion of the pool of hemopoietic precursors and impairment of EP production in the kidneys.

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