Mechanisms of Regulation of Hemopoiesis during Experimental Cytostatic Myelosuppression Induced by Carboplatin

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The mechanisms of suppression and recovery of the bone marrow erythroid stem were studied on the model of myelosuppression induced by administration of carboplatin in the maximum tolerated dose. Single administration of the cytostatic led to the development of long-term hypoplasia of hemopoiesis. Despite enhanced proliferation of erythroid precursor caused by increased erythropoietic activity of blood plasma and bone marrow cells, inhibition of cell maturation prevented recovery of the content of morphologically recognizable erythrokaryocytes in the bone marrow.

Key Words: *carboplatin; erythropoietic activity; erythropoiesis*

Functional studies of hemopoietic tissue always involve a variety of experimental models of pathological processes. A large body of evidence indicates that cytostatic-induced myelosuppression is a convenient model for evaluation of the mechanisms of myelosuppression syndrome and development of new methods for stimulation of suppressed hemopoiesis. Significant differences exist in the degree and duration of hemopoietic stem suppression induced by cytostatics with various mechanisms of action. For example, the erythroid stem is most sensitive to the inhibitory effect of anthracycline antibiotics. The sensitivity of other stems to this antibiotic decreases in the following order: lymphoid stem>myeloid stem>thrombocytic stem. Alkylating agent cyclophosphamide is primarily toxic to granulocytopoiesis and erythropoiesis [2].

Complex platinum compounds (*e.g.*, carboplatin) is a promising group of cytostatics with a wide range of chemotherapeutic activity [1,9]. Previous studies showed that administration of these drugs in high doses induced inhibition of bone marrow hemopoiesis (especially erythropoiesis) in animals [5]. Here we studied the mechanisms of destruction and recovery of the hemopoietic tissue during experimental myelosuppression induced by carboplatin.

MATERIALS AND METHODS

Experiments were performed on 150 CBA/CaLac mice (class I conventional strain) aging 2 months and obtained from the nursery of Institute of Pharmacology (Tomsk Research Center). The mice received single intraperitoneal injection of carboplatin in a maximum tolerated dose (100 mg/kg). The animals were sacrificed by cervical dislocation or decapitation under ether anesthesia on days 1-15. The function of the peripheral erythron (hemoglobin level, erythrocyte count, hematocrit, and hemoglobin concentration) was studied on an ABACUS

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automatic blood analyzer (Diatron) under veterinary conditions. The count of peripheral blood reticulocytes and leukocytes and total number and qualitative composition of bone marrow myelokaryocytes were estimated routinely [6]. The number of committed precursors of erythropoiesis (CFU-E) in the bone marrow tissue was measured by *in vitro* cloning in methylcellulose [3]. The study of erythropoietic activity (EPA) in conditioned media from adherent and nonadherent cells of the hemopoiesis-inducing microenvironment (HIM) was performed in semisolid medium with myelokaryocytes from intact mice [3]. Blood erythropoietin concentration in experimental animals was evaluated by enzyme immunoassay with Sangui Bio Tech Inc. kit according to manufacturer's recommendations. The results were analyzed by Student's *t* test [7].

RESULTS

Carboplatin induced significant changes in the blood system. The signs of suppression of bone marrow hemopoiesis were revealed on day 1 after administration of this drug. The total number of myelokaryocytes remained low throughout the observation period; the minimum values were attained on days 3 and 12, while on day 8 this parameter significantly surpassed the baseline values. Erythrokaryocyte count was $(0.27\pm0.04)\times10^6$ on day 3 (*vs.* $(1.64\pm0.20)\times10^6$ per femur in the control); this parameter returned to normal on day 15 (Fig. 1, *a*). Similar changes were observed in the peripheral blood. Reticulocyte count decreased on days 3-12 and reticulocytosis developed on day 15 days after cytostatic treatment (433% of the basal level; Fig. 2, *a*). Erythrocyte count varied within the normal

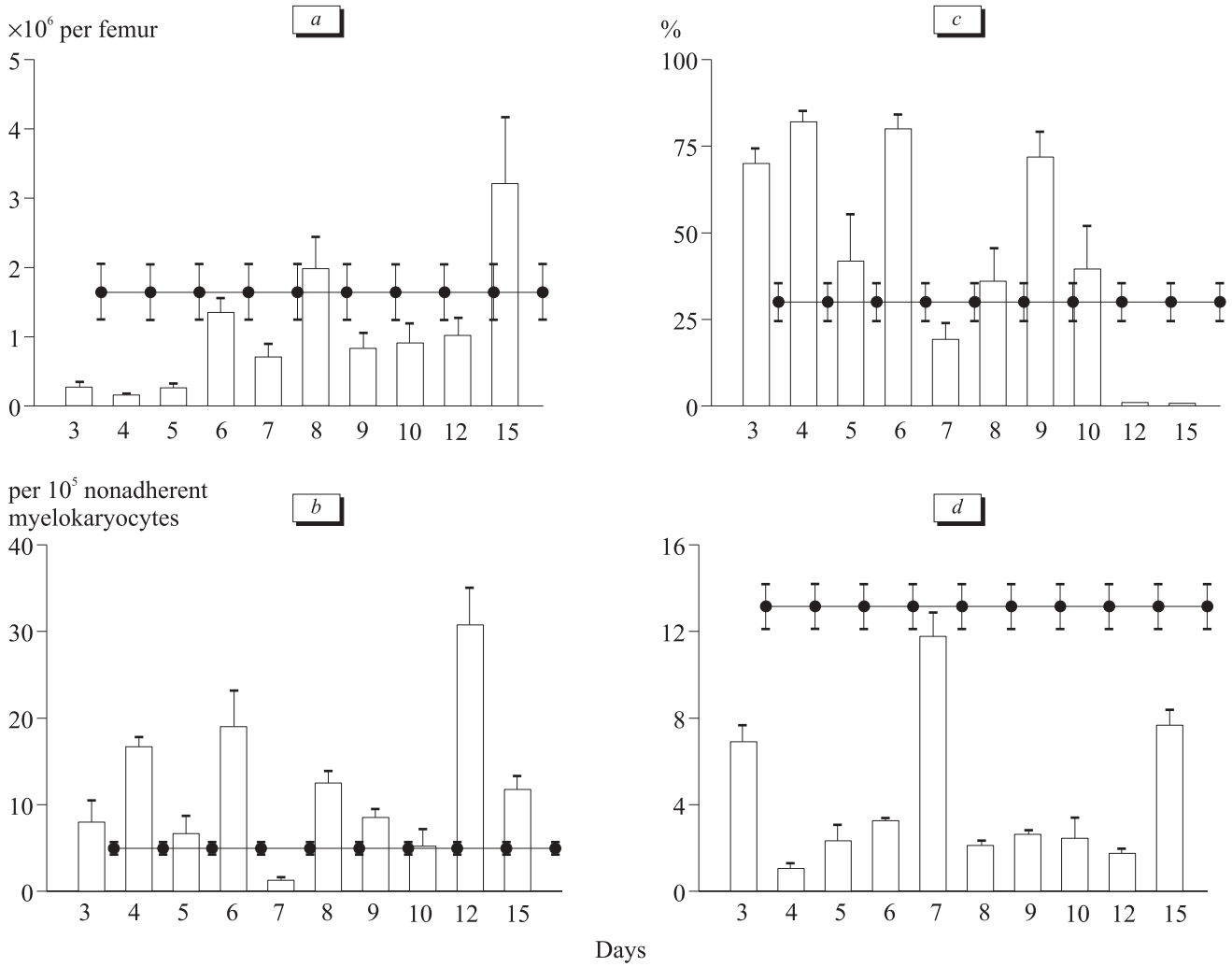

Fig. 1. Number of erythrokaryocytes ($\times10^6$ cells per femur, *a*) and erythroid precursor cells in the bone marrow (per 10^5 nonadherent myelokaryocytes, *b*) ratio of CFU-E in S-phase of the mitotic cycle (%, *c*), and maturation of CFU-E (*d*) in CBA/CaLac mice after single carboplatin treatment. Here and in Fig. 2: solid line, basal level. Confidence intervals at p=0.05.

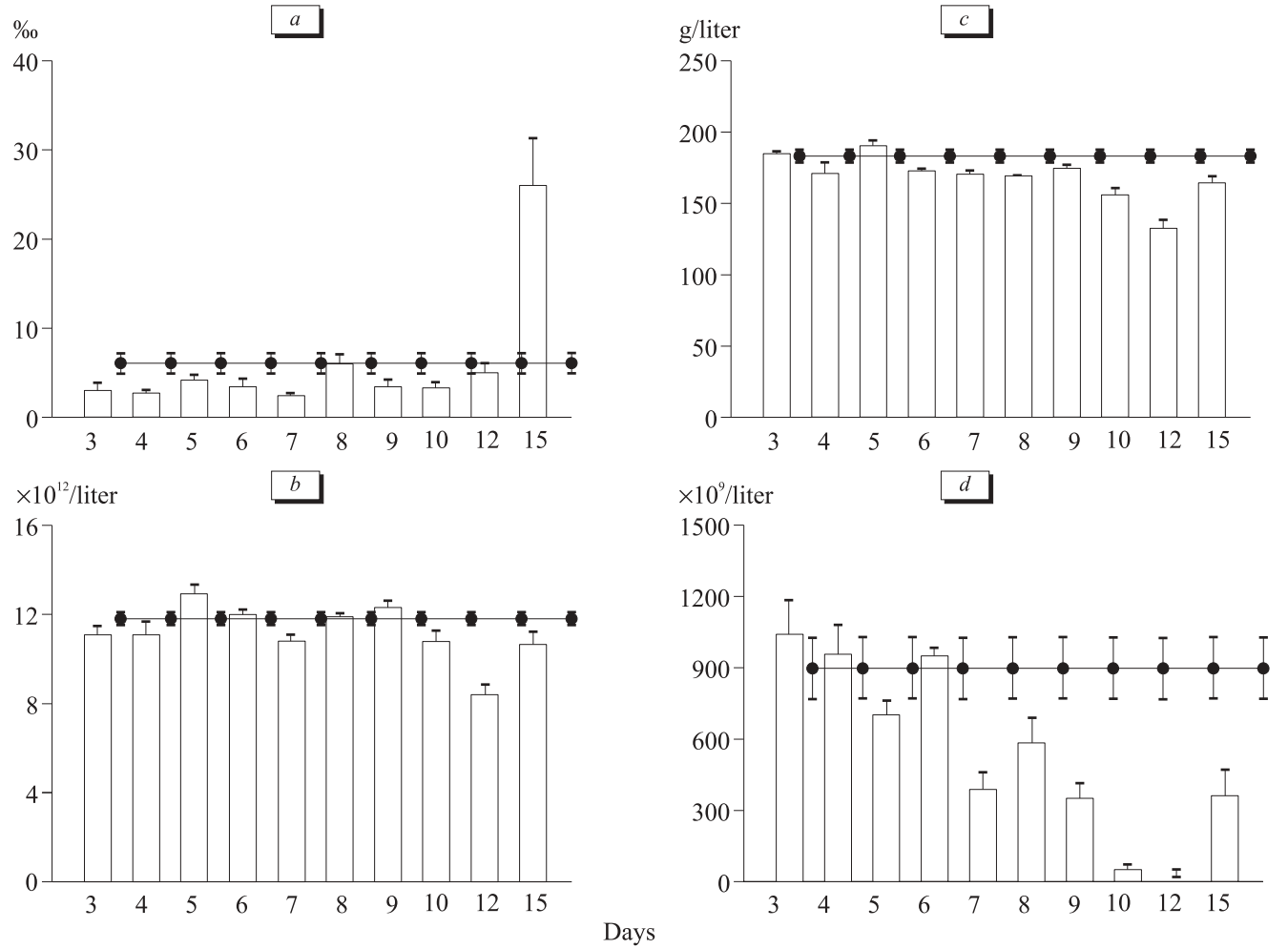

Fig. 2. Content of reticulocytes (‰, *a*), erythrocytes (×10¹²/liter, *b*), hemoglobin (g/liter, *c*), and platelets (10⁹/liter, *d*) in the peripheral blood of CBA/CaLac mice after single treatment with carboplatin.

range (not more than 10%; Fig. 2, *b*). Hemoglobin content was below the normal throughout the experiment, except day 5, when hemoglobin level surpassed the normal by 4.12% (Fig. 2, *c*). The number of peripheral blood platelets in mice was minimum on days 10 and 12 after carboplatin administration (49.86±10.96 and 36.66±5.46, respectively, *vs.* 895.70±65.38 G/liter in the control; Fig. 2, *d*).

Study of CFU-E function showed that carboplatin decreases the number of erythroid colonies in 3-day-old cultures only on day 7 (1.25×10^5 *vs.* 5.00×10^5 nonadherent myelokaryocytes in control animals; Fig. 1, *b*). In other period of the study, this parameter was above the basal level. Proliferative activity of CFU-E increased on days 3-6 and 8-10, but decreased on days 7 and 12-15. The intensity of differentiation remained low at various periods, but underwent a compensatory increase on days 7 and 15. These changes contributed to an increase in the number of morphologically recognizable erythroid precursors in the bone marrow (Fig. 1, *a*, *d*).

The study of humoral factors circulating in peripheral blood and secreted by HIM cells is important for evaluation of the intensity of proliferation and differentiation of hemopoietic cells [4,8,10]. Cytostatic treatment increased EPA of adherent bone marrow cells, especially on days 4 and 15 (Table 1). Carboplatin had little effect on the content of EPA factors in supernatants from nonadherent bone marrow cells (Table 1). Plasma erythropoietin concentration (additionally measured by ELISA) significantly decreased on 3 day, progressively increased in the follow-up period, and exceeded the basal level starting from day 5 after carboplatin administration (Table 1).

Studying the ratio between morphologically recognizable erythrokaryocytes and CFU-E showed that carboplatin significantly decreases the number of bone marrow erythroid cells, but has little effect on colony-forming activity. The increase in CFU-E proliferation results from the production of EPA factors released from adherent bone marrow cells

TABLE 1. EPA of Conditioned Medium from Bone Marrow Cells ($\times 10^5$ Karyocytes) and Plasma Erythropoietin Concentration (U/ml, EIA) in CBA/CaLac Mice after Single Treatment with Carboplatin ($X \pm m$)

Period, days	EPA		Erythropoietin
	adherent cells	nonadherent cells	
Before treatment	0.71±0.19	0.71±0.19	1.24±0.05
3	1.00±0.14	0.57±0.20	0.21±0.02*
4	1.57±0.20*	0.71±0.24	1.35±0.08
5	0.57±0.14	1.29±0.29	1.35±0.22*
6	0.86±0.27	0.57±0.20	2.42±0.21*
7	1.14±0.27	0.43±0.14	2.26±0.17*
8	0.29±0.13	0.43±0.20	4.33±0.11*
9	1.14±0.23	0.86±0.18	2.81±0.48*
10	1.00±0.15	0.43±0.20	33.39±1.15*
12	0.29±0.13	0.57±0.14	35.82±0.23*
15	1.86±0.18*	0.86±0.23	15.34±0.45*

Note. *$p<0.05$ compared to initial level.

in the early period of the study. The observed changes probably prevent the decrease in plasma erythropoietin concentration during this stage. The increase in the number of CFU-E during the late stage is determined by synergistic effect of this hemopoietin and humoral factors produced by HIM cells. Despite increased proliferative activity of CFU-E, cytostatic treatment was followed by impairment of their differentiation. These changes are the major cause of hypoplasia of the erythroid hemopoietic stem.

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