Mechanisms of Regulation of Erythropoiesis during Hemolytic Anemia

G. N. Zyuz'kov, E. V. Abramova, A. M. Dygai, and E. D. Gol'dberg

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 10, pp. 378-381, October, 2004
Original article submitted March 10, 2004

We studied changes in the erythroid hemopoietic stem during phenylhydrazine-induced hemolytic anemia. Stimulation of erythropoiesis was associated with increased functional activity of erythroid precursors, which resulted from changes in feeder capacity of hemopoiesis-inducing microenvironmental cells and erythropoietic activity of the plasma. The development of encephalopathy induced by a hemolytic poison was accompanied by a decrease in hyperplasia of bone marrow erythropoiesis. It was related to a decrease in the number of proliferating erythroid precursor cells. These changes accompanied the increase in the secretory function of adherent myelokaryocytes, rise in erythropoietic activity of the plasma, enhanced formation of erythroid hemopoietic islets, and accelerated maturation of hemopoietic cells.

Key Words: *erythropoiesis; hemolytic anemia; encephalopathy; phenylhydrazine*

The blood system maintains gas homeostasis in the organism [2]. Many diseases are related to the reduction of blood oxygen capacity. These pathological disorders include disturbances induced by industrial and natural poisons with hemolytic activity [9]. Adequate compensatory-and-adaptive reaction of hemopoietic organs can modulate the course of recovery processes in the organism. Local and distant regulation of hemopoiesis during induced hemolysis is poorly understood. Moreover, the effect of encephalopathy accompanying hemolytic anemia on the development of blood changes remains unknown. Published data show that dysfunction of the central nervous system (CNS) plays a role in disadaptation of hemopoietic tissue during hypoxic hypoxia [3,4,7].

Here we studied the mechanisms of regulation of erythropoiesis during hemolytic anemia induced by phenylhydrazine.

MATERIALS AND METHODS

Experiments were performed on 526 CBA/CaLac mice (class I conventional mouse strain) weighing 18-20 g and obtained from the nursery of the Department of Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center). Blood hypoxia was induced by intraperitoneal injection of phenylhydrazine hydrochloride in single doses of 30 and 150 mg/kg. Treatment with the substance in a dose of 30 mg/kg did not cause pronounced changes in the psychoneurological status. Administration of phenylhydrazine hydrochloride in high dose was followed by the development of encephalopathy, which was confirmed by amnesia of a conditioned passive avoidance response [1] and impairment of orientation and exploratory activities in the open field [1,13]. Peripheral blood indexes were recorded on days 1-10 using an ABACUS automatic blood analyzer (Diatron) under veterinary conditions. The state of bone marrow hemopoiesis was estimated by standard hematological methods [8]. We studied the number of erythroid precursor cells (CFU-E) in the bone marrow, proliferative activity and intensity of differentiation, production of erythropoietically active compounds by fractions of the hemopoiesis-inducing microenvironment (HIM), erythropoietic activity (EPA) of the plasma, and structural and functional organization of the bone marrow [5]. The results were analyzed by methods of varia-

Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences

0007-4888/04/1384-0334

tional statistics using Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

Injection of phenylhydrazine in a dose not inducing brain pathology was followed by a decrease in erythrocyte number (days 1-9) and hematocrit (days 1-5) and development of peripheral blood reticulocytosis (days 1-10). Qualitative study of formed elements revealed a decrease in the volume of erythrocytes on days 1-3 after treatment, which was probably related to destruction of large cells during passage over the microcirculatory bed. The increase in the volume of erythrocytes on days 8-10 was associated with the influx of a considerable number of young erythrocytes into the circulation and increase in the degree of anisocytosis (days 4-7, 9, and 10). Induced hemolysis was accompanied by hyperplasia of bone marrow erythropoiesis. It was manifested in an increase in the count of erythrokaryocytes (days 1-10) by 252% compared to the control (day 5, Fig. 1). These changes were preceded by the increase in colony-forming activity of hemopoietic tissue, acceleration of CFU-E division, and rise in differentiation capacity of erythroid precursors (Fig. 2). We revealed increased production of erythropoietically active compounds by adherent (days 4, 5, 8, and 9) and nonadherent bone marrow cells (days 3, 5, and 6) and rise in erythropoietic activity of the plasma (Fig. 3). Study of structural and functional organization of the bone marrow showed an increase in the ability of macrophages to form erythroid hemopoietic islets (days 1-10, Fig. 1, *f*).

Our results are consistent with published data on blood changes during regenerative anemia [6,10-12].

We evaluated whether dysfunction of CNS during hemolytic anemia can be considered as a possible mechanism for dysregulation of hemopoiesis. Administration of phenylhydrazine hydrochloride in a dose inducing brain pathology modified the reaction of hemopoietic tissue. Hyperplasia of the erythroid stem became less pronounced on days 3-5 and 7, which resulted from a decrease in the number of bone marrow CFU-E (days 4, 6, 7, and 9). Proliferative activity

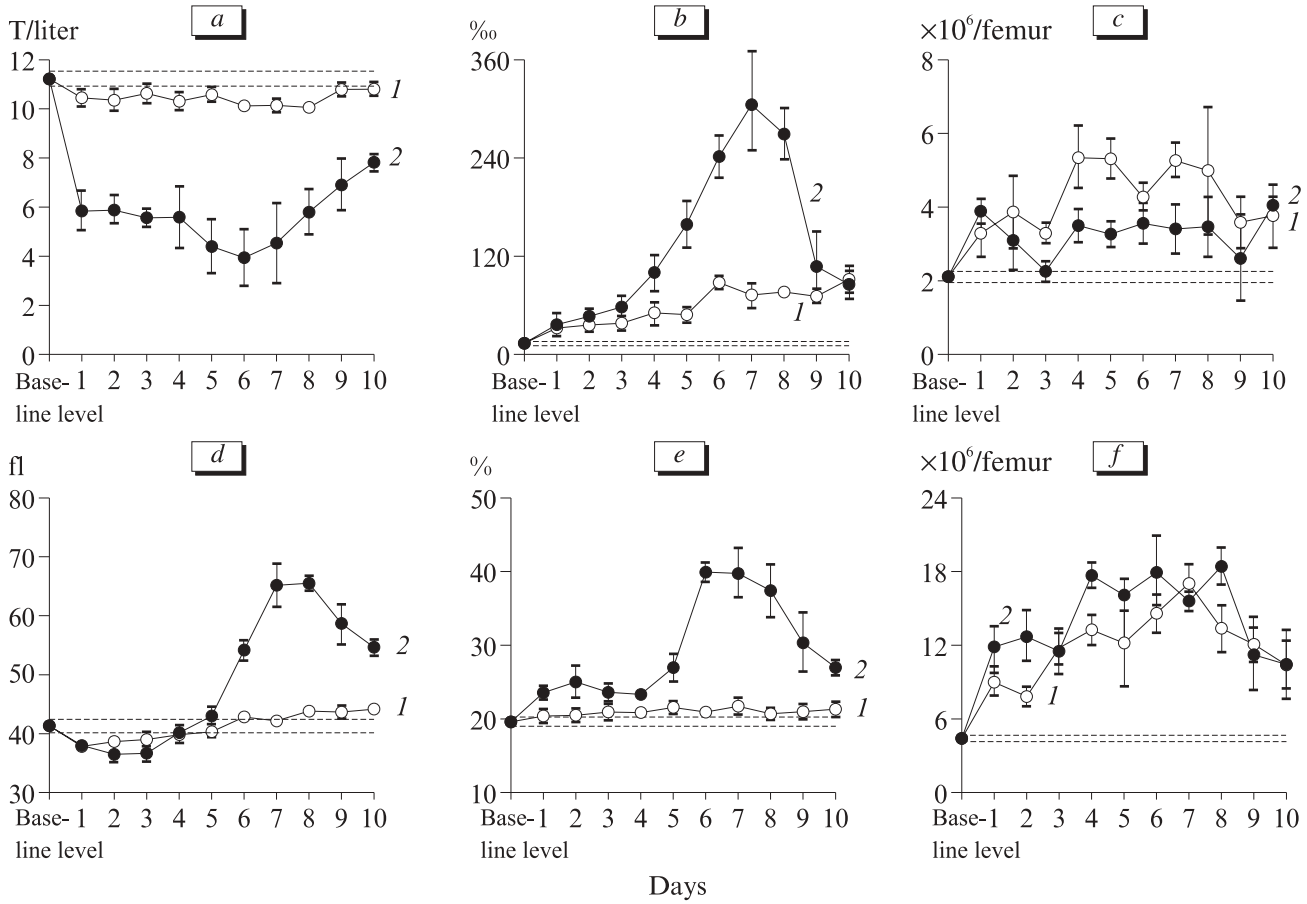
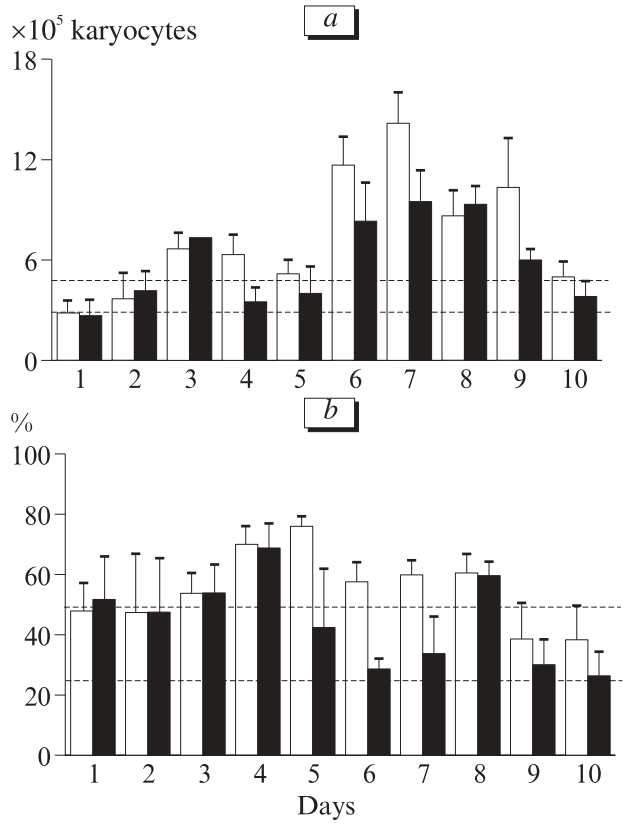


Fig. 1. Number of peripheral blood erythrocytes (*a*) and reticulocytes (*b*) and bone marrow erythrokaryocytes (*c*), volume of erythrocytes (*d*), degree of anisocytosis (*e*), and count of erythroid hemopoietic islets in the bone marrow of CBA/CaLac mice (*f*) during administration of 30 mg/kg phenylhydrazine hydrochloride (*1*) and development of encephalopathy induced by 150 mg/kg phenylhydrazine hydrochloride (*2*). Here and in Figs. 2 and 3: confidence intervals at $p<0.05$.



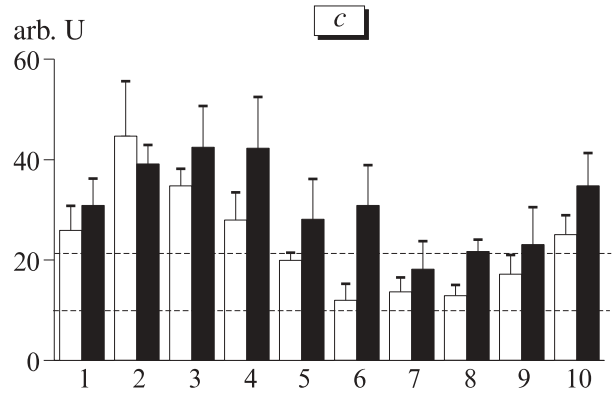
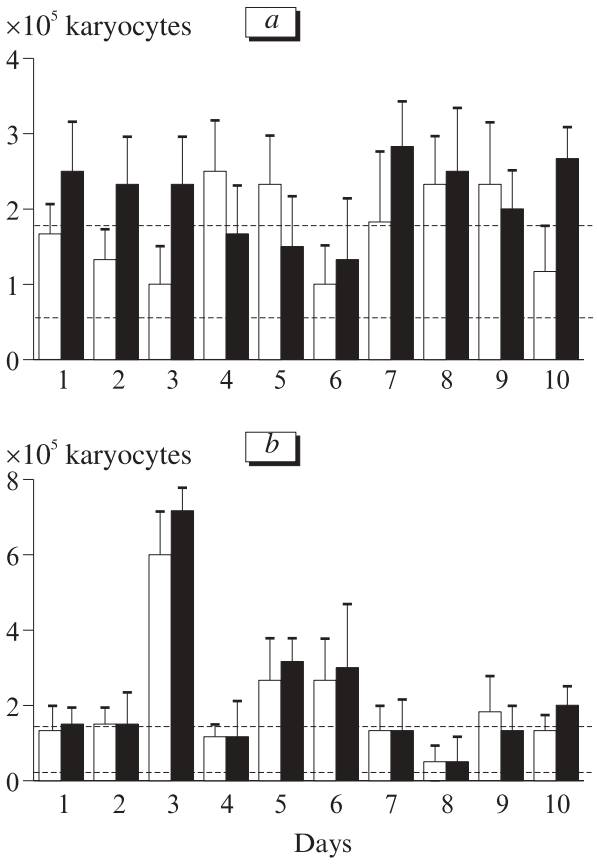


Fig. 2. Number of bone marrow CFU-E (*a*) in CBA/CaLac mice receiving phenylhydrazine hydrochloride in doses of 30 (light bars) or 150 mg/kg (dark bars, encephalopathy), ratio of CFU-E in S-phase of the mitotic cycle (*b*), and intensity of maturation (*c*).



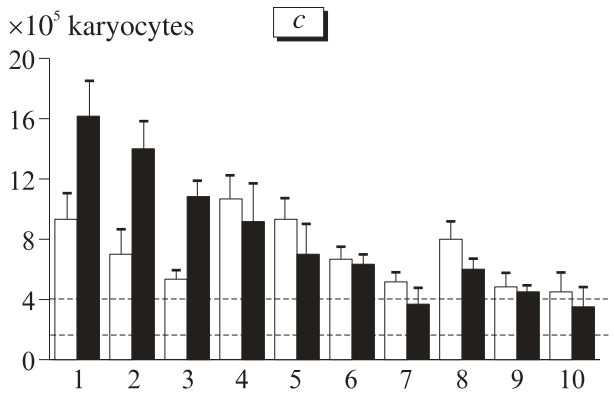


Fig. 3. Erythropoietic activity of conditioned media from adherent (*a*) and nonadherent myelokaryocytes (*b*) and blood plasma (*c*) in CBA/CaLac mice receiving phenylhydrazine hydrochloride in doses of 30 (light bars) or 150 mg/kg (dark bars, encephalopathy).

of cells decreased on days 5-7. It should be emphasized that the count of erythrocytes decreased despite compensatory activation of erythroid precursor maturation (Fig. 2). These changes were associated with increased secretory function of adherent myelokaryocytes (days 2, 3, and 10) and plasma EPA (days 1-3) and enhanced formation of erythroid cells in the bone marrow (Fig. 1, *f*). Long-term and severe anemia developed on days 1-10 after administration of phenylhydrazine in high dose and was manifested in a decrease in erythrocyte count and hematocrit. The degree of reticulocytosis increased 3-8 days after treatment and reached 2231% on day 7. We observed a significant increase in the size of mature red blood cells on days 6-10 (158.6% on day 8) and development of pronounced anisocytosis (Fig. 1, *d*, *e*). These changes probably aggravated anemia due to dieresis of macro- and megalocytes formed during intensive erythropoiesis under conditions of severe blood hypoxia.

Blood changes during hemolytic anemia accompanied by encephalopathy are similar to dysfunction of erythropoiesis observed under conditions of severe hypoxic hypoxia [3,4,7]. Our previous studies revealed a relationship between dysfunction of CNS and damage to hemopoietic precursors, which results from an increase in feeder capacity of stromal HIM cells and production of functionally abnormal erythrocytes in the posthypoxic period.

These data indicate that changes in the blood system resulting from failure of compensatory and adaptive mechanisms of hemopoiesis during severe hypoxia (*e.g.*, massive hemolysis) can be considered as "erythropoietic distress". This state is manifested in disadaptation of hemopoietic tissue and formation of abnormal erythrocytes.

REFERENCES

1. Ya. Buresh, O. Bureshova, G. P. Houston, *Methods and Main Experiments in Studies of the Brain and Behavior* [in Russian], Ed. A. S. Batuev, Moscow (1991)
2. *Hypoxia; Adaptation, Pathogenesis, and Clinics* [in Russian], Ed. Yu. L. Shevchenko, St. Petersburg (2000).
3. E. D. Gol'dberg, A. M. Dygai, and G. N. Zyuz'kov, *Eksperimental'na Klinichna Meditsina*, No. 2, 30-35 (2003).
4. E. D. Gol'dberg, A. M. Dygai, G. N. Zyuz'kov, *et al*., *Byull. Eksp. Biol. Med.*, **134**, No. 8, 142-145 (2002).
5. E. D. Gol'berg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
6. Yu. M. Zakharov and A. G. Rassokhin, *Erythroblastic Islet* [in Russian], Moscow (2002).
7. G. N. Zyuz'kov, L. A. Gur'yantseva, N. I. Suslov, *et al*., *Byull. Eksp. Biol. Med*., **134**, No. 10, 379-382 (2002).
8. *Manual on Laboratory Studies in Clinical Practice* [in Russian], Ed. V. V. Men'shikov, Moscow (1987).
9. *Toxicology, Hygiene, and Occupational Diseases* [in Russian], Ed. S. D. Zaugol'nikov, Moscow (1982), pp. 260-263.
10. Y. Fujii, N. Terada, H. Ueda, *et al*., *J. Electron Microsc. (Tokyo)*, **46**, No. 6, 477-484 (1997).
11. M. F. McMullin, T. R. Lappin, G. E. Elder, *et al*., *Biochem. Med. Metab. Biol.*, **41**, No. 1, 30-36 (1989).
12. P. A. Redondo, A. I. Alvarez, C. Diez, *et al*., *Lab. Anim. Sci*., **45**, No. 5, 578-583 (1995).
13. R. N. Walsh and R. A. Cummins, *Psychol. Bull*., **83**, 482-504 (1976).