Mechanisms of Changes in the Erythroid Hemopoietic Stem during Hypoxias of Different Severity

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Brain pathology (acute hypoxia and posthypoxic encephalopathy) is associated with less pronounced hyperplasia of the bone marrow erythroid stem (due to decreased count of proliferating committed precursors) and hemolytic anemia, while secretory activity of stromal cells of the hemopoiesis-inducing microenvironment is not impaired. Severe oxygen deficiency affects erythroid precursors and impairs production of functionally normal erythrocytes in the posthypoxic period.

Key Words: *erythropoiesis; hypoxia; encephalopathy*

Hypoxia is a general pathological process that accompanies various diseases. The damaging effects of oxygen deficiency were extensively studied. However, the pathogenesis of changes resulting from anoxia is poorly understood. Oxygen deficiency leads to severe energy starvation, induces considerable structural and functional changes in integration systems (*e.g.*, central nervous system, CNS) and the formation of qualitatively new pattern of their relationships. Exhaustion of adaptive capacities produces pathological changes in other organs maintaining homeostasis. This contributes to the development of various disturbances in vital activity of the organism after clinical death [6]. Taking into account high lability of the hemopoietic tissue and its important role in the maintenance of homeostasis, changes in the blood system during hypoxia of different severity are of considerable importance.

Here we studied the mechanisms of changes in the erythroid stem during the posthypoxic period.

MATERIALS AND METHODS

Experiments were performed on 390 male 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).

Hypoxia was produced in a 500-ml sealed chamber. The animals were removed from the chamber after generalized convulsions and/or visual respiratory arrest for 10-15 sec. No considerable psychoneurological changes were observed after short-term hypoxia (single exposure). Long-term hypoxia (two hypoxia session with a 10-min interval) causes encephalopathy starting from day 1, which was estimated by amnesia in the conditioned passive avoidance test and impairment of exploratory and locomotor activity in the open field test (days 1-10) [1,9]. Parameters of the peripheral blood, intensity of bone marrow hemopoiesis [7], osmotic resistance of erythrocytes [2], count of bone marrow erythroid precursors (CFU-E), their proliferative activity, CFU-E differentiation, and production of humoral hemopoietic factors by individual fractions of the hemopoiesis-inducing microenvironment (HIM) [4] were assayed on days 1-10.

The results were analyzed by Student's *t* test.

RESULTS

Hypoxic exposure sharply stimulated the erythroid hemopoietic stem. The count of bone marrow ery-

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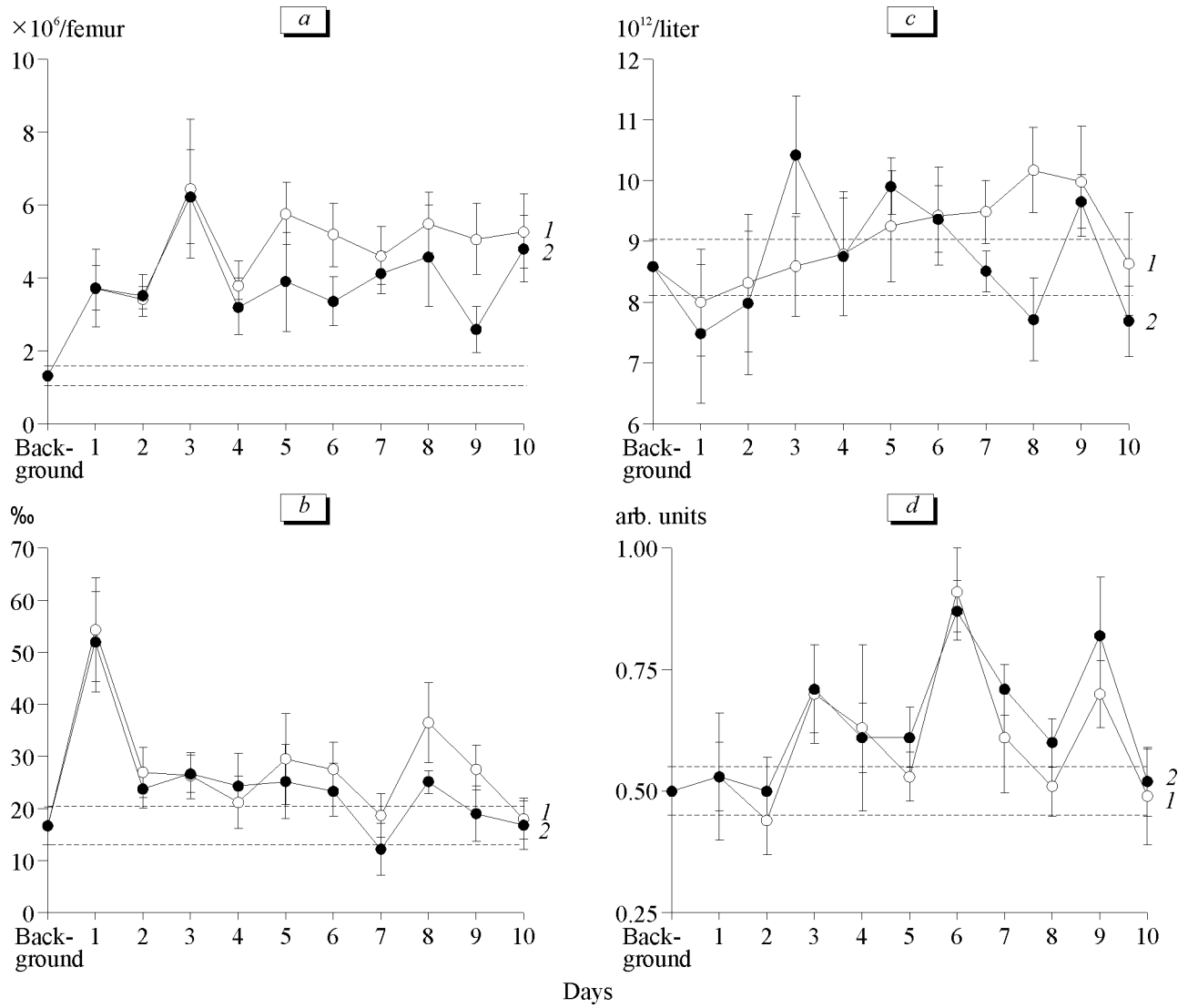

Fig. 1. Dynamics of the contents of bone marrow erythrokaryocytes (*a*) and peripheral blood reticulocytes (*b*) and erythrocytes (*c*). Hemolysis of erythrocytes (*d*) in male CBA/CaLac mice after acute hypoxia (*1*) and during the development of posthypoxic encephalopathy (*2*). Here and in Figs. 2 and 3 (dotted lines): confidence intervals at $p<0.05$.

throkaryocytes increased on days 1-10 ($p<0.05$), which was accompanied by the development of peripheral blood reticulocytosis (days 1-6, 8, and 9, $p<0.05$) and erythrocytosis (days 7, 8, and 9, $p<0.05$, Fig. 1).

Colony-forming activity of the hemopoietic tissue increased on days 1-6 and 9 ($p<0.05$). Studies of proliferation and differentiation of committed erythroid precursors showed rapid division of CFU-E and increased maturation index of these cells (Fig. 2). Erythropoietic activity (EPA) of media conditioned by adherent and nonadherent bone marrow cells increased, hence these changes resulted from secretory functions of HIM cells. Moreover, we observed an increase in plasma EPA (Fig. 3).

These reactions were probably induced by not only passage of erythrocyte destruction products into the vascular bed due to their decreased osmotic resistance (Fig. 1, *d*), but also the effects of hypoxia, activation of sympathoadrenal and pituitary-adrenal systems, and migration of T lymphocytes regulating hemopoiesis (peripheral blood lymphocytosis, days 1-10, $p<0.05$) into the hemopoietic tissue (day 3, $p<0.05$) [3].

Most complications developed in the postresuscitation period directly depend on the severity of CNS damages [6,8]. We studied the effects of posthypoxic encephalopathy on hemopoiesis. CFU-E count in 3-day-old cultures increased less significantly, which was primarily associated with a decrease in the number of proliferating cells (Fig. 2) due to inhibition of secretion of erythropoiesis-stimulating substances by nonadherent HIM cells (day 4, $p<0.05$). By contrast,

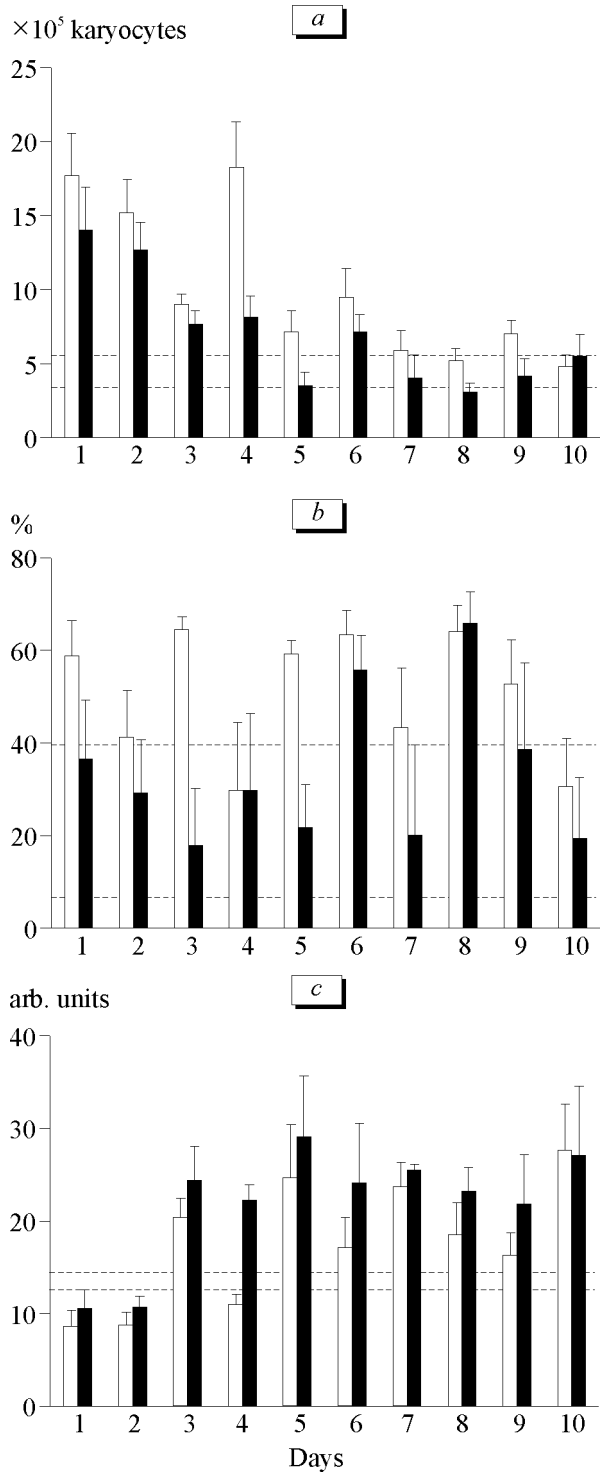

Fig. 2. Dynamics of the contents of bone marrow CFU-E in male CBA/CaLac mice after acute hypoxia (light bars) and during the development of posthypoxic encephalopathy (dark bars, *a*); percentage of S-phase cells (*b*) and intensity of their maturation (*c*).

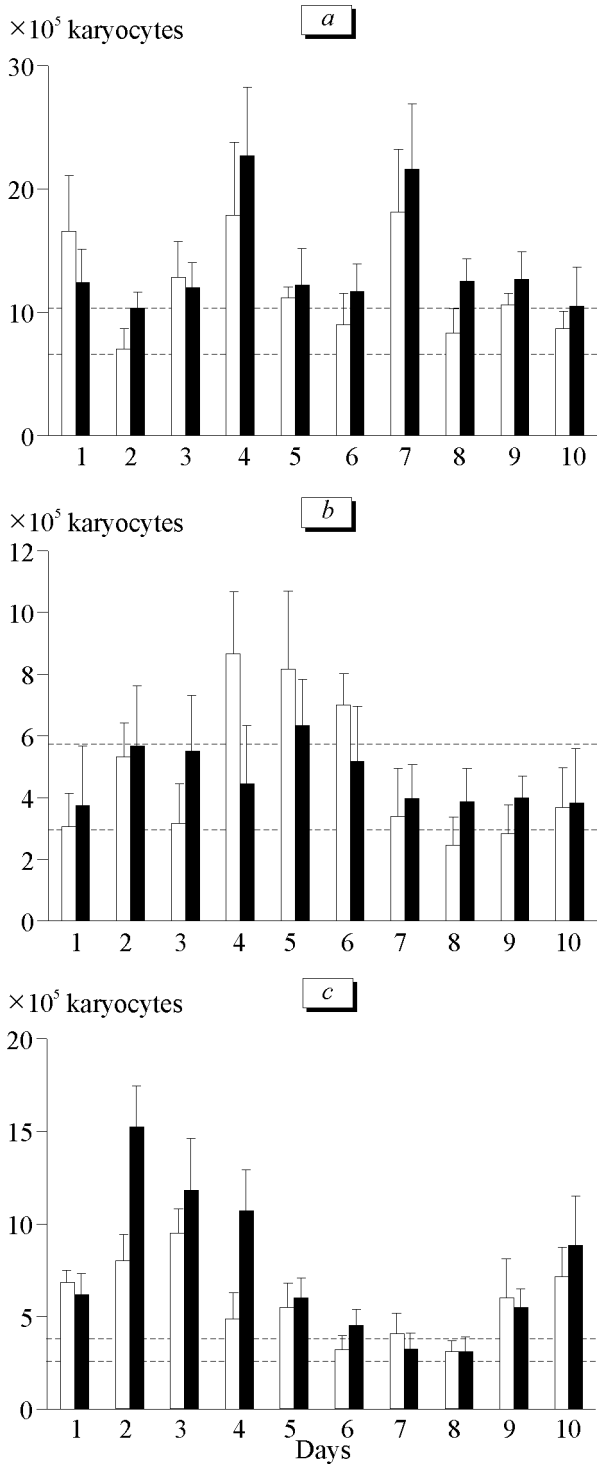

Fig. 3. Changes in erythropoietic activity of conditioned media from adherent (*a*) and nonadherent myelokaryocytes (*b*) and plasma (*c*) in male CBA/CaLac mice after acute hypoxia (light bars) and during the development of posthypoxic encephalopathy (dark bars).

EPA in adherent myelokaryocytes and plasma increased (Fig. 3). The increase in the concentration of humoral regulators in biological fluids (EPA) was manifested in compensatory acceleration of precursor differentiation. A statistically significant increase in this parameter was observed on day 4 ($p<0.05$). However, these changes were insufficient for maintaining the count of erythroid nucleated cells in the bone mar-

row at the level of the hypoxic control on days 5, 6, and 9. Moreover, the content of erythrocytes in the peripheral blood decreased and anemia developed (Fig. 1, *c*). This resulted from functional abnormalities of mature red blood cells (decreased osmotic resistance on days 5 and 8, $p<0.05$) and intensification of erythrodieresis in mononuclear phagocytes.

The disturbances in the mechanisms of hemopoiesis during severe global hypoxia are accompanied by damage to mitotically active CFU-E in the posthypoxic period. However, resistant stromal HIM cells retain their functional activity. These processes reduce the passage of normal specialized cells into the vascular bed. Oxygen deficiency that contributes to the formation of damages to brain structures markedly impairs adaptive reactions of the blood system, which aggravates the symptoms of oxygen starvation in tissues after clinical death.

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