Local Mechanisms Underling the Regulatory Effect of Kropanol on Hemopoiesis during Paradoxical Sleep Deprivation

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We studied the effect of Kropanol on local regulatory mechanisms of hemopoiesis during paradoxical sleep deprivation. The regulatory effect of Kropanol on granulomonocytopoiesis was determined by increased binding capacity of stromal cells in relation to granulocyte-macrophage precursors and increased formation of mixed hemopoietic islets leading to accelerated maturation of granulocyte-macrophage precursors and hyperplasia of the granulomonocytic hemopoietic stem. Stimulation of erythropoiesis with Kropanol was associated with increased formation of erythroid hemopoietic islets, accelerated differentiation of erythroid precursors, and high content of erythroid cells in the bone marrow. Kropanol increased proliferative activity of erythroid precursors. Stimulation of these processes depended on enhanced production of short-distance humoral regulators of erythro- and granulomonocytopoiesis.

Key Words: *paradoxical sleep deprivation; hemopoiesis; hemopoiesis-inducing microenvironment; hemopoietic islets; Kropanol*

Our previous studies showed that various natural preparations, including extracts of Siberian ginseng, bergenia, *Rhodiola rosea*, and ginseng and pantohematogen, can be used for the correction of blood changes during paradoxical sleep deprivation (PSD) [7,8]. Published data show that Baikal skullcap extract and pantohematogen affect hemopoiesis during cytostatic-induced myelosuppression, which is mediated by modulation of local regulatory mechanisms [2,3]. Natural preparations possess hematotropic activity, but their use for the treatment of various pathologies is based on empirical data. Here we evaluated the role of hemopoiesis-inducing microenvironment (HIM) in the regulatory effect of Kropanol on hemopoiesis during PSD.

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MATERIALS AND METHODS

Experiments were performed on 100 CBA/CaLac mice (conventional mouse strain) aging 2-2.5 months and obtained from the collection of the Laboratory for Experimental Biological Modeling (Institute of Pharmacology, Tomsk Research Center). PSD for 48 h served as a model of experimental neurosis [14]. Officinal preparation Kropanol was synthesized from dry pantohematogen (Altai elk blood subjected to low-temperature vacuum drying) at the Institute of Pharmacology (Tomsk Research Center). The preparation was *ex tempore* dissolved in distilled water and administered through a gastric tube in a daily dose of 50 mg/kg for 5 days (once a day). Control mice received an equivalent volume of distilled water. On days 1-7 the animals were euthanized by cervical dislocation under ether anesthesia. We estimated the contents of colony- (CFU) and cluster-forming units (ClFU) for granulo-

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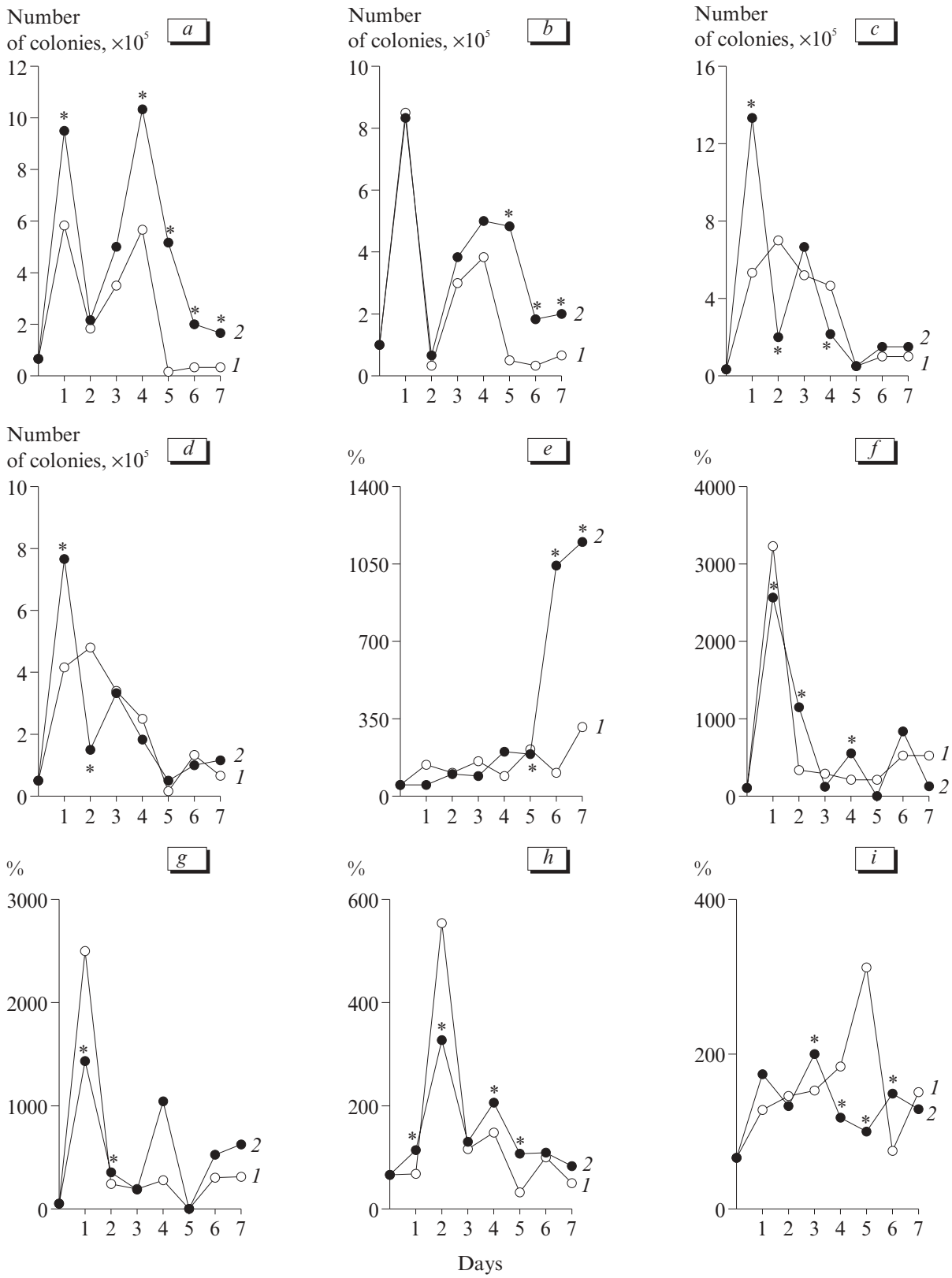

Fig. 1. Content of erythroid ClFU (E, *a*), CFU-E (*b*), granulocyte-macrophage ClFU (GM, *c*), and CFU-GM (*d*), ratio of S-phase CFU-E (*e*), ClFU-GM (*f*), and CFU-GM (*g*), and maturation of CFU-E (*h*) and CFU-GM (*i*) in the bone marrow of CBA/CaLac mice receiving physiological saline (*1*) or Kropanol (*2*) during paradoxical sleep deprivation (PSD). Ordinate: colony-forming ability of the bone marrow (*a-d*), ratio of S-phase hemopoietic precursors (*e-g*), and index of maturation (*h*, *i*). Here and in Fig. 2: *$p<0.05$ compared to animals with PSD receiving physiological saline.

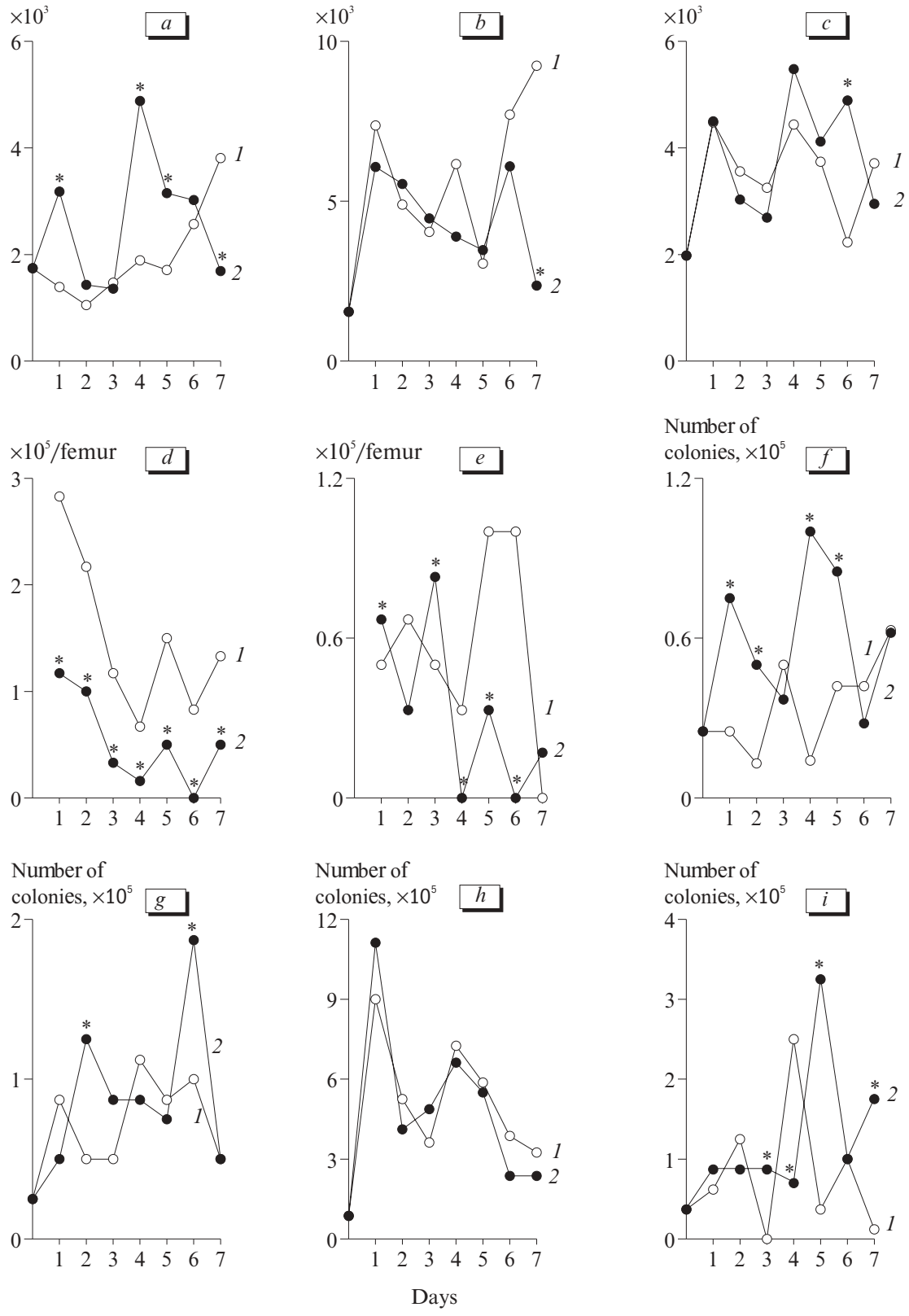

Fig. 2. Count of erythroid (*a*), granulocytic (*b*), and mixed hemopoietic islets in the bone marrow (*c*). Binding capacity of myelokaryocytes in relation to CFU-E (*d*) and CFU-GM (*e*). Erythropoietic activity (EPA) in supernatants of adherent (*f*) and nonadherent myelokaryocytes (*g*). Colony-stimulating activity (CSA) of adherent (*h*) and nonadherent myelokaryocytes (*i*) from CBA/CaLac mice receiving physiological saline (*1*) or Kropanol (*2*) during PSD. Ordinate: count of hemopoietic islets in the bone marrow (*10^3, *a-c*), binding capacity of myelokaryocytes (*d*, *e*), and EPA or CSA (*f-i*).

monocytopoiesis (GM) and erythropoiesis (E), intensity of hemopoietic precursor differentiation, and proliferative activity of precursors [4]. The quantitative and qualitative composition of hemopoietic islets was determined by the method of P. R. Crocker and S. Gordon with modifications of E. D. Gol'dberg *et al*. [4]. Erythropoietic (EPA) and colony-stimulating activities (CSA) of conditioned media from adherent and nonadherent HIM elements were estimated in a semisolid culture medium with intact mouse myelokaryocytes [4]. We studied the ability of bone marrow stromal cells to bind hemopoietic precursors [4]. The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by Student's *t* test and Wilcoxon nonparametric rank test [6].

RESULTS

PSD was accompanied by hyperplasia of the granulomonocytic hemopoietic stem and suppression of bone marrow erythropoiesis. It was related to changes in local regulatory mechanisms of hemopoiesis [5,7,8]. The count of CFU-GM and ClFU-GM increased on days 1-4, while the number of erythroid precursors decreased on days 5-7 of PSD (Fig. 1, *a-d*). We observed an increase in proliferative activity of CFU-GM (days 1, 2, 4, and 6, 7) and ClFU-GM (days 1-3 and 6, 7, Fig. 1, *f*, *g*) and accelerated maturation of granulocyte-macrophage precursors (Fig. 1, *e*).

PSD affected structural and functional organization of the bone marrow. The count of granulocytic and erythrogranulocytic hemopoietic islets increased throughout the experiment (Fig. 2, *b*). However, the number of erythroid colonies markedly decreased on day 2 of PSD (Fig. 2, *a*). It should be emphasized that the ability of adherent cells from mouse bone marrow to bind CFU-GM (days 1, 2, 5, and 6) and CFU-E (days 1-3 and 5-7, Fig. 2, *d*, *e*) increased. EPA tended to decrease, while CSA of adherent myelokaryocytes increased at the early stage of PSD (Fig. 2, *f-h*).

Then we studied local regulatory mechanisms underlying the effect of Kropanol on hemopoiesis during PSD. Kropanol administered during PSD stimulated accumulation of CFU-GM and ClFU-GM (day 1). These changes were followed by a decrease in the count of CFU-GM (day 2) and ClFU-GM (days 2 and 4, Fig. *c*, *d*). However, Kropanol increased the number of CFU-E (days 5-7) and ClFU-E (days 1 and 4-7, Fig. 1, *a*, *b*).

The preparation affected proliferative activity of committed hemopoietic precursors. The rate of division of CFU-GM and ClFU-GM decreased on day 1 and increased on days 2 and 4 (Fig. 1, *f*, *g*). By contrast, the rate of CFU-E proliferation increased after treatment with Kropanol (days 6 and 7, Fig. 1, *e*).

Administration of Kropanol induced wave-like changes in the rate of differentiation of granulocyte-macrophage precursors. The rate of maturation increased on day 3, decreased on days 4-5, and again surpassed the baseline level on day 6 (Fig. 1, *i*). The preparation stimulated differentiation of erythroid precursors on days 1, 4, and 5 (Fig. 1, *h*).

Kropanol affected structural and functional organization of the bone marrow during PSD. This preparation increased the number of erythroid (days 1, 4, and 5) and mixed hemopoietic islets (day 6, Fig. 2, *a*, *c*). The observed changes were probably associated with an increase in the binding capacity of adherent bone marrow cells in relation to CFU-GM (days 1 and 3, Fig. 2, *e*). However, Kropanol inhibited binding of myelokaryocytes to CFU-E throughout the experiment (Fig. 2, *d*). Moreover, Kropanol increased EPA of media conditioned by adherent (days 1, 2, 4, and 5) and nonadherent bone marrow cells (days 2 and 6) and CSA of nonadherent myelokaryocytes (days 3, 5, and 7; Fig. 2, *f*, *g*, *i*).

Thus, the regulatory effect of Kropanol on granulomonocytopoiesis during sleep disorders was manifested in improved binding capacity of stromal cells in relation to granulocyte-macrophage precursors and increased formation of mixed hemopoietic islets. These changes were followed by accelerated maturation of granulocyte-macrophage precursors, decrease in the count of these cells in the bone marrow, and increase in the number of mature morphologically verified granulocytes. Kropanol-induced stimulation of erythropoiesis during PSD was associated with the increased formation of erythroid hemopoietic islets and accelerated differentiation of erythroid precursors. Moreover, Kropanol increased proliferative activity of erythroid precursors. Therefore, the count of committed erythroid precursors increased in the bone marrow. The regulation of hemopoiesis depended on the increase in production of short-distance humoral regulators for erythropoiesis and granulomonocytopoiesis.

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