PHARMACOLOGY AND TOXICOLOGY

Mechanisms of Hemostimulating Effects of Granulocytic CSF and Pantohematogen under Conditions of Cytostatic Myelosuppression

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We compared hemostimulating activity of pantohematogen and granulocytic CSF under conditions of 5-fluorouracil-induced cytostatic myelosuppression. It was found that activation of hemopoiesis regeneration under the effect of the test preparations was accompanied by the development of hyperplasia of the granulocytic and monocytic hemopoietic bone marrow lineages and more rapid recovery of the count of pholymorphonuclear leukocytes and monocytes in the peripheral blood (more marked under the effect of pantohematogen) followed by neutrophilia and monocytosis.

Key Words: *pantohematogen; granulocytic colony-stimulating factor; 5-fluorouracil; myelosuppression*

We previously demonstrated hemostimilating activity of preparations of various origins, including recombinant granulocytic CSF (G-CSF) and a preparation from maral blood pantohematogen (PH) on the model of cyclophosphamide-induced myelosuppression. G-CSF more markedly stimulated granulocytopoiesis [5]. The presence of an indirect (via elements of hemopoiesis-inducing microenvironment) component in the mechanisms of action of both preparations [1,4] attests to possible existence of other regularities in regeneration of the hemopoietic tissue after treatment with other cytostatics differing from cyclophosphamide by the mechanism of action. This information is very important for extending our knowledge on the mechanisms of hemopoiesis regulation and substantiation of the use of various hemopoiesis-stimulating drugs in clinical practice.

Here we studied the effects of PH and G-CSF on leukocyte production on the model of cytostatic myelosuppression induced by 5-fluorouracil (5-FU) treatment.

MATERIALS AND METHODS

Experiments were carried out on 2-2.5-month-old female CBA/CaLac mice (n=200) obtained from the nursery of Institute of Pharmacology, Tomsk Research Center. The animals received intraperitoneal injection of 5-FU in MTD (228 mg/kg). After injection of the cytostatic, the experimental mice received officinal drugs: PH (Pantoproekt) *per os* in a dose of 50 mg/kg 7 times (experimental group 1) and G-CSF (Vek-

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tor) in a dose of 125 μg/kg once a day for 5 consecutive days (experimental group 2). Controls received an equivalent volume (0.2 ml) of vehicle (distilled water). On days 3-6, 8, 10, 12, 14, and 16 we determined leukocyte count in the peripheral blood, myelokaryocyte count and their qualitative composition in the bone marrow [7], content of granulomonocyte precursor cells (CFU-GM) in the bone marrow, their proliferative activity, and intensity of differentiation, and colony stimulating activity of adherent and non-

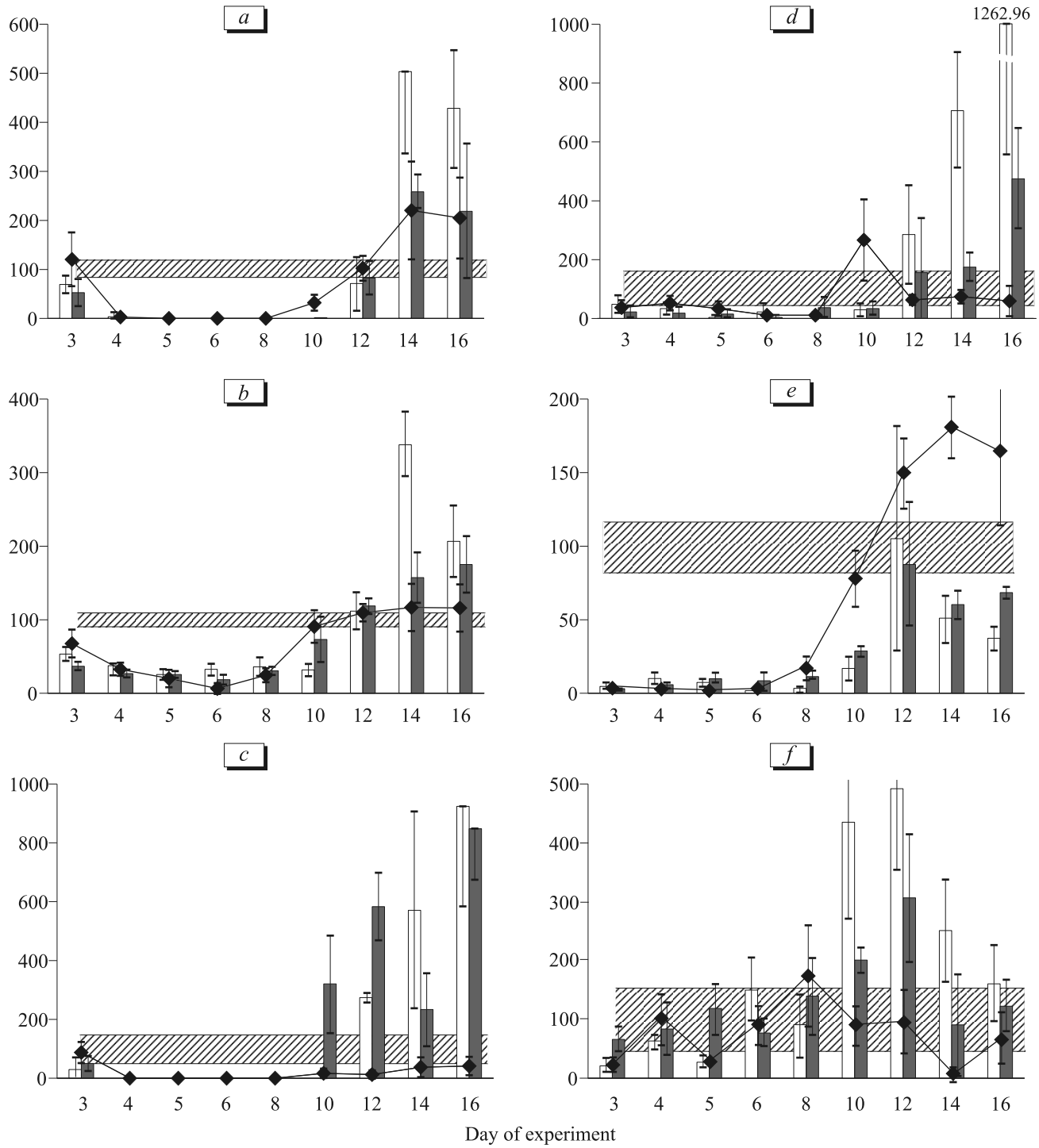

Fig. 1. Dynamics of the content of segmented neutrophil granulocytes (*a*), total leukocyte count (*b*), stab neutrophil granulocytes (*c*), monocytes (*d*) in the peripheral blood and immature neutrophil granulocytes (*e*) and monocytes (*f*) in the bone marrow of mice receiving 5-FU (curve), 5-FU and PH (open bars), and 5-FU and G-CSF (dark bars). Ordinate: cell count in the peripheral blood and bone marrow (% of background values). Confidence intervals at p=0.05.

adherent cells of the hemopoiesis-inducing microenvironment and blood serum [3].

The data were expressed in percents of the initial level. The results were processed using Student *t* test.

RESULTS

The experiments revealed the increase in total leukocyte count in the peripheral blood of animals receiving PH (on days 6, 14, and 16 of the experiment) and G-CSF (on days 6 and 16) compared to the control (Fig. 1, *b*). The observed differences were determined by higher content of stab and segmented neutrophils, monocytes, and lymphocytes. For instance (on days 10-16 of the experiment), the content of stab neutrophils in mice receiving recombinant cytokine was considerably higher than in animals receiving cytostatic alone (Fig. 1, *c*). In experimental group 1, significant differences in this parameter were noted on days 12-16 of the experiment (Fig. 1, *c*). Maximum differences in the count of these cells after treatment with G-CSF were observed on days 12 and 16 and PH, respectively (Fig. 1, *c*). Blood content of segmented neutrophils was significantly increased only in mice receiving PH on days 14 and 16 of the experiment (Fig. 1, *a*). At the same time, treatment with G-CSF was followed by transient leukopenia (Fig. 1, *a*). Of principal importance is the fact that administration of hemopoiesis stimulating preparations produced a pronounced effect on the dynamics of recovery of not only neutrophil granulocytes, but also monocytes of the peripheral blood. After treatment with PH, the recovery of monocyte count was more pronounced than in the control and even in mice receiving G-CSF (1262.96±229.63% from the background value on day 16 of the experiment, Fig. 1, *d*).

Analysis of hemopoiesis parameters showed that administration of both PH and G-CSF to animals with cytostatic myelosuppression significantly increased total cellularity of the bone marrow (on days 4, 5, and 12 in experimental group 1 and on days 3-5 in mice treated with G-CSF). The count of immature neutrophil granulocytes in the bone marrow significantly increased on days 4 and 5 after PH treatment and on days 4, 5, and 6 after G-CSF treatment and then decreased to the end of the experiment in both experimental group (Fig. 1, *e*) The mean count of mature neutrophils significantly surpassed the corresponding parameter in control mice on day 12 in experimental group 1 and on days 10 and 12 in experimental group 2. The absolute number of lymphoid lineage cells after treatment with PH and G-CSF significantly surpassed the corresponding parameter in animals receiving the cytostatic alone on days 3, 4, and 5 of the experiment. Treatment with PH and G-CSF significantly increased

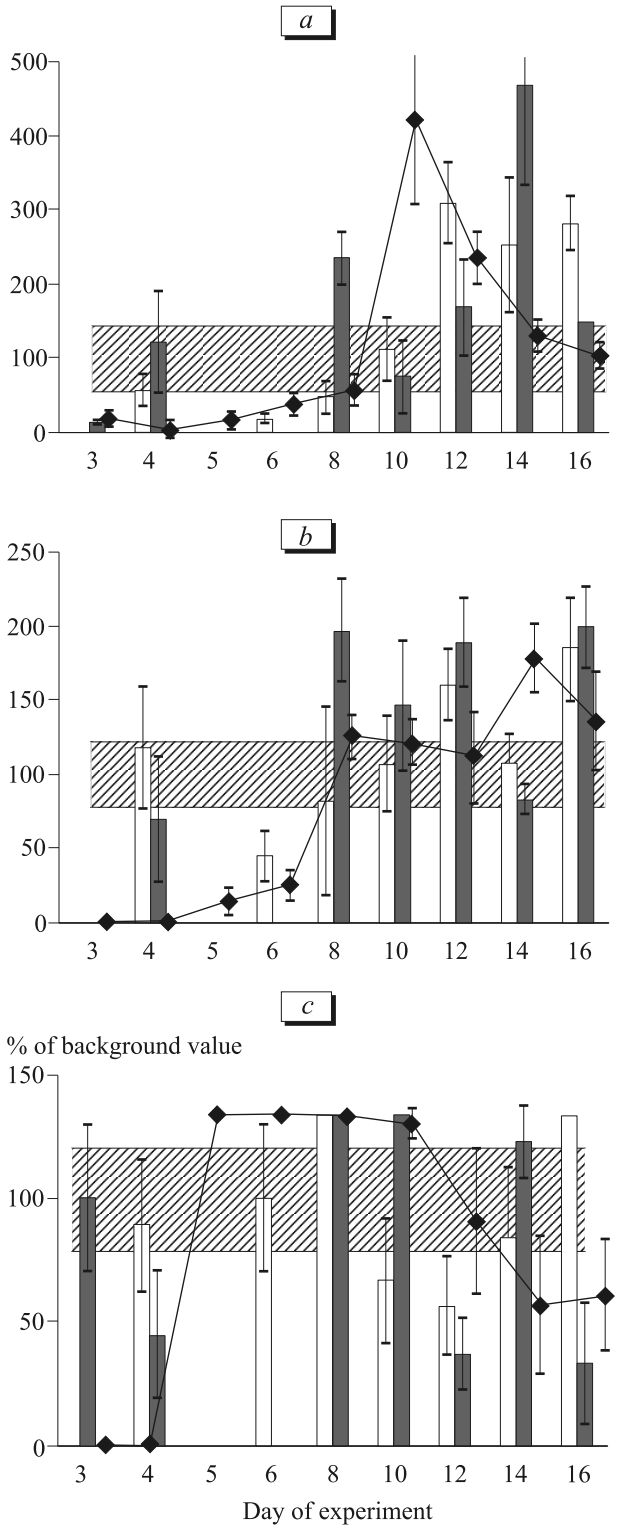

Fig. 2. Dynamics of the content of G-CSF in mouse bone marrow (*a*), changes in the rate of maturation of granulomonocytopoietic precursors (*b*), and relative content of S-phase granulomonocytopoietic precursors (*c*) in the bone marrow of mice receiving 5-FU (curve), 5-FU and PH (open bars), and 5-FU and G-CSF (dark bars). Ordinate: content of CFU-GM per 10^5 nuclears (*a*), coefficient of differentiation (*b*) Confidence intervals at p=0.05.

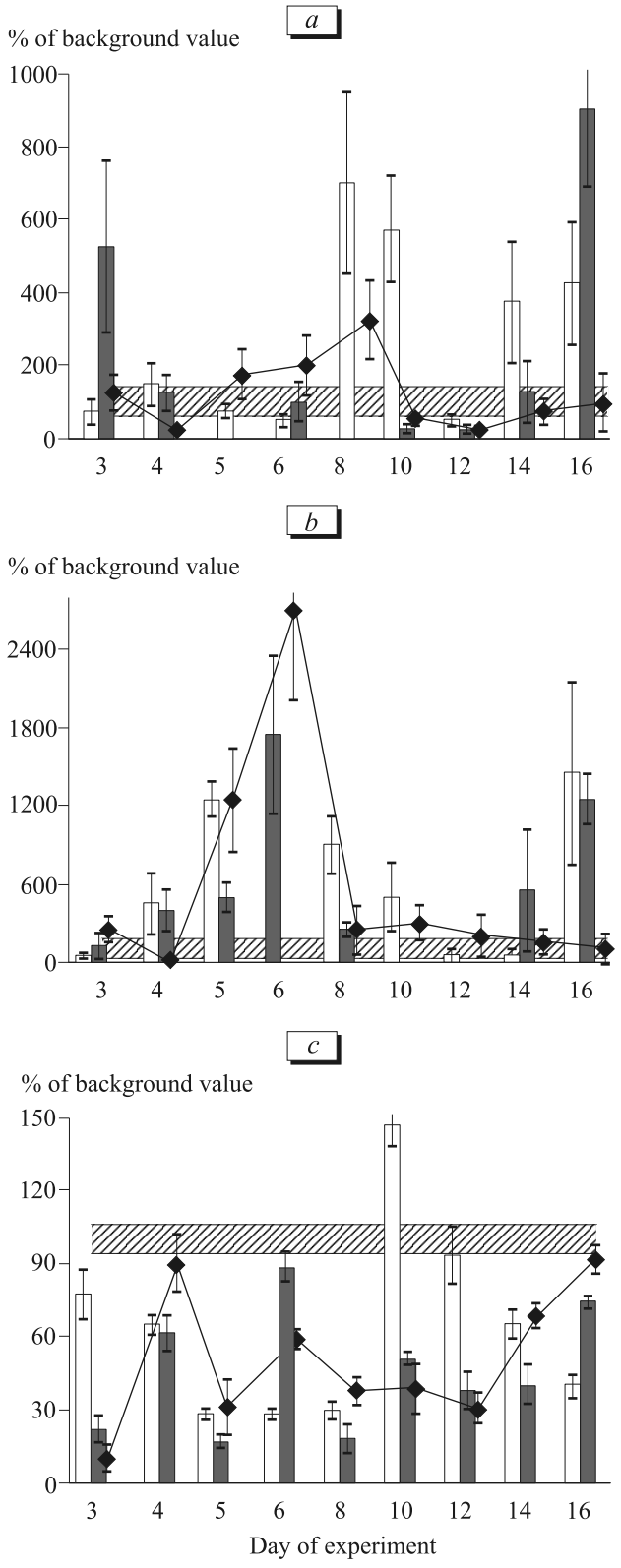

Fig. 3. Dynamics of colony-stimulating activities in supernatants of adherent (*a*) and nonadherent (*b*) bone marrow karyocytes and blood serum (*c*) in mice receiving 5-FU (curve), 5-FU and PH (open bars), and 5-FU and G-CSF (dark bars). Ordinate: colony-stimulating activity. Confidence intervals at p=0.05.

monocyte count in the bone marrow on days 3, 5, 10, 12, 14, and 16 (these changes were more pronounced after PH treatment); this parameter peaked on day 14 of the experiment, when it 45- and 16-fold surpassed the corresponding value in the control group (Fig. 1, *f*). The hemostimulants were little effective in restoring 5-FU-suppressed erythroid hemopoietic lineage. The increase in the number of erythrokaryocytes was observed only in mice receiving PH and only by the end of the experiment (day 14).

Analysis of colony-forming capacity of the bone marrow in mice receiving the hemostimilants against the background of single cytostatic treatment revealed the following changes. The yield of CFU-GM after treatment with PH increased compared to the control group on days 4, 12, and 16 of the experiment being maximum on day 12 (308.99±28.09% from the background value, Fig. 2, *a*). The coefficient of differentiation considerably increased to 117.65 and 160% from the background values on days 4 and 12 of the experiment, respectively (Fig. 2, *b*). The relative content of S-phase hemopoietic precursors was higher than in the control group only on days 4 and 16 (Fig. 2, *c*).

G-CSF considerably increased the content of granulocytic precursors in the bone marrow. The number of CFU-GM in the hemopoietic tissue of experimental animals significantly surpassed the corresponding parameter in control mice on days 4, 8, 14, and 16 by 6.5, 4.2, 3.6, and 1.5, respectively (Fig. 2, *a*). Significant acceleration of CFU-GM maturation after cytokine treatment was observed on days 4, 8, 12, and 16 (Fig. 2, *b*), while the increase in proliferative activity was noted on days 3, 4, and 14 of the experiment (Fig. 2, *c*).

Further studies showed that hemostimulants activate functions of the hemopoiesis-inducing microenvironment cells. For instance, combined treatment with 5-FU and PH led to stimulation of secretion of colony-stimulating activity by adherent (days 4, 8, and 16) and nonadherent (days 4, 8, and 16) elements of the bone marrow (Fig. 3, *a*, *b*). In mice treated with G-CSF after single cytostatic treatment, we observed an increase in the production of colony-stimulating activity by adherent cells (days 3, 4, and 16) and nonadherent myelokaryocytes (days 4 and 16, Fig. 3, *a*, *b*). Colony-stimulating activity of blood serum also surpassed the corresponding control value on days 3, 10, and 12 in mice receiving PH and on days 3 and 6 in animals treated with G-CSF (Fig. 3, *c*).

Thus, administration of G-CSF against the background of myelosuppression induced by 5-FU treatment promotes recovery of primarily granulocytic lineage, while administration of PH stimulated recovery of granulocytic and monocytic hemopoietic lineages. The activating effect of these preparations on

processes of bone marrow hemopoiesis are based on activation of colony-forming capacity and increase in functional activity of hemopoiesis-inducing microenvironment. The effect of G-CSF is primarily determined by direct stimulation of proliferation and differentiation of hemopoietic precursors and morphologically discernible myelokaryocytes [6,8]. The effect of PH is mediated by primarily adherent cells of the microenvironment, which probably determines its later manifestation. The hemostimulating activity of G-CSF cannot be fully realized under conditions of dysfunction of hemopoiesis-inducing microenironment (caused by potent destructive effect of 5-FU) [2]. This can be explained by impaired release of mature cells, primarily granulocytes, from the bone marrow into the peripheral blood.

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