Mechanisms of Myelopoiesis Stimulation with Pantohematogen and Granulocyte Colony-Stimulating Factor during Cytostatic Myelosuppression

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 130, No. 11, pp. 512-515, November, 2000
Original article submitted June 7, 2000

We compared hemopoiesis-stimulating activities of dry pantohematogen and recombinant granulocyte colony-stimulating factor under conditions of cyclophosphamide-induced myelosuppression. These preparations stimulated regeneration of bone marrow granulomonocytopoiesis by various mechanisms.

Key Words: *pantohematogen; granulocyte colony-stimulating factor; cyclophosphamide; granulomonocytopoiesis*

Most drugs used for the therapy of malignant tumors produce toxic effects on proliferating cells, in particular, on the hemopoietic tissue [5]. The search for new drugs acting as natural hemopoietic regulators is of considerable importance. Dry pantohematogen obtained from red deer blood (PH, Pantoproekt, Biysk) and recombinant granulocyte colony-stimulating factor (G-CSF, Hoffman-LaRoche) are of particular interest in this respect.

Here we compared the mechanisms of hemopoiesis stimulation with a biological response modifier PH and recombinant G-CSF on the model of cytostatic myelosuppression.

MATERIALS AND METHODS

Experiments were performed on 200 CBA/CaLac mice aging 2-2.5 months (collection of the Laboratory of Experimental Biological Modeling, Institute of Pharmacology, Tomsk Research Center). The animals received single intraperitoneal injection of cyclophosphamide (CP) in maximum permissible dose (250 mg/kg). After injection of this cytostatic, experimental mice were daily administered with PH (50 mg/kg perorally) or G-CSF (125 μg/kg subcutaneously) for 7 and 5 days, respectively. Control mice received an equivalent volume of distilled water (0.2 ml). The animals were euthanized by cervical dislocation on days 5, 6, 7, 8, 10, and 12 after injection of CP. The count of peripheral blood leukocytes, total number of bone marrow myelokaryocytes, and their composition were estimated by routine hematological tests. The content of committed granulomonocytopoiesis precursors (GM-CFU) in the bone marrow was determined by *in vitro* culturing in methyl cellulose [6]. Colony-stimulating activity (CSA) of culture media of adherent and nonadherent cells of the hemopoietic microenvironment was tested on intact mouse myelokaryocytes cultured in a semisolid medium [6]. Differentiation of hemopoietic precursors was estimated by the index of maturation. Proliferative activity of GM-CFU was studied by the method of cell suicide using hydroxyurea [6].

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

RESULTS

Both test preparations stimulated CP-suppressed granulomonocytopoiesis. On days 8-12 after cytostatic

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0007-4888/00/0011-1051$25.00

administration, the cellularity of the bone marrow surpassed the control due to accumulation of immature and mature neutrophilic granulocytes (Fig. 1, *c*, *d*). G-CSF accelerated regeneration of granulomonocytopoiesis and increased the count of mature bone marrow granulocytes on days 5-7 (Fig. 1, *d*). The differences were most pronounced on day 6, when PH produced no stimulatory effect, while G-CSF elevated the content of these cells by 2.8 times.

The absolute number of segmented neutrophilic granulocytes in the peripheral blood increased on days 8 and 10 after cytostatic treatment, while in animals injected with G-CSF this parameter surpassed the control on days 5-8 (Fig. 1, *b*). Both preparations increased the count of peripheral blood monocytes and normalized the content of neutrophilic leukocytes in CP-treated mice.

The state of bone marrow hemopoiesis depends on functional activity of hemopoietic precursors [2]. Our experiments showed that PH-induced changes are related to more pronounced accumulation of GM-CFU in the hemopoietic tissue on days 7, 8, and 10 (Fig. 1, *a*). PH increased the number of GM-CFU in S phase of the mitotic cycle 6 days after CP injection, but inhibited maturation of these cells. By contrast, G-CSF 6-fold decreased colony-forming capacity of mouse bone marrow 5 days after CP injection (Fig. 1, *a*). These changes were related to accelerated maturation of GM-CFU in experimental mice: the index of maturation increased on days 5-6. At the same time, our findings confirm the ability of recombinant G-CSF to stimulate migration of hemopoietic precursors into peripheral circulation [4,9].

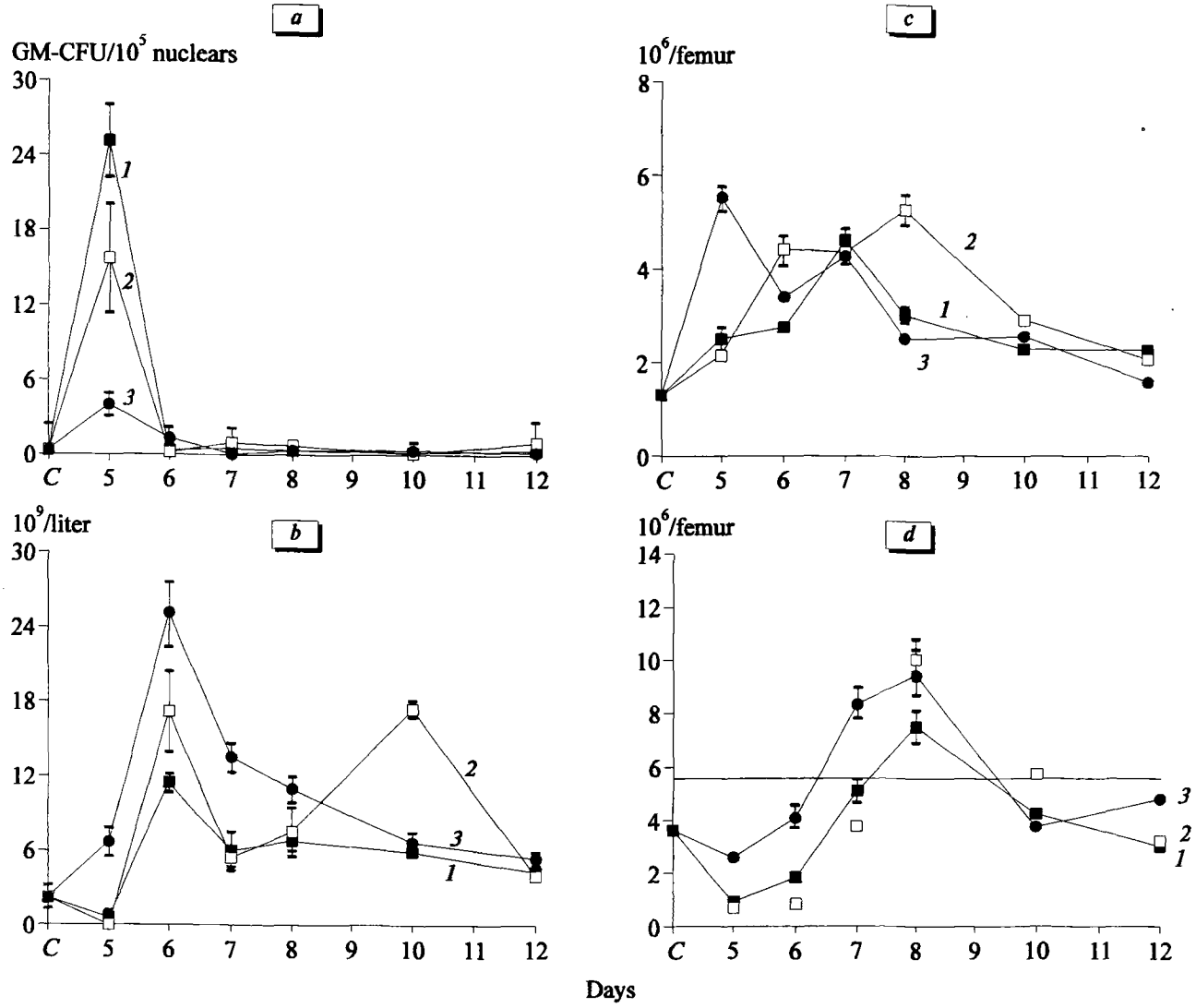

Fig. 1. Contents of granulocyte-macrophage colony-forming units in the bone marrow (*a*), segmented granulocytes in the peripheral blood (*b*), and immature (*c*) and mature (*d*) neutrophilic granulocytes in the bone marrow of mice treated with cyclophosphamide (*1*), cyclophosphamide and pantohematogen (*2*), or cyclophosphamide and granulocyte colony-stimulating factor (*3*). Confidence intervals at p=0.05.

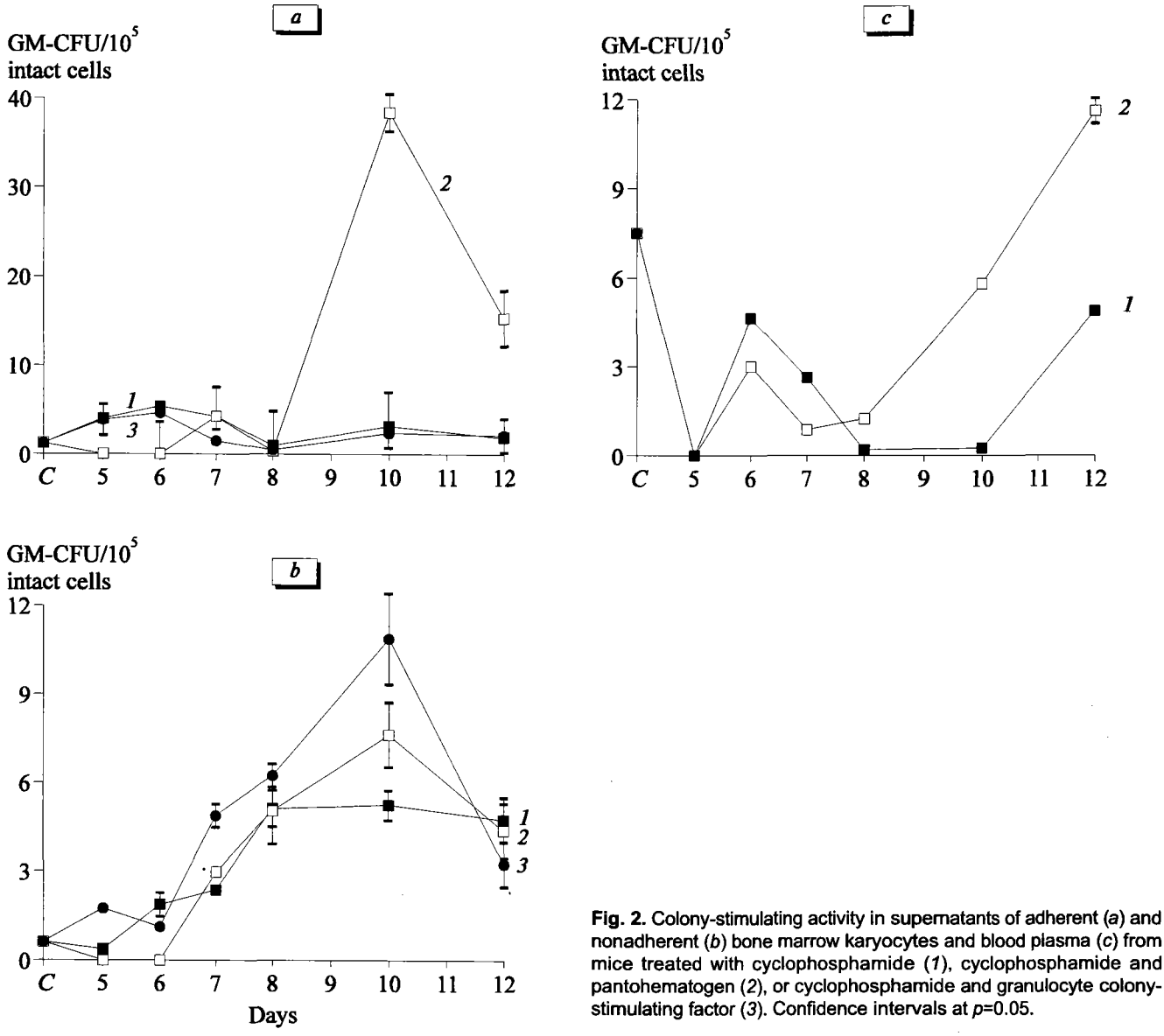

Fig. 2. Colony-stimulating activity in supernatants of adherent (*a*) and nonadherent (*b*) bone marrow karyocytes and blood plasma (*c*) from mice treated with cyclophosphamide (*1*), cyclophosphamide and pantohematogen (*2*), or cyclophosphamide and granulocyte colony-stimulating factor (*3*). Confidence intervals at p=0.05.

The hemopoietic microenvironment plays an important role in the regulation of hemopoiesis [2,8]. To evaluate the mechanisms of PH- and G-CSF-induced stimulation of granulomonocytopoiesis regeneration, we studied CSA in supernatants of bone marrow nuclears.

Recombinant G-CSF increased CSA of nonadherent myelokaryocytes 5, 7, 8, and 10 days after CP injection, but did not change this parameter in adherent bone marrow cells (Fig. 2, *a*, *b*). By contrast, PH increased CSA of adherent bone marrow cells on days 10 and 12, but had no effect on nonadherent cells (Fig. 2, *a*, *b*). PH also increased plasma CSA in CP-treated mice on days 8-12 (Fig. 2, *c*).

Our results show that in animals with CP-induced hemopoietic disturbances, PH and G-CSF stimulate hemopoiesis by various mechanisms. G-CSF not only stimulates maturation of hemopoietic cells, but also triggers blood redistribution [5], while PH primarily activates myelokaryocyte proliferation. Since PH has nootropic properties [5], this stimulation of proliferation is probably related to its modulatory effect on the central neuroendocrine mechanisms of hemopoiesis regulation. Different stimulatory effects of G-CSF and PH on suppressed hemopoiesis attest to their various effects on functional activity of hemopoietic microenvironmental cells.

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