PHARMACOLOGY AND TOXICOLOGY

Modulation of the Blood-Stimulating Effect of Immobilized Granulocyte Colony-Stimulating Factor by Immobilized Hyaluronidase

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We evaluated whether immobilized hyaluronidase can modify the hematotropic effect of immobilized granulocyte CSF (G-CSF). The preparation of immobilized hyaluronidase (50 arb. units per mouse) potentiated the specific effect of immobilized G-CSF on granulomonocytopoiesis. The preparation was shown to facilitate the indirect effect of immobilized G-CSF on hemopoiesis (stimulation of the erythroid and lymphoid hemopoietic stems). These changes were accompanied by an increase in functional activity of hemopoietic precursor cells, secretion of humoral factors by bone marrow myelokaryocytes, and concentration of hemopoietins in the serum.

Key Words: *hemopoiesis; immobilized granulocyte colony-stimulating factor; immobilized hyaluronidase*

The preparations on the basis of recombinant granulocyte CSF (G-CSF) are extensively used in medical practice. This cytokine is produced by cells of the hemopoiesis-inducing microenvironment (HIM) [2,13]. G-CSF is usually prescribed for patients with abnormal granulocytopoiesis [3,9]. Previous experiments showed that hyaluronidase (HD) has a modulatory effect on the blood system [4,5,9]. This enzyme catalyzes conversion of hyaluronic acid into polymers with various molecular weights [14]. There are data on combined effect of G-CSF and HD on hemopoiesis. It was shown that each of these factors can modify specific properties of another factor [6]. However, the use of these preparations in medical practice is limited due to several reasons. For example, G-CSF produces a toxic effect, has immunogenic properties, and can be administered only parenterally [15]. It is difficult to achieve systemic effect of HD, since this enzyme can be rapidly inactivated by inhibitors [12].

The blood-stimulating, mobilizing, and hepatotropic properties of immobilized G-CSF (imG-CSF) obtained by conjugation of proteins with low-molecular-weight polymeric carrier were studied at the Institute of Pharmacology (Siberian Division of the Russian Academy of Medical Sciences) and specific activity of the preparation was demonstrated [6,8].

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Coadministration of standard preparations from G-CSF and HD has a modulatory effect. It can be suggested that combined treatment with immobilized forms of these substances will produce a strong therapeutic effect.

Here we studied the effect of combined treatment with immobilized forms of G-CSF and HD on the blood system.

MATERIALS AND METHODS

Experiments were performed on 140 CBA/CaLac mice (class I conventional strain) aging 2 months, weighing 18-20 g, and obtained from the nursery of the Institute of Pharmacology (certified animals).

Myelosuppression was induced by a single intraperitoneal injection of cyclophosphamide (CP) in the maximum permissible dose (MPD 250 mg/kg, probit analysis). Animals of the treatment groups received intragastrically imG-CSF (100 μg daily for 7 days; Scientific Features Management Company), immobilized HD (imHD, 50 arb. units daily for 2 days; Scientific Features Management Company), or imG-CSF and imHD (the same regimen of treatment) [14]. Control mice received distilled water (corresponding regimen of treatment).

The animals were decapitated under ether anesthesia on days 3, 5, 7, and 10 after cytostatic treatment. The peripheral compartment of the erythron (hemoglobin concentration, erythrocyte number, hematocrit level, and average corpuscular concentration of hemoglobin) was studied on an ABACUS automatic blood analyzer (Diatron) under veterinary conditions. The count of various forms of peripheral blood leukocytes and parameters of bone marrow hemopoiesis were evaluated by standard blood tests [10]. The number of erythroid (CFU-E) and granulomonocytic precursor cells (CFU-G) in the bone marrow and erythropoietic (EPA) and colony-stimulating activity (CSA) of conditioned media from adherent and nonadherent cells of HIM and blood serum were estimated by culture methods [7].

The results were analyzed by the Student *t* and nonparametric Mann–Whitney tests [11].

RESULTS

Studying the peripheral compartment of the erythron in mice receiving distilled water after CP treatment revealed a significant decrease in the number of erythrocytes, hematocrit (day 5), and hemoglobin concentration (from day 7 to the end of the experiment; Fig. 1, *a*, *b*). On days 3 and 5, blood platelet count in control mice was much lower than in intact animals (baseline; Fig. 1, *c*).

The recovery of peripheral erythropoiesis in treated mice (as compared to the baseline) was observed starting from day 5. The number of erythrocytes, hemoglobin concentration, and hematocrit level in animals of the imHD and imHD+imG-CSF group increased significantly on day 5 (Fig. 1, *a*). The count of peripheral blood platelets in animals of all groups increased in the same period (Fig. 1, *c*). A significant increase in platelet count and hemoglobin concentration was also observed on day 7 of combined treatment with immobilized preparations (Fig. 1, *a*, *c*). At the final stage of the study, a significant increase in hemoglobin concentration, hematocrit, and platelet count was revealed in animals of the combined treatment group. The number of erythrocytes increased significantly in imHD-receiving mice. Administration of imHD in combination with imG-CSF had a modulatory effect on the peripheral blood system, including the number of erythrocytes, count of platelets, concentration of hemoglobin (days 5, 7, and 10), and hematocrit (days 5 and 10; Fig. 1, *a*-*c*). The number of leukocytes and individual morphological forms of these cells was measured in the peripheral blood from experimental animals with CP-induced myelosuppression. Leukopenia was observed until the 7th day and resulted from a significant decrease in the number of stab neutrophils, segmented neutrophils, and lymphocytes (Fig, 2, *a*, *b*). An increase in the count of leukocytes (segmented neutrophils) was observed starting from day 7 of the study.

The test preparations were shown to accelerate the recovery of peripheral white blood cells. It was manifested in a significant increase in the total number of leukocytes (days 7 and 10). This parameter peaked on days 7 (imG-CSF group) and 10 (imG-CSF+imHD group; Fig. 2, *a*). imHD modulated the stimulating effect of G-CSF on stab neutrophils (day 5; Fig. 2, *b*).

Combined treatment with immobilized preparations had a stimulating effect on the erythroid stem during suppression of bone marrow hemopoiesis (day 3; Fig. 2, *e*). Regeneration of the bone marrow hemopoiesis on day 5 of the study was accompanied by a significant increase in the total number of nucleated cells (immature neutrophilic granulocytes, monocytes, and erythrokaryocytes) after individual or combined administration of immobilized preparations (Fig. 2, *c*-*e*). The total number of nucleated cells (immature neutrophilic granulocytes and lymphoid cells) increased most significantly on day 7 of combined treatment with immobilized preparations. The potentiating effect of combined treatment with the test preparations (*i.e.*, rapid recovery of bone marrow hemopoiesis) was manifested in an increase in the number of lymphoid cells and monocytes (day 5), total number of nucleated cells and immature neutrophilic granulocytes (day 7), and count of erythroid cells (day 10; Fig. 2, *c*-*e*).

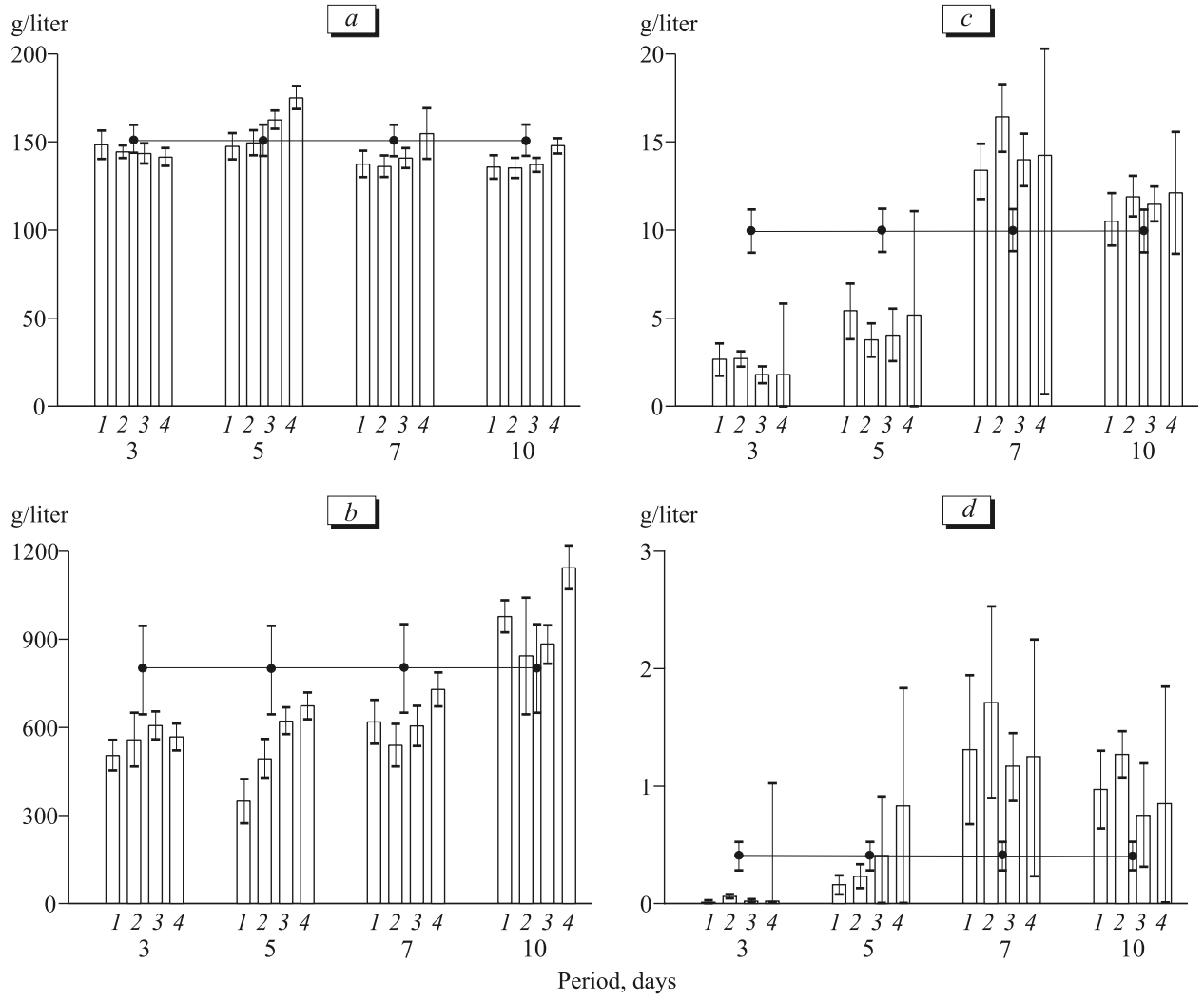

Fig. 1. Concentration of hemoglobin (*a*), count of platelets (*b*), and total number of leukocytes (*c*) and stab neutrophils (*d*) in the peripheral blood from CP-receiving (single dose 250 mg/kg) CBA/CaLac mice after single administration of the solvent (*1*), imG-CSF (*2*), imHD (*3*), or imG-CSF+imHD (*4*). Here and in Figs. 2 and 3: solid line, baseline level. Confidence intervals at $p \leq 0.05$.

CP-induced variations in hemopoietic precursor cells were associated with a compensatory response of the blood system to cytostatic treatment. It was manifested in the accumulation of erythroid precursors (day 3) and granulocyte precursors in the bone marrow (days 3, 5, and 7; Fig. 3, *a*, *b*). Administration of the test preparations to animals with cytostatic-induced myelosuppression was followed by an increase in the number of bone marrow CFU-E on day 5 of the experiment (except for mice receiving imG-CSF). Erythropoietic activity (EPA) of the bone marrow increased most significantly after combined administration of immobilized preparations. This effect persisted on day 10 of the study, which confirms a modulatory action of imHD on the blood-stimulating effect of imG-CSF (days 3 and 5; Fig. 3, *a*).

The granulocytic stem recovered after 3 days. It manifested in a significant increase in the number of CFU-G after imHD treatment. imHD had a modulatory action on the stimulating effect of imG-CSF on days 3 and 5 of the study (Fig. 3, *b*).

Hemopoietic humoral growth factors of HIM cells play a major role in the regulation of hemopoiesis. EPA was measured in conditioned media of cells from various fractions of the bone marrow [7]. EPA production by adherent myelokaryocytes was increased in animals of all groups on day 3 of the study (as compared to the baseline). This parameter increased significantly in animals of the imHD group and imHD+imG-CSF group (compared to the cytostatic control; Fig. 3, *a*). In animals of all groups, CSA of cell supernatants from the adherent fraction of bone marrow nucleated cells was maximum on days 3 and 7 (as compared to the baseline and cytostatic control; Fig. 3, *d*). The production of EPA by nonadherent myelokaryocytes increased significantly on days 3 and 7 of imHD treatment (alone or in combination with imG-CSF; Fig. 3, *b*). Admin-

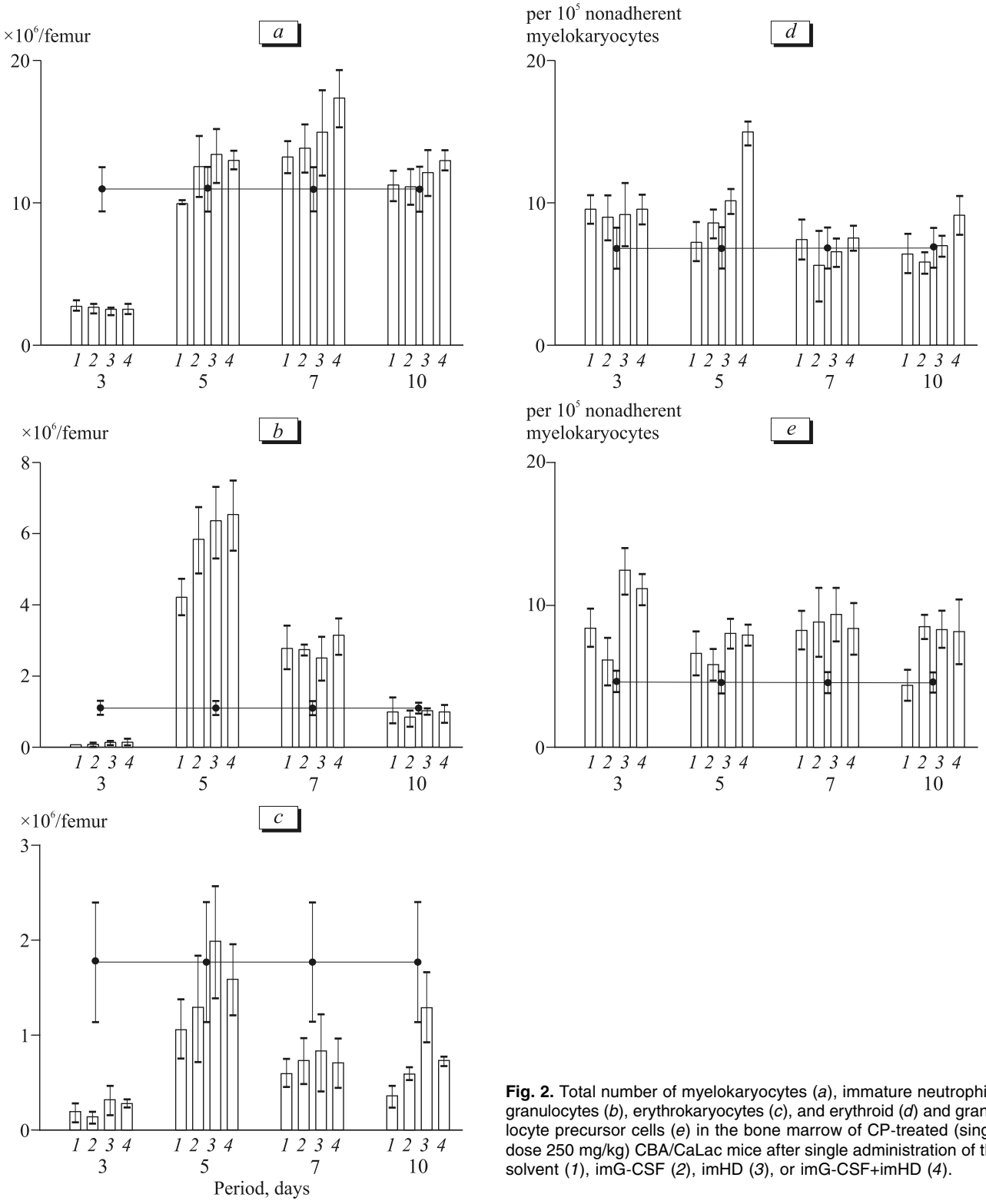

Fig. 2. Total number of myelokaryocytes (*a*), immature neutrophilic granulocytes (*b*), erythrokaryocytes (*c*), and erythroid (*d*) and granulocyte precursor cells (*e*) in the bone marrow of CP-treated (single dose 250 mg/kg) CBA/CaLac mice after single administration of the solvent (*1*), imG-CSF (*2*), imHD (*3*), or imG-CSF+imHD (*4*).

istration of imG-CSF alone or in combination with imHD was followed by a significant increase in CSA of nonadherent nucleated cells from CP-treated mice (day 3; Fig. 3, *e*). CSA of the serum from animals of the treatment group remained above the baseline at various terms of the study. The increase in EPA of treated mice was observed on days 3-7 (Fig. 3, *f*). A significant increase in EPA of the serum was revealed on days 5 and 7 after administration of imHD alone or in combination with imG-CSF (compared to the

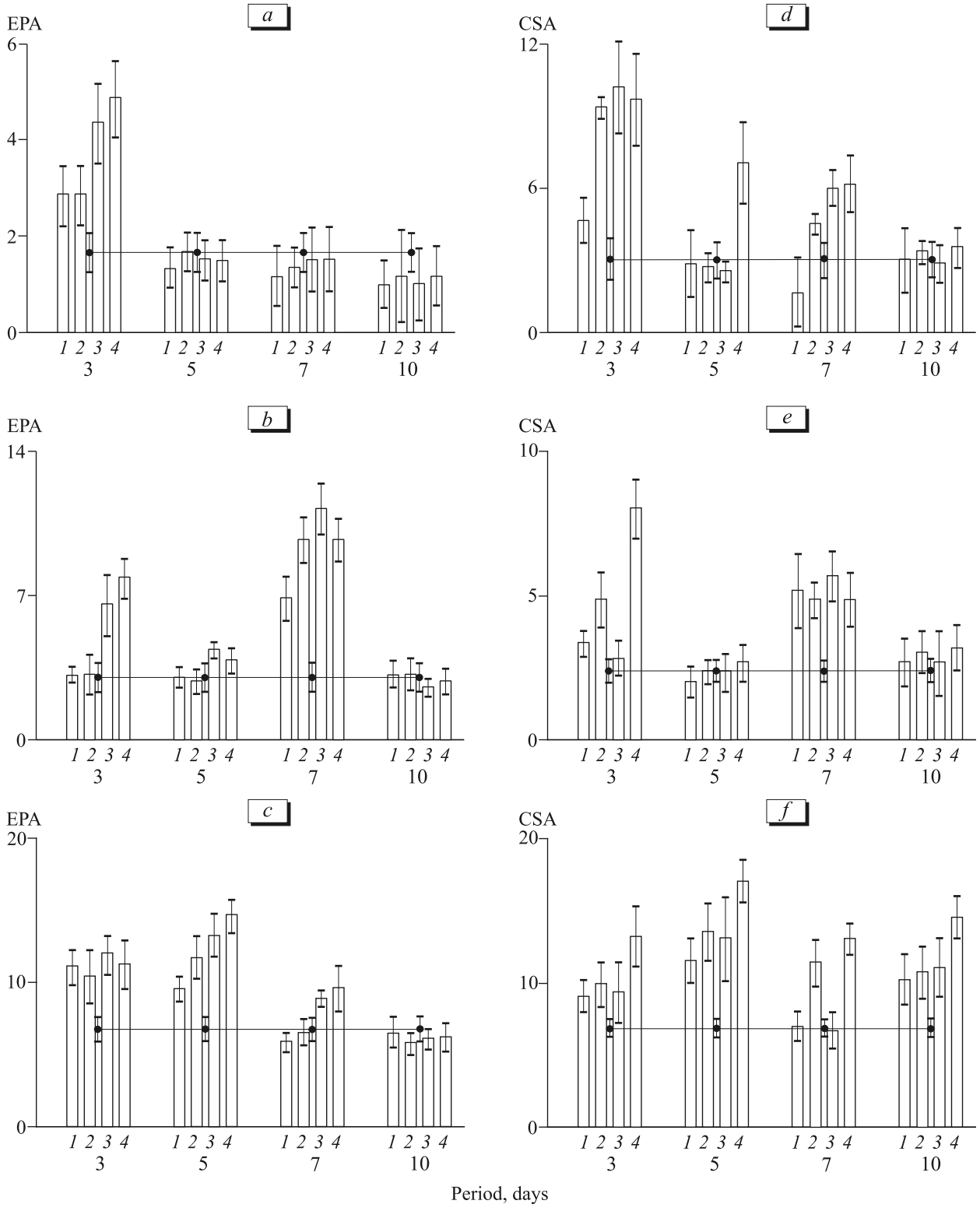

Fig. 3. EPA (*a*, *b*, *c*) and CSA (*d*, *e*, *f*; per 10^5 cells) in supernatants of nonadherent (*a*, *d*) and adherent cells of the bone marrow (*b*, *e*) and peripheral blood serum (*c*, *f*) from CP-receiving (single dose 250 mg/kg) CBA/CaLac mice after single administration of the solvent (*1*), imG-CSF (*2*), imHD (*3*), or imG-CSF+imHD (*4*).

cytostatic control; Fig. 3, *c*). CSA of the serum from cytostatic-receiving animals was shown to increase on days 3 and 5 of combined treatment with immobilized preparations. In the follow-up period (day 7), an increase in CSA was found only in mice of the imG-CSF group. At the final stage of the study,

these differences were observed only after combined treatment with immobilized preparations.

Analysis the secretory activity of conditioned media from the bone marrow and peripheral blood serum showed that imHD had an indirect modulatory action on the blood-stimulating effect of imG-CSF during cytostatic-induced myelosuppression. This conclusion is derived from a significant increase the following parameters after combined administration of immobilized preparations: EPA and CSA of adherent myelokaryocytes (days 3 and 5, respectively); EPA (days 3 and 5) and CSA of nonadherent myelokaryocytes from the bone marrow (day 3); and EPA (days 5 and 7) and CSA of the serum (days 3 and 5).

Our results indicate that the preparation of imG-CSF (oral administration) possesses a specific granulocytopoiesis-stimulating activity. It should be emphasized that the preparation of HD also has an activating effect on various components of the erythroid hemopoietic stem. It is probably related to the production of considerable amounts of hyaluronic acid fragments in the bone marrow. Published data show that these substances can stimulate cell proliferation [4,9].

We conclude that the preparation of imHD potentiates the effect of imG-CSF on the blood system. Enzyme preparations have a modulatory effect on the central, peripheral, and regulatory compartments of the blood system. They modify the direct (activation of granulocytopoiesis) and indirect effects (stimulation of other stems of hemopoiesis) of hemopoietic growth factor.

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