Mechanisms of Hemostimulating Effect of Glycyrram

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Glycyrram is a plant preparation containing 2 D-glucuronic acid residues, a component of extracellular matrix of the bone marrow. Glycyrram is shown to stimulate restoration of granulomonocyto- and erythropoiesis under conditions of cytostatic myelosuppression induced by 5-fluorouracil or cyclophosphamide. The preparation elevates the contents of both morphologically discernible elements and committed precursors. Myelotropic effect of glycyrram is not related to its direct action on hemopoietic cells but depends on stimulation of hemopoietic microenvironment.

Key Words: *5-fluorouracil; cyclophosphamide; hemopoietic environment; myelosuppression; glycyrram*

The development of blood system disturbances induced by cytostatics is related not only to direct damage to hemopoietic cells but also to the toxic effect of antitumor drugs on the regulatory apparatus, in particular, on the hemopoiesis-inducing microenvironment (HIM) [2]. Glycosaminoglycans, an important component of the extracellular matrix of HIM, contain glucuronic acid [6]. It has been previously found [1] that D-glucuronic acid effectively stimulates hemopoiesis in mice under conditions of cytostatic myelosuppression. We assumed that substances containing glucuronic acid can also exert a protective effect with respect to HIM. For instance, glycyrram containing 2 glucuronic acid residues was found to exhibit hemostimulating activity [4].

The aim of the present study was to examine the possibility of using glycyrram as a hemopoietic activator in myelosuppression induced by various cytostatics and to elucidate the mechanisms of hemostimulating activity of this drug.

MATERIALS AND METHODS

Experiments were performed on 370 male CBA mice weighing 18-20 g (Rassvet Nursery, Tomsk). The animals were singly injected with either 5-fluorouracil (Darnitsa Chemopharmaceutic Association) or cyclophosphamide (Biokhimic Plant, Saransk) in maximally permissible doses of 228 and 250 mg/kg, respectively. Glycyrram (50 mg/kg/day) was then administered orally for 5 days. Control animals received an equivalent volume of water (0.2 ml). The mice were sacrificed by cervical dislocation under ether narcosis at different times after injection of antitumor agents. Peripheral blood erythrocytes, reticulocytes, and leukocytes were routinely counted. The total number of karyocytes in the bone marrow was determined, and their qualitative composition was assessed on bone marrow smears stained by the Nocht—Maksimov method [5]. The content of committed precursors of granulomonocytopoiesis (CFU-GM) and erythropoiesis (CFU-E) was determined by cloning nonadherent myelokaryocytes in methylcellulose medium *in vitro* [3]. Colony-stimulating and erythropoietic activities in the conditioned medium from adherent and nonadherent myelokaryocytes and in the serum of experimental and control mice were tested as described elsewhere [3]. The effect of glycyrram on the growth of CFU-GM and CFU-E in the bone marrow obtained from intact animals was assessed *in vitro*. To this end, the preparation (10^{-12}, 10^{-10}, 10^{-8}, 10^{-6}, and 10^{-4} M) was added to RPMI-1640 medium

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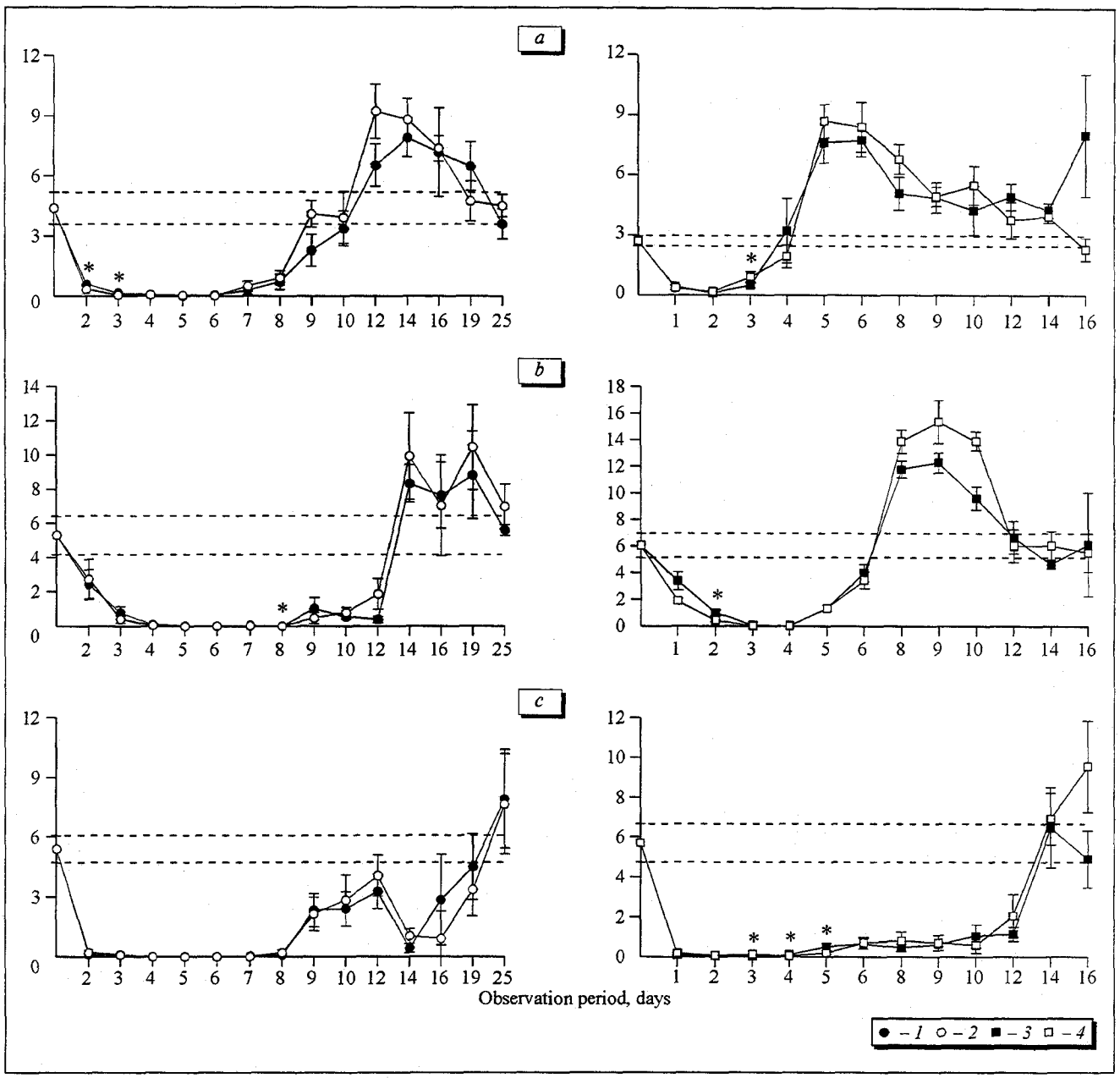

Fig. 1. Content of immature (*a*) and mature (*b*) neutrophilic granulocytes and erythroid cells (*c*) in the bone marrow from CBA mice treated with 5-fluorouracil (*1*), 5-fluorouracil+glycyrram (*2*), cyclophosphamide (*3*), and cyclophosphamide+glycyrram (*4*). Ordinate: cell content, $\times 10^6$/ femur. Confidence intervals are indicated at p=0.05.

(Sigma) containing 20% fetal calf serum (Serva), 280 mg/ml L-glutamine (Sigma), 5×10^{-5} M 2-mercaptoethanol, and 1% methylcellulose (Sigma). Total suspension of bone marrow cells from intact animals and its different fractions were used as the test system.

RESULTS

Glycyrram stimulated restoration of bone marrow cellularity due to an increase in the number of immature and mature neutrophilic granulocytes. Bone marrow hyperplasia was observed on days 12-14 after injection of 5-fluorouracil and on days 7-10 after injection of cyclophosphamide (Fig. 1). In both cases a considerable rise of peripheral blood reticulocytes was noted. In the group treated with 5-fluorouracil and glycyrram this parameter 30-35% surpassed the control values on days 12-25 of the experiment. The test preparation twice increased the reticulocyte count in cyclophosphamide-treated mice on days 7-8 after the cytostatic challenge.

Changes in the content of morphologically discernible precursors resulted from enhanced accumulation of granulomonocyte and erythroid pre-

cursors in the bone marrow during a period preceding the restoration of cellularity of the corresponding hemopoietic cell lineages (Fig. 2).

The study of the hemostimulating effect of glycyrram revealed higher colony-stimulating and erythropoietic activities in supernatants of adherent bone marrow cells at the early stage after administration of cytostatics (Fig. 3, *a*, *c*), while secretory activity of nonadherent HIM fraction increased on days 6-8 and 8-12 for cyclophosphamide- and 5-fluorouracil-induced hemodepression, respectively (Fig. 3, *c*, *d*). For both cytostatics, no significant differences in both colony-stimulating and erythropoietic activities of the blood were found between the control and experimental mice.

In vitro experiments showed that the addition of 10^{-4} M glycyrram to the medium stimulates the growth of both granulocyte and erythroid colonies from nonfractionated bone marrow cells in the presence of exogenous growth factors (by 71 and 100%, respectively). Lower glycyrram concentrations had no effect on the intensity of colony formation. Glycyrram in working concentrations did not stimulate colony formation from nonadherent myelokaryocytes. Pretreatment of these cells with glycyrram had no effect on the efficiency of colony formation occurring in the presence of either colony-stimulating or erythropoietic activity. However, preincubation of adherent bone marrow cells with a maximum concentration of glycyrram (10^{-4} M) enhanced colony formation from co-cultured nontreated nuclears.

Thus, the test preparation exhibited no direct effect on erythro- and granulomonocytopoiesis precursors. Stimulation of colony formation from nonfractionated bone marrow cells *in vitro* is an indirect effect of glycyrram and results from functional activation of adherent HIM elements. The last phenomenon also determines the earlier rise in secretion of humoral factors by adherent HIM fraction in comparison with nonadherent cells in the bone marrow from glycyrram-treated mice.

These data imply the possibility of using glycyrram for correction of hematological disorders induced by cytostatics.

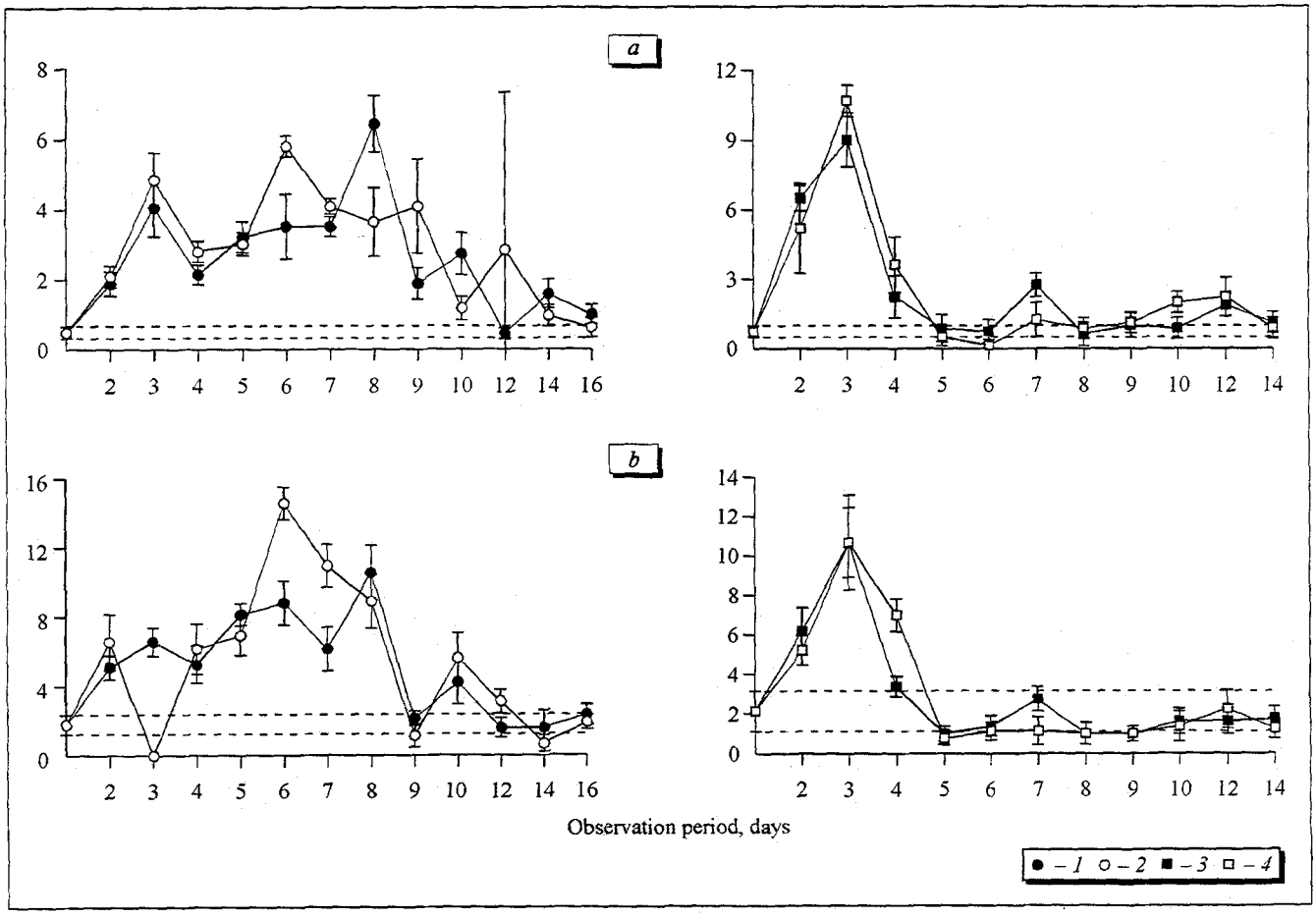

Fig. 2. Content of granulocytomacrophagal (*a*) and erythroid (*b*) precursors in the bone marrow from CBA mice treated with 5-fluorouracil (*1*), 5-fluorouracil+glycyrram (*2*), cyclophosphamide (*3*), and cyclophosphamide+glycyrram (*4*). Ordinate: content of hemopoietic precursors (per 10^5 myelokaryocytes). Confidence intervals are indicated at p=0.05.

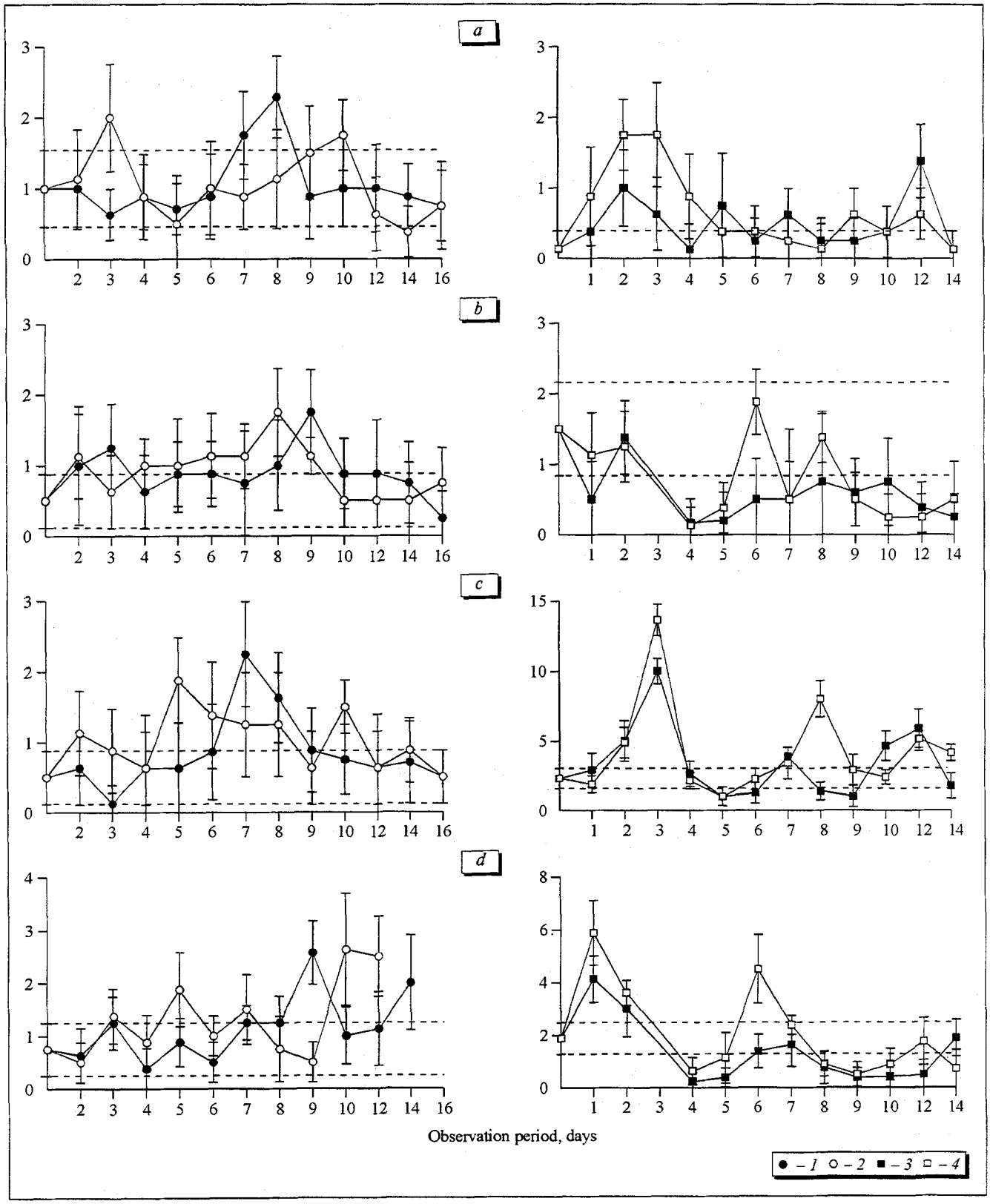

Fig. 3. Colony-stimulating activity of conditioning media from adherent (*a*), nonadherent (*b*) and erythropoietic activity of conditioning media from adherent (*c*), nonadherent (*d*) bone marrow cells from CBA mice treated with 5-fluorouracil (*1*), 5-fluorouracil+glycyrram (*2*), cyclophosphamide (*3*), and cyclophosphamide+glycyrram (*4*).

Ordinate: number of granulomonocytopoiesis (*a*, *b*) or erythropoiesis (*c*, *d*) precursors per 10^5 intact myelokaryocytes. Confidence intervals are indicated at p=0.05.

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