Mechanisms of Protective Effect of Dicarbamin on the Blood System in Cytostatic Treatment

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The effect of Dicarbamin preparation on hemopoiesis suppressed with cyclophosphamide was studied in animal experiments. It was shown that Dicarbamin produced a protective effect on granulocytic hemopoietic steam. This property of the preparation is determined by both protection of immature granulocytic cells at early terms after cytostatic treatment and more active maturation of neutrophils in the bone marrow due to enhanced secretion of humoral factors by elements of hemopoietic environment at late terms of the experiment.

Key Words: *Dicarbamin; granulocytopoiesis; granulocytic precursors; cytostatic myelosuppression*

Practically all known cytostatics even in therapeutic doses produce a suppressive effect on hemopoiesis, which manifests in reduced cellularity of the bone marrow, activation of apoptosis, and deceleration of differentiation of young hemopoietic elements into mature cells. This necessitates regular control of blood parameters during chemotherapy of tumor patients and interruption of treatment in case of necessity [7,8].

The disturbances in the blood system caused by chemotherapy are corrected by unspecific protectors of hemopoiesis: biopreparations, polysaccharide, vitamins, and nucleic acid precursors. However, their use in experiments and clinical practice showed that they are little effective in pronounced myelosuppressions or induce exhaustion of the bone marrow [14]. From pathogenetic point of view, preparations on the basis of endogenous regulators of hemopoiesis (CSF, IL, erythropoietin) seem to be more promising for clinical medicine. However, their use is limited due to high incidence of side effects and impossibility of preventive administration [10-13].

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At the same time, protection of the hemopoietic tissue from the effects of cytostatic preparations is more rational way of myelosuppression correction than stimulation of suppressed hemopoiesis. Therefore, the search for effective hemoprotectors among preparations characterized by low toxicity and the absence or minor side effects suitable for long-term administration and preventive treatment is an urgent problem.

Dicarbamin, a peptide amine of pentanedioic-1,5 acid 2-(imidazol-4-yl)-ethanamide, a biogenic amine aminoacyl derivative, is very interesting in this respect. We previously demonstrated that the preparation produced a positive effect on some parameters of the blood system under conditions of treatment with myeloinhibiting agents in both experimental studies and clinical practice [1,8].

Here we studied hemostimulating activity and mechanisms of action of Dicarbamin on the model of cyclophosphamide (CP)-induced myelosuppression.

MATERIALS AND METHODS

Experiments were carried out on 2-month-old male CBA/CaLac mice (n=120), conventional mouse strain

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obtained from the nursery of Institute of Pharmacology, Siberian Division of Russian Academy of Medical Sciences. The animals were maintained under standard vivarium conditions with free access to food and water. The mice were divided into 3 groups. Myelosuppression in experimental mice was modeled by single intraperitoneal injection of cyclophosphamide in MTD (250 mg/kg, according to probit-analysis). Of them, 56 mice additionally received Dicarbamin (Valenta Farm Company) in a dose of 0.5 mg/kg through a gastric tube (0.2 ml/mouse); the treatment was started 5 days before and continued to day 12 of cytostatic treatment. Controls (n=56) received distilled water *per os* in a volume of 0.2 ml/mouse. Baseline values were measured in intact mice (n=8).

The animals were decapitated under ether narcosis on days 2, 3, 4, 6, 8, 10, and 12 after cytostatic treatment. The count of leukocytes and their morphological forms in the peripheral blood and parameters of bone marrow hemopoiesis (total and differential counts of myelokaryocytes) were evaluated by routine methods. The intensity of maturation of morphologically recognizable cells of the granulocytic lineage in the bone marrow was evaluated by the ratio of mature (stab and segmented neutrophils) to immature neutrophils (myeloblasts, promyelocytes, myelocytes, and metamyelocytes). The content of committed granulocytic precursors (CFU-G) in the bone marrow and colony-stimulating activity (CSA) in conditioned media from adherent and non-adherent elements of the hemopoiesis-inducing microenvironment were studied *in vitro* by cloning nonadherent myelokaryocytes in semisolid culture [5].

The data were processed by methods of variation statistics using Student t test. In case of deviation of data distribution from normal law, significance of differences was evaluated by nonparametric Mann–Whitney test [6]

RESULTS

In mice of the control group, severe leukopenia was observed against the background of CP treatment. These changes were most pronounced on day 3 of the experiment and were accompanied by a decrease in the content of all leukocyte forms, especially segmented neutrophils, in the circulation (Fig. 1, *a*). Starting from day 4, leukocyte count returned to the baseline level and even surpassed it at the expense of accumulation of leukocytes (day 4), neutrophilic granulocytes and monocytes (days 6-8) followed by a trend to normalization of these parameters by day 10 of observation.

Course administration of Dicarbamin against the background of CP treatment markedly increased leukocyte count by accelerating recovery of segmented neutrophil count in the peripheral blood on days 8 and 10 (Fig. 1, *a*). By day 12, the total leukocyte count overshoot (above the background values) was similarly pronounced in the two groups.

As soon as on day 2 after cytostatic treatment we observed severe suppression of hemopoiesis processes. The total count of myelokaryocytes and cellularity of some hemopoietic stems of the bone marrow were minimum on day 3 after CP injection in the control group and in mice additionally receiving Dicarbamin (up to 7.8% of the control level). Then, hemopoiesis rapidly recovered and on days 4-6 the cellularity of the bone marrow returned to the initial level. Delayed decrease (day 2) in the content of neutrophilic promyelocytes and myelocytes in the bone marrow was observed in animals receiving Dicarbamin. Then, the count of neutrophil promyelocytes, myelocytes, and metamyelocytes in the bone marrow of mice receiving CP with and without Dicarbamin significantly surpassed that in intact animals (for instance, the count of myelocytes increased by 5-7 times on day 6; Fig. 1, *c*). At this term, the count of mature neutrophils (segmented) in the hemopoietic tissue also increased, while the number of promyelocytes decreased (Fig. 1, *b*). Similar processes were noted for erythroid cells. On day 8 of the experiment, we observed a repeated decrease in the total number of myelokaryocytes in the two specified groups (primarily due to depletion of the erythroid hemopoietic stem of the bone marrow), which is a typical phenomenon for single cytostatic treatment and attests to temporal (abortive) nature of the first elevation of bone marrow cellularity [4]. Later, the cell count in the bone marrow completely recovered and was followed by an overshoot (day 12), which was more pronounced in animals receiving CP with Dicarbamin. In these animals this phenomenon was determined by a considerable increase in the content of segmented neutrophils in the bone marrow compared to that in mice receiving CP alone (Fig. 1, *b*). Calculation of maturation indices for cells of the neutrophil lineage showed that the increase in the count of segmented neutrophils under the effect of Dicarbamin observed on days 6 and 10-12 is determined by accelerated maturation of immature morphologically identifiable bone marrow elements into mature cells. In the experimental group this parameter surpassed the control value from day 6 to 12, differences observed on day 6 and 10 being statistically significant (Fig. 1, *d*).

Evaluation of the mechanisms of granulocytopoiesis suppression and regeneration involving progenitor cells revealed changes similar to those in morphologically identifiable bone marrow cells. Administration of CP suppressed granulocyte colony-forming capacity of the bone marrow at early terms of the experiment (Fig. 2, *a*). Course treatment with Dicarbamin in combina-

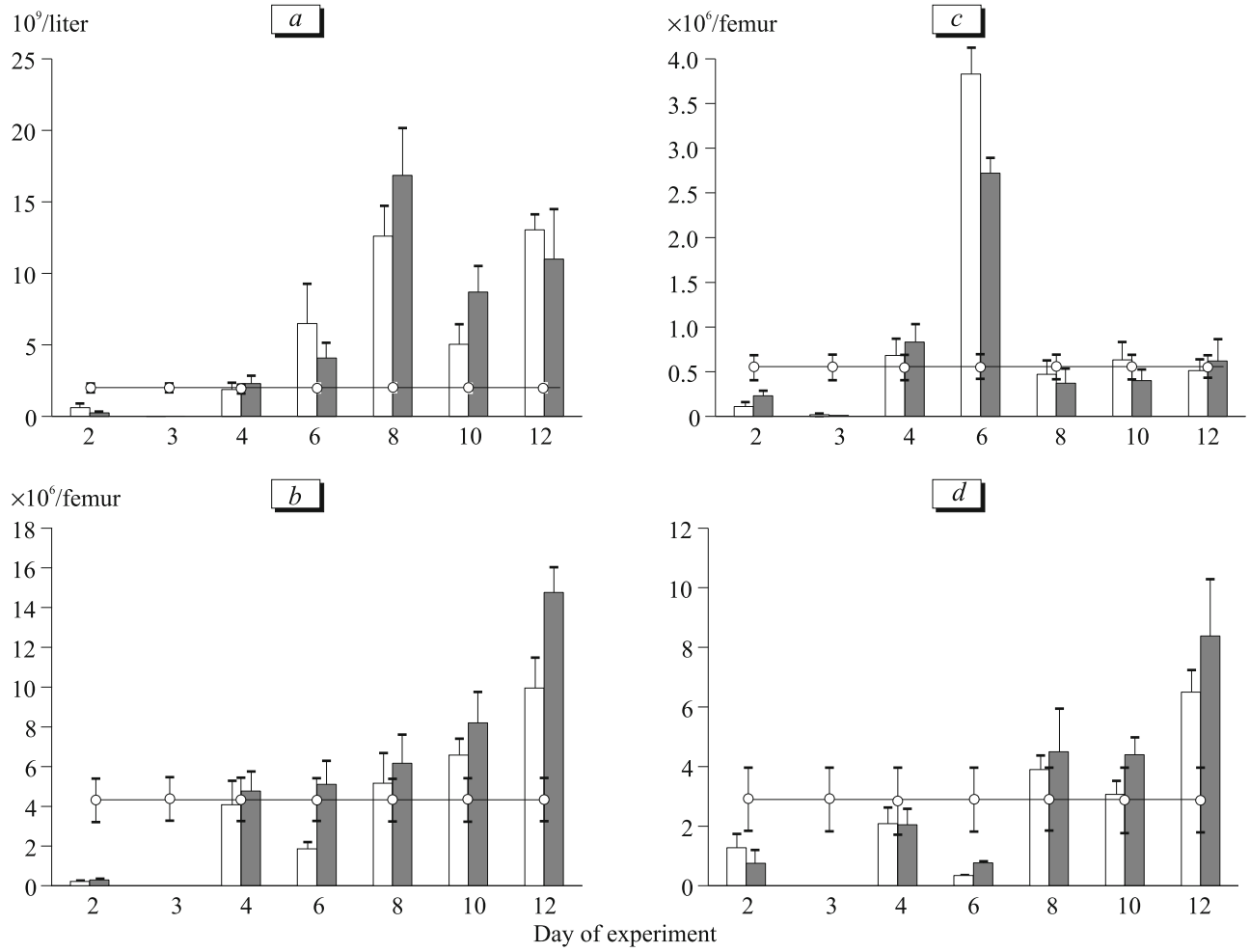

Fig. 1. Content of segmented neutrophils in the peripheral blood (*a*), segmented neutrophilic granulocytes (*b*) and neutrophilic myelocytes (*c*) and intensity of maturation of neutrophilic granulocytes (*d*, index of maturation) in the bone marrow of CBA/CaLac mice receiving single injection of CP in MTD (light bars) or single injection of CP in MTD against the background of course treatment with Dicarbamin in a dose of 0.5 mg/kg (dark bars). Solid line: level in intact animals. Confidence intervals at p=0.05.

tion with CP promoted more rapid recovery of CFU-G content in the hemopoietic tissue (on day 3 *vs*. 4 in the control), while the index of CFU-G maturation remained unchanged at this term (Fig. 2, *a*, *b*). All these findings confirm the protective effect of Dicarbamin on granulocytic precursors under conditions of cytostatic treatment. Then, changes in their content in the hemopoietic tissue were parallel in the two compared groups (on days 6-10 the corresponding values were similar and significantly surpassed the background values). This phenomenon represents a natural reaction of the hemopoietic tissue to an extreme exposure, *e.g.* cytostatic treatment [3]. By day 12, the content of CFU-G in the bone marrow of mice receiving CP alone returned to the control level, while in mice additionally receiving Dicarbamin, the colony-forming capacity of the hemopoietic tissue was still increased despite accelerated maturation of precursors during this and preceding periods of the experiment (Fig. 2, *a*, *b*). This can be related to enhanced proliferation of CFU-G or with accelerated maturation of earlier hemopoietic precursors.

Since humoral hemopoietic growth factors play a crucial role in hemopoiesis regulation, we studied CSA of conditioned media from different fractions of the bone marrow.

CSA of supernatants of adherent cells (stromal cells and macrophages are the most secretory active in this fraction) from mice receiving CP with Dicarbamin surpassed the background values on day 2 of the experiment, in contrast to that in control mice (Fig. 2, *c*). This probably explains higher content of promyelocytes and myelocytes on day 2 in mice of this group (according to differential bone marrow cell count, Fig. 1, *c*) and accelerated recovery of CFU-G content in the bone marrow on day 3 (Fig. 2, *a*). The next significant rise of CSA production by adherent cells under the effect of the test preparation (day 10 of the experiment)

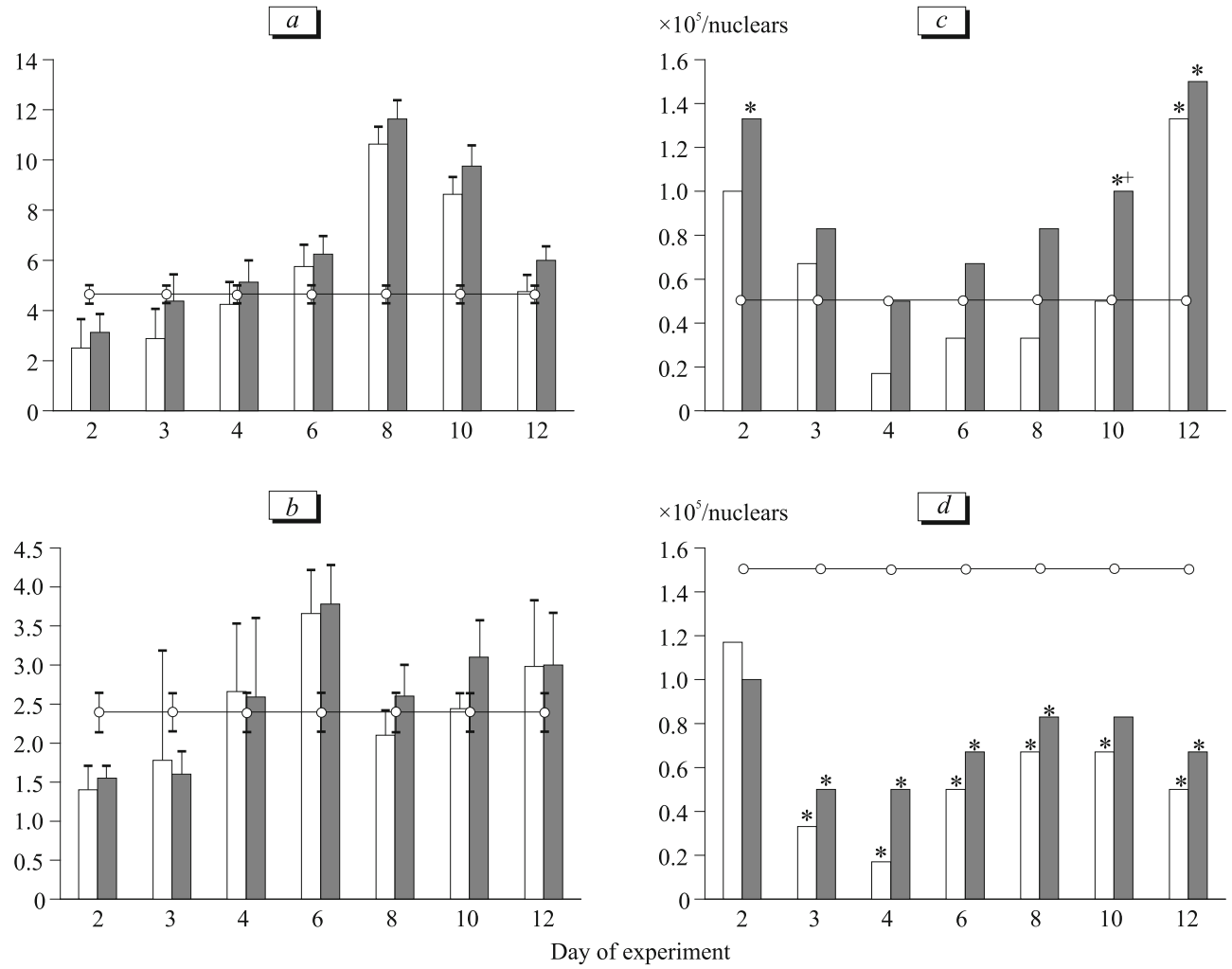

Fig. 2. Content of granulocytic precursors (*a*, per 10^5 nonadherent myelokaryocytes), dynamics of CFU-G maturation (*b*, index of maturation), CSA in supernatants from adherent (*c*) and nonadherent (*d*) bone marrow cells of CBA/CaLac mice receiving single injection of CP in MTD (light bars) or single injection of CP in MTD against the background of course treatment with Dicarbamin in a dose of 0.5 mg/kg (dark bars). Solid line: level in intact animals. Confidence intervals at p=0.05. p_U<0.05 compared to: *background, +control group.

preceded higher content of granulocytic precursors and segmented neutrophils in the hemopoietic tissue.

Changes in the production of factors constituting CSA by nonadherent cells of the bone marrow (presented primarily by lymphoid elements) seemed had no marked effect on the dynamics of bone marrow parameters throughout the experiment, except day 4 (Fig. 2, *d*). The increase in secretion of growth factors under the action of Dicarbamin during this period (compared to the control) probably determined accumulation of mature neutrophils in the bone marrow on day 6 of the experiment (Fig. 1, *b*).

These findings confirm pronounced protective effects of Dicarbamin preparation on granulocytic hemopoietic stem. This property is determined by protective effects of the preparation on granulocytic precursors and immature neutrophilic cells detected at early stages of hemopoiesis recovery after CP treatment and manifests in accelerated maturation of neutrophils in the bone marrow at late terms of the cytostatic disease. Granulocyte differentiation is intensified primarily due to more active secretion of hemopoietically active substances by cells of hemopoietic microenvironment under the effect of Dicarbamin accumulated in the hemopoietic tissue by this term as a result of repeated injections [1,2,8].

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