Potentiation of the Stimulatory Effect of Erythropoietin on Erythropoiesis by Antiserotonin Drug during Cytostatic Myelosuppression

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Combined stimulatory effect of antiserotonin drug and erythropoietin on the bone marrow erythropoiesis was studied under conditions of cytostatic myelosuppression. The stimulatory effect of erythropoietin on regeneration of the hemopoietic erythroid stem increased, if serotonin mediation in the CNS was disordered before induction of cytostatic myelosuppression. The stimulatory effect of a combination of cyproheptadine and epocrine (experimental group) significantly surpassed that of cyproheptadine and epocrine monotherapies. The increase in the content of erythroid elements in the blood system of experimental animals was due to recovery of the structural and functional integrity of the hemopoietic tissue (at the expense of reduction of the suppressive effect of CNS serotonin on the formation of erythroid hemopoietic islets) and simultaneous stimulation of division and maturation of erythroid precursors with epocrine.

Key Words: *erythropoiesis; erythropoietin; serotonin; hemopoietic islets; 5-fluorouracyl*

Recombinant human erythropoietin preparations are used for the treatment of anemias of different origin, including those caused by cytostatics [2,8]. However, long-term therapy with these drugs leads to inevitable exhaustion of the pool of erythropoiesis precursors and, hence, to the development of complications [9]. This fact largely restricts the use of drugs on the basis of erythropoietin, endogenous regulator of erythron.

It should be noted that the development of cytostatic anemia is caused by not only suppression of mitotic processes and maturation of erythroid precursors. Stubborn destructive changes in the hemopoietic tissue, disorders in cell-cell interactions, and suppression of the erythropoietin system contribute to the development of anemias [3]. Study of the distant mechanisms of hemopoiesis regulation revealed a relationship between activity of cells of the hemopoiesis-inducing microenvironment (HIM) and monoaminergic structures of the CNS [5,6]. Tropism of the erythroid hemopoietic stem to drugs modifying serotoninergic structures was found. These data suggest that recovery of the structural and functional integrity of the hemopoiesis erythroid compartment can appreciably potentiate the effect of synthetic analogs of endogenous erythropoietin on the erythron suppressed by cytostatics.

We studied the possibility of potentiation of the stimulatory effect of epocrine towards erythropoiesis suppressed by 5-fluorouracyl by cyproheptadine (antiserotonin drug).

MATERIALS AND METHODS

Experiments were carried out on 2-2.5 month-old female CBA/CaLac mice (n=550; certified first-category conventional inbred mice from breeding center of Institute of Pharmacology).

Cytostatic myelosuppression was induced by a single intraperitoneal injection of 5-fluorouracyl (fluo-

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ropyrimidine antimetabolite) in $^1/_3$ MTD (76 mg/kg). Cyproheptadine (antiserotonin drug; Serva) in a dose of 30 mg/kg was intraperitoneally injected 30 min before the cytostatic. The drug was dissolved in sterile saline directly before injection. Starting from the next day after cytostatic injection, the animals received daily subcutaneous injections of epocrine (recombinant human erythropoietin, epoietin-α; Institute of Extrapure Biopreparations) in a dose of 10 U/mouse for 5 days. Controls were injected with saline in an equivalent volume (0.2 ml) in all experimental series according to similar protocols. Intact animals served as the reference group (basal values).

Reticulocyte count in the peripheral blood was measured on days 2-7 and 12 after injection of the cytostatic. The animals were then sacrificed by cervical dislocation under ether narcosis and erythrokaryocytes in the bone marrow were counted [4]. Structural and functional organization of the bone marrow was studied by enzymatic isolation of hemopoietic islets (HI) and subsequent evaluation of their quantitative and qualitative composition [11].

The data were statistically processed by standard methods of variation statistics. The significance of differences was evaluated using Student *t* test or nonparametric Mann—Whitney *U* test.

RESULTS

The content of erythrokaryocytes in the bone marrow was reduced after 5-fluorouracyl injection throughout the entire period of observation; the drug caused the development of pronounced reticulocytopenia in the peripheral blood on days 2-6 of experiment (Fig. 1). Suppression of the erythroid stem was caused by inhibited formation of macrophage-positive, erythroid, and erythrogranulocytic (mixed) HI over the entire period of the study (Fig. 2). Secretion of humoral regulators of erythropoiesis by adherent cells of HIM was disordered, the erythropoietin system was suppressed, and serotonin-dependent proliferative activity and intensity of differentiation of erythroid precursors were inhibited under these conditions [3,6]. It is noteworthy that addition of recombinant human erythropoietin to the culture of nonadherent murine myelokaryocytes treated with cytostatics stimulated production of colony-forming erythroid units (CFU-E) [6]. Obviously, erythroid precursors retain high reactivity to erythropoietin (one of the leading regulators of the erythron).

Treatment with epocrine after 5-fluorouracyl led to a statistically significant increase in the count of erythroid cells in hemopoietic tissue (days 4, 6, 7, 12; Fig. 1). A significant increase in reticulocyte count in the peripheral blood was observed starting from day 2 until the end of the experiment. It is noteworthy that the cell composition of hemopoietic erythroid stem normalized on days 6, 7, and 12 of experiment. According to modern concepts, burst-forming erythroid units (BFU-E) and CFU-E cannot proliferate without erythropoietin, and hemoglobin synthesis in erythroblasts does not start without it [1,10,12,13]. No doubt, the stimulatory effect of epocrine in our experiments is linked with stimulation of functional activity of

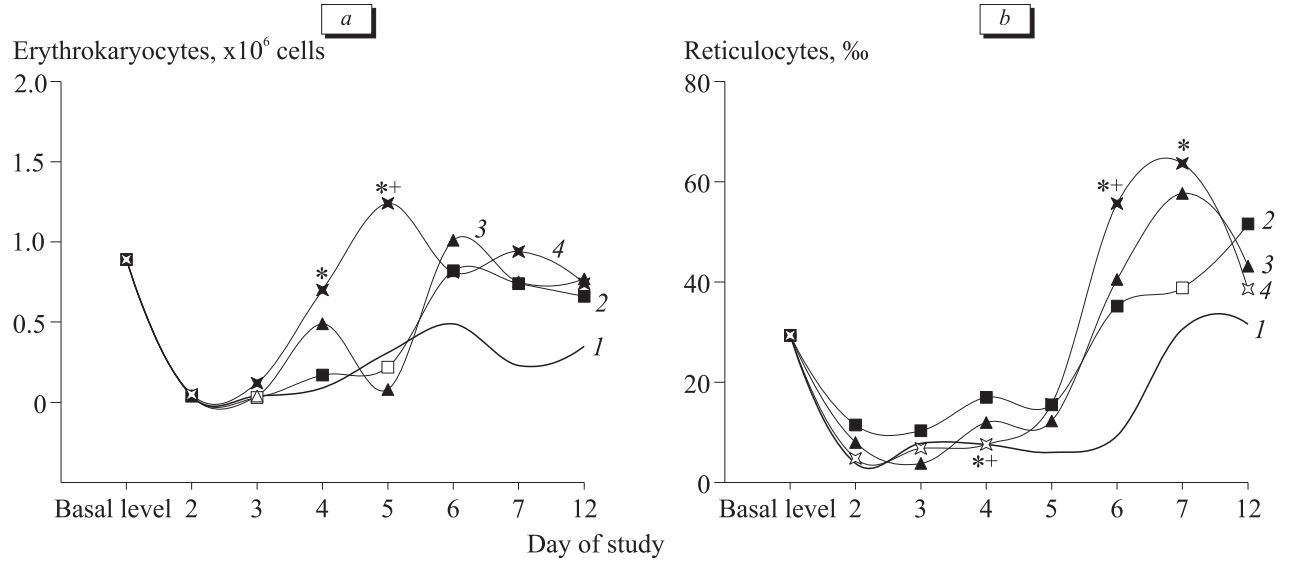

Fig. 1. Effects of cyproheptadine, epocrine, and a combination of both on the time course of erythrokaryocyte count in the bone marrow (*a*) and reticulocyte count in the peripheral blood (*b*) in CBA/CaLac mice surviving after 5-fluorouracyl injection. Here and in Fig. 2: *1*) injection of 5-fluorouracyl with saline; *2*) course of epocrine after 5-fluorouracyl; *3*) 5-fluorouracyl after cyproheptadine; *4*) combined treatment with cyproheptadine and epocrine under conditions of 5-fluorouracyl treatment. Here and in Fig. 2: dark symbols: $p<0.05$ compared to *1*; $^*p<0.05$ compared to *2*; $^+p<0.05$ compared to *3*.

erythropoiesis precursors. The fact that erythropoietin preparation did not influence the structural and functional integrity of the bone marrow throughout the entire study supports this conclusion (Fig. 2).

Cyproheptadine also stimulated erythropoiesis regeneration after 5-fluorouracyl treatment. This was seen from accumulation of erythrokaryocytes in the bone marrow (days 4, 6, 7, 12) and reticulocytes in the peripheral blood (days 2, 4-7, 12; Fig. 1). In contrast to epocrine, the effect of antiserotonin drug was mainly due to *de novo* formation of cell complexes with central macrophage element (days 2-4, 7), as well as formation of erythroid (days 2-4, 12) and mixed HI (days 2-4, 6, 7, 12; Fig. 2). Cyproheptadine suppression of CFU-E growth in culture of nonadherent myelokaryocytes of animals injected with 5-fluorouracyl confirms the key role of this mechanism [6].

Blocking of postsynaptic serotonin C2 receptors followed by erythropoietin injection resulted in a more intense recovery of the erythroid stem cell count in comparison with that in animals treated with epocrine and cyproheptadine (days 3-12; Fig. 1). As early as on day 4, the content of erythroid cells in the bone marrow virtually did not differ from the initial level, and erythropoiesis hyperplasia developed by day 5. More active release of reticulocytes into peripheral blood resulted in the development of manifest reticulocytosis on days 6, 7.

The study of local mechanisms of combined effect of cyproheptadine and epocrine showed active recovery of the structural and functional integrity of the bone marrow in mice injected with 5-fluorouracyl. The drugs promoted accumulation of macrophage-positive (days 2, 4-7), erythroid (days 2, 7), and less so of erythrogranulocytic cell complexes (days 2, 4, 6, 7, 12; Fig. 2). The formation of additional foci of erythroid hemopoiesis was followed by the development of bone marrow erythropoiesis hyperplasia and blood reticulocytosis. It is noteworthy that erythroid HI cells in the experimental group were presented by medium and late polychromatophilic normoblasts, oxyphilic normoblasts, and reticulocytes. Interestingly that the counts of nuclears in HI in control groups was significantly lower than in the experiment, no reticulocytes were detected in mice treated with cyproheptadine.

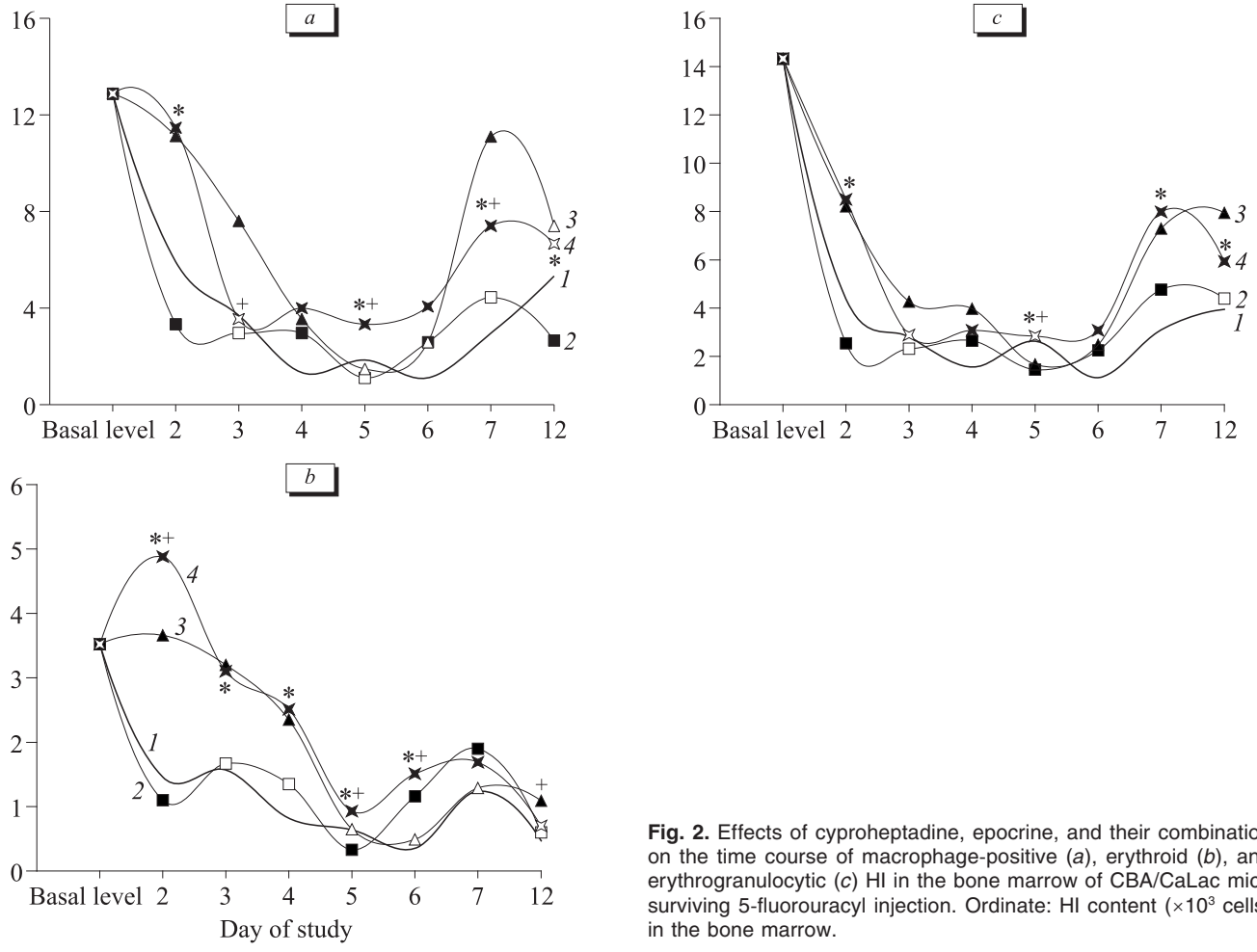

Fig. 2. Effects of cyproheptadine, epocrine, and their combination on the time course of macrophage-positive (*a*), erythroid (*b*), and erythrogranulocytic (*c*) HI in the bone marrow of CBA/CaLac mice surviving 5-fluorouracyl injection. Ordinate: HI content ($\times 10^3$ cells) in the bone marrow.

Hence, violation of serotonin mediation in CNS before induction of cytostatic myelosuppression by 5-fluorouracyl and subsequent treatment with erythropoietin abolished suppression of the bone marrow erythropoiesis and promote intensive recovery of reticulocyte count in the peripheral blood and even development of reticulocytosis. The stimulatory effect in experiment (combination of cyproheptadine and epocrine) was higher in comparison with recovery of suppressed erythropoiesis in animals treated with epocrine or cyproheptadine.

The effect of CNS serotonin on HI under conditions of 5-fluorouracyl treatment is "destructive". Our previous study demonstrated a suppressive effect of serotonin *in vitro* on mitotic activity of animal erythroid precursors under conditions of 5-fluorouracyl treatment [6]. Hence, we hypothesize a "serotonin" mechanism of anemia development in cytostatic treatment. It is realized at the level of committed hemopoietic precursors and local regulation system (HI formation). The use of drugs reducing activity of the serotoninergic system in therapy of cytostatic anemias seems to be advisable.

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