Dependence of the Proliferation of Hemopoietic Adrenergic Precursors Under the Influence of Cytostatics

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After administration of 5-fluorouracil in a high dose (114 mg/kg), the proliferation of committed precursors of erythro- and granulomonocytopoiesis is controlled by sympatheticoadrenal system. On the one hand, catecholamines facilitate, predominantly via β-structures, the migration of Thy-1$^+$-cells that stimulate hemopoietic precursors for the S-phase of cell cycle. On the other hand, sympatheticoadrenal transmitters potentiate via β- and α-adrenoreceptors the suppressor activity of the cytostatic on the growth and maturation of erythropoietic and, to a lesser extent, granulocytopoietic precursors. These factors may contribute to the process of long restoration of the granulocyte and, to a higher extent, erythrocyte stem cells in the bone marrow after a high dose of 5-fluorouracil.

Key Words: *Thy-1$^+$-cells; committed precursors of hemopoiesis; S-phase; adrenergic structures; 5-fluorouracil*

The search for effective, pathogenetically reasonable ways of overcoming hypoplastic state of the bone marrow caused by various damaging factors, such as cytostatics, ionizing radiation, hematotropic toxins, etc., is an urgent problem in practical medicine [2]. Restoration of hemopoiesis strongly depends on the functional state of viable hemopoietic precursors [8]. Therefore, the investigation into the mechanisms of local and remote regulation of proliferation and differentiation of hemopoietic cells in extreme state is of interest. It is known that neurotransmitters control the rate of cell division [10]. Catecholamines modulate proliferative status of various cells [7,10], including hemopoietic [4,14]. Direct (via receptors) and indirect (via neuroendocrinic factors of the microenvironment) control is the key factor in division and maturation of hemopoietic precursors [14]. Nevertheless, the possibility of autonomic regulation of the mitotic cycle of hemopoietic precursors in myelosuppressive states practically has not been explored.

In the present study we evaluated the role of sympathic structures in the regulation of DNA-synthesizing precursors in the hemopoietic tissues of mice treated with a cytostatic agent.

MATERIALS AND METHODS

Experiments were performed on 269 male CBA mice weighing 18-20 g. Myelosuppression was modeled by a single intraperitoneal injection of 5-fluorouracil (5-FU) in a dose of 114 mg/kg (half of the maximum tolerated dose, MTD). The α-adrenolytic agent dihydroergotamine (3.9 mg/kg) or the β-adrenolytic agent propranolol (5 mg/kg) was injected subcutaneously 3-5 min before and 5 h after injection of the cytostatic. Control mice were injected with an equivalent volume of normal saline (0.2 ml). The animals were sacrificed under ether anesthesia by cervical dislocation at various periods after the cytostatic injection. The contents of T cells in the bone marrow

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TABLE 1. Changes in the Content of Thy-1$^+$ Cells (% Cytotoxic Index) in the Bone Marrow of Mice Given 5-FU and α- or β-Adrenolytic ($X \pm m$)

Observation period, days	5-FU	5-FU+α-adrenolytic	5-FU+β-adrenolytic
Before injection	4.09±1.01	4.09±1.01	4.09±1.01
4	10.91±1.69*	6.97±2.82	8.13±1.45*
5	16.44±2.51**	9.21±2.99	6.33±2.53*
6	22.76±2.74**	13.40±3.44*	7.42±3.50*
7	4.49±1.35	12.40±2.05*,*	5.42±1.78
8	12.23±3.63*	9.27±1.48*	16.58±2.34**
9	14.96±1.52**	10.44±1.45*	1.78±0.85**
10	2.81±1.61	0.82±0.53*	2.38±1.00

Note. Here and in Table 2: *$p<0.05$, **$p<0.001$ compared with initial level; *$p<0.05$, **$p<0.001$ compared with the level after administration of 5-FU (t test for Table 1, U test for Table 2).

was determined by the cytotoxic test [3] with DT-anti-Thy-1 monoclonal antibodies ("DiagnoTech", Moscow). Nontoxic undiluted rabbit serum served as the source of complement.

Proliferative activity of hemopoietic precursors was assessed as described elsewhere [3]. Viable nuclear cells from the bone marrow (5×10^6 cells/ml RPMI-1640) were incubated with 1.5 mg/ml hydroxyurea (Sigma) for 2 h in a thermostat. The precursors were cloned in 96-well plastic plates (Corning) in a methylcellulose culture of myelokaryocytes (2×10^5 cells/ml semisolid culture medium) [3]. The pools of colony-forming units-erythrocyte and granulocyte/monocyte (CFU-E and CFU-GM, respectively) in the S-phase of mitotic cycle were calculated from the following formula: $N=[(a-b)/2]\times10^5$ cultured cells, where a and b are the numbers of precursors grown from myelokaryocytes not treated and treated with hydroxyurea, respectively.

Direct effects of α- and β-adrenomimetics (mesaton and alupent, respectively) on the growth of CFU-E and CFU-GM in a methylcellulose culture of nonadherent myelokaryocytes isolated on day 3 after administration of 5-FU were examined in the concentration range 1 nM-10 μM against the background of β- or α-adrenolytic added *in vitro* in a concentration 0.1 mM.

Statistical analysis of the results was carried out with the use of Student's t test and Wilcoxon U test.

RESULTS

During the bone marrow depletion caused by 0.5 MTD 5-FU (days 1-9) [11], the mechanisms providing for postcytostatic restoration are formed. Specifically, the content of Thy-1$^+$ cells increases on day 4, reaching the maximum on days 6-9 of the investigation (557 and 366% of the original level, Table 1). The absolute number of lymphoid cells and cytokine production by nonadherent bone marrow nuclear cells on days 7-10 also increased [6]. Consequently, these results can be interpreted as accumulation of T cells in the bone marrow of mice treated with a high dose of 5-FU.

TABLE 2. Changes in the Contents (per 10^6 cells) of Committed Hemopoietic Precursors in the S-Phase in Bone Marrow of Mice Given 5-FU and α- or β-Adrenolytic

Observation period, days	CFU-E			CFU-GM		
	5-FU	5-FU+ α-adrenolytic	5-FU+ β-adrenolytic	5-FU	5-FU+ α-adrenolytic	5-FU+ β-adrenolytic
Before administration	0.3	0.3	0.3	0	0	0
4	0	0	0	—	—	—
5	0	0.9	1.3	0.6	3.4*,*	0
6	1.3	3.1	5.0*,*	1.3	2.1	3.8*
7	4.4*	4.3*	0*	4.4*	2.6	1.2
8	5.0*	0*	2.6	3.8*	2.4	0*
9	5.0*	0*	0.6*	7.5*	9.4*	3.8**
10	3.1	0.6	0*	5.0*	1.9	0*

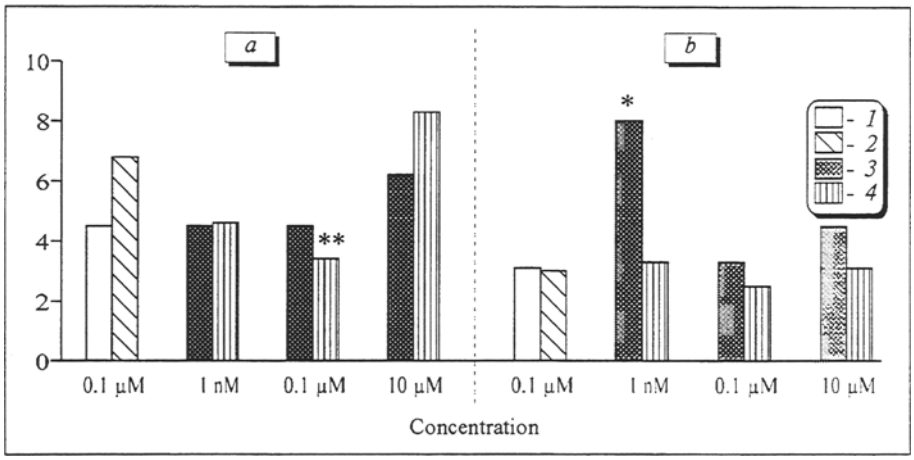

Fig. 1. Effects of adrenergic agonists on the contents of CFU-E (*a*) and CFU-GM (*b*) in a culture of nonadherent myelokaryocytes isolated from bone marrow of mice 3 days after administration of 5-fluorouracil. Ordinate: number of precursors per 10^5 cells. *1*) propranolol; *2*) dihydroergotamine; *3*) propranolol+mesaton; *4*) dihydroergotamine+alupent. Statistically significant (*U* test) in comparison with *propranolol and **dihydroergotamine.

It was established that thymocytes migrating into the bone marrow after administration of 5-FU are involved in proliferation and differentiation of splenic colony-forming units [9], the population of which is restored on days 5-7 [8]. In this case the synthesis of DNA in committed precursors of CFU-E and CFU-GM has been observed on days 7-10 after administration of 5-FU (Table 2). At the same time, the ability of adherent myelokaryocytes to secrete erythropoietic and colony-stimulating activities in response to 5-FU was restored only on days 10-11 [6]. Presumably, in 5-FU-treated mice the transition of CFU-E and CFU-GM towards the S-phase is determined by the spectrum of lymphokines produced by T cell migrating into the bone marrow.

These findings agree with the ability of activated T cells to stimulate proliferation and differentiation of committed precursors [13] under extreme conditions [15]. It was interesting to assess the ability of α- and β-adrenergic antagonists to control the number of hemopoietic precursor cells in the S-phase in 5-FU-treated mice. In fact, on days 7-10 of the experiment the content of DNA-synthesizing CFU-E (α-, β-adrenolytic) and CFU-GM (β-adrenolytic) decreased (Table 2).

These processes are determined predominantly by the ability of β-adrenolytic injected together with 5-FU to reduce the content of Thy-1^+ cells in the bone marrow (Table 1) and their secretory activity on days 5-6 and 9 of the experiment [6]. It was reported that β-adrenergic structures participate in the migration of lymphocytes into the bone marrow [5], and stimulation of the sympatheticoadrenal system for several (up to 4) days precedes changes in the activity of lymphocytes [1].

After administration of α-adrenoblocker, the number of T cells in the bone marrow remained practically unchanged. Moreover, by the 7th day, it increased by 203 and 176%, respectively, over the original and control (one cytostatic) levels (Table 1), which may account for the absence of the inhibitory effect of the preparation on the cell cycle of CFU-GM (Table 2).

It was demonstrated [12] that low doses of 5-FU (15-30 mg/kg) increase the content of the bone marrow cells in the S-phase by the 3rd-6th day after administration of the cytostatic, which is likely to result from the predominance of stimulating effect of 5-FU mediated via the neuroendocrine system over its direct genotoxic effect. 5-Fluorouracil activates the sympatheticoadrenal system [1]. Catecholamines increase the content of DNA-synthesizing cells [7]. However, when the cytostatic dose is increased to 100 mg/kg and higher, prolonged suppression of DNA production [12] has been observed in granulocyte and, to a greater extent, in erythropoietic precursors [8].

Since *in vivo* under the background of 114 mg/kg 5-FU α- or β-adrenergic antagonist caused a transient rise of DNA production by granulomonocytopoietic (day 5) and erythrocytopoietic precursors (day 6, Table 2), it was hypothesized that catecholamines potentiate direct damaging effect of 5-FU on hemopoietic cells. Under conditions of immobilization stress, α- (1 nM-0.1 μM) or β-adrenomimetic (10 μM) increases the yield of CFU-GM and CFU-E, respectively, stimulate in a culture of nonadherent myelokaryocytes [4].

In a culture of nonadherent bone marrow cells isolated on day 3 after administration of 5-FU, the β-adrenolytic+α-adrenomimetic combination (stimulation of α-adrenoreceptors) caused a significant 3-fold increase in the CFU-GM content (*U* test, $p<0.05$, compared with one blocker) only at low concentration of the mimetic (1 nM, Fig. 1).

On the other hand, the α-adrenolytic+β-adrenomimetic combination (activation of β-adrenoreceptors) at an agonist concentration of 0.1 μM suppressed 2-fold (*U* test, $p<0.05$, compared with one blocker) the formation of erythroid colonies by nonadherent

myelokaryocytes (Fig. 1). Thus, in contrast to immobilization stress, the stimulatory effect of low concentration of mesaton and inhibiting effect of alupent in 5-FU-treated mice indicate that catecholamines potentiate the suppressing effect of cytostatics on CFU-E and, to a lesser extent, on CFU-GM. Presumably, this is associated with intracellular modifications of the cyclic nucleotide contents, which to a certain degree determine proliferation and differentiation processes [10].

The above-mentioned effects of adrenergic antagonists and agonists on hemopoiesis indicate that the response of sympatheticoadrenal system to a hemopoiesis-suppressing agent stimulates (via migration of T cells into the bone marrow) the proliferation of erythropoietic and, to a greater extent, granulomonocytopoietic precursors. On the other hand, direct inhibiting effect of catecholamines on the growth and maturation of hemopoietic precursors (CFU-E>CFU-GM) has been revealed against the background of cytostatic effect. Thus, the ambiguity of sympathic influences on the mitotic cycle of hemopoietic cells is one of the causes [9,11] of prolonged restoration of the granulocytic and predominantly erythroid stem cells in the bone marrow after administration of high doses of 5-FU.

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