PHARMACOLOGY AND TOXICOLOGY

Catecholamine Regulation of Stromal Precursors and Hemopoietic Stem Cells in Cytostatic Myelosuppression

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Effects of a sympatholytic drug on bone marrow stromal and hemopoietic precursors were studied on the model of cyclophosphamide-induced myelosuppression. Sympatholytic treatment increased the content of hemopoietic stem cells of different classes in the bone marrow. Selective stimulation of differentiation of polypotent precursors into granulocyte-macrophage precursors was noted. Acceleration of proliferation and maturation of granulocytic precursors was observed at later terms during regeneration of the hemopoietic tissue. The sympatholytic inhibited proliferation of stromal precursors and reduced feeder activity of fibroblasts for granulocyte precursors.

Key Words: *stromal precursors; hemopoietic stem cells; granulocyte precursors; catecholamines; cyclophosphamide*

Neurohumoral processes are the basis of structural and functional organization of the blood system in extreme exposures (cytostatic treatment, immobilization stress, experimental neuroses) [2,3,8]. Under conditions of cytostatic treatment (cyclophosphamide, 5-fluorouracil), catecholamines via adrenergic receptors directly inhibit committed hemopoietic precursors of the granulocyte-macrophage (CFU-GM) and erythroid (CFU-E) lineages [3,6]. Catecholamines also inhibit recovery of damaged hemopoietic microenvironment elements, which aggravated uncoupling of CFU-GM and CFU-E proliferation and differentiation processes and resulted in delayed normalization of the cellularity of the erythroid and granulocytic lineages.

Regeneration of hemopoiesis under conditions of myelosuppression cannot be completed without hemopoietic stem cells [1,5] and mesenchymal stem cells capable of transferring and creating hemopoietic microenvironment [1,9]. At the same time, the interaction of hemopoietic and mesenchymal stem cells and regulation of their activity by central neurotransmitter structures under conditions of blood system pathology are poorly studied.

Here we studied the role of CNS catecholamines in the regulation of stromal and polypotent hemopoietic precursors under conditions of cyclophosphamide treatment.

MATERIALS AND METHODS

The experiments were carried out on 2-2.5-month-old female CBA/CaLac mice (n=105, conventional mouse strain obtained from the nursery of Institute of Pharmacology, Tomsk Research Center, Siberian Division of the Russian Academy of Medical Sciences).

Cytostatic myelosuppression was induced by single intraperitoneal injections of alkylating cytostatic cy-

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clophosphamide in a dose of 83 mg/kg. The animals of experimental group received single intraperitoneal injection of sympatholytic reserpine (Polfa) in a dose of 2 mg/kg 30 min before cytostatic treatment. Intact animals served as the background (intact control, IC). On days 1-7 after cytostatic treatment, the mice were sacrificed by CO_2 overdose under ether narcosis and mature and immature forms of neutrophilic granulocytes in the bone marrow were counted. The content of polypotent hemopoietic precursors in the bone marrow that formed colonies consisting of non-differentiated hemopoietic cells (CFU-N) was determined by the method of limiting dilutions [4,5]. Then, differentiation of polypotent hemopoietic precursors under the effect of granulocytic CFU (CFU-G, neupogen, Hoffman-La Roche Ltd) into more mature cells, CFU-GM and CFU-G, was studied [5]. To this end, CFU after the 3rd passage were dissociated and transferred to culture media containing G-CSF (2 ng/ml) for growth of CFU-GM and CFU-G. The formed CFU were counted after 7 days.

The intensity of growth of granulocyte-erythroid-macrophage-megakaryocyte colonies (CFU-GEMM) consisting of 4 types of hemopoietic cells (erythrokaryocytes, granulocytes, macrophages, and megakaryocytes) and granulocytic colonies was evaluated [11]. Proliferative activity of CFU-G was evaluated by the method of cell suicide using hydroxyurea and the intensity of cell differentiation was determined by the index of maturation (ratio of clusters to colonies in the same well) [4]. We also evaluated the content of stromal precursor cells forming fibroblast colonies in culture of adherent myelokaryocytes (CFU-F) and feeder activity of fibroblast cells for CFU-GEMM and CFU-G [4].

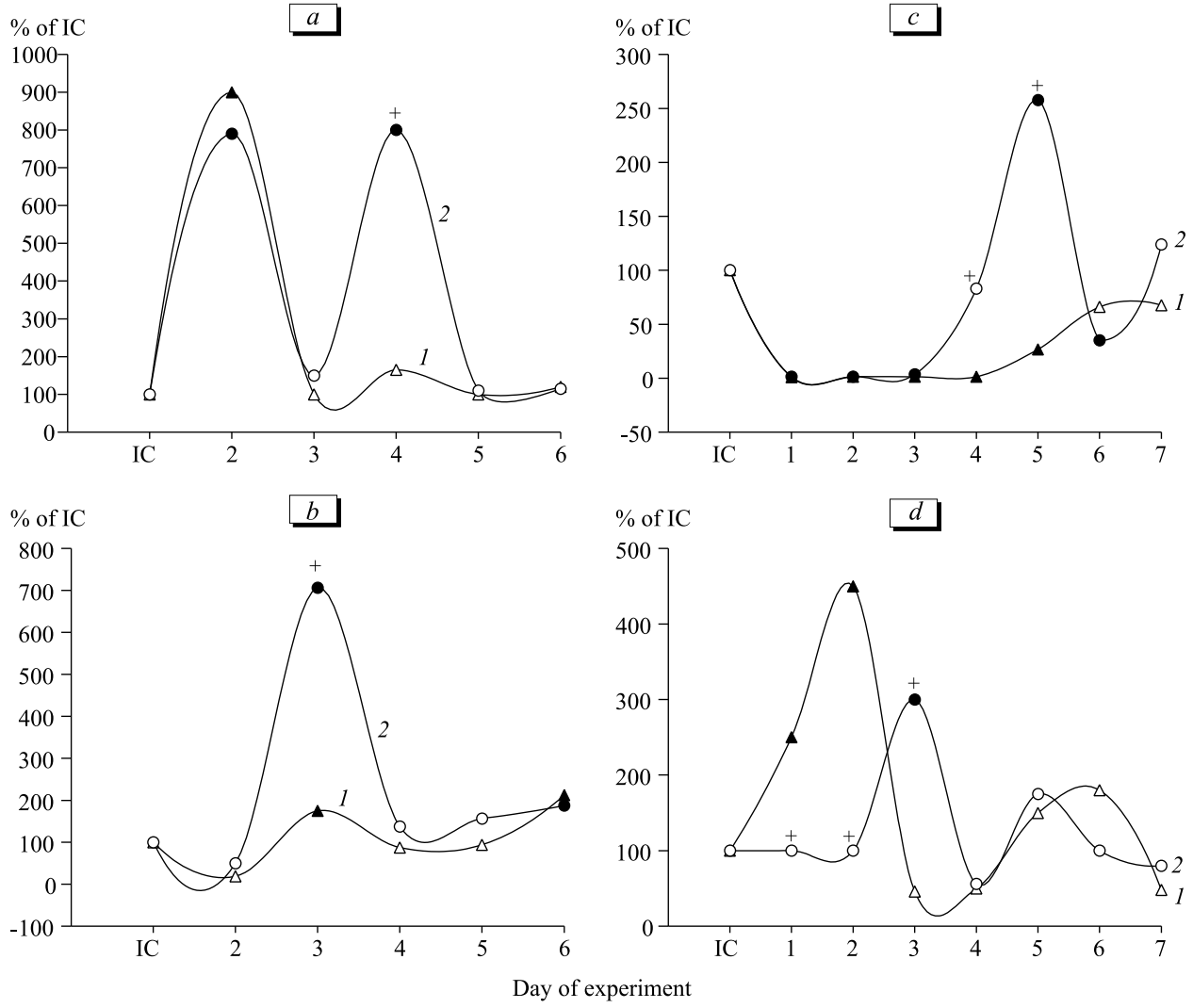

Fig. 1. Effect of sympatholytic on the formation of CFU-N (*a*), CFU-GEMM (*b*), CFU-G (*c*), and CFU-F (*d*) in culture of bone marrow cells from CBA/CaLac mice treated with cyclophosphamide. Ordinate: intensity of CFU growth. Here and in Fig. 2, 3: *1*) solvent, *2*) sympatholytic. Dark symbols: $p<0.05$ in comparison with IC; $^{+}p<0.05$ in comparison with mice receiving solvent.

The data were processed by standard methods of variation statistics. Significance of differences was evaluated using parametric Student's *t* test and nonparametric Mann–Whitney *U* test. For data expressed in fractions, Fisher exact test was used.

RESULTS

At the first stage of the experiment, the reactions of hemopoietic and stromal precursors to cyclophosphamide treatment were evaluated. The concentration of CFU-N in the hemopoietic tissue on day 2 after cytostatic treatment increased by 9 times in comparison with the level of intact control (Fig. 1, *a*). At later terms (days 3-7) their content practically did not differ from the control. G-CSF increased the intensity of formation of CFU-GM and CFU-G on day 2 in the culture of undifferentiated hemopoietic cells by 4.6 and 7.7 times, respectively, in comparison with IC (Fig. 2, *a*, *b*). On days 3-6, activity of this process decreased (primarily for granulocytic precursors). Significant increase in the count of CFU-CEMM was observed on days 3 and 6 after cytostatic treatment: to 175-213% from IC (Fig. 1, *b*).

In contrast to immature hemopoietic cells, the growth of CFU-G was suppressed on days 1-5 and proliferation of granulocytic precursors was inhibited on days 1-4 after cytostatic treatment (Fig. 1, *c*; Fig. 2, *c, d*). The number of mitotically active CFU-G increased (by 1.5 times in comparison with IC, $p<0.05$) starting from day 5. However, despite acceleration of proliferation the content of CFU-G in the hemopoietic tissue at these terms was 67% of the corresponding value in IC. The rate of differentiation of granulocytic precursors into morphologically discernible neutrophilic granulocytes also increased on days 5-7 after cytostatic

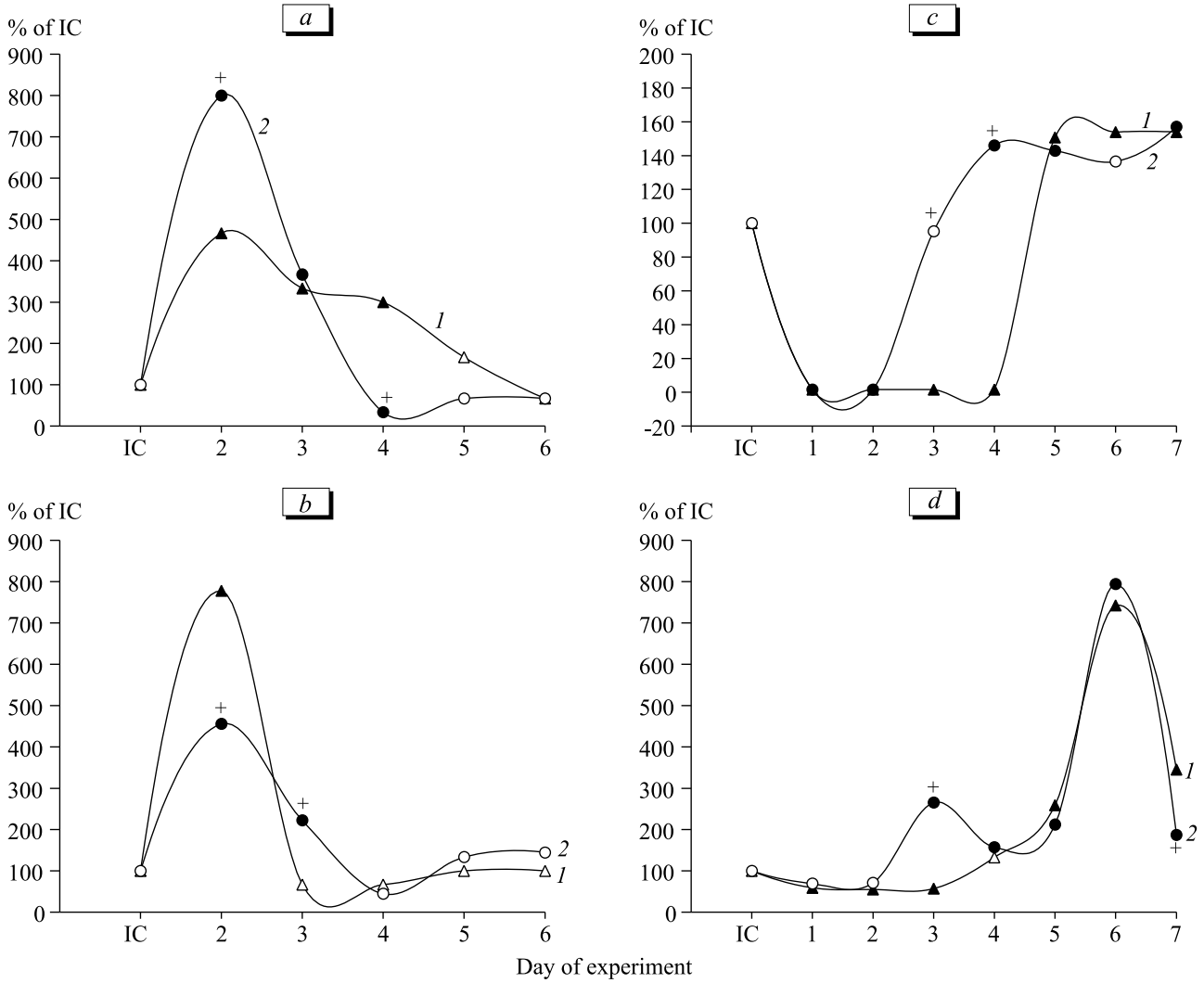

Fig. 2. Effect of sympatholytic on the growth of CFU-GM (*a*) and CFU-G (*b*) in cultures of polypotent hemopoietic precursors and proliferative activity (*c*) and intensity of differentiation (*d*) of CFU-G in CBA/CaLac mice treated with cyclophosphamide. Ordinate: intensity of CFU growth, proliferative activity, and intensity of CFU-G differentiation.

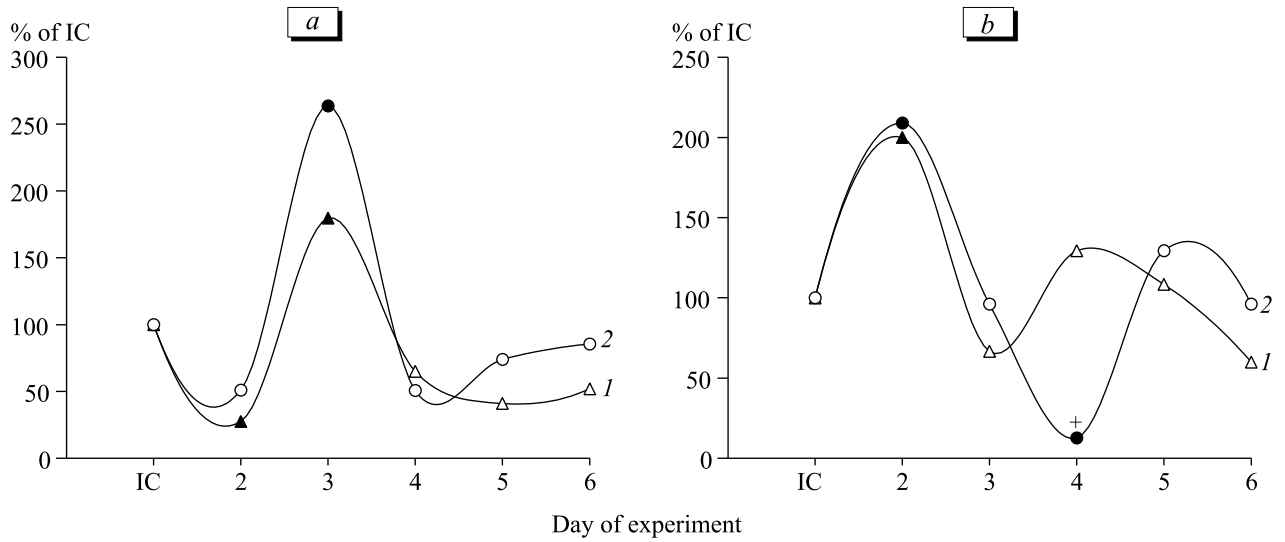

Fig. 3. Effect of sympatholytic on fibroblast-like cell-mediated stimulation of the formation of CFU-GEMM (*a*) and CFU-G (*b*) in CBA/CaLac mice treated with cyclophosphamide. Ordinate: intensity of CFU growth.

treatment and peaked on day 6 attaining 742% ($p<0.05$) of IC, *i.e.* the intensity of CFU-G differentiation considerably surpassed their proliferative activity.

Thus, replenishment of CFU-G loss under conditions of cytostatic-induced myelosuppression is associated with mobilization and targeted differentiation of polypotent hemopoietic cells into CFU-GM. It is difficult to imagine that the compartment of committed hemopoietic precursors in the hemopoietic tissue after cytostatic exposure is formed exclusively from the descendants of mobilized hemopoietic passing multistage differentiation. According to current views, hemopoiesis in myelopoiesis-inhibiting influences is maintained at the expense of a cytostatic-resistant population of hemopoietic precursors [1]. This is also confirmed by our findings that the rate of CFU-GM proliferation and intensity of their differentiation sharply increased starting from day 3 after cytostatic treatment [6]. We

TABLE 1. Effect of Sympatholytic on the Content of Neutrophilic Granulocytes ($\times 10^6$ cells/femur) in the Bone Marrow of CBA/CaLac Mice Treated with Cyclophosphamide

Parameter	IC	Day of experiment						
		day 1	day 2	day 3	day 4	day 5	day 6	day 7
Immature neutrophilic granulocytes								
physiological saline	2.48±0.28	0.60±0.11 $p_1<0.05$	0.46±0.03 $p_1<0.05$	0.66±0.08 $p_1<0.05$	2.84±0.25	1.86±0.41	3.20±0.31	2.51±0.14
sympatholytic	2.48±0.28	0.58±0.06 $p_1<0.05$	0.76±0.12 $p_1<0.01$	1.86±0.08 $p_2<0.001$	2.88±0.23	2.65±0.33 $p_2<0.05$	2.93±0.25	2.63±0.17
Mature neutrophilic granulocytes								
physiological saline	6.35±0.49	2.23±0.26 $p_1<0.05$	1.10±0.08 $p_1<0.05$	0.77±0.09 $p_1<0.05$	3.09±0.28 $p_1<0.05$	3.09±0.65 $p_1<0.05$	9.10±0.43 $p_1<0.05$	8.64±0.52
sympatholytic	6.35±0.49	1.89±0.20 $p_1<0.001$	1.51±0.28 $p_1<0.001$	2.45±0.08 $p_1<0.05$ $p_2<0.001$	3.37±0.30 $p_1<0.05$	6.31±0.65 $p_2<0.05$	9.55±0.57 $p_1<0.05$	9.24±0.51 $p_1<0.01$

Note. p_1 compared to IC, p_2 compared to cytostatic control.

believe that the observed uncoupling phenomenon in the CFU-G compartment towards their accelerated differentiation is an essential condition for postcytostatic regeneration of the hemopoietic tissue.

The development of cytostatic myelosuppression was accompanied by stimulation of stromal precursors in the bone marrow: on days 1-2 their content increased to 250-350% ($p<0.05$) of IC (Fig. 1, *d*). The cytostatic reduced the rate of CFU-GEMM growth on stromal cell feeder on day 2 (to 28% of the initial level). However, feeder activity of fibroblasts for CFU-GEMM and CFU-G sharply increased on days 3 and 2 (by 1.8 and 2 times, respectively; Fig. 3). Preserved contacts of stromal elements with primarily immature hemopoietic precursors during myelosuppression development probably provides rapid recovery of the content of granulocytic hemopoietic islets previously observed by us [7].

Sympatholytic promoted the recovery of neutrophilic granulocyte content in the bone marrow on days 3 and 5 after cytostatic treatment (Table 1). The observed effects were accompanied by additional increase in the content of polypotent cells (day 4) and GEMM precursors (day 3) in the hemopoietic tissue (Fig. 1, *a*, *b*). However, the effect of the drug on G-CSF-induced differentiation of CFU-N was ambiguous: enhanced growth of CFU-GM in culture of undifferentiated cells (day 2) was followed by a decrease in the rate of colony formation (day 4). Opposite dynamics was observed for CFU-G (Fig. 2, *a*, *b*).

Exhaustion of catecholamine depots accelerated recovery of the granulocyte precursor pool, which was seen from more rapid (compared to cytostatic control) appearance of CFU-G in methylcellulose medium (days 4-5; Fig. 1, *c*). Further analysis of granulocytic precursors revealed increased count of actively proliferating CFU-G starting from day 3 (*vs.* day 5 in cytostatic control). On day 3, the index of CFU-G maturation increased and surpassed that in cytostatic control by 196% ($p<0.05$; Fig. 2, *c, d*).

The sympatholytic decelerated the growth of CFU-F. The formation of colonies containing fibroblast-like cells was observed one day later (day 2) than in the cytostatic control (day 3; Fig. 1, *d*). Moreover, the sympatholytic reduced feeder activity of fibroblast-like cells for CFU-G (Fig. 3, *b*).

These findings suggest that the mechanism of granulocytopoiesis-inducing effect of the sympatholytic includes activation of mature and immature precursors. The content of buffer pool cells (granulocyte-macrophage precursors) considerably increased under these conditions. In the hierarchy of hemopoietic cells, granulocyte-macrophage precursors are CFU-G precursors and largely determine their content in the hemopoietic tissue under extreme exposures [1]. The increased production of CFU-G in the culture of polypotent cells observed by us attests to accelerated differentiation of committed precursors into more mature cells of granulocytopoiesis. At the same time, the intensity of stromal precursor proliferation and feeder activity of fibroblasts for granulocyte precursors decreased.

Taking into account the mechanism of sympatholytic effect (exhaustion of catecholamine depots), we can hypothesize that under conditions of cyclophosphamide treatment catecholamines of CNS along with growth and inhibitory factors produce a suppressive effect on hemopoietic stem cells of various classes and CFU-G. In contrast, the content and functional activity of stromal precursors increased under the effect of catecholamines. According to current views, stromal cell population of the bone marrow is extremely heterogeneous [9,10,12,13]. Apart from stromal precursors, it contains undifferentiated cells capable of self-renewal and differentiation. In light of this, we can hypothesize that the function of mesenchymal stem cells depends on activity of catecholamines.

REFERENCES

1. A. I. Vorob'ev, *Manual in Hematology* [in Russian], Ed. A. I. Vorob'ev, Vol. 1, Moscow (2002).
2. E. D. Goldberg, A. M. Dygai, V. V. Zhdanov, *et al., Pharmacological Regulation of the Blood System in Experimental Neuroses* [in Russian], Tomsk (2007).
3. E. D. Goldberg, A. M. Dygai, and I. A. Khlusov, *Role of Autonomic Nervous System in the Regulation of Hemopoiesis* [in Russian], Tomsk (1997).
4. E. D. Goldberg, A. M. Dygai, and V. P. Shakhov, *Methods of Tissue Culture in Hematology* [in Russian], Tomsk (1992).
5. A. M. Dygai, E. G. Skurikhin, O. V. Pershina, *et al., Byull. Eksp. Biol. Med.*, **148**, No. 4, 400-404 (2010).
6. A. M. Dygai, E. G. Skurikhin, O. V. Pershina, *et al., Byull. Eksp. Biol. Med.*, **145**, No. 4, 383-388 (2008).
7. A. M. Dygai, E. G. Skurikhin, O. V. Pershina, *et al., Byull. Eksp. Biol. Med.*, **147**, No. 5, 540-543 (2008).
8. Yu. M. Zakharov, *Ross. Fiziol. Zh.*, **90**, No. 8, 987-1000 (2004).
9. I. L. Chertkov, N. I. Drize, and A. I. Vorob'ev, *Ter. Arkhiv*, No. 7, 5-12 (2006).
10. P. Bianco, M. Riminucci, S. Gronthos, and P. G. Robey, *Stem Cells*, **19**, No. 3, 180-192 (2001).
11. G. J. Ventura, J. P. Hester, E. S. Buescher, *et al., Exp. Hematol.*, **18**, No. 8, 878-882 (1990).
12. P. S. In't Anker, W. A. Noort, S. A. Scherjon, *et al., Hematologica*, **88**, No. 10, 845-852 (2003).
13. M. Owen and A. J. Friedenstein, *Giba Found Symp*. **136**, 42-60 (1988).