Dopaminergic Regulation of Granulocytopoiesis during Cytostatic-Induced Myelosuppressions

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The role of the dopaminergic system in the regulation of the granulocytic hemopoietic stem was studied after administration of cyclophosphamide and 5-fluorouracil. Dopamine in the central nervous system promotes the development of cytostatic-induced myelosuppressions. The inhibitory effect of dopaminergic structures on granulocytopoiesis after administration the fluoropyrimidine antimetabolite was of shorter duration compared to that observed in experiments with the alkylating agent. The inhibitory effect of brain dopamine is associated with a decrease in proliferative activity and differentiation of granulomonocytic precursors, prevention of hemopoietic islet formation, and impairment of secretion of colony-stimulating activity in adherent cells of the hemopoiesis-inducing microenvironment. Cyclophosphamide and 5-fluorouracil had different effects on the rate of hemopoietic tissue regeneration, which was related to specific interaction between distant regulatory structures and hemopoiesis-inducing microenvironment and differences in the influence of cytostatics on hemopoietic and stromal cells.

Key Words: *dopamine; granulocytopoiesis; hemopoiesis-inducing microenvironment; precursor cells; cytostatics*

The monoaminergic system plays a major role in the regulation of hemopoiesis. Receptors for catecholamines and serotonin on hemopoietic and stromal cells play a role in plastic reconstruction of the blood system under extreme conditions (immobilization stress, cytostatic treatment, and neuroses) [1,2,4-6]. Activity of peripheral monoaminergic mechanisms is regulated by the central nervous system (CNS). After administration of cytostatic drugs, the regulatory effect of dopaminergic structures on proliferation and differentiation of erythroid cells is realized via dopamine receptors on erythroid precursors and cells of the hemopoiesis-inducing microenvironment. This effect is also mediated by changes in activity of the erythropoietin system [5]. However, the regulatory effect of brain monoamines on hemopoietic tissue during cytostatic-induced myelosuppressions is poorly understood. The mechanisms for dopaminergic regulation of granulocytopoiesis remain unknown.

Here we studied the role of the dopaminergic system in the regulation of granulocytopoiesis during cytostatic-induced myelosuppressions.

MATERIALS AND METHODS

Experiments were performed on 620 female CBA/CaLac mice (class I conventional mouse strain) aging 2-2.5 months and obtained from the nursery of the Institute of Pharmacology.

Cytostatic myelosuppression was induced by single intraperitoneal injection of alkylating agent cyclophosphamide (one-third of the maximum tolerated dose [MTD], 83 mg/kg) and fluoropyrimidine antimetabolite 5-fluorouracil (76 mg/kg). The ani-

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mals of the treatment groups received intraperitoneal injection of neuroleptic drug haloperidol in a single dose of 30 mg/kg (Gedeon Richter A.O.) 30 min before cytostatic treatment. Control animals received an equivalent volume of physiological saline (0.2 ml) under similar conditions. The number of neutrophilic leukocytes in the peripheral blood was measured on days 1-7 after cytostatic treatment. The animals were euthanized by cervical dislocation under ether anesthesia. The count of mature and immature neutrophilic granulocytes in the bone marrow was evaluated. The content of granulocyte-macrophage colony-forming (CFU-GM) and cluster-forming units (ClFU-GM) in the bone marrow was estimated by *in vitro* culturing of myelokaryocytes in methylcellulose [3]. Proliferative activity of granulomonocytopoietic precursors was studied by the method of cell suicide using hydroxyurea. Cell differentiation was assayed by the index of maturation (cluster/colony ratio in the well) [3]. Structural and functional characteristics of the bone marrow were estimated by enzymatic isolation of hemopoietic islets and study of their quantitative and qualitative composition [7]. Colony-stimulating activity (CSA) of conditioned media from adherent and nonadherent cells of the hemopoiesis-inducing microenvironment and blood plasma was tested using intact mouse myelokaryocytes [3].

The final concentrations of dopamine (Sigma) and recombinant granulocyte colony-stimulating factor (CSF, Neipogen, Hoffman-La Roche) in the bone marrow culture were 10^{-8} M and 5 ng/ml, respectively.

The results were analyzed by standard methods of variational statistics. The significance of differences was evaluated by parametric Student's *t* test and nonparametric Wilcoxon—Mann—Whitney *U* test.

RESULTS

Cyclophosphamide and 5-fluorouracil caused suppression of bone marrow granulocytopoiesis (days 1-7 and 1-6, respectively) and neutrophilic leukopenia in the peripheral blood (days 1-5, and days 2 and 4-7, respectively; Fig. 1, Table 1). The rate of division and maturation of granulocytic precursors practically did not differ in treated and control animals (Fig. 2). The exceptions were days 5 and 7 after administration of the alkylating agent and fluoropyrimidine antimetabolite, respectively. Addition of granulocyte CSF under these conditions was followed by CFU-GM growth in the culture of nonadherent bone marrow cells.

The development of severe myelosuppression was preceded by dysfunction the local regulatory system. The alkylating agent and fluoropyrimidine antimetabolite impaired the formation of fibroblastoid (days 1-6) and granulocytic hemopoietic islets (days 1-4 and 6, and days 1-5, respectively; Fig. 1). Cyclophosphamide and 5-fluorouracil decreased the secretion of CSA by adherent (days 1, 3, 5, and 7, and days 1, 3, and 7, respectively) and nonadherent cells of the hemopoietic microenvironment (days 1, 3, 5, and 7). They also caused deficiency of CFU-GM growth factors in blood plasma on days 3, 5, and 7. This conclusion was derived from a sharp decrease in activity of biological fluids (up to 80% of the basal level).

Cytostatic-induced suppression of granulocytopoiesis is associated with changes in the structural and functional integrity of the bone marrow, CSA deficiency in biological media, and inhibition of proliferation and differentiation of granulomonocytic precursors. However, neutrophilic leukocytosis in the peripheral blood developed on days 6 and 7 after cyclophosphamide treatment. The increase in neutrophil count was preceded by the formation of granulocytic hemopoietic islets and acceleration of division and maturation of granulocyte-macrophage precursors in the bone marrow (day 5). Activation of colony formation and accumulation of cell complexes were observed at a later period after administration of 5-fluorouracil (day 7). Stimulation of regeneration after administration of the alkylating agent develops more rapidly than in experiments with the antimetabolite. These differences are probably associated with a higher toxicity of 5-fluorouracil due to the impairment of RNA synthesis, dysfunction of RNA [8,9,11,12], and blockade of DNA synthesis [10].

Study of the peripheral dopaminergic mechanisms underlying the function of granulomonocytopoietic precursors showed that dopamine treatment after administration of 5-fluorouracil *in vitro* stimulated the growth of CFU-GM and increased proliferative activity of precursor cells (day 7, Fig. 3). The intensity of granulomonocytic colony formation and rate of precursor cell division in animals with cyclophosphamide-induced myelosuppression did not differ from those in the intact control. However, the maturation index significantly increased on day 5 after cyclophosphamide administration.

Hence, dopaminergic structures on granulomonocytic precursors are involved in the regulation of proliferation and differentiation during cytostatic-induced myelosuppression. Dopamine primarily stimulates precursor cell maturation and accelerates precursor cell division after treatment with cyclophosphamide and 5-fluorouracil, respectively.

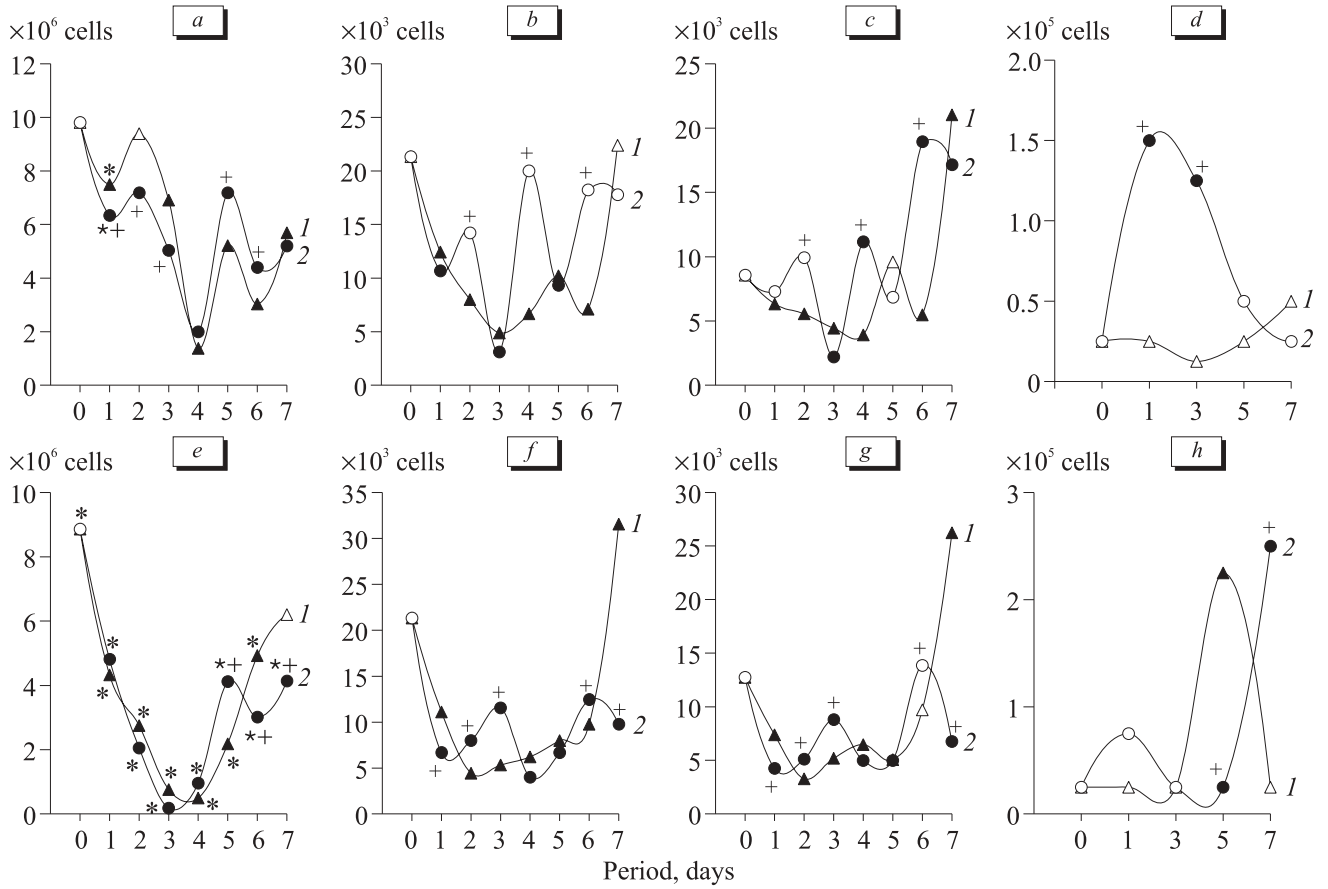

Fig. 1. Number of neutrophilic granulocytes (*a*, *e*), content of fibroblastoid (*b*, *f*) and granulocytic hemopoietic islets in the bone marrow (*c*, *g*), and CSA in supernatants of adherent myelokaryocytes (*d*, *h*) from CBA/CaLac mice receiving cyclophosphamide (*a-d*) and 5-fluorouracil (*e-h*). Physiological saline (*1*) and haloperidol (*2*). Dark symbols: $p<0.05$ compared to the pretreatment parameter. $p<0.05$: *compared to the parameter after cytostatic treatment and administration of haloperidol; +compared to physiological saline.

The haloperidol-induced decrease in activity of brain dopaminergic structures had different effects on the cytostatic inhibition of granulocytopoiesis. The neuroleptic decreased the count of neutrophilic granulocytes in the bone marrow on days 1-3 after administration of cyclophosphamide (Fig. 1). Cell number increased in the follow-up period (days 5 and 6). The degree of neutrophilic leukopenia (day 5) and neutrophilia (days 6 and 7) in the peripheral blood under these condition was lower compared to the control (untreated animals, Table 1). Haloperidol prevented the development of 5-fluorouracil-induced myelosuppression on day 5, but on days 6 and 7 the number of bone marrow granulocytes was 61 and 67% of the control level, respectively (Fig. 1). Progressive leukopenia in the peripheral blood was observed on days 4-6. However, leukopenia was not found on days 2 and 7 (Table 1).

After consecutive treatment with the neuroleptic drug and cyclophosphamide, addition of CSF to the culture of nonadherent bone marrow cells significantly stimulated the formation of granulocyte-macrophage colonies (days 5 and 7). These changes were related to activation of granulomonocytic precursor proliferation (Fig. 2). However, the ClFU-GM/CFU-GM ratio decreased on days 3, 5, and 7. CFU-GM release (days 5 and 7) in the methylcellulose medium in the presence of dopamine results from accelerated division (days 5 and 7) and maturation (day 7), of precursor cells (Fig. 3). Blockade of postsynaptic dopamine D_2 receptors in the brain and administration of 5-fluorouracil were followed by an increase in differentiation of granulomonocytopoietic precursors induced by granulocyte CSF (day 3) and dopamine (day 1; Figs. 2 and 3). The intensity of colony formation did not exceed the control level. Colony formation and proliferative activity of precursor cells increased in the follow-up period (day 5), but decreased by the end of the study.

Haloperidol treatment after administration of the alkylating agent prevented the development of structural and functional disturbances in the bone marrow on days 2, 4, and 6. This conclusion was derived from the increase in the number of fibro-

blastoid and granulocytic hemopoietic islets (Fig. 1). During antimetabolite-induced myelosuppression this neuroleptic drug not only increased the number of hemopoietic islets (days 2, 3, and 6), but also inhibited the formation of cell complexes (day 7).

The blockade of dopamine D_2 receptors in the brain had no effect on the ability of nonadherent cells in the hemopoiesis-inducing microenvironment to produce CSA during cytostatic treatment. However, secretory activity of adherent myelokaryo-

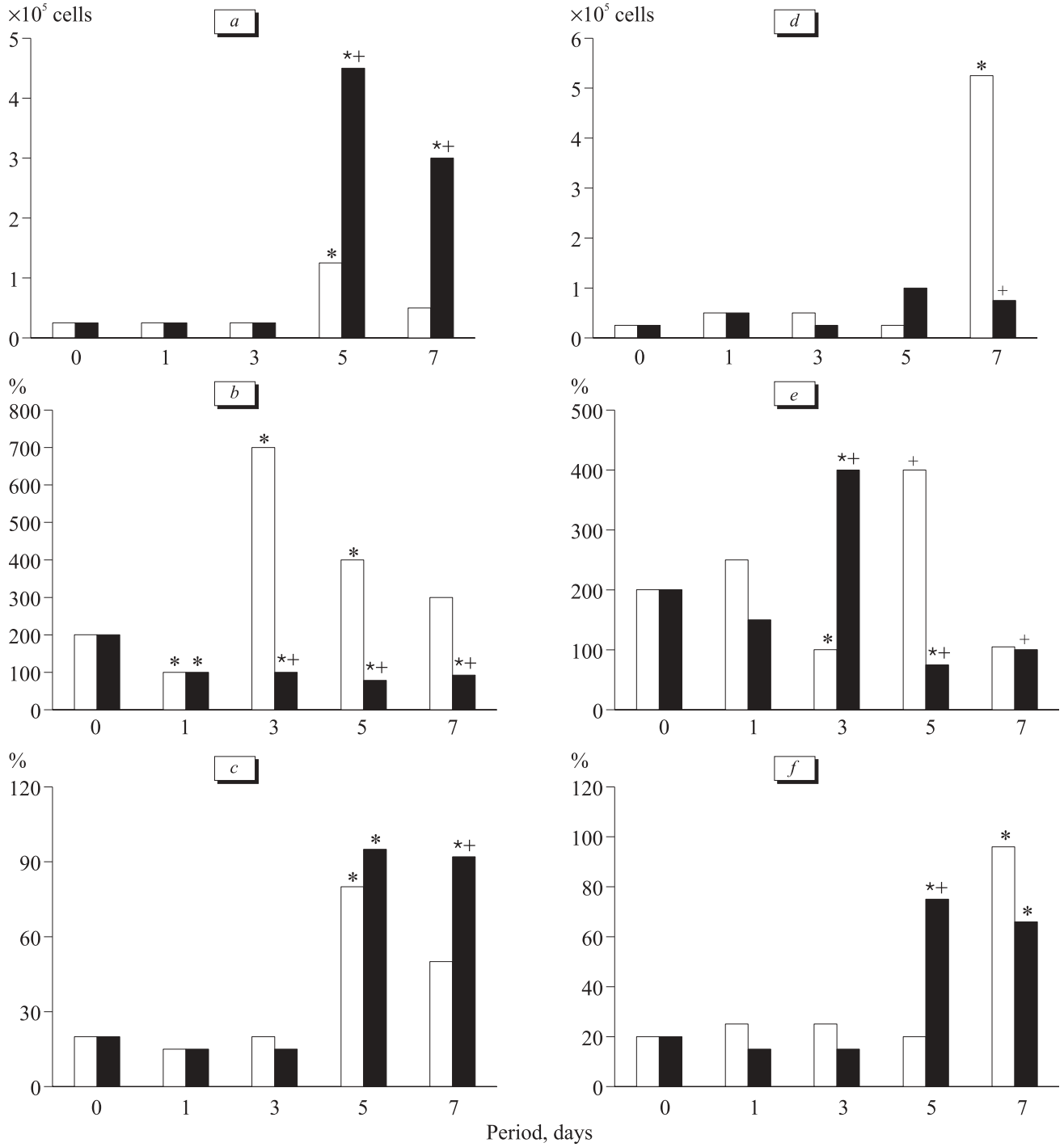

Fig. 2. Number of CFU-GM (*a*, *d*), ratio of CFU-GM in S phase of the mitotic cycle (*c*, *f*), and maturation of granulocytic precursors (*b*, *e*) in the bone marrow of CBA/CaLac mice receiving cyclophosphamide (*a-c*) and 5-fluorouracil (*d-f*). Stimulation with granulocyte CSF. Here and in Fig. 3: light bars, physiological saline; dark bars, haloperidol. $p<0.05$: *compared to the parameter after haloperidol administration; +compared to physiological saline.

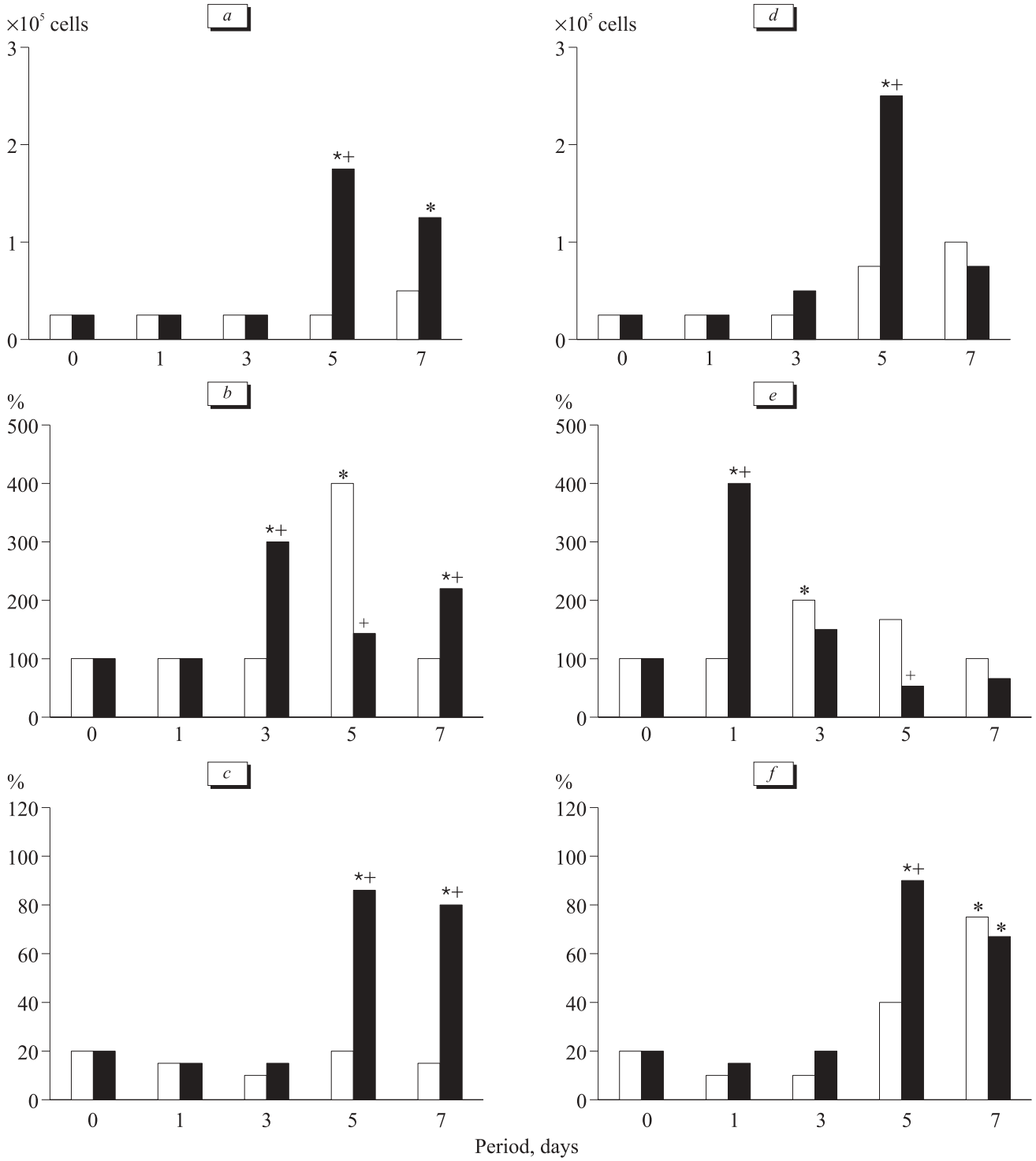

Fig. 3. Number of CFU-GM (*a*, *d*), ratio of CFU-GM in S phase of the mitotic cycle (*c*, *f*), and maturation of granulocytic precursors (*b*, *e*) in the bone marrow of CBA/CaLac mice receiving cyclophosphamide (*a-c*) and 5-fluorouracil (*d-f*). Stimulation with dopamine.

cytes increased after administration of cyclophosphamide (days 1 and 3) and 5-fluorouracil (day 7, Fig. 1). After antimetabolite treatment, haloperidol abolished the increase in activity in supernatants of adherent nucleated cells (day 5) and blood plasma (day 1).

Our results indicate that the rate of division and maturation of granulomonocytic precursors, activity of adherent cells in hemopoiesis-inducing microenvironment, and CSA of blood plasma during cytostatic-induced myelosuppressions are regulated by the dopaminergic system. The inhibito-

TABLE 1. Effect of Haloperidol on the Number of Segmented Neutrophils ($\times 10^9$ cells/liter) in the Peripheral Blood of CBA/CaLac Mice after Cytostatic Treatment (n=12, $X \pm m$)

Period, days	*In vivo* preparation	Cyclophosphamide	5-Fluorouracil
Intact control	Physiological saline	4.514±0.441	3.679±0.389
1	Physiological saline	1.364±0.114*	3.134±0.372
	Haloperidol	1.481±0.147*	2.502±0.432*
2	Physiological saline	0.968±0.085*	1.366±0.268*
	Haloperidol	1.104±0.167*	3.144±0.381+
3	Physiological saline	0.147±0.059*	2.914±0.329
	Haloperidol	0.206±0.037*	2.433±0.326
4	Physiological saline	0.099±0.023*	1.144±0.168*
	Haloperidol	0.077±0.011*	0.277±0.043*+
5	Physiological saline	0.511±0.224*	0.523±0.072*
	Haloperidol	0.886±0.061*	0.196±0.025*+
6	Physiological saline	8.064±0.468*	0.401±0.057*
	Haloperidol	5.203±0.496+	0.175±0.018*+
7	Physiological saline	11.553±0.912*	1.106±0.167*
	Haloperidol	9.086±0.779*	2.571±0.309+

Note. $p<0.05$: *compared to the intact control; +compared to cytostatic-treated animals with no haloperidol administration.

ry effect of brain dopaminergic structures on proliferation and differentiation is realized via receptors for granulocyte CSF and monoamine on granulomonocytopoietic precursors and mediated by adherent cells of the microenvironment. However, cytostatic drugs regulate several functions of hemopoietic tissue that depend on the mechanism of their action. For example, peripheral dopaminergic structures have various effects on division and maturation of precursor cells during myelosuppressions induced by 5-fluorouracil and cyclophosphamide. Blockade of postsynaptic dopaminergic D_2 receptors in the brain after treatment with the alkylating agent restores the formation of hemopoietic islets in the bone marrow, increases CSA production by adherent myelokaryocytes, and stimulates CFU-GM growth. Under these experimental conditions the dopaminergic system increases the severity of cytostatic damage to adherent nucleated cells of the hemopoiesis-inducing microenvironment, suppresses division and maturation of granulocyte-macrophage precursors, and initiates dissociation of precursor and stromal cells in the microenvironment. However, the restoration of cell complex formation, stimulation of CSA production by adherent nucleated cells, and increase in proliferative activity of granulomonocytopoietic precursors in 5-fluorouracil-treated mice under the influence of haloperidol are followed by the inhibition of CFU-GM growth and development of severe damage to the local regulatory system. It may be suggested that the inhibitory effect of CNS dopamine on the hemopoietic tissue decreases after antimetabolite administration.

REFERENCES

1. E. D. Gol'dberg, A. M. Dygai, N. V. Provalova, *et al.*, *Role of the Nervous System in the Regulation of Hemopoiesis* [in Russian], Tomsk (2004).
2. E. D. Gol'dberg, A. M. Dygai, and I. A. Khlusov, *Role of the Autonomic Nervous System in the Regulation of Hemopoiesis* [in Russian], Tomsk (1997).
3. E. D. Gol'dberg, A. M. Dygai, and V. P. Shakhov, *Tissue Culture Methods in Hematology* [in Russian], Tomsk (1992).
4. Yu. M. Zakharov, *Ros. Fiziol. Zh.*, **90**, No. 8, 987-1000 (2004).
5. M. Yu. Minakova, E. G. Skurikhin, and O. V. Pershina, *Byull. Eksp. Biol. Med.*, Suppl. 1, 79-86 (2007).
6. M. Yu. Minakova, E. G. Skurikhin, O. V. Pershina, and E. V. Udut, *Ibid.*, Suppl. 1, 86-91 (2007).
7. P. R. Crocker and S. Gordon, *J. Exp. Med.*, **162**, No. 3, 993-1014 (1985).
8. P. V. Danenberg, C. C. Shealuke, and K. Danenberg, *Cancer Res.*, **50**, No. 6, 1757-1763 (1990).
9. C. Heidelberger, *Ibid.*, **30**, 1549-1569 (1970).
10. S. Kondo, *J. Radiat. Res.*, **36**, No. 1, 56-62 (1995).
11. H. J. Lenz, D. J. Manno, K. D. Danenberg, and P. V. Danenberg, *J. Biol. Chem.*, **269**, No. 50, 31,962-31,968 (1994).
12. E. Rojas, L. A. Herrera, M. Sordo, *et al.*, *Anticancer Drugs*, **4**, No. 4, 637-640 (1993).