Benz(a)pyrene is a mutagen requiring metabolic activation for the manifestation of its effect. As coumarins inhibit its mutagenic effect, one could suppose that the antimutagenic effect of coumarins is related to their interference with the processes of metabolic activation of mutagens. However, thioTEP used in our experiments does not require metabolic activation for the realization of its effect. ThioTEP possesses prooxidant properties [1]: it is therefore plausible to assume that the antimutagenic effect of coumarins is connected with their capacity to bind free radicals. The disclosed antioxidant properties of coumarins [5] may serve as indirect confirmation of this assumption.

It follows from our results that aqueous and alcohol extract of medicinal angelica possess antimutagenic activity, as manifested by their ability to reduce the frequency of thioTEP-induced micronuclei in mouse bone marrow and peripheral blood. Most probably, the antimutagenic effect of the extracts is due to their antioxidant properties. Thus, medicinal angelica contains antimutagens that, according to the classification offered by G. G. Poroshenko and S. K. Abilev [4], belong to the free radical-binding antimutagens.

## REFERENCES

1. A. D. Durnev, *Antimutagens as a New Class of Pharmacological Agents* [in Russian]: M. D. Degree Thesis, Moscow (1991).
2. G. F. Lakin, *Biometry* [in Russian], Moscow (1990).
3. N. V. Maslova, *Botanical Research in the Urals* [in Russian], Sverdlovsk (1990), p. 63.
4. G. G. Proshenko and S. K. Abilev, *Itogi Nauki i Tekhniki, Seriya Obshchaya Genetika, Moscow*, **12**, 5-208 (1988).
5. K. Georgieff, Science, **173**, 537-539 (1971).
6. D. L. Morris and J. B. Ward, *Environm. Molec. Mutagen.*, **19**, 132-138 (1992).
7. Y. Sakaia, H. Nagase, Y. Ose, *et al.*, *Mutat. Res.*, **206**, 327-334 (1988).
8. W. Schmid, *Ibid.*, **31**, 9-15 (1975).

# The Role of the Sympatheticoadrenal Structures in Hematopoiesis Regulation under Cytostatic Myelodepression

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As has been demonstrated previously, the neuroendocrine system markedly affects the hemopoietic response under extreme conditions which do not cause myelodepression [2,3,8,9]. At the same time, the role neurohormonal signals play in the proliferation and differentiation of the hemopoietic cells during various hemodepressive states remains unclear. It is known from the fragmentary data that glucocorticoids are able to reduce the toxic effects of antitumor drugs on hemopoiesis [1], and a mild halothane anesthesia significantly decreases the sensitivity of CFUs to cytostatics [7].

The aim of the present investigation was to establish the role of the sympathetic nervous system in

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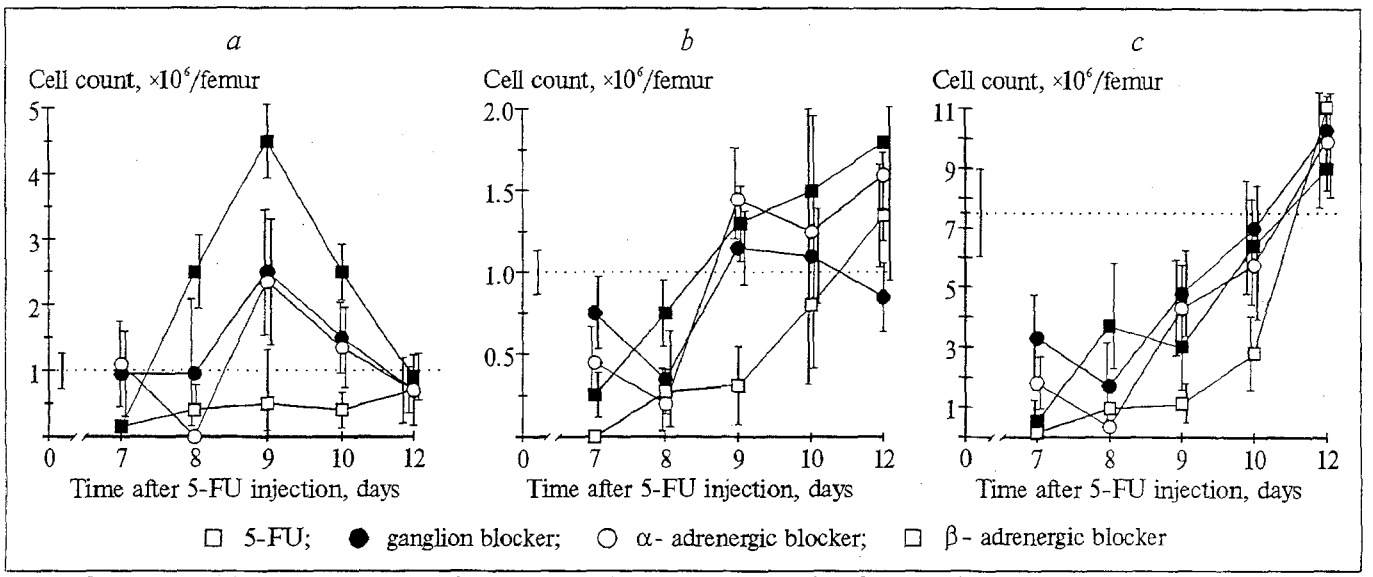


**Fig. 1.** Time course of content of nucleated erythrocytes (*a*) and immature (*b*) and mature (*c*) neutrophilic leukocytes in the bone marrow of CBA mice treated with sympathetic antagonists on the third day after 5-FU injection.

hemopoiesis regulation during 5-fluorouracil-induced (5-FU) myelodepression.

## MATERIALS AND METHODS

Experiments were carried out on 670 male CBA mice ( Rassvet nursery, Tomsk) weighing 18-20 g. A single i.p. injection of 5-FU in a dose of 114 mg/kg was performed to induce myelodepression.

Each experimental series consisted of four groups. All experimental animals were treated with 5-FU. In the first experimental series the combined action of 5-FU and sympatholytic drugs was studied. For this purpose the ganglioblocking drug pentamine (6 mg/kg), the α-adrenergic blocker dihydroergotamine (3.9 mg/kg), or the β-adrenergic blocker propranolol (5 mg/kg) was injected twice: 3-5 min before and 5 h after 5-FU. The animals of the second series were injected twice at an interval of 5 h with the same sympatholytic drugs 3 days after 5-FU injection. Control animals were injected with an equal volume of saline according to the corresponding scheme. Mice were killed by dislocation of the neck at different times after 5-FU treatment. The total number of nucleated bone marrow cells of the femur and leukocytes of the peripheral blood were counted. The white cell count and total blood cell count were performed using smears and expressed in parts per 1000. In experiments *in vitro* bone marrow cells were flushed out of the femur using 1 ml of complete culture medium: 90 % RPMI-1640 (Serva, Germany); 10% BES (Flow, Great Britain ); 10 mmol HEPES (Flow, Great Britain); 80 mg/liter gentamicin (Serva, Germany); 280 mg/liter L-glutamine (Sigma, USA); $5\times10^{-5}$ M 2-mercaptoethanol (Sigma, USA). 5-FU in a concentration of $10^{-7}$ M was added to the suspended bone marrow cells.

**TABLE 1.** Micronucleated Erythrocytes Count (%) in the Peripheral Blood of Mice Treated with Sympathetic Antagonists on the Third Day after 5-FU Injection ($M\pm m$)

| Time of obcervation, days | 5-FU | *p* | Ganglion blocker | *p* | *p** | α-adrenergic blocker | *p* | *p** | β-adrenergic blocker | *p* | *p** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Prior to treatment | 0.73±0.10 | – | 0.73±0.10 | – | – | 0.73±0.10 | – | – | 0.73±0.10 | – | – |
| 1 | 0.97±0.07 | | – | | – | | – | | | | |
| 2 | 0.63±0.11 | | – | | – | | – | | | | |
| 3 | 1.93±0.28 | 0.01 | – | | – | | – | | | | |
| 4 | 2.04±0.14 | 0.001 | 0.95±0.29 | | 0.01 | 1.20±0.24 | | 0.05 | 1.30±0.24 | | 0.05 |
| 5 | 2.46±0.16 | 0.001 | 1.22±0.28 | | 0.01 | 1.25±0.18 | 0.05 | 0.001 | 1.14±0.18 | | 0.001 |
| 6 | 1.89±0.25 | 0.01 | 1.18±0.18 | 0.05 | 0.05 | 0.86±0.18 | | 0.01 | 1.11±0.17 | | 0.05 |
| 7 | 0.89±0.18 | | 0.96±0.16 | | | 0.93±0.19 | | | 1.05±0.15 | 0.05 | |
| 8 | 1.07±0.14 | | 0.92±0.20 | | | 0.80±0.12 | | | 0.63±0.21 | | |
| 9 | 0.71±0.08 | | 0.54±0.11 | | | 0.64±0.10 | | | 0.58±0.08 | | |
| 10 | 0.46±0.08 | | 0.39±0.07 | 0.05 | | 0.54±0.16 | | | 0.43±0.07 | 0.05 | |
| 12 | 0.61±0.10 | | 0.43±0.07 | 0.05 | | 0.42±0.08 | 0.05 | | 0.54±0.11 | | |

**Note:** *p*: significance of differences compared with intact animals, *p**: significance of differences compared with control (mice treated with 5-FU).

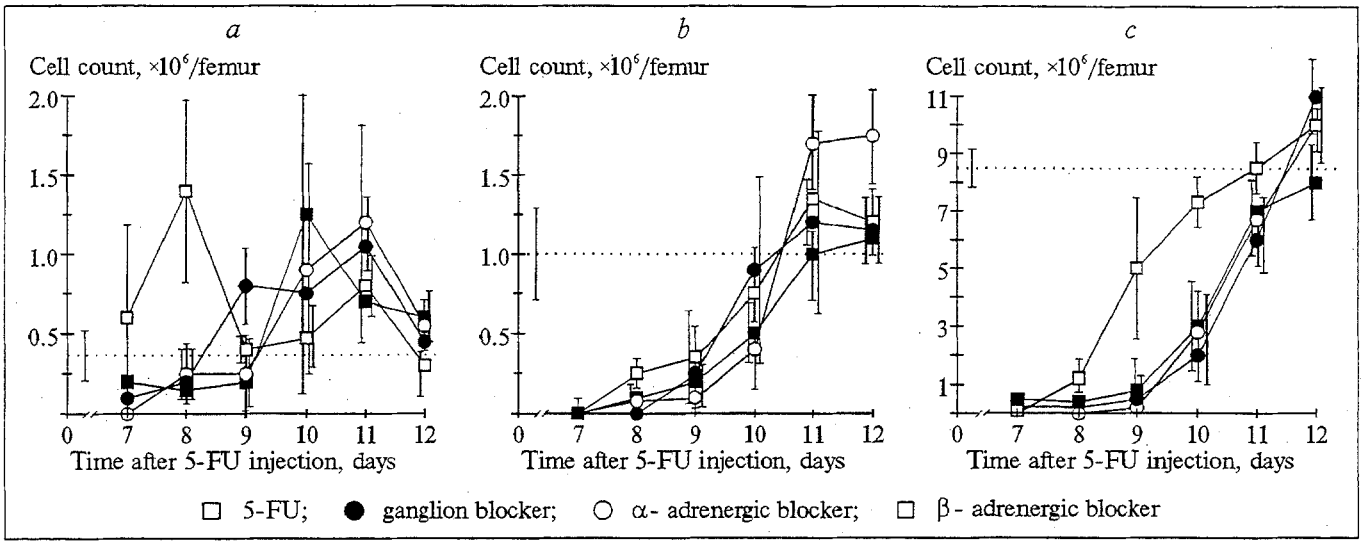


**Fig. 2.** Time course of content of nucleated erythrocytes (*a*) and immature (*b*) and mature (*c*) neutrophilic leukocytes in the bone marrow of CBA mice after combined injection of sympathetic antagonists and 5-FU.

Pentamine, dihydroergotamine, or propranolol in the corresponding concentrations was added twice 3-5 min before and 5 h after 5-FU treatment. The cell suspension was then incubated in a $CO_2$ incubator at 37°C, 5% $CO_2$, and 100% humidity. A count of viable karyocytes stained with Trypan Blue was performed in a Goryaev chamber [6]. The data were processed statistically using Student *t* test and Wilcoxson *T* test [4].

## RESULTS

Mice treated with 5-FU in a dose of 114 mg/kg exhibited a pronounced decrease of erythro- and granulocytopoiesis to the absolute disappearance of immature neutrophilic leukocytes and nucleated precursors of erythrocytes in the bone marrow slides. The depression of granulocytopoiesis was accompanied by a decrease of the mature neutrophil count in the bone marrow (1-10 days) and in the blood (4-9 days). Up to the fifth day the number of polymorphonuclear leukocytes was minimal - 0.13% and 0.8% of the initial level, respectively. These results are in good agreement with those we obtained previously [5].

**TABLE 2.** Bone Marrow Cell Count *in Vitro* 24 h after Combined Injection of Sympathetic Antagonists with 5-FU in a Concentration of $10^{-7}$ M.

| Drug | Cell count, $10^6$ ml | TP |
|---|---|---|
| Prior to treatment | 13.30 | – |
| 5-FU | 3.35 | – |
| Ganglion blocker | 2.03 | 0.01 |
| α-adrenergic blocker | 1.55 | 0.01 |
| β-adrenergic blocker | 1.03 | 0.01 |

**Note:** TP: significance of differences compared with control (5-FU alone) according to Wilcoxson *T* test.

The dynamics of the hemopoietic cell recovery was similar for all sympatholytic drugs used in the period of the maximal depletion of hemopoietic tissue. Treatment with the ganglion blocker, or the α- or β-adrenergic blocker on the third day after 5-FU injection caused by the ninth day a rise of the number of erythroblasts in the bone marrow (2.5-4.5 times compared to the intact animals). On the other hand the animals treated only with 5-FU exhibited a decreased level of nucleated erythrocyte precursors in comparison with the initial level during the whole period of the study (Fig. 1). The described phenomenon is probably due in part to the cytoprotective properties of the sympathetic antagonists and results in a significant decrease of the micronucleated erythrocyte output into the blood in the period (4-6 days) preceding active hemopoiesis repair (Table 1).

The sympathetic blocking agents markedly enhanced the proliferation of neutrophilic granulocytes in the bone marrow (Fig. 1) and their differentiation to mature forms (9-10 days) with an increase of their output to the blood (12th day) 1.5 (ganglionic blocker) and 2.5 (α- or β-adrenergic blocker) times compared to the animals treated with 5-FU only.

Combined injection of 5-FU and the sympathetic antagonists slowed the hemopoietic repair processes in comparison with the control animals. The number of bone marrow nucleated erythrocytes and immature neutrophilic granulocytes significantly decreased (Fig. 2) and their differentiation was disrupted on the 8th and 10th day of the experiment. Thus, on the 8th day the number of mature bone marrow neutrophilic leukocytes was 8-9 times lower in the groups of animals administered the ganglion blocker and α- or β-adrenergic blocker in comparison with those which received 5-FU only. At the same time there was a

corresponding decrease of stab neutrophils and segment-nuclear leukocytes in the blood.

It should be noted that combined addition of 5-FU and sympathetic antagonists *in vitro* resulted in a decrease of the bone marrow cell count 1.5 times compared to the control 24 h after incubation (Table 2). There was a significant increase (174-210% of the control level) in the number of immature (with some nuclear material) erythrocytes in the blood on the first day of the experiment *in vivo*, which can be probably related to disturbances of the cellular adaptive mechanisms.

Thus, the joint injection of 5-FU and sympathetic antagonists has a depressive effect on hemopoiesis in mice. It should be emphasized that we obtained similar data under immobilization stress, when sympathetic blocking agents were used according to the same scheme. In this case the phenomenon of hemopoietic hyperplasia was absent, and hypoplasia of erythropoietic tissue developed [2,3]. The sympatheticoadrenal system may control the adaptive response of hemopoietic tissue by affecting the aplastic and repair processes. The negative regulative effects of sympathetic neurotransmitters on hemopoietic tissue repair after 5-FU treatment seem to be beyond question. Sympathetic antagonists used 2-4 days after 5-FU injection accelerate the bone marrow reparative processes and increase the mature cell output to the blood. The findings provide a basis for the correction of hemopoiesis disturbances induced by antitumor therapy.

## REFERENCES

1. M. L. Gershanovich, *Complications with Chemo- and Hormone Therapy of Malignant Tumors* [in Russian], Moscow (1982).
2. E. D. Gol'dberg, A. M. Dygai, I. A. Khlusov, *et al., Pat. Fiziol.*, № 3, 14-17 (1991).
3. A. M. Dygai, I. A. Khlusov, V. P. Shakhov, *et al., Ibid.*, pp. 17-20.
4. G. F. Lakin, *Biometry* [in Russian], Moscow (1990).
5. E. V. Melik-Gaikazyan, *Probl. Gematol.*, № 8, 48-50 (1980).
6. G. Frimel', *Immunological Methods* [in Russian], Moscow (1987).
7. I. L. Chertkov and A. Ya Fridenshtein, *Cellular Principles of Hemopoiesis* [in Russian], Moscow (1977).
8. A. M. Dygai, V. P. Shakhov, A. V. Mikhlenko, *et al., Biomed. Pharmacother.*, **45**, 9-14 (1991).
9. E. D. Gol'dberg, A. M. Dygai, O. Yu. Zakharova, *et al., Folia. Biol. (Praha)*, **36**, 319-331 (1990).

# Effect of Nifedipine and Ruthenium Red on the Contractile Function and Oxidative Metabolism of the Myocardium

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The myocardial adrenoreceptors have been shown to participate in metabolism, being involved not only in glycolysis but in the processes of oxidative phosphorylation as well [2]. These effects are mediated by the adenylate cyclase system, this attesting to a specific role of $Ca^{2+}$ in this mechanism. The available data on the protective role of $Ca^{2+}$ blockers on the cardiac function during hypoxia argue in favor of this assumption [1,4,5]. It is known that the $Ca^{2+}$ sup-

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