ONCOLOGY

The Impact of Standard and Autohemochemotherapy for Catecholamine Levels in the Spleen and Thymus of Rats with Sarcoma-45

I. V. Kaplieva

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The aim of this study was to detect specific features of the effects of the blood–drug complex on catecholaminergic activity of the thymus and spleen. Catecholamine content was quantitatively measured by modified highly sensitive fluorometric method. Standard cyclophosphamide therapy led to tissue "adrenalization" against the background of reduced concentrations of norepinephrine (in the thymus) and dopamine (in both organs). Administration of cyclophosphamide by the autohemochemotherapy method stabilized catecholamine levels in the thymus and spleen of rats. Hence, cyclophosphamide autohemochemotherapy is more effective due to limitation of the destructive "stress" effect of the cytostatic on organs of the immune system.

Key Words: *catecholamines; immune system organs; autohemochemotherapy; cyclophosphamide*

New chemotherapeutic methods characterized by higher antitumor and lower toxic effects on normal tissues are effectively used at the clinical department of Rostov Institute of Oncology. One of these methods is autohemochemotherapy (AHCT): injection of cytostatics preincubated with autoblood. The method has been proposed by Yu. S. Sidorenko, Academician of the Russian Academy of Sciences and Russian Academy of Medical Sciences. Study of the mechanisms of the protective effect of the blood–drug system on the immune system is an interesting trend of research.

The sympathoadrenal system is one of the main components of the stress-realizing system. It plays the key role in adaptation to tumor process and to cytostatic therapy [6]. Catecholamines (CA), hormones and mediators of this system, are compounds with numerous functions maintaining the work of many systems, involved, among other things, into immunological regulations [2,7,9,11,12]. Traditional cytostatic therapy, in addition to suppression of immune reactions, modifies hormone status and disorders the function of the sympathoadrenal system [10].

We studied the effects of cytostatic (cyclophosphamide) on CA activities of the thymus and spleen during standard chemotherapy (SCT) and AHCT.

MATERIALS AND METHODS

The study was carried out on 95 outbred albino rats (170-200 g). Group 1 were intact rats (control 1; n=24). Group 2 were rats with sarcoma-45 (control

Laboratory of Experimental Hormone Therapy of Tumors, Rostov Research Institute of Oncology, Federal Agency for Health Care and Social Development, Rostov-on-Don, Russia. ***Address for correspondence:*** kaplirina@yandex.ru. I. V. Kaplieva

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2; n=19). Group 3 were rats with sarcoma after SCT (n=26) and group 4 were animals with sarcoma after AHCT (n=26).

Cyclophosphamide was injected on days 1 and 4 of the experiment. Standard chemotherapy was carried out by infusion of a single dose (50 mg/kg) of the drug, AHCT by reinfusion of 1 ml blood preincubated (45 min, 37°C) with the single dose of the drug into the jugular vein. Epinephrine, norepinephrine, dopamine levels in the thymus and spleen were measured 2 weeks after repeated injection of cyclophosphamide. The animals were sacrificed by decapitation.

Quantitative analysis of CA was carried out by a modified highly sensitive micromethod consisting in direct extraction of CA from tissues by an organic solvent (butanol) with their subsequent redistribution in inorganic phase with alteration of the organic eluate polarity. All steps of CA isolation were carried out strictly on ice. Fluorometric analysis of CA was carried out after oxidation of the resultant inorganic eluate with ferricyanide (for epinephrine) and iodine solution in ethanol (for norepinephrine and dopamine) with consideration for specific wavelengths of fluorescence stimulation and emission, forming during fluorophore chemical reaction.

The concentrations of all CA fractions in the studied biological material were calculated by comparing the fluorescence intensities in experimental and respective reference samples after all steps of the method. The fluorescence intensities of experimental and reference samples were measured on an MPF-4 spectrofluorometer (Hitachi).

The data were processed statistically using parametric Student–Fisher t test and nonparametric Mann–Whitney test [1]. The differences were considered significant at $p<0.05$.

RESULTS

The dynamics of CA concentrations in organs of the immune system depended on the chemotherapy method.

Epinephrine concentration in thymic tissues of rats with sarcoma-45 did not differ from that in intact animals, while hormone concentration in the spleen increased 3-fold. Norepinephrine concentrations increased in both organs more than 2-fold after SCT, while AHCT virtually did not change its level. For epinephrine, the difference between the two experimental groups was 2.5 times in the thymus and 2.2 times in the spleen (Tables 1, 2).

The development of sarcoma and SCT were paralleled by significant shifts in norepinephrine and dopamine concentrations in the thymus. The level of these CA decreased more than 5-fold in rats with sarcoma in comparison with intact animals. Cyclophosphamide SCT led to a still greater decrease in monoamine concentrations: a 3.1 times drop of norepinephrine and a 16-fold drop of dopamine, while AHCT stabilized their levels in the organ. Hence, the differences between two chemotherapy methods were 5.6 times for norepinephrine and 22 times for dopamine (Tables 1, 2). In the spleen, sarcoma growth caused just a slight reduction of dopamine concentration (1.48 times). These changes persisted after SCT, while after AHCT dopamine concentration did not differ from the intact control level (Tables 1 and 2).

It seems that this dynamics of pyrocatechine monoamines in organs of the immune system reflects stress reaction of the organism to tumor growth. Standard cyclophosphamide therapy augments its manifestation, because it promotes an increase of epinephrine level and exhausts norepinephrine and dopamine reserve in the thymus and dopamine reserve in the spleen. By contrast, AHCT decreases manifestation of stress reaction. The maintenance of norepinephrine concentration in the thymus at the level of tumor control after AHCT is presumably achieved due to stimulation of mediator release from adrenergic terminals under the effect of high serotonin level, which was detected in parallel studies [4]. On the other hand, epinephrine whose level increases in SCT stimulates acetylation processes leading to lesser accumulation in tissues of stress-limiting trophotropic mediators (serotonin and histamine) [6] playing an important role in the immune process. No doubt, stabilization of CA content in AHCT is a positive factor preventing the catastrophe occurring in these organs during the development of cancer process and augmenting under the effect of SCT.

In addition, epinephrine stimulation of adrenergic receptors leads to attenuation of immune reactions. Epinephrine reduces lymphocyte cytotoxicity and chemotaxis, inhibits their migration, reduces the counts of rosette-forming T_1, T_2, and B cells, decelerates the passage of B cells through the cell cycle, and presumably modifies mobility of B cells. On the other hand, norepinephrine-induced increase in cAMP level correlates with intensification of immune processes.

Increased level of dopamine in rats after AHCT is a positive factor characterizing dopamine as an indicator of CA resynthesis and as a mediator directly modulating the immune status. It is known that dopamine stimulates lymphocyte adhesion. Injection of DOPA to immunized animals stimulates the immune reaction.

Our data on the protective effect of the blood–cyclophosphamide complex reducing manifestation of the stress reaction are in line with the data of other authors. Those studies showed that chemotherapy on autologous liquid tissues leads to positive shifts in the immune status irrespective of the process location and

TABLE 1. Content of CA in Thymus Tissues (ng/ml) in Rats with Sarcoma-45 under Conditions of Cyclophosphamide Therapy by Different Methods (*M*±*m*)

Group	Epinephrine	Norepinephrine	Dopamine	CA sum
Intact	0.068±0.012	0.29±0.07	0.90±0.19	1.26±0.18
Sarcoma-45	0.075±0.026	0.053±0.02*	0.16±0.08*	0.29±0.08*
SCT	0.150±0.024*+	0.017±0.017*	0.010±0.007*+	0.18±0.04*
AHCT	0.060±0.037o	0.095±0.047*o	0.22±0.07*o	0.37±0.10*

Note. Here and in Table 2: $p<0.05$ compared to: *intact, +sarcoma-45, oSCT.

TABLE 2. Content of CA in Splenic Tissues (ng/ml) in Rats with Sarcoma-45 under Conditions of Cyclophosphamide Therapy by Different Methods (*M*±*m*)

Group	Epinephrine	Norepinephrine	Dopamine	CA sum
Intact	0.029±0.007	0.27±0.03	0.80±0.08	1.10±0.11
Sarcoma-45	0.088±0.020*	0.23±0.05	0.54±0.14	0.86±0.14
SCT	0.200±0.035*+	0.18±0.08	0.47±0.11*	0.85±0.21
AHCT	0.091±0.012*o	0.22±0.07	0.69±0.21	1.00±0.26

initial immunosuppression level, protects the lymphostructure of the thymus and spleen, prevents serious toxic effects on the immunity organs, and elevates extremely low membrane potential of immunocompetent cells [3,8].

Hence, cyclophosphamide treatment by the AHCT method is preferable to SCT, because it limits manifestation of the destructive "stress" effect on the immune system organs, which is characteristic of the classical cyclophosphamide chemotherapy method.

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