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DISTURBANCE OF MEDULLARY HEMATOPOIESIS IN THE LATE STAGES AFTER EXPOSURE TO CYTOSTATICS

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Clinical observations and experimental studies in recent years have fully confirmed the correctness of our theoretical ideas regarding the true nature of late effects of the damaging action of antitumor preparations on healthy (not affected by a tumor) organs and tissues [4]. Under these circumstances disturbances in the nervous, endocrine, and cardiovascular systems are comparatively easily diagnosed, whereas changes in the blood system may be latent in character and may be discovered only as a result of penetrating investigations or after additional hematopoiesis-inducing procedures (blood loss, stress, inflammation, etc.) [1, 2, 7]. It is considered that depression of the reserve capacity for granulocytopoiesis and erythropoiesis after treatment with cytostatics is linked with exhaustion of the pool of hematopoietic stem cells with different degrees of maturity [8]. It has also been suggested that disturbances of hematopoiesis discovered in the late stages after treatment with cytostatic drugs may be linked with changes in the hematopoiesis-inducing microenvironment [9], which plays an important role in the regulation of proliferation and differentiation of hematopoietic stem cells (HSC) [3].

The aim of this investigation was to study the state of bone marrow hematopoiesis and functional activity of adherent bone marrow cells in mice in the late period after administration of cytostatic drugs widely used in clinical practice: doxorubicin, vinblastine, and cyclophosphamide.

EXPERIMENTAL METHOD

Experiments were carried out on CBA mice aged 8-12 weeks (from the "Rassvet" nursery, Tomsk). Standard preparations of doxorubicin (Pharmorubicin, India), vinblastine (Hungary), and cyclophosphamide (USSR) were dissolved immediately before use in isotonic solution and given as a single intraperitoneal injection in the maximally tolerated dose (MTD), based on the results of graphic probit analysis, of 6 mg/kg for doxorubicin, 2.2 mg/kg for vinblastine, and 250 mg/kg for cyclophosphamide. The mice were killed by cervical dislocation 6 months after administration of the cytostatics. The total number of karyocytes (TNK) and the myelogram were counted (on bone marrow films stained by the method of Nocht and Maximow). The number of committed precursor cells of the granulocytic-monocytic series in the hematopoietic tissue was determined by cloning bone-marrow nuclears in methylcellulose in vitro by the method in [6]. A suspension of bone marrow cells in a concentration of $2 \cdot 10^7$ cells/ml was incubated in medium RPMI-1640 with 5% fetal calf serum for 30 min on plastic Petri dishes to separate the adhesive fraction of myelokaryocytes. Nonadherent cells were then collected, their viability estimated, and added in a concentration of $3 \cdot 10^5$ cells/ml to culture medium with the following composition:

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TABLE 1. Total Number of Myelokaryocytes and Absolute Number (·10/femur) of Individual Bone Marrow Cell Types 6 Months after Injection of Doxorubicin, Vinblastine, and Cyclophosphamide in MTD

Preparation	Total number of myelokaryocytes	Undifferentiated blast cells	Immature neutrophils	Mature neutrophils	Lymphoid cells	Monocytes	Macrophages	Erythroid cells
Control	21,7±0,9	0,07±0,01	3,7±0,3	6,9±0,3	4,4±0,4	0,20±0,03	0,08±0,02	4,2±0,5
Doxorubicin	19,9±2,0	0,04±0,01*	5,0±0,6*	8,7±1,2	2,6±0,5*	0,47±0,12*	0,02±0,02*	1,9±0,2*
Vinblastine	18,3±1,6	0,07±0,03	3,7±0,3	9,3±1,3*	2,0±0,5*	0,31±0,04*	0,13±0,005	1,6±0,5*
Cyclophosphamide	25,3±1,5*	0,17±0,03	3,8±0,2	10,6±0,4*	5,1±0,9	0,5±0,04*	0,1±0,03	3,5±0,4

Legend. Asterisk indicates significant differences from control values ($p < 0.05$).

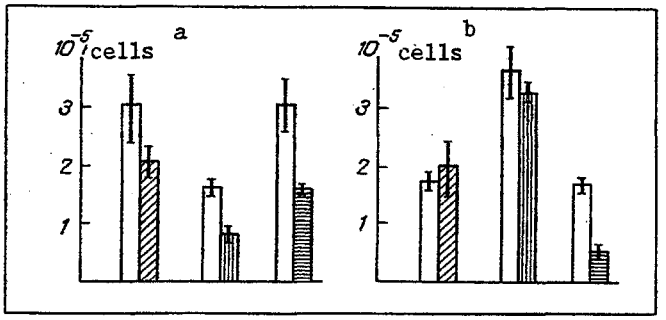

Fig. 1. a) Ability of adherent bone marrow cells of intact mice 6 months after injection of cytostatics to maintain growth of colonies of normal bone marrow cells; b) number of colony-forming units (·10) in bone marrow of intact mice 6 months after injection of doxorubicin (▨), cyclophosphamide (■), and vinblastine (□); control animals (▤).

80% medium RPMI-1640, 10% fetal calf serum, 10% conditioning medium from stimulated spleen cells, 280 mg/kg glutamine, 50 mg/kg gentamicin, and 0.4·10 M 2-mercaptoethanol. The cells were incubated in 24-well plastic planchets for 7 days at 37°C in an atmosphere of 5% CO_2. The number of colonies was then counted. By colonies was meant the number of foci of hematopoiesis containing more than 50 cells in the culture. To assess the functional activity of the adherent cells, 10^5 nonadherent myelokaryocytes from intact mice were added to each well of the 24-well planchet containing the test cells. Further culture was carried out as described above. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

The results of the study of medullary hematopoiesis are given in Table 1. They show that the total number of bone marrow cells in mice 6 months after injection of doxorubicin and vinblastine was virtually identical with the corresponding values in intact animals, whereas the relative numbers of the various morphologic forms of cells still remained considerably disturbed. The absolute number of lymphocytes and erythrokaryocytes was sharply reduced: by 41% and 55% after administration of doxorubicin and by 55% and 62% after injection of vinblastine respectively. The number of monocytes in the two cases, on the contrary, was significantly higher (by 135% and 55%) than the control values. Furthermore, the low (compared with the control) number of undifferentiated blast cells and macrophages in mice receiving doxorubicin and the increased number of mature neutrophils after exposure to vinblastine (Table 1) will be noted. The total number of myelokaryocytes in the mice 6 months after injection of cyclophosphamide was significantly higher than the control values, mainly due to an increase in the absolute number of mature neutrophilic leukocytes (by 53.6%), of undifferentiated blast cells (by 142.9%), and of monocytes (by 150%).

To assess the ability of the adherent bone marrow cells to maintain growth of committed hematopoietic precursor cells, 6 months after injection of the drugs syngeneic nonadherent intact bone marrow cells were incubated on a monolayer of adherent bone marrow cells from the experimental mice for 7 days in a culture medium containing methylcellulose. It

will be clear from Fig. 1 that adherent cells obtained from mice after exposure to cytostatics maintain growth of colonies of normal bone marrow cells less effectively. The ability of mouse bone marrow cells to form colonies in vitro in conditioning medium with methylcellulose, 6 months after injection of doxorubicin and cyclophosphamide, however, did not differ from that in control animals. The number of colony-forming cells in mice receiving vinblastine under identical experimental conditions, however, was significantly lower than the control values.

Thus a single injection of doxorubicin, vinblastine, and cyclophosphamide, in MTD, causes a long-lasting phenomenon of disorganization of the cell composition of the bone marrow in mice. The disturbances discovered are most probably associated not only with exhaustion of the pool of hematopoietic stem cells, but also with disturbance (judging by the parameters of the myelograms) of processes of their differentiation and change in functional activity of the stromal cells. The slow rate of cell renewal which was observed and the resistance of the stromal cells to cytostatic action (including irradiation) [5], suggests that elimination of "pathological" clones of stromal cells under these circumstances proceeds much more slowly than elimination of injured hematopoietic cells, and this in turn may lead to the appearance of stromal cells with altered functional activity in the late stages of the investigation. Considering the determinant role of stromal elements of the hematopoiesis-inducing microenvironment in the mobilization of medullary hematopoiesis [3], it can be asserted that one of the possible late effects of the damaging action of cytostatics may be profound decompensation of hematopoiesis (up to and including the development of severe hypoplastic states), arising in response to additional disturbing procedures (blood loss, stress, inflammation).

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