PARTICULAR FEATURES OF HEMATOPOIESIS IN MICE PROTECTED BY DEOXYCYTIDINE AGAINST THE LETHAL EFFECT OF CYTOSAR

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The study of changes observed in the tissues of normal animals is used for screening and investigation of the affinity of antitumor compounds and their combinations. If the necessary dosetime conditions are satisfied, we know that deoxycytidine (dC, 2'-deoxycytidine hydrochloride, from Reanal, Hungary) specifically weakens the various manifestations of activity of cytosar (araC, from Upjohn, USA) in systems both in vitro and in vivo [1-5]. However, the present writers have found that the number of lymphocytes in the bone marrow falls equally toward the end of a lethal course of araC both in mice dying from toxicosis and treated with araC alone and in outwardly healthy mice protected by dC [1]. Experiments conducted on the basis of these observations showed that dC, while protecting the animals against death, does not affect the depression by araC of antibody production in response to immunization by sheep's erythrocytes [3] and creates the conditions for manifestation of a powerful antitumor effect on developed lymphoblastic L 1210 leukemia [2]. In connection with the promising prospects of this combination for clinical use as an antitumor and immunoregulatory agent, a more penetrating study was required of the changes in hematopoiesis that develop in response to its administration, and the investigation described below was undertaken for that purpose.

EXPERIMENTAL METHOD

Female C57BL/6j (B6) mice weighing 20-25 g, from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, were used. The preparations were dissolved in sterile physiological saline and injected simultaneously in doses of 0.2 ml per mouse intraperitoneally and by mouth, respectively. The mice of group 1 received dC + araC, those of group 2 received araC only, and the animals of group 3 were intact. Since according to data in the literature and our own observations, administration of dC alone has no effect on the state of hematopoiesis, there was no corresponding control group of animals. On definite days six mice of each group were killed so that the cell composition of the main hematopoietic organs could be studied. To determine the total number of nucleated bone marrow cells one femur was fragmented in 5% acetic acid and bone marrow was expressed from the other femur into a drop of bovine serum, and films were prepared. The spleen and thymus were weighed and cut into two parts; squash preparations were obtained from one part at different levels, the other part was weighed, cut into small pieces in 5% acetic acid, and the total number of nucleated cells in these organs was counted in a Goryaev's chamber. The number of nucleated cells in 1 μl peripheral blood also was determined and the cell composition was studied in films. Films and squash preparations, fixed in methanol, were stained by the Romanovsky–Giemsa method. In each blood film 200 cells, in each bone marrow film 500 cells, and in each squash preparation from the spleen 1000 cells were identified. On the basis of the results the number of the corresponding types of cells contained in 1 μl blood, in the femoral marrow, and in the thymus and spleen was calculated. In a special experiment set up in accordance with a similar scheme on (CBA × B6)F_1 mice weighing 20 g (from the Stolbovaya nursery the number of polypotent hematopoietic stem cells in the bone marrow and spleen was determined by the method of exocolony production in the spleen (CFU_S) of lethally irradiated (1200 R) mice. The animals' general condition was monitored by periodic inspection and weighing. Mortality was determined on specially isolated groups of mice. The results were subjected to statistical analysis, with calculation of mean values and the 95% confidence interval. Differences between the means were taken to be significant at $P < 0.05$.

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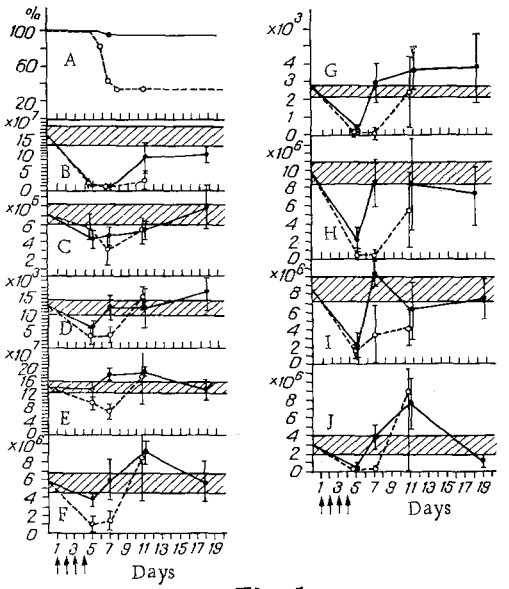

Fig. 1

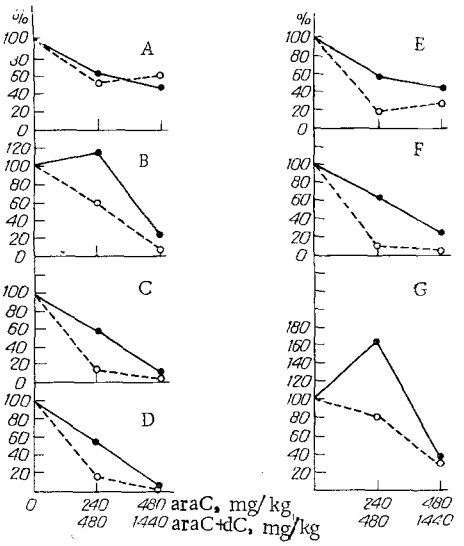

Fig. 2

Fig. 1. Effect of the combination araC + dC on hematopoiesis. Abscissa, days of observation; ordinate, in A, number of surviving animals (%), six mice in each group. In other figures: ordinate, absolute number of corresponding types of cells; B) thymus lymphocytes, C) femoral bone marrow lymphocytes, D) lymphocytes in 1 μl blood, E) splenic lymphocytes; F) postmitotic bone marrow granulocytes, G) granulocytes in 1 μl blood, H) postmitotic splenic granulocytes; I) erythroid cells in femoral marrow; J) erythroid cells in spleen. Broken line – mice receiving araC only (20 mg/kg 3 times a day for 4 days); continuous line – individuals receiving araC (20 mg/kg 3 times a day for 4 days) in combination with dC (40 mg/kg 3 times a day for 4 days); shaded region represents 95% confidence interval in control. Arrows indicate days of administration of compounds.

Fig. 2. Effect of pattern of administration of araC + dC combination on hematopoiesis. Abscissa, dose of araC per course (240 = 20 mg/kg 3 times a day for 4 days and 480 = 40 mg/kg 4 times a day for 3 days) in combination with dC (480 = 40 μg/kg 3 times a day for 4 days and 1440 = 120 mg/kg 4 times a day for 3 days); ordinate, relative number (in %) of different types of cells in bone marrow (A, B, C, D) and in peripheral blood (E, F, G). Broken line represents araC, continuous line araC + dC. A and D – lymphocytes; B) mitotic granulocytes; C and F) postmitotic granulocytes; G) monocytes; H) erythroid cells.

EXPERIMENTAL RESULTS

Injection of dC into mice in doses 2 or 3 times greater than those of araC administered simultaneously prevented the development of toxic effects in most cases (Fig. 1A). However, protection of the hematopoietic tissue was more effective when araC was given in a dose of 20 mg/kg 3 times a day for 4 days (program 1) than under more intensive dosage conditions: 40 mg/kg 4 times a day for 3 days (program 2) (Fig. 2). Administration of dC prevented the development of lethal toxicosis even when araC was given by program 2 in 3 courses with intervals of 4 days. After the end of the last course regeneration of hematopoiesis began and was excessive in character. For instance, 1 week later the mean weight of the spleen had increased from 120 to 260 mg, the number of mature granulocytes in the bone marrow had increased by 1.5 times, and the number of cells of the erythroid series was 1.9 times greater than in the control. The particular features of the changes in hematopoiesis during protection by dC against toxicosis induced by araC were manifested more clearly when program 1 was used. Cells of the granulocytic and erythroid hematopoietic series were sufficiently protected against the harmful effect of araC by means of dC: after an initial fall in the number of cells, toward the end of the course their level was quickly restored to normal after only 3 days (Fig. 1F, G, H, I, J). The pattern of change of these cells was identical in type in the bone marrow, spleen, and blood. Changes in the lymphocyte level in the protected mice were qualitatively and quantitatively different in different organs. In the blood and spleen these changes were similar to those observed in cells of the granulo-

TABLE 1. Protection by dC of Polypotent Hematopoietic Stem Cells against the Toxic Action of araC

Group	Bone marrow		Spleen
	$CFU_s/10^5$ cells	CFU_s/ femur	$CFU_s/10^5$ cells
Intact control	20±4	7051	5±1
araC	28±2	2576	23±3
dC + araC	34±5	15 706	24±2

Note. Each group consisted of six donor mice and 14 recipients. No endocolonies were found in the lethally irradiated recipients. The donors received intraperitoneal injections of araC in a dose of 20 mg/kg 3 times a day for 4 days, and dC simultaneously with the araC, also intraperitoneally in a dose of 40 mg/kg 3 times a day for 4 days. The donors were killed on the day after the end of the course and recipients on the 9th day after transplantation of donors' cells.

cytic and erythroid series (Fig. 1D, E). Meanwhile, in the thymus and bone marrow inhibition of lymphocytopoiesis was identical in mice protected and unprotected against toxicosis. Under these circumstances lymphocytes disappeared virtually completely from the thymus, and not until 1-2 weeks after the end of the course was their incomplete recovery observed in this organ. A temporary reduction in the number of lymphocytes in the bone marrow by half was observed 3 days after the end of the course (Fig. 1B, C). The number of polypotent hematopoietic stem cells, estimated from the number of CFU_S, was reduced in the unprotected mice. Administration of dC gave complete protection to these cells. In the spleen the relative number of CFU_S was increased fivefold both in mice receiving araC alone and in those receiving a combination of araC with dC. Since the total number of cells in the spleen of the unprotected mice was only half of that in the intact control, whereas in the protected mice it varied from a little below to twice as high as the control level, it can be concluded that the absolute number of CFU also was higher in this organ (Table 1).

The experiments thus showed that dC does not influence the cytotoxic effect of araC on lymphocytes of thymus and bone marrow; inhibition of lymphocytes by araC was more marked in the thymus than in the bone marrow. This may be connected both with the special sensitivity of definite lymphocyte populations to araC and also with the dissemination of cells to the periphery, or to the action of both factors together. The special sensitivity to araC can be explained by the ability of certain lymphocytes to assimilate exogenous dC. It has also been shown for some clones of malignant mouse lymphocytes that their sensitivity to araC is inversely proportional to the intracellular dC level [5].

Simultaneous injection of deoxycytidine into B6 female mice in doses twice or three times greater than those of araC (20 mg/kg 3 times a day for 4 days) prevents the development of lethal toxicosis due to cytosar, protects the polypotent hematopoietic stem cells of the bone marrow and spleen and myeloid and erythroid cells of the bone marrow, spleen, and blood, and also lymphocytes of the spleen and blood against the toxic action of cytosar and does not protect lymphocytes of the thymus and bone marrow.

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