ACTION OF HEMOLYSATE OF POLYCYTHEMIC ANIMALS ON PROLIFERATIONS OF BONE MARROW CELLS

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Erythrocytic inhibitor from polycythemic rats depressed mitotic activity of cells of of the erythroid series in mice by 40%. The inhibitory effect lasted about 12 h. The inhibitor acted on the G_2 period. The "points of application" of the inhibitor were not only blast forms of the erythron but also hematopoietic stem cells.

KEY WORDS: *erythrocytic inhibitor; erythropoiesis; stem cells; splenic colonies.*

There are isolated reports in the literature on the inhibitory action of hemolysates or of extracts from erythrocytes of polycythemic animals on erythropoiesis [1, 4]. It is suggested that this inhibitor has an inhibitory effect on erythropoiesis by its direct action on hematopoietic stem cells or indirectly by blocking erythropoietin [4].

By analogy with erythropoietin, erythrocytic inhibitor can evidently exert its effect on various processes of hematopoiesis. The possibility cannot be ruled out that some inhibitors act mainly on proliferation and others on differentiation and maturation of the cells and their liberation into the blood stream.

This paper gives data on the action of an inhibitor present in the hemolysate of polycythemic animals on the dividing cells of the erythron and on proliferation of hematopoietic stem cells.

EXPERIMENTAL METHOD

Experiments were carried out on 55 noninbred albino rats weighing 20-25 g into which a hemolysate from polycythemic rats was injected and its action studied after 25-27 h. Polycythemia was produced in large rats weighing 170-220 g by the blood transfusion method. The rats were killed 48 h later and distilled water was added in the ratio of 1:1 to the packed erythrocytes separated from leukocytes. The protein concentration was determined by the IRF-22 refractometer in the lysate after removal of the cell membranes.

The working dose of hemolysate (32 mg/100 g body weight) giving a reliable inhibitory effect on erythropoiesis was determined in preliminary experiments. The inhibitory action of the inhibitor on mitosis was almost completely abolished by heating. Physiological saline was injected into control mice. Allowing for the diurnal rhythm of mitotic activity of the bone marrow cells [5] the control mice were killed at intervals throughout the experiment. The criterion of the inhibitory action of the hemolysate was mitoses stopped by colchicine (C-mitoses) in the erythroid series of the femoral marrow. The mitotic index was calculated for the proliferating cells (proerythroblasts, basophilic erythroblasts, young polychromatophilic erythroblasts) and expressed as a percentage. To stop mitosis, a 0.1% solution of colchicine was injected into the animals in a dose of 0.5 ml/100 g body weight 2 h before sacrifice. Bone marrow films were stained by the Romanovsky–Giemsa method and 500 proliferating cells were counted at each time.

The effect of the hemolysate on the stem cells was studied by the method of Till and McCulloch [12].* Experiments were carried out on 64 noninbred albino mice. The animals

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TABLE 1. Effect of Hemolysate of Polycythemic Animals on Mitotic Activity (C-mitoses) of Mouse Bone Marrow Cells

Group of mice	Time of observation, h					
	2½	4	8	10	16	27
Control	16,5±1,56	15±1,09	—	16±2,02	—	18±2,43
Experimental *P*	12±1,07 <0,05	9±1,54 <0,05	8±1,22 <0,05	10±1,30 <0,05	13±1,73 p>0,05	19±2,07 >0,05

TABLE 2. Effect of Hemolysate of Polycythemic Animals on CFU Formation in Spleen after Exposure of Bone Marrow Cells to It for 4 h (M ± m)

Index studied	Group 1 (background)	Group 2 (control)	Group 3 (experimental)
Number of colonies per spleen	7±1,70 (9)	18,6±3,03 (10)	10±1,61 (8)
Weight of spleen, mg	30±5,62	56±8,70	36±6,15
	Experiment No. 2		
Number of colonies per spleen	10,6±1,55 (8)	23,7±2,36 (11)	17±1,85 (8)
Ratio of number of large colonies to total number of medium-sized and small colonies	$\frac{6}{4,6}=1,3$	$\frac{13,1}{10,6}=1,2$	$\frac{7}{10}=0,7$

Legend. Number of animals in parentheses.

were irradiated with ^{137}Cs γ rays on the GUPOS apparatus in a dose of 550 R (dose rate 478 R/min) and next day they were given an intravenous injection of $2.6 \cdot 10^5$ bone marrow cells taken from five mice in Eagle's medium. Altogether three groups of mice were studied: 1) the background group for counting the number of endocolonies (irradiation without injection of bone marrow), 2) the control group (irradiation followed by injection of bone marrow from intact mice), and 3) the experimental group (irradiation followed by injection of bone marrow from mice previously treated *in vivo* with polycythemic hemolysate for 4 h).

The number of colony-forming units (CFU) was counted 9 days after injection of the cells. The spleens were fixed in Bouin's fluid and the number of colonies in them was estimated visually. Morphometric measurements of the newly formed colonies also were made and the fixed spleens were weighed.

The mitotic cycle of cells of the erythroid series was studied by an autoradiographic method based on the curve of labeled mitoses. The results showed that the duration of the mitotic cycle (T) of cells of the erythroid series was 14 h, the G_1 period 2 h, the S period 7 h, and the G_2 period 4 h. The duration of mitosis determined by the colchicine method was 0.7 h. According to data in the literature the duration of the mitotic cycle of cells of the erythroid series varies from 14 to 18-26 h [2, 9].

EXPERIMENTAL RESULTS

The observations showed that 2.5 h after the action of the erythrocytic inhibitor mitotic activity in the experimental group was inhibited by roughly 30%. The inhibitory action of the hemolysate still continued 4-8 h later, but this was followed by gradual recovery of mitotic activity to its original level which was reached after 16 h (Table 1). These results indicate that the duration of the inhibitory action of the hemolysate in a dose of 32 mg/100 g body weight on cells of the erythroid series is about 12 h.

Comparison of the results showing inhibition of mitosis by the action of the hemolysate with the duration of the individual phases of the cell cycle in the erythroid series leads to the conclusion that the hemolysate inhibits the mitotic cycle in the G_2 phase by blocking the transition into the phase of mitosis. This is confirmed by the results showing a decrease in the mitotic index 2.5 and 4 h after treatment with the inhibitor. Considering that cell division was asynchronous, it can be concluded that mitosis during this period was represented

by cells which, at the moment of treatment, were in early prophase and at the end of the G_2 period. The fact that mitoses were present after longer treatment with the inhibitor indicates that the block in the G_2 period was incomplete.

The disturbance of erythropoiesis in the bone marrow under the influence of the hemolysate took place not only through the depression of proliferation of erythroid blast forms but also through a decrease in the number of stem cells. The results given in Table 2 show that treatment of bone marrow cells by erythrocytic inhibitor for 4 h followed by their injection into irradiated recipients leads to a marked decrease in the number of hematopoietic stem cells capable of forming CFU in the spleen. The decrease in weight of the spleens studied (compare groups 2 and 3 in Table 2) also indirectly indicates a decrease in the number of CFU in the experimental group.

Different types of colonies, of unequal size [6], are known to be formed in the spleens of irradiated mice. The largest colonies in the spleen (0.8-0.9 mm and over) are considered to be erythroid, the smallest (0.2-0.3 mm and under) megakaryocytic, and colonies of intermediate size are regarded as myeloid. With this in mind, in one of the experiments the number of large colonies and the number of medium and small colonies were determined per spleen and expressed as a ratio of each other.

The results are given in Table 2 (experiment No. 2), which shows that the ratio between the number of large (erythroid) colonies and the combined number of medium and small (myeloid and megakaryocytic) colonies was 1.2 in the control and 0.7 in the experimental group. The reduction by almost half in this ratio is indirect evidence of the selective depression of proliferation of erythroid colonies in the spleen by the erythrocytic inhibitor.

It can thus be concluded from these results that hemolysate from polycythemic animals acts on blast forms of erythropoiesis and also on polypotent stem cells. Considering the rapidly developing reaction of inhibition of proliferation of splenic CFU under the influence of the hemolysate, it can be postulated that it is due to the direct action of the hemolysate on the stem cells.

The nature of the inhibitor present in the hemolysate of the polycythemic animals is not yet clear. There is reason to suppose that the inhibitor belongs to the chalone class. In recent years much experimental evidence that inhibitors with a specific action, known as chalones, can be extracted from differentiated cells of various tissues has been published [8, 11]. There are as yet no standard methods for the isolation of chalones. Buffer systems or distilled water is most frequently used to extract them [3, 7, 10]. Consequently, considering the method of isolation and the biological effect (inhibition mainly of the large colonies) of the inhibitor present in the hemolysate from polycythemic animals it seems likely that it is an inhibitor of the chalone type.

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