EFFECT OF ERYTHROCYTIC G_2-CHALONE ON THE KINETICS OF ERYTHROPOIESIS IN MICE

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Erythrocytic inhibitor (G_2-chalone) was shown to act on both proliferating and differentiating cells of the erythroid series in mouse bone marrow. The effect of the chalone was observed as early as 3 h after injection and continued for 8 to 48 h thereafter depending on the degree of cell differentiation. After a single injection of chalone, despite considerable disturbances of erythropoiesis, the number of erythrocytes counted after 8-12 days remained within the control limits. After repeated injections of the chalone the erythrocyte count fell in the late stages of the experiment.

KEY WORDS: erythrocytic inhibitor; G_2-chalone; bone-marrow cells.

Investigations by several workers have demonstrated the important role of erythrocytic chalone in the regulation of cell division in the erythron [12]. Erythrocytic chalone has been shown to be present in both normal and polycythemic serum, but its content in the latter is much higher [13]. Subsequent investigations showed that the inhibitor is also present in a lysate of erythrocytes from intact and polycythemic animals and it evidently enters the plasma following destruction of the erythrocytes [4, 11].

The principal property of inhibitors of chalone type is their marked tissue specificity [9, 10]. Experiments on various objects have suggested that several types of chalones (G_1- and G_2-chalones), which act on different phases of the cell cycle, are present in all tissues of the body [8, 14]. Two types of chalones – G_1 and G_2 – also are known to participate in proliferation of the erythron [5, 12].

The object of this investigation was to study the kinetics of the initial disturbances of the principal stages of erythropoiesis in the bone marrow of animals during the first hours after injection of erythrocytic G_2-chalone. Insufficient attention has been paid to the study of the quantitative changes arising in the system of the erythron after exposure to the inhibitor. There are only isolated reports on the effect of erythropoiesis inhibitor on the reticulocyte and erythroblast counts in the late stages of the experiment (3rd day) after repeated injections of the inhibitor into animals [3].

EXPERIMENTAL METHOD

Experiments were carried out on 190 noninbred albino mice weighing 20-22 g. The kinetics of the numbers of erythrocytes, erythroblast-normoblasts, proerythroblasts, and colony-forming units (CFU) after injection of G_2-chalone was investigated. The inhibitor was obtained from the blood of polycythemic rats (100 animals) 24 h after blood transfusion [5]. The protein concentration in the hemolysate was determined on the IRF-22 refractometer and the hemolysate itself was injected intraperitoneally at various times in doses of 16 and 64 mg/100 g body weight. Physiological saline was injected into the control mice.

To determine the number of the various cells of the erythroid series the total number of myelocytes was counted in the femur, bone marrow films were stained by Romanovsky's method, and the absolute number of cells was then calculated by analysis of the myelograms. The number of CFU on the surface of the spleens was determined by the method of Till and McCulloch [15]. For this purpose the recipients were irradiated on a "Gupos" apparatus with ^{137}Cs γ rays in a dose of 750 R at a dose rate of 478 R/min and, 4 days later, donors' bone marrow cells were injected into them [1]. The cell suspension used in each case was obtained from five donors after preliminary injection of erythrocytic chalone. The number of cells injected was $0.2 \cdot 10^6$. The animals were killed 8 days later, the spleens were fixed, and the total number of colonies on the surface of the

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TABLE 1. Dynamics of Quantitative Changes in Proliferating and Differentiating Cells of Erythroid Series of Mouse Bone Marrow after Single Injection of Chalone (64 mg/100 g body weight) (M ± m)

Cells	Time of investigation after injection of chalone, h					
	0	3	8	16	25	48
Erythrocytes (· 10^6)	9,8±0,375	8,1±0,21	8,5±0,185	9,4±0,29	8,9±0,47	9,5±0,84
Erythroblast-normoblasts (· 10^6)	2,7±0,22	1,8±0,63	1,3±0,14	1,7±0,095	1,9±0,11	2,5± 0,125
Proerythroblasts (· 10^4)	12±0,84	4,4±0,25	2,2±0,22	3,6±0,24	5±0,30	10,2±0,47
Myelokaryocytes (· 10^6)	14,6±1,01	11,3±0,41	11±1,15	12±0,66	11,5±0,67	13,8±0,75
CFU_S	32±3,20 (18)	24±3,17 (10)	8±1,95 (3)	19±1,93 (8)	20±1,55 (8)	22±2,41 (10)

Legend. Number of large colonies (0.8-1 mm) shown in parentheses. Background (number of endocolonies) 0.8 per spleen.

TABLE 2. Dynamics of Changes in Erythrocyte Count in Peripheral Blood of Mice after a Single Injection of Chalone (64 mg/100 g body weight) (M ± m)

Group of animals	Initial erythrocyte count	Erythrocyte count (· 10^6) at different times after injection of chalone		
		8th day	10th day	12th day
Control	9,3±0,25	9,2±0,40	9,5±0,44	9,35±0,49
Experiment	9,3—0,25	9±0,5	9,2±0,49	8,95±0,74

spleens and the number of large colonies (0.8-1 mm) were determined; according to data in the literature, the latter are erythroid colonies [7].

The erythrocytes were counted in a Goryaev's chamber by the usual method. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

After a single injection of chalone the general pattern of the changes in the number of erythrocytes, erythroblast-normoblasts, proerythroblasts, and erythroid colonies was uniform in type (Table 1).

Irrespective of the degree of maturity and differentiation of the cells of the erythroid series, a decrease in the number of all types of cells of the erythron, of myelokaryocytes, and of CFU was observed during the first few hours after injection of the inhibitor, followed by a return to the original level. Differences affecting individual types of cells of the erythroid series extended only to the degree or duration of these changes: the lower the level of differentiation of the cell, the more severely and the longer it was damaged. For instance, for erythrocytes a significant deviation ($P < 0.05$) from normal values was of short duration (from 3 to 8 h) throughout the duration of the experiment, for erythroblast-normoblasts it was observed for 3 to 25 h, for proerythroblasts from 3 to 25 h, and for CFU (including erythroid) from 3 to 48 h.

On the other hand, the severity of injury, i.e., the maximal deviation of the experimental from the control values, amounted to 17% for erythrocytes, 50% for erythroblast-normoblasts, 80% for proerythroblasts, and 85% for CFU (0.8-1 mm).

The severest disturbances of erythropoiesis were thus found in stem cells and proerythroblasts. Considering these results, on theoretical grounds a reduction in the number of erythrocytes in the peripheral blood would be expected at a time after injection of the inhibitor equal to the transit time of the erythrocytes. This period for erythrocytes developing from the stem cell and proerythroblast is known to be 10-14 and 5-8 days respectively [22]. With these data in mind, the number of erythrocytes in the peripheral blood was counted after a single injection of chalone (Table 2).

As Table 2 shows, at all stages of the experiment the erythrocyte count remained relatively constant. Differences between the erythrocyte count in the experimental and control animals were not significant ($P > 0.05$).

It can thus be concluded from an analysis of the results of the experiments of series I and II (Tables 1 and 2) that, despite considerable disturbances of hematopoiesis in the stages of stem cells and proerythroblasts, no significant decrease in the number of normoblasts was observed during the 2-12 days that the experi-

TABLE 3. Dynamics of Changes in Erythrocyte Count in Peripheral Blood of Mice after Repeated Injections of Chalone (16 mg/100 g body weight) (M ± m)

Group of animals	Initial erythrocyte count (· 10^6)	Erythrocyte count (· 10^6) at different times after injection of chalone								
		1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	8th day	9th day
Control	9,3±0,25	+	+	+	+	+	+	—	9±0,32	9,4±0,5
Experiment	9,3—0,25	+	+	+	+	+	+	—	6,9±0,51	9,6±0,39

Legend. +) Injection of erythrocytic chalone into experimental animals and of physiological saline into controls.

ments lasted. This was evidently due to the presence of powerful reserve mechanisms, controlling erythrocyte production, in the hematopoietic system. The subsection of committed stem cells, performing the role of buffer system, and "terminal" erythropoiesis, playing the role of "sluice" in erythrocyte production during exposure to various factors, can be included among the number of these regulators of the normocyte population [2, 6].

Evidently a reduction in the number of erythrocytes in the peripheral blood on account of depression of the proliferative pool of cells of the erythroid series can be obtained only after prolonged administration of erythrocytic chalone. In the next series of experiments, the inhibitor was accordingly injected repeatedly into the experimental mice in a dose of 16 mg/100 g body weight. The intervals between injections were 10-14 h, for according to data in the literature the inhibitory action of the chalone on proliferative activity of the cells varies within these limits [5, 8, 12].

The scheme of this series of experiments and their results are given in Table 3.

As Table 3 shows, on the 8th day of the experiments a significant ($P < 0.05$) decrease was found in the number of erythrocytes in the animals of the experimental group, followed by a return to the initial level 3 days after administration of the inhibitor ceased. Later in the course of the investigation (10th-12th days) the difference between the erythrocyte count in the experimental and control groups was not significant.

It is thus only during prolonged administration of erythrocytic chalone that proliferation of stem cells and erythroblasts can be inhibited to a sufficient degree to cause an eventual reduction in the erythrocyte count in the peripheral blood. After the repressive influence is removed the erythrocyte count gradually returns to its original values, evidently on account of an increase in the activity of the proliferative pool of the erythroid series of the bone marrow.

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