EFFECT OF HEPARIN ON REGENERATION OF THE THYMUS AFTER IRRADIATION IN MICE WITH DIFFERENT RADIOSENSITIVITY

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An important system of the body responsible for the restoration of homeostasis under the influence of extremal factors is the hematopoietic tissue, whose function necessitates migration of cells [2]. The migration phenomenon assumes particular importance in the case of irradiation. By modifying cell migration it is possible to influence the course of regeneration and, consequently, the radioresistance of the body [6, 9, 11].

The object of this investigation was to study the effect of heparin, a humoral regulator of migration [3, 6], on regeneration of the thymus and bone marrow in irradiated mice of different lines.

EXPERIMENTAL METHOD

To discover genotypic differences experiments were carried out on inbred BALB/c, (CBA × C57BL)F_1 hybrid, and noninbred albino mice weighing 19-21 g. The animals were exposed to a single whole-body irradiation from a ^{60}Co source (dose rate 0.2 Gy/min). To obtain a roughly equal degree of radiation injury, the BALB/c and noninbred mice were irradiated in a dose of 4 Gr and the hybrid mice in doses of 5 and 6 Gy. To study the effect of bone marrow on regeneration of the thymus, BALB/c mice also were irradiated with x rays in a dose of 4 Gy on the RUM-17 apparatus (180 kV, 17 mA, 0.5 mm Cu + 1.0 mm Al, 0.3 Gy/min). Animals of the experimental group were given heparin (from Richter, Hungary) by intraperitoneal injection once daily from the 5th through the 9th days after irradiation in a dose of 250 units/kg; control mice received physiological saline. The effect of heparin was judged from the change in weight of the thymus and number of myelokaryocytes in the femoral marrow [8, 13, 14].

EXPERIMENTAL RESULTS

In agreement with data in the literature [1, 8, 13, 14] restoration of the weight of the thymus in mice after irradiation was biphasic in character. Although the degree of the primary decrease in weight of the thymus (5th day) was practically identical in mice of the different lines, the secondary decrease in weight on the 20th day after irradiation was greater in mice of the radio-sensitive BALB/c line than in the radioresistant hybrids (Table 1).

According to one hypothesis, the secondary fall in weight of the thymus is attributable to migration of thymocytes into the bone marrow, where they cause activation of myeloid hematopoiesis [4, 10]. Another hypothesis links the second phase of regeneration with the need to colonize the thymus with stem cells migrating from bone marrow [12, 13, 15]. Whatever the case the degree of decrease in weight of this central lymphoid organ depends on the state of the bone marrow. This hypothesis was confirmed by the results of the present experiments. For instance, at the time of maximal depopulation of the bone marrow (2nd day after irradiation) the number of myelokaryocytes in BALB/c, noninbred, and hybrid mice was 26, 32, and 35% of the initial level respectively.

Screening the hind limb (10% of the total number of myelokaryocytes) during irradiation completely prevented the secondary decline in weight of the thymus but had no effect on the primary depopulation (Fig. 1).

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TABLE 1. Effect of Heparin on Change in Weight of Thymus in Irradiated Mice (in % of initial level)

Line of mice	$LD_{50/30}$, Gy	Dose of irradiation, Gy	Time after irradiation, days											
			5	9		12		16		20		30		
				control	heparin	control	heparin	control	heparin	control	heparin	control	heparin	
BALB/c	6	4	24,3±2,6 (10)	56,6±6,1 (9)	44,0±5,0 (10)	68,8±7,1 (10)	57,0±6,1 (10)	44,0±4,8 (10)	53,4±5,6 (10)	32,0±3,4 (10)	49,2±5,2* (10)	59,7±6,2 (10)	82,2±9,0* (10)	
Noninbred	6,3	4	28,5±3,0 (9)	61,3±5,9 (17)	52,1±5,3 (16)	70,8±6,3 (14)	59,1±6,0 (14)	50,5±5,2 (10)	62,1±5,3 (9)	42,5±4,3 (9)	60,0±5,9* (10)	72,5±7,0 (10)	83,0±9,1 (10)	
(CBA×C57BL)F_1	7	5	31,4±2,8 (6)	53,4±5,1 (5)	50,5±5,3 (5)	62,3±5,9 (8)	63,1±6,1 (8)	62,8±6,1 (8)	62,8±5,9 (10)	50,7±6,0 (10)	49,0±5,8 (10)	82,7±9,1 (6)	80,0±9,5 (6)	

Legend. Here and in Table 2, asterisk denotes $P < 0.05$ compared with control; number of animals given in parentheses.

TABLE 2. Effect of Heparin on Change in Weight of Thymus and Number of Myelokaryocytes in (CBA × C57BL)F_1 Hybrid Mice Irradiated in a Dose of 6 Gy

Parameter studied, % of initial level	Time after irradiation, days								
	5	9		14		16		20	
		control	heparin	control	heparin	control	heparin	control	heparin
Weight of thymus	23,5±1,9 (20)	35,5±3,6 (10)	35,5±3,1 (10)	55,0±4,8 (10)	55,0±3,9 (10)	56,9±5,3 (10)	52,6±4,9 (9)	47,3±4,1 (9)	40,4±4,2 (8)
Number of myelokaryocytes	15,7±1,1 (20)	26,2±2,3 (10)	25,0±2,1 (10)	61,3±5,8 (10)	84,8±7,8* (10)	70,4±6,3 (9)	106±8,9* (10)	116,1±10,5 (10)	131,3±12,5 (10)

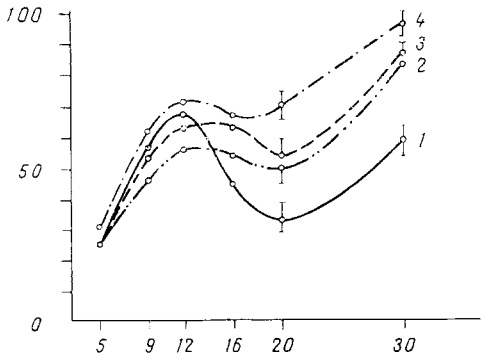

Fig. 1. Time course of restoration of weight of thymus in irradiated BALB/c and (CBA × C57BL)F_1 hybrid mice. Abscissa, time after irradiation (in days); ordinate, weight of thymus (in % of initial level). 1) BALB/c mice: whole-body irradiation, physiological saline; 2) BALB/c mice: whole-body irradiation, heparin; 3) (CBA × C57BL)F_1 mice: whole-body irradiation, physiological saline; 4) BALB/c mice: irradiation with screening of one hind limb.

Comparison of the time course of regeneration of the thymus in mice of the experimental and control groups showed that injection of heparin after irradiation, like transplantation of bone marrow and chemical protection [8, 14], prevented the second decline in weight of the thymus (Table 1). On the 20th day after irradiation of the noninbred and BALB/c mice receiving heparin the weight of the thymus was significantly higher than in animals of the control group. Furthermore, in experiments on BALB/c mice receiving heparin, restoration of the weight of the thymus to its original values took place more rapidly (Table 1).

In experiments on radioresistant hybrid mice irradiated in a dose of 5 Gy heparin did not affect regeneration of the thymus (Table 1). Assuming that irradiation in this dose did not produce the necessary degree of depopulation of the bone marrow, in the next series of experiments hybrid mice were irradiated in a dose of 6 Gy (Table 2). In this case also, however, even though the number of myelokaryocytes fell to 16%, no distinct decrease in weight of the thymus could be obtained on the 20th day, nor did heparin give a positive effect. Meanwhile in the hybrid mice heparin led to more rapid recovery of the number of myelokaryocytes than in the control (Table 2).

The writer showed previously that administration of heparin is followed by intensification of myeloid hematopoiesis, accompanied by reciprocal inhibition of erythropoiesis in the bone marrow of both intact and irradiated mice [5, 7]. Consequently, when planning the experiments it was assumed that if thymocytes are essential for the change in direction of differentiation of hematopoietic stem (committed) cells, injection of heparin ought to reduce the decrease in weight of the thymus due to migration of cells into the bone marrow. In fact, the experimental results showed that injection of heparin caused marked inhibition of cell migration from the thymus in the more radio-sensitive BALB/c and noninbred mice. In experiments on radioresistant hybrid mice, in which there was virtually no secondary decline in weight of the thymus, heparin administration was followed by more rapid recovery of the number of myelokaryocytes (Table 2). The absence of a secondary fall in weight of the thymus in the radioresistant hybrid mice was probably due to the more rapid repair processes, including in the bone marrow.

It can be concluded from the results that heparin is a regulator of regenerative and compensatory processes after radiation injury. The lower the level of the protective and adaptive responses of the animal, the more clearly its regulating role is exhibited.

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