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DIFFERENTIATION AND RADIOSENSITIVITY OF HEMOPOIETIC STEM CELLS OF MICE DURING HYPOKINESIA

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DIFFERENTIATION AND RADIOSENSITIVITY OF HEMOPOIETIC STEM CELLS OF MICE DURING HYPOKINESIA

by

V. N. Shvets

Currently the problem of hypokinesia is acquiring general biological /199* and medical importance due to scientific and technical progress resulting in a reduction of muscle loads in daily human activity; in clinical practice when the patient is strictly confined to bed for long periods; in space medicine where hypokinesia is an integral part. The main etiological factor of hypokinesia is the sharp reduction in the volume of muscle activity [1] leading to a decrease in the oxygen demand by the tissues [2]. A change in the respiration by the muscle tissue during hypokinesia can affect the erythropoietic and compensatory function of the bone marrow. It is generally acknowledged that the process of hemopoiesis is based on the proliferation and differentiation of the stem hemopoietic cells. The reaction of the latter under conditions of hypokinesia to the effect of ionizing radiatior is not known. In addition, hypokinesia (as a unique form of immobilization) can serve as a model of stress which, as a rule, occurs in the first two weeks of hypokinesia [1]. Taking into consideration the

*Numbers in margin indicate pagination in original foreign text.

described peculiarities of hypokinesia the task was set of studying the potential for differentiation and radiosensitivity of the stem hemopoietic cells (KOE) under conditions of initial hypokinesia when the changes in the blood system are characteristic for stress, and later hypokinesia when the changes in many tissues of the organism are determined by hypokinesia strictly [1]. For this purpose the method of exogenous cloning of the stem cells in the organism of lethally-irradiated recipient mice [3].

Material and Technique

Hypokinesia was created (non-rigid) by placing mice of the strain (SVA x S 57 V1) F_1, females, 2-3 months old in small-sized box cages. On the third and 30th days of hypokinesia bone marrow was removed from the mice and suspended. Part of the cellular bone marrow suspension was irradiated in vitro with γ-rays of ^{137}Cs (power of dose 35-37 R/min). The standard number of cells irradiated in different doses (100-600 R) were injected intravenously to lethally irradiated (950 R) recipients of the same strain. The control was bone marrow of mice maintained under vivarium conditions, irradiated in the same doses as the bone marrow of experimental mice, then transplanted to the lethally irradiated recipients. On the ninth day after transplanting the cells of the experimental and control animals the spleen and femur were removed from the recipients. After fixing in Bouin's fluid the macroscopically distinguishable cell colonies were counted in the spleen. The femurs after decalcination in 5% nitric acid were cast in /200
paraffin. On series histological sections of the femoral bone marrow 5 μm thick stained with hematoxylin-eosin the colonies of cells of different types were counted, erythroid, myeloid, megakaryocytic, nondifferentiated, and mixed. The last two types of colonies were not entered into the table. The method of least squares was used to compute the magnitude of dose D_0 and the value of the extrapolation number n.

In part of the experiments conducted in the N. F. Gamaleya Institute of Epidemiology and Microbiology of the USSR Academy of Medical Sciences* an analysis was made of the number of T-lymphocytes in the thymus and

*The author is deeply grateful to N. V. Latsinik for help in setting up the cytotoxic reaction.

bone marrow of mice who spent three days under conditions of hypokinesia. The T-lymphocytes were identified according to the test of cytotoxicity of the standard anti-T-serum whose index is the magnitude of the cytotoxic index (CI) in percents, computed according to the formula [4]: $CI = \frac{N_1 - N_2}{100 - N_2}$

where N_1--percentage of dead cells with anti-T-serum, N_2--percentage of dead cells with normal serum.

Results and Discussion

As follows from the data given in the table the intact and irradiated cells of the bone marrow of normal mice primarily form myeloid colonies in the bone marrow. The ratio of the number of erythroid colonies to the number of myeloid (E/M) equals 0.6-0.7. At the same time the maintenance of mice under conditions of 3-day hypokinesia results in the primary formation in the bone marrow of the recipients of erythroid colonies (E/M=1.3-1.6) regardless of the magnitude of the dose of irradiation of the transplanted cells. On the 30th day of hypokinesia the nature of the KOE differentiation in the direction of the erythro- and myelopoiesis significantly varies (E/M=0.7-2.0). The deviation from the usual type of KOE differentiation on the third day of the experiment, apparently, can be linked to the effect of stress developing in the mice in these periods [1]. This is indicated by the data on the development of atrophy of the thymico-lymphatic apparatus, and the increase in the level of corticosteroids in the blood plasma [1, 2]. One can think about the two paths for the hormone affecting the KOE population. It can be direct or mediated through the other cell-targets necessary for KOE proliferation, and primarily through the T-lymphocytes. It seems to us that intensification in the formation of erythroid colonies in the bone marrow of the recipient after transplanting of cells of hypokinetic mice is linked to the redistribution under conditions of hypokinesia of the T-lymphocytes that occurs during hypercorticism [5-7]. Based on this one can hypothesize that at the early periods of hypokinesia redistribution of the lymphocytes occurs as in the exogenous introduction of corticosteroids [5-7]. The evaluation of the T-system of immunity with the help of anti-T-serum showed the increase in the number of T-cells in the bone marrow of the mice during immobilization [8]. The

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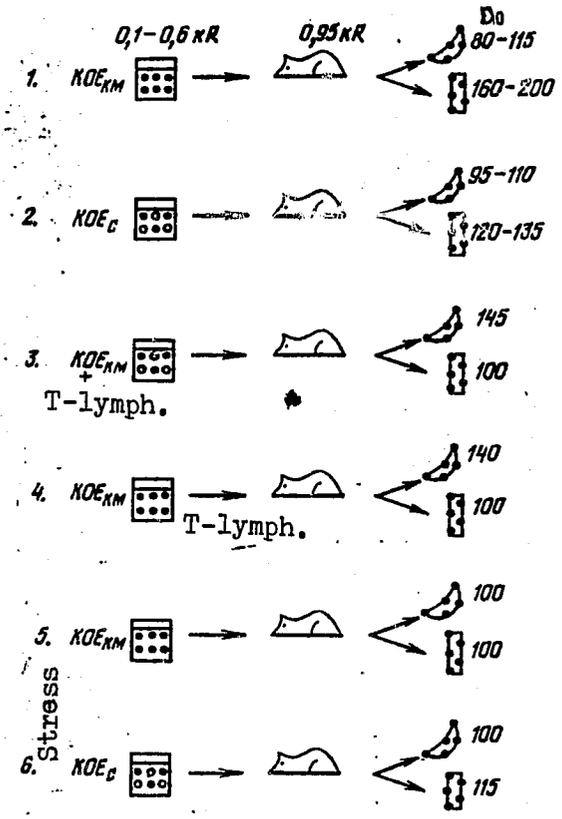
irrad. dose, in R	number of injected cells, $\times 10^6$	duration of hypokinesia, in days	number of recepient mice	type of homopoietic colonies in femur marrow, in %			E/M*	average number of colonies per 100 000 injected cells	
				erythroid	myeloid	megakaryocytic		in femur	in spleen
0	0,1	0	15	31,2±1,0	41,6±2,0	27,1±2,0	0,7	4,8±0,09	16,0±0,7
		3	10	43,7±3,1	30,0±2,0	15,6±1,0	1,4	3,2±0,2	13,0±0,8
		30	9	40,0±4,0	40,0±4,0	20,0±2,0	1,0	5,0±0,6	28,0±1,8
100	0,2	0	14	27,75±1,0	41,4±1,5	30,85±3,0	0,6	2,27±0,05	10,0±0,5
		3	11	45,0±5,0	30,0±2,5	25,0±1,2	1,5	2,0±0,2	9,1±0,6
		30	9	57,1±8,2	28,6±3,0	14,3±3,0	2,0	1,75±0,12	12,6±0,3
200	0,5	0	14	26,4±1,0	43,0±2,0	30,55±5,0	0,6	1,44±0,04	4,6±0,3
		3	9	47,0±4,2	31,25±2,1	21,75±1,2	1,5	0,96±0,03	4,6±0,6
		30	8	46,0±6,0	41,67±6,2	12,21±3,4	1,1	0,98±0,12	6,0±0,34
300	1,0	0	16	30,0±1,2	41,7±1,2	28,3±2,5	0,7	0,91±0,02	2,0±0,1
		3	10	50,0±4,3	30,0±2,0	20,0±2,0	1,6	0,51±0,01	2,4±0,2
		30	9	35,1±5,5	47,3±5,5	17,6±3,2	0,7	0,57±0,04	2,06±0,16
400	2,0	0	15	30,16±1,0	44,4±2,0	25,44±2,0	0,6	0,63±0,01	0,85±0,05
		3	12	42,1±1,2	26,3±1,1	31,6±2,0	1,6	0,19±0,01	1,0±0,1
		30	11	37,5±3,5	46,5±3,5	16,0±2,4	0,8	0,32±0,02	1,1±0,05
500	5,0	0	13	30,3±2,0	42,4±2,2	27,3±2,0	0,7	0,33±0,005	0,4±0,02
		3	10	50,0±3,2	34,4±1,6	15,6±1,6	1,4	0,064±0,004	0,36±0,02
		30	9	39,0±4,0	46,2±5,0	14,8±1,0	0,8	0,195±0,008	0,51±0,008
600	10,0	0	11	30,0±2,0	43,0±2,6	27,0±3,0	0,6	0,21±0,005	0,16±0,004
		3	9	50,0±4,0	38,4±4,0	11,6±2,0	1,3	0,026±0,001	0,1±0,001

* E/M - ratio of erythroid to myeloid colonies

same also occurs in the mice who spent three days in a state of hypokinesia. Thus, in the control mice the cytotoxic index for the bone marrow is 0.08 (8%), and in the hypokinetic mice it equals 0.26-0.34 (with thinning of the anti-T-serum 1:8), i.e., the T-lymphocytes comprise 26-34%. In the thymus of normal and hypokinetic mice respectively 98-100 and 77-84% T-cells are found. The results of these experiments indicate that an intensification of KOE differentiation in the direction of erythropoiesis during hypokinesia is linked to the redistribution of T-lymphocytes. Proof of this can also be the experiments with addition of corticoresistant T-lymphocytes to the transfusion of the bone marrow of normal mice [9]. In this case in the bone marrow of the recipient colonies of the erythroid type are primarily formed in the same way as in the transplanting of bone marrow from the hypokinetic mice. The described changes in the differentiation of KOE in hypokinesia are more clearly pronounced in the period of development of the stress reaction and do not depend on the dose of irradiation. At the later periods of hypokinesia the direction of KOE differentiation approaches the level of the control. /202

The data we obtained with the help of the method of cloning KOE in the spleen on the radiosensitivity of the stem cells of normal mice whose D_0 magnitude was equal to 112 R, and n=1.5 did not differ from those established in the literature. During cloning in the bone marrow the values of these parameters significantly differ: D_0=208 R, and n=0.8. At the same time the stay of the animals under conditions of 3-day hypokinesia results in the change in KOE radiosensitivity. The values of D_0 and n in this case are respectively 113 R and 1.8 as compared to 208 R and 0.8 in the control. According to the spleen test the value of these parameters is not altered in relation to the control. On the 30th day of hypokinesia the KOE radiosensitivity remains on the level of the norm: D_0=180 R, n=0.6 and D_0=115 R, n=1.2 according to the bone marrow and spleen tests respectively. The modification of KOE radiosensitivity with 3-day hypokinesia, evidently, in the same way as KOE differentiation is linked to the effect of the T-lymphocytes. As follows from the figure, depending on the conditions for conducting the experiment (6 variants of experiments) and the cloning method (in the spleen or bone marrow) different values are recorded for the



Change in Values of Dose D_0 Depending on Conditions of Irradiation and Method of Cloning

KOE_{km}--KOE from bone marrow; KOE_c--KOE from spleen; T-lymph.--thymocytes; 1-6--variants of experiment. Colonies were studied in the spleen and bone marrow of the femur on the 8-9th day after transplantation of the cells.

parameter D_0 [10-12]. Thus, according to the cloning test in the spleen the amount D_0 for the KOE from the bone marrow or spleen (first and second variants) equals 80-115 R; according to the cloning test in the bone marrow /203
the amount D_0 for the same KOE significantly differs from that in cloning in the spleen (D_0=160-200 R for KOE from the bone marrow and 120-135 R for the KOE from the spleen). Based on the fact that the cellular suspension from the spleen is distinguished from that of the bone marrow by the presence of T-lymphocytes one can think about the modifying effect of T-cells on the KOE radiosensitivity from the spleen. The latter can be verified

experimentally if T-lymphocytes are added to the bone marrow cells. Such a mixture as if simulates the population of spleen cells of normal mice. As a result of the corresponding experiments it was established that under the influence of the exogenous thymocytes the amount D_0 for the KOE from the bone marrow is modified regardless of whether the T-cells were irradiated jointly with the KOE (third variant of experiment) or they were added later to the irradiated bone marrow cells (fourth variant of experiment). Since under conditions of stress redistribution of the T-lymphocytes occurs, the number of which increases in the bone marrow [5-7] experiments were undertaken to introduce into the mice hydrocortisone (fifth and sixth variants of the experiment). As a result the endogenous T-lymphocytes that migrated into the bone marrow after injection of the hydrocortisone also modify the radiosensitivity of the KOE from the bone marrow like the exogenous T-lymphocytes. A similar pattern is observed with the injection of bone marrow from mice who spent three days under conditions of hypokinesia. Thus, in a comparison of the results of the experiments given both in this work and in the previous studies [10-12] one can consider that the T-lymphocytes modify the reaction of the KOE to irradiation.

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