EFFECT OF MECHANICAL INJURY TO BONE MARROW IN IRRADIATED MICE ON DISPERSION OF ENDOGENOUS COLONY-FORMING UNITS

E. A. Zherbin, V. M. Dorofeev, M. A. Kir'yakov, and A. S. Sysoev

UDC 617-001.28-092.9-06:616.419-001-07:616.155-007.1-076.5

The femur was fractured in mice irradiated in a dose of 600 R and the fragments united by a metal pin. Under these conditions, when part of the bone marrow was destroyed, the number of endogenous colonies in the spleen was increased.

KEY WORDS: mice; irradiation; bone marrow; colony-forming units.

There is much evidence to show that the outcome of radiation sickness is largely determined by the severity of damage to the stem cell population [3]. It has been calculated that preservation of 0.1% of undamaged bone marrow cells enables 50% of irradiated animals to survive [2]. It has also been shown that, despite considerable radiation injury to bone marrow cells, hematopoiesis can be stimulated by injecting DNA and thymidine [8], by giving biogenic stimulators [9], or by treating the bone marrow in various ways – by puncture, injection of physiological saline, and so on [4].

The fact that treatment of fractures by internal fixation with metal pins destroys large quantities of bone marrow tissue suggests that this must lead to stimulation of hematopoiesis and to the more intensive migration of bone marrow cells. The possibility of using this method of treatment for fractures of the long bones in conjunction with irradiation makes it imperative to study the effect of this procedure on the state of hematopoiesis and, in particular, on the population of hematopoietic stem cells.

The object of this investigation was to study the possibility of colony formation by stem cells in totally irradiated mice with mechanical injury to the bone marrow tissue.

EXPERIMENTAL METHOD

The experimental animals were 162 noninbred mice weighing 19-24 g and irradiated in a dose of 600 R on the GUB-20,000 apparatus with Co^{60} γ rays (dose rate 70 R/min). In the course of 1-2 h after irradiation the femur was fractured under pentobarbital anesthesia. In some animals the fragments were fixed by a metal pin. Irradiated mice without internal fixation and irradiated mice on which no other procedure was carried out were used as the controls. On the ninth day the mice were decapitated, after which their spleens were removed, weighed, and placed in Bouin's solution. The number of endogenous colonies on the surface of the spleen was counted 3-4 h after fixation [12]. Statistical analysis was carried out with the aid of Student's t test.

EXPERIMENTAL RESULTS

As Table 1 shows, all mice of the experimental groups survived compared with only 85% of mice in the control group. In irradiated mice that were simply anesthetized the number of colonies and the weight of the spleen were not significantly changed. Fracture of the femur inflicted after irradiation led to an

Department of General Radiology, Institute of Medical Radiology, Academy of Medical Sciences of the USSR, Obninsk. (Presented by Academician of the Academy of Medical Sciences of the USSR G. A. Zedgenidze.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 1, pp. 70-72, January, 1976. Original article submitted March 28, 1975.

TABLE 1. Effect of Mechanical Destruction of Part of the Bone Marrow on Endogenous Colony Formation in the Spleen of Irradiated Mice

Experimental conditions	Weight of spleen (in mg)	Number of colonies	Mice surviving until 30th day, %
Irradiation 600 R (control)	23±2,0	11,2±1,8	85
Irradiation 600 R+ anesthesia	25,8±3,0	10,4±2,4	—
Irradiation 600 R+ fracture without pinning	43,8±2,5*	15,0±2,6	100
Irradiation 600 R+ fracture with pinning	33,7±2,7*	20,4±2,0*	100

*P < 0.01 compared with the control.

increase in the weight of the spleen by 87%, and a tendency was observed in these mice for the number of colonies to be increased although the differences was not statistically significant.

After destruction of the bone marrow by pinning the weight of the spleen was increaed by 44%. The number of colonies was almost twice as great as in the control and 1.5 times greater than in the mice in which the bone fragments were not fixed.

Injury to bone marrow in mice irradiated in doses causing minimal mortality thus leads to an increase in colony formation by stem cells and to an increase in the survival rate of the animals. This may probably be explained, on the one hand, by spontaneous dispersion of stem cells following mechanical injury to the bone marrow, and on the other hand, by the action of humoral factors on the migration and proliferation of polypotent cells. Stem cells are known to be constantly circulating in the blood of healthy animals, but under the influence of radiation, even in small doses, they cannot be found [11]. The work of Strelin and co-workers [5-7] showed that transfusion of bone marrow cells taken from a completely or partly screened area of bone marrow into the peripheral blood stream accelerates the recovery of hematopoiesis and increases the survival rate of the irradiated animals, which confirms the first hypothesis. Presumably pinning the fracture similarly promotes spontaneous dissemination of the bone marrow stem cells of mice totally irradiated in small doses.

Some evidence in support of the validity of the second hypothesis is given by data [8-10] showing an increase in the number of splenic colonies and an increase in the survival rate of irradiated mice after administration of biogenic stimulators or treatment aimed at the pituitary–adrenal system. Fractures and manipulations associated with their treatment lead to considerable humoral changes which must evidently affect the cellular and vascular interrelations, as a result of which favorable conditions are created for migration of stem cells into the blood stream and for their settling subsequently in other zones of hematopoiesis.

The results thus show that mechanical injury to the bone marrow may have a beneficial action on colony formation by stem cells and, ultimately, on the restoration of hematopoiesis and the course of acute radiation sickness.

LITERATURE CITED

1. E. N. Kabakov and A. G. Konoplyannikov, Radiobiologiya, No. 2, 205 (1972).
2. V. I. Korogodin, Yu. V. Korogodina, L. A. Kharlamova, et al., in: Radiation and the Organism [in Russian], Part One, Obninsk (1967), p. 9.
3. E. P. Cronkite, in: Textbook of Radiation Hematology [Russian translation], Moscow (1974), p. 163.
4. I. A. Gudakov, S. F. Rudakova, and V. M. Zyablitskii, Byull. Éksperim. Biol. Med., No. 10, 98 (1970).
5. G. S. Strelin, N. K. Shmidt, and N. N. Sil'chenko, Probl. Gematol., No. 2, 38 (1966).
6. G. S. Strelin, Radiobiologiya, No. 7, 751 (1967).
7. G. S. Strelin, V. S. Baibara, A. V. Bogatyrev, et al., in: Regenerative and Compensatory Processes after Radiation Injuries [in Russian], Leningrad (1973), p. 39.
8. R. M. Khaitov, N. K. Evseeva, and L. S. Seslavina, Radiobiologiya, No. 2, 210 (1972).
9. "The effect of the protein fraction of mouse spleen on the kinetics of hematopoietic stem cells in the irradiated organism," in: Regenerative and Compensatory Processes after Radiation Injuries [in Russian], Leningrad (1973), p. 41.
10. V. Yurashkova, Med. Radiol., No. 10, 51 (1974).
11. D. Barnes and J. F. Loutit, Nature, 213, 1142 (1967).
12. J. E. Till and E. A. McCulloch, Radiat. Res., 18, 96 (1963).