USE OF AUTOLOGOUS BONE MARROW TAKEN FROM A SHIELDED AREA AT VARIOUS TIMES AFTER IRRADIATION

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Shielding the leg saves the life of 32% of animals irradiated with x rays in an absolutely lethal dose (850 R). Autografting of bone marrow from the protected area, if carried out immediately after irradiation, greatly increases the shielding effect. This protective action on autografting continues if it is carried out during the first 5 days after irradiation. Later (on the 7th to 10th day after irradiation) autografting of bone marrow from the shielded area becomes less effective.

KEY WORDS: irradiation; shielding; autografting; bone marrow.

The advantages of autologous over homologous bone marrow transplantation for the treatment of acute radiation sickness are widely known. However, the necessity of obtaining marrow before irradiation and the difficulties of its keeping and preservation make the use of this method in practice much more complicated.

Experiments on rats [7], mice [6], monkeys [1], and dogs [8] in the writers' laboratory have shown that bone marrow autografting can be successful even after irradiation of animals if the marrow is taken from a shielded region. When this method has been used in clinical practice for the treatment of complications of radiotherapy in cancer patients favorable results have also been obtained [3, 4].

Since autografting of bone marrow taken from the shielded region immediately after irradiation was used in all previous investigations, it was decided to investigate the effect of transplantation of autologous bone marrow taken from the shielded area at longer times after irradiation. Besides its practical importance, the study of this problem can shed light on the state of the shielded bone marrow in the irradiated animal.

EXPERIMENTAL METHOD

Experiments were carried out on 269 albino rats weighing 180-200 g. The animals were irradiated on the RUM-17 apparatus (15 mA, 200 kV, skin-focus distance 50 cm, filters 0.5 mm Cu + 1 mm Al). The dose of irradiation in all the experiments was 850 R. The rats were divided into the following groups: 1) whole-body irradiation, 2) whole-body irradiation with shielding of the leg, 3) irradiation with screening of the leg followed by autografting of bone marrow from the shielded area at different times after irradiation. The animals were kept in specially made transparent plastic boxes during irradiation, and the left hind limb of the animal was abducted and a lead sleeve with walls 5 mm thick was placed over the leg. The control rat, whose leg was unprotected by a shield, was kept in a similar sort of box under the same conditions.

Bone marrow was taken in the various series of experiments by means of a special needle under superficial ether anesthesia immediately or on the 3rd, 5th, 7th, and 10th days after irradiation by puncture of the proximal part of the leg. A suspension of bone marrow cells was prepared in 1 ml physiological saline to which sodium citrate was added to prevent clotting. Before injection, the number of nucleated cells in the suspension was counted. A bone marrow suspension containing $20 \cdot 10^6$ nucleated cells was injected intravenously

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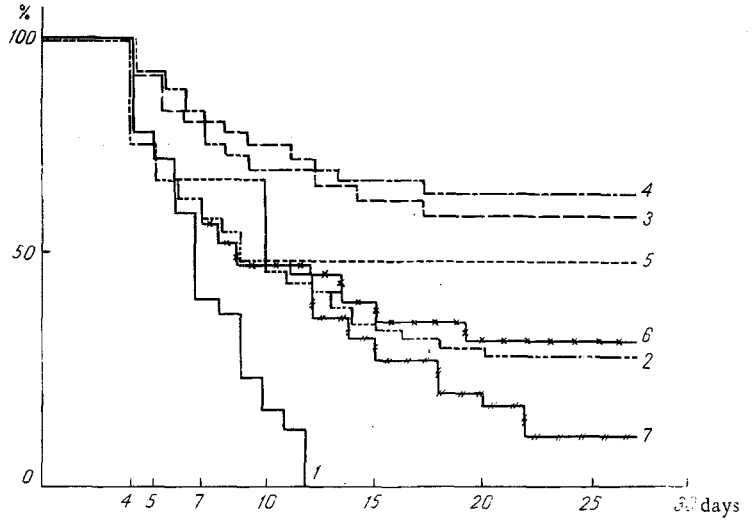

Fig. 1. Survival rate of rats after irradiation and shielding of the leg: 1) whole-body irradiation; 2) shielding of leg; 3) autografting of bone marrow immediately after irradiation; 4) on 3rd day after irradiation; 5) on 5th day; 6) on 7th day; 7) on 10th day. Ordinate, survival rate (in %); abscissa, days after irradiation.

into the experimental animals. Rats not receiving bone marrow also were anesthetized, their legs were punctured, and they were given an intravenous injection of physiological saline. Repair of radiation damage was assessed on the basis of survival of the animals for 30 days.

EXPERIMENTAL RESULTS

Animals of all groups began to die on the 4th day after irradiation (Fig. 1); in the case of whole-body irradiation (curve 1) the mortality was higher and by the 12th day after irradiation all 50 rats of this group had died. The mortality curve of the shielded animals (curve 2) coincided with that of the unshielded animals until the seventh day after whole-body irradiation. The two curves then diverged, and by the 30th day 24 of the 75 shielded animals, i.e., 32%, still remained alive. Migration of bone marrow cells autografted from the shielded region immediately after irradiation had a marked protective action (curve 3) compared with animals which were simply shielded. By the 30th day 62% of the animals (25 of 40) survived. This result agrees completely with previous observations under similar experimental conditions [7]. Autografting of bone marrow from the shielded area on the third day after irradiation (curve 4) was just as effective: 24 of the 36 animals (66%) survived. Bone marrow transplantation 5 days after irradiation also increased the survival rate of the animals considerably compared with shielding alone (curve 5): 6 of the 12 rats (50%) survived. As regards the effectiveness of later transplantation, if carried out on the seventh day after irradiation it did not increase the protective action of shielding, for only 10 of the 28 rats (35%) survived. Transplantation on the 10th day not only did not increase the survival rate of the animals compared with shielding alone, but it actually increased their mortality: only 4 of the 28 rats (14%) survided; i.e., the mortality rate among these animals was close to that in the group of animals receiving whole-body irradiation.

It can be concluded from the results of these experiments that transplantation of bone marrow from an uninjured region after subtotal irradiation can be carried out to good advantage not only immediately after irradiation, but also during the next 5 days. There is evidence in the literature of the beneficial action of injecting isologous (mouse), homologous, and heterologous (rats) bone marrow into animals 24 h and also on the third, fifth, and eighth day after whole-body irradiation on their survival rate [5, 9, 10]. Transplantation of autologous bone marrow in large animals (dogs and monkeys) has a better protective effect if carried out within 24 h after irradiation. With homologous bone marrow transplantations in dogs an increase in the survival rate was found if the transplantation was carried out on the sixth and seventh days after irradiation, an effect regarded as due predominantly to the stimulating action of transplantation on development of the recipients' own hematopoietic cells [2]. The present investigation demonstrated the positive action of transplantation of autologous bone marrow, taken from a shielded area at different times after irradiation, in animals subjected to subtotal irradiation. The results also indicate that normal bone marrow is preserved for a considerable time (up to 7 days) in the region shielded during irradiation. This conclusion is in agreement with

results obtained in the writers' laboratory on mice by Sil'chenko. In her experiments bone marrow taken from the shielded region, when transplanted into irradiated recipients (by the method of Till and McCulloch) preserved its ability to produce exogenous colonies in the spleen.

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