TRANSPLANTATION OF HOMOLOGOUS BONE MARROW INTO RATS AFTER ADMINISTRATION OF TOXIC DOSES OF THIO-TEPA

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Damage to the hemopoietic system limits the use of existing antitumor preparations. Attempts have been made to overcome this complication by using bone marrow transplantation [1-5].

The object of the present investigation was to study the most favorable conditions for transplantation of marrow into rats after administration of toxic doses of thio-tepa.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats weighing 150-200 g. Bone marrow for transplantation was obtained by the method described previously [1] and injected intravenously in a dose of (100-150) · 10^6 nucleated cells 24, 48, and 72 h after injection of thio-tepa. The preparation was injected intraperitoneally in single doses varying from the maximal tolerated dose (MTD) to the absolutely lethal dose (LD_{100}) and above (6-13 mg/kg body weight).

In the course of the experiments the general condition of the rats, their body weight, changes in the principal hematological indices of the blood (leukocytes, erythrocytes, platelets) and the survival rate of the animals were studied.

EXPERIMENTAL RESULTS

The results of the experiments showed that transplantation of bone marrow is not always effective. Administration of marrow after injection of thio-tepa in superlethal doses did not prevent death of the animals on the 3rd-4th day with signs of severe damage to the bone marrow and the gastro-intestinal tract. If the marrow was transplanted after injection of thio-tepa in doses of LD_{100} or less, the survival rate of the rats could be increased by 20-80%. The highest survival rate (30-80%) was observed in cases when the marrow was transplanted 48 h after injection of thio-tepa.

The study of the bone marrow and of the principal blood indices in the surviving animals receiving bone marrow 24 h after injection of thio-tepa showed that even if the thio-tepa was given in a dose of LD_{100}, the development of leukopenia and aplasia of the bone marrow could be arrested, and the processes of normalization of hemopoiesis could be accelerated (Fig. 1). At the same time, all the animals of the control group died from aplasia of the bone marrow.

In an attempt to find the most favorable time for transplantation of the donor's marrow, the interval between the last injection of thio-tepa and transplantation of the bone marrow was increased to 48 and 72 h. A further increase in this interval was impossible, for the animals of the experimental and control groups began to die on the 4th-5th day (in some cases even on the 3rd day) after injection of thio-tepa. The results of the blood investigations of the rats receiving bone marrow 72 h after injection of thio-tepa showed that the changes in the total leukocyte count in their blood during the first 6-8 days after injection of the preparation differed only slightly from those in the control group (Figs. 2 and 3). Starting with the 9th day the changes in the leukocyte count were generally similar to those in the animals receiving the marrow 48 h after the injections.

The changes in the leukocyte count in the blood of the rats receiving homologous bone marrow 48 h after injection of the preparation showed some special features. In these animals a further decrease in

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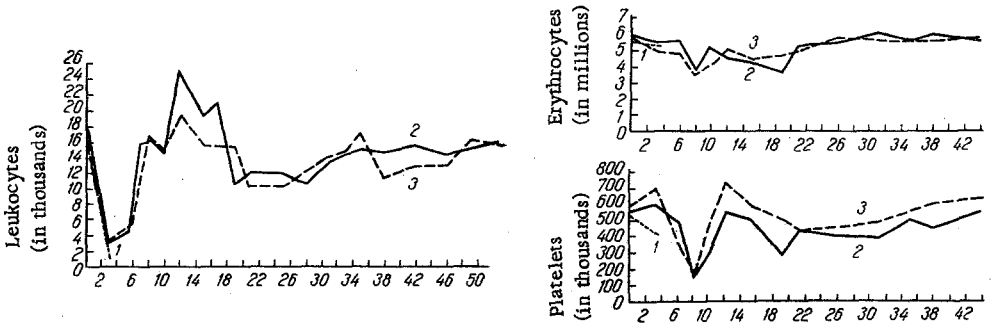

Fig. 1. Changes in peripheral blood indices of rats receiving thio-tepa in a single dose of 11 mg/kg body weight (LD_{100}) followed after 24 and 48 h by transplantation of bone marrow. Legend here and in Figs. 2 and 3: 1) thio-tepa; 2) thio-tepa + bone marrow after 24 h; 3) thio-tepa + bone marrow after 48 h; 4) thio-tepa + bone marrow after 72 h. Abscissa – days of experiment.

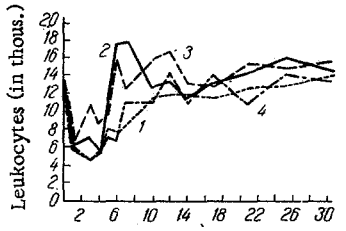

Fig. 2. Changes in the leukocyte count in the blood of rats receiving thio-tepa in a single dose of 6 mg/kg body weight (MTD) followed by transplantation of bone marrow after an interval of 24, 48, and 72 h.

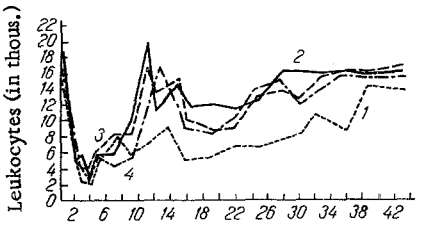

Fig. 3. Changes in the leukocyte count in the blood of rats receiving thio-tepa in a single dose of 10 mg/kg body weight (LD_{80}) followed by transplantation of bone marrow after an interval of 24, 48, and 72 h.

the leukocyte count could be prevented more rapidly than in the rats receiving bone marrow 24 h after thio-tepa.

On the following days, when the leukocyte count in the blood of all the experimental animals with transplanted bone marrow fell below the initial level, in the blood of the rats receiving bone marrow 48 h after thio-tepa this decrease was more marked.

Probably this temporary decrease in the leukocyte count was associated with loss of function of the donor's bone marrow, which was subsequently replaced (in the 2nd-3rd week) by the recipient's hemopoietic tissue. Consequently, the temporary decrease in the blood leukocyte count may be regarded as the recipient's reaction to the donor's genetically incompatible hemopoietic tissue. Another fact confirming this hypothesis was the decrease in the rate of growth of the animals, coinciding in time with the change in the blood leukocyte count. This slowing of the rate of growth was slightly more marked in the animals receiving bone marrow 48 h after thio-tepa.

The possibility of survival of homologous bone marrow after administration of large doses of thio-tepa has been demonstrated cytologically in experiments on rabbits [4]. Comparison of the hematological data obtained in rabbits with the results of the present experiments on rats shows that there is much in common in the character of the changes in the leukocyte count of the peripheral blood. This suggests that in the present experiments, because large doses of thio-tepa were used, the immunologic system of the rats was depressed and in this way the donor's bone marrow was able to survive.

Prolonged observations (for 2-4 months) on the state of the surviving animals showed no clear signs of "secondary disease." In investigations on mice [2] protected against lethal doses of thio-tepa by transplantation of homologous bone marrow, the absence of signs of "secondary disease" also was observed,

and this suggests that death of animals from toxic doses of chemical substances can be prevented by means of homologous bone marrow without the risk of development of "secondary disease."

Very probably the absence of signs of "secondary disease" in the present investigations was attributable also to the weakened antigenic difference between donors and recipients in rats of the Wistar line.

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