TRANSPLANTATION OF BONE MARROW IN RATS WITH SARCOMA M-1 AFTER THE USE OF TOXIC DOSES OF ThioTEPA

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The problem of bone marrow transplantation is of definite interest in the chemotherapeutic treatment of malignant diseases. The possibility of curing aplasia caused by various chemotherapeutic preparations has been proved in the works of certain investigators [4-7].

The purpose of our research was to study the use of bone marrow transplantation in rats with sarcoma M-1 after the use of toxic doses of ThioTEPA for the purpose of increasing the survivability of animals and, possibly, curing tumors.

EXPERIMENTAL METHOD

The experiments were carried out on rats of the Wistar line weighing 130-190 g. Treatment began on the 6th day after transplantation of sarcoma M-1. The animals were divided into 3 groups: the animals of the first group after injection of ThioTEPA received bone marrow transplantation; the animals of the 2nd group were injected with ThioTEPA in the same doses as the 1st group, but transplantation was not used; only transplantation of sarcoma M-1 was done on the animals of the 3rd group.

To treat the tumors we used tablets containing 10 mg of ThioTEPA which were dissolved in a physiological salt solution immediately before intraperitoneal injection into the animals.

The bone marrow for transplantation was obtained from the tibia and femur of donor animals of the same line and injected intravenously in a quantity of $41 \cdot 10^6$-$217.5 \cdot 10^6$ nucleus-containing cells into recipient rats 24 h after the last injection of ThioTEPA.

Two series of experiments were carried out. In the I series (6 experiments) ThioTEPA was injected repeatedly in a quantity of 16.8-3 mg per 1 kg of weight (the total dose was from 45.1 to 27.3 mg/kg) with subsequent transplantation of bone marrow in a quantity from $41 \cdot 10^6$ to $217.5 \cdot 10^6$ nucleus-containing cells. The II series (5 experiments) ThioTEPA was injected once in doses from 53.3 to 11 mg/kg with subsequent transplantation of $87.5 \cdot 10^6$-$171.5 \cdot 10^6$ nucleus-containing bone marrow cells.

EXPERIMENTAL RESULTS

The results of these experiments are shown in the table. At large doses of ThioTEPA (29.1-45.1 mg/kg in the I series of experiments and 13-53.3 mg/kg in the II series) the transplantation of bone marrow did not lead to an increase in rat survival.

However, with the injection of smaller doses of ThioTEPA in the I series of experiments (total dose 27.3 mg/kg, single dose 6.1 and 3 mg/kg) we were able to obtain a certain increase in the survivability of rats (3 out of 4 rats survived) after the first bone marrow transplantation in a quantity of $82 \cdot 10^6$ nucleus-containing cells. Transplantation of $41 \cdot 10^6$ cells and repeated transplantation of $145 \cdot 10^6$ cells was unsuccessful. The survivability of rats in this case did not differ from that of the animals in the group where only ThioTEPA was injected (without transplantation of bone marrow).

Effect of Bone Marrow Transplantation on the Survivability of Rats Treated with ThioTEPA

No. of Expt.	Total dose (In mg/kg)	Single dose (In mg/kg)	No. of rats that died before transplantation	No. of transplanted nucleus-containing cells multiplied by 10^6	No. of rats that survived†
		I Series of experiments			
1	45,1	16,8×2	0(10)	130,0	0(10)
		11,5×1	0(10)		0(10)
2	37,8	6,3×6	2(10)	127,0+150,0	1(8)
			2(10)	—	1(8)
3	30,9	6,9×3	2(10)	160,0	1(8)
		3,4×3	4(10)	—	1(6)
4	29,8	5,9×2	1(6)	217,5	4(5)
		3,0×6	0(3)	—	2(3)
5	29,1	7,3×3	0(6)	81,3	2(6)
		3,6×2	0(4)	—	1(4)
6	27,3	6,1×3	1(10)	41,0+145,0	1(9)
		3,0×3	1(10)	—	1(9)
6	27,3	6,1×3	0(4)	82,0+145,0	3(4)
		3,0×3	1(10)	—	1(9)
		II Series of experiments			
1	53,3—28,7	—	0(6)	155,0	0(6)
			0(3)	—	0(3)
2	23,6—13,5	—	0(12)	125,0+152,5	0(12)
			0(3)	—	0(3)
3	13,0		0(10)	87,5	0(10)
			0(10)	—	0(10)
4	12,5	—	0(15)	150,0	12(15)
			0(10)	—	5(10)
5	11,0	—	0(10)	110,0	10(10)
			0(10)	—	10(10)

* The number of rats used in the experiment is indicated in parentheses.

† The number of rats treated in indicated in parentheses.

The results of the I series of investigations of the peripheral blood of animals showed that in all experiments after the course of injections of ThioTEPA there was an appreciable drop in the number of formed elements of the peripheral blood (leukocytes and erythrocytes) of the rats. In 2 experiments where ThioTEPA was injected in a total dose of 45.1 and 37.8 mg/kg, the number of formed elements of the peripheral blood continued to drop even after bone marrow transplantation until the moment of animal death.

In experiments Nos. 3, 4, 5 normalization of the indexes of the peripheral blood (leukocytes and erythrocytes) occurred similarly in the animals of the 1st and 2nd groups that survived.

In experiment No. 6 where 82 · 10^6 nucleus-containing cells were injected into the animals of the first group during the first bone marrow transplantation, normalization of the number of leukocytes of the peripheral blood occurred more quickly than in animals which with the first bone marrow transplantation were injected with half the ber of cells (Fig. 1). Thus, on the 3rd day after the first transplantation of bone marrow the number of leukocytes in 1 cc of peripheral blood of rats that received 82 · 10^6 nucleus-containing cells was 7600, whereas in rats that had received half the number of bone marrow cells it increased to 3900, i.e., approached the number of leukocytes in animals of the 2nd group (3500).

The number of erythrocytes in rats of all groups continued to drop for 5 days after the first bone marrow transplantation, reaching 3,100,000 in the 1st group and 1,700,000 in the 2nd. After the second transplantation of 145 · · 10^6 nucleus-containing bone marrow cells, the number of luekocytes in the rats that survived was as follows on the 3rd day: in the 1st group, 34,400 (in rats that received during the 1st transplantation 82 · 10^6 cells) and 22,700 in 2 rats that survived that had received half the number of bone marrow cells in the first transplantation; in the 2nd group, where only ThioTEPA was injected, the number was 5500.

The number of erythrocytes began to increase starting with the fifth day after the 2nd bone marrow transplantation, and reached a normal level in all rats that survived of both groups by the 10-14th day after the 2nd bone marrow transplantation.

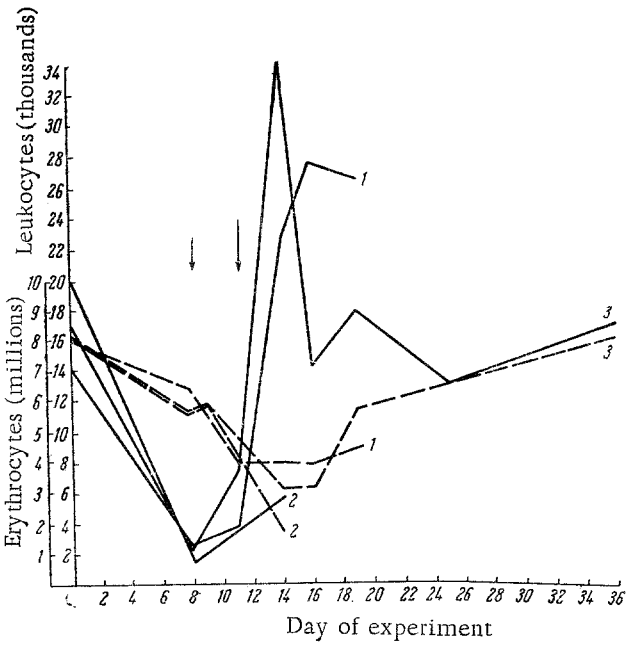

Fig. 1. Change in the peripheral blood indexes of rats in experiment No. 6, I series. 1) Rats that received ThioTEPA in a total dose of 27.3 mg/kg with subsequent transplantation of $41 \cdot 10^6$ bone marrow cells; 2) rats that received ThioTEPA in the same doses but without bone marrow transplantation; 3) rats that received ThioTEPA in the same doses with subsequent transplantation of $82 \cdot 10^6$ bone marrow cells. Solid line) Leukocytes; dashed line) erythrocytes. Arrow) Transplantation of bone marrow.

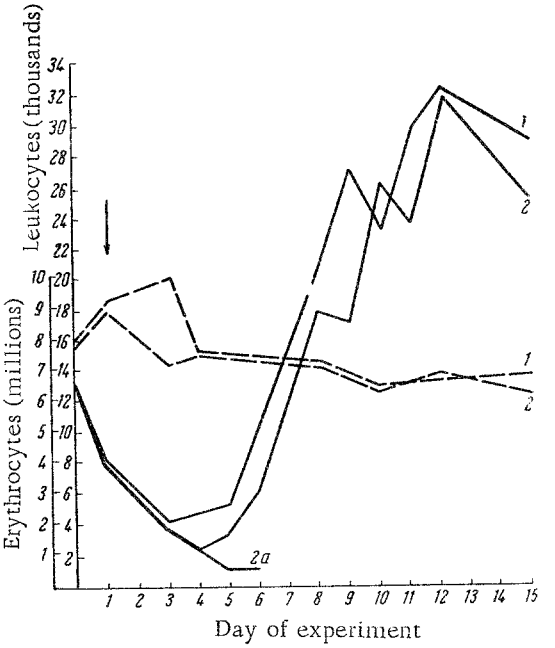

Fig. 2. Change of peripheral blood indexes of rats in experiment No. 4 (II series). 1) Rats that received once 1.2 mg/kg ThioTEPA with subsequent bone marrow transplantation; 2) rats that received once 12.5 mg/kg ThioTEPA without bone marrow transplantation; 2a) rats that died after one injection of 12.5 mg/kg ThioTEPA but without bone marrow transplantation. Solid line) Luekocytes; dashed line) erythrocytes. Arrow) Transplantation of bone marrow.

In the II series of experiments with the injection of ThioTEPA we were able to achieve an increase in the survival rate of rats by 30% in the group where, after injection of the preparation in a dose of 12.5 mg/kg, $150 \cdot 10^6$ nucleus-containing bone marrow cells were transplanted (see table, Expt. No. 4).

However, in the animals which were injected with 11 mg/kg ThioTEPA, bone marrow transplantation promoted acceleration of spontaneous normalization of hematopoiesis in rats of the 1st group.

A single injection of ThioTEPA, just as with the multiple injection, led to appreciable changes of the peripheral blood of the animals. On injecting the preparation in doses from 53.3 to 13 mg/kg (which led to 100% death of the animals of both groups) the number of erythrocytes and leukocytes continued to drop even after transplantation of bone marrow to the same extent as in animals which were injected with only ThioTEPA. In Expt. No. 4 (Fig. 2) the drop in the number of leukocytes to 4000 continued for 2 days after bone marrow transplantation, whereas in the 2nd group the number of leukocytes during the same period dropped to 3800-3000 and continued to decline in 50% of the rats for the next 2 days (to 2000-1000). On the fifth day after bone marrow transplantation in animals of the first group the number of leukocytes increased to the initial level (13,000). On the 11th day the number of leukocytes in the animals of the first group and 50% of the rats of the 2nd group increased to 32,200-36,000. The number of erythrocytes in the animals of the 1st and 2nd groups during this period did not substantially change.

A picture of aplasia was elicited in bone marrow smears of the dead animals in both series of experiments. The bone marrow of the rats that survived, killed 2 months after transplantation, had a normal cell composition.

As the works of a number of authors have shown [1-3] and the results of our investigations, high doses of ThioTEPA, in addition to affecting the hematopoietic function of the bone marrow, have a toxic effect on various internal organs and most of all the gastrointestinal tract.

As an analysis of the efficacy of treating sarcoma M-1 with ThioTEPA shows, regression of the tumor in all animals that survived was achieved only by multiple injection of the preparation in total doses of 30.9-27.3 mg/kg.

SUMMARY

A study was made of the effect of the bone marrow transplantation in rats of the Wistar line with sarcoma M-1 treated with toxic ThioTEPA doses.

The bone marrow transplantation contributed to the survival of rats after a single dose of thioTEPA—12.5 mg/kg—or following its repeated administration in a total course dose of 27.3 mg/kg body weight. Tumor regression was noted after a repeated administration of the preparation, whereas single doses caused but temporary inhibition of the tumor growth.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
