EFFECT OF DNA ON RESTORATION OF HEMATOPOIESIS IN MICE WITH MYLERAN HYPOPLASIA

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KEY WORDS: myleran; DNA administration; colony-forming units.

Preparations of high-polymer DNA promote resotration of hematopoiesis in animals with hypoplasia induced by ionizing radiation [1, 2, 7, 9]. In cytostatic-induced hypoplasia, both experimental [8] and clinical [11], it has also been noted that DNA, when used therapeutically, reduced the development of leukemia. However, the effect of DNA on recovery of hematopoiesis in hypoplasia induced by cytostatic agents has not been adequately studied and there are no data on restoration of colony-forming units (CFU_c).

In the investigation described below the action of heterologous DNA on recovery of hematopoiesis wasstudied in mice after repeated administration of myleran.

EXPERIMENTAL METHOD

Male CBA mice aged 3-3.5 months were used. Chronic cytostatic hypoplasia of hematopoietic tissues was induced by repeated administration of myleran [10]: 10 mg/kg intraperitoneally, daily for 5 weeks. Preparations of high-molecular-weight DNA obtained from the testes of sturgeons were kept in 70° ethanol, dissolved in standard SSC, and injected intraperitoneally in a dose of 15 mg/kg once a week. Injections of DNA began 24 h after the last injection of myleran and continued for 9 weeks. The number of CFU_c, the number of nucleated cells in the femoral bone marrow and spleen, and peripheral blood indices were determined once a week in the treated and untreated animals (eight mice in each group). Cells and karyocytes were counted by the usual methods. The number of CFU_c in the bone marrow and spleen was determined by the exogenous colonies method. Female CBA mice, irradiated in a dose of 9.5 Gr on an experimental gamma-source with ^{60}Co isotope (dose rate 1.6-1.8 Gr/min), were used as recipients. With this dose of irradiation the number of endogenous colonies on the surface of the spleen did not exceed 0.1. A suspension of bone marrow or spleen cells was injected intravenously into the recipient mice 2-2.5 h after irradiation. In each case the number of cells injected into the recipient mice was chosen experimentally depending on the CFU_c concentration in the hematopoietic tissues, reckoning that on average 10-15 colonies formed on the surface of the spleen of recipient mice 9 days after injection. At each time of investigation 10 recipient mice were selected for injection of bone marrow and spleen cells. Death of the treated and untreated mice was recorded for 15 weeks in two separate groups (25 animals in each group). The results were subjected to statistical analysis by Student's t test ($P = 0.05$ level of significance).

EXPERIMENTAL RESULTS

Repeated injection of myleran in a dose of 10 mg/kg caused a gradual decrease in the number of all cells in the hematopoietic tissues of the mice. After the first injection of myleran the total number of CFU_c in the femoral marrow fell to 7% of its initial value, and in the spleen to 9% ($P < 0.01$; Fig. 1). With each successive injection of myleran the disturbances in the hematopoietic tissues progressed, and 1 week after the last injection of the cytostatic the total number of CFU_c was 0.5% in the bone marrow and 1% in the spleen ($P < 0.01$).

The decrease in the number of hematopoietic stem cells was reflected in the number of nucleated cells in the bone marrow and spleen and also in the number of peripheral blood

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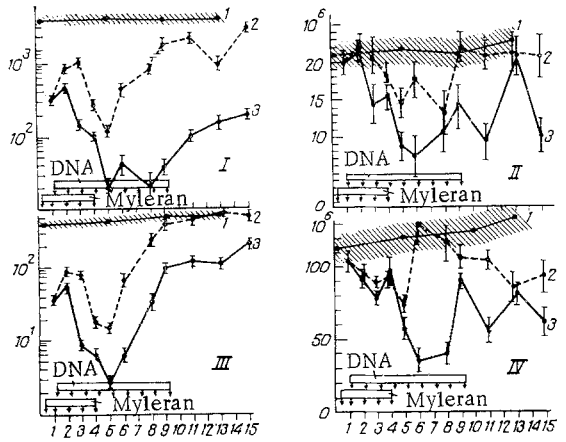

Fig. 1

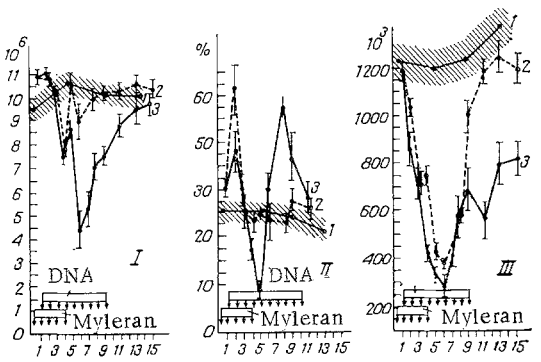

Fig. 2

Fig. 1. Number of CFU_c and nucleated cells in hematopoietic tissues of intact mice (1), mice receiving myleran and DNA (2), and mice receiving myleran only (3). I) CFU_c in femoral marrow; II) nucleated cells in femoral marrow; III) CFU_c in spleen; IV) nucleated cells in spleen. Abscissa, time of investigation (in weeks); ordinate, number of cells studied.

Fig. 2. Number of erythrocytes (I), reticulocytes (II), and platelets (III) in 1 mm^3 peripheral blood of intact mice (1), mice receiving myleran and DNA (2), and mice receiving myleran alone (3). Legend as to Fig. 1.

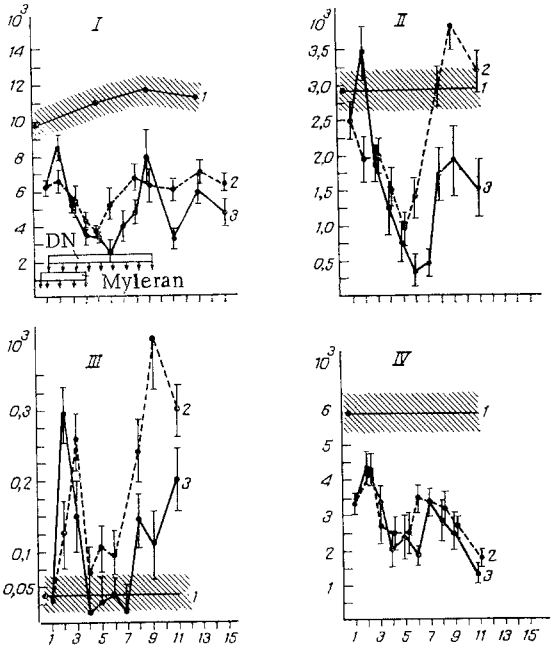

Fig. 3. Number of leukocytes (I), neutrophils (II), stab cells (III), and lymphocytes (IV) in 1 mm^3 peripheral blood in intact mice (1), mice receiving myleran and DNA (2), and mice receiving myleran alone (3). Legend as to Fig. 1.

cells (Figs 2 and 3). After administration of myleran ceased an increase was found in the number of CFU_c in the spleen, and their number in the femoral marrow increased rather more slowly and later. The number of nucleated cells in the hematopoietic tissues increased and the erythrocyte, platelet, and leukocyte counts recovered. In the untreated mice, however, recovery of hematopoiesis was incomplete. For instance, the total number of CFU_c and nucleated cells in the bone marrow and spleen, and also the number of leukocytes and platelets in the peripheral blood, had not reached their initial level 15 weeks after the beginning of the investigation. The prolonged and severe hypoplasia of the hematopoietic tissues was the main cause of death of the animals, which began after the last injection of myleran and continued for 3-5 weeks. During this period 68% of animals in the control group died.

Injections of preparations of heterologous DNA had a significant effect on the state of hematopoiesis in the animals receiving myleran. Even in the first stage of the investigation the number of CFU_c in the treated mice was 1.5-9 times greater than in the untreated animals ($P < 0.01$; Fig. 1). Subsequent injections of the cytostatic, however, led to a decrease in the number of cells. In the treated mice the number of CFU_c 1 week after the end of myleran injections was 5 times greater than in the control animals ($P < 0.01$). Continuation of the DNA injections after the end of the course of myleran led to a faster increase in the number of CFU_c in the bone marrow and spleen. By the time of the last injection of DNA the number of CFU_c in the spleen was back to its initial level, and in the femoral marrow it reached 50% of the level in intact animals. In untreated mice at this time the number of CFU_c in the bone marrow was only 0.5%, but in the spleen it was 20% of the initial values. Injection of DNA into mice receiving myleran also affected the number of nucleated cells in the hematopoietic tissues. For instance, during the period of injection of the cytostatic, the total number of myelokaryocytes in the bone marrow of the treated animals was maintained for 3 weeks at the level established in intact mice. The decrease in the number of nucleated cells in the treated animals was less than in the untreated animals. The number of myelokaryocytes and splenocytes in the experimental animals 1 week after the last injection of myleran was 50% greater than in the control mice ($P < 0.05$). Subsequent injections of DNA after discontinuation of myleran administration led to a faster increase in the number of nucleated cells in the hematopoietic tissues. The total number of hematopoietic cells in the bone marrow and spleen after the end of DNA injections in the treated animals returned to the original values. In the treated animals there was a smaller decrease in the number of peripheral blood cells. After the end of the course of myleran the experimental mice had no signs of anemia, and the platelet count was 1.5 times greater ($P < 0.05$) than in the untreated animals (Figs. 2 and 3). These differences continued later. Under the influence of DNA the neutrophil count in the mice regained the original values, and the number of stab cells was actually several times greater than that observed in intact animals. However, the total leukocyte count reached only 65% of its initial level, due to the slower recovery of the lymphocyte count. This action of the DNA preparations on the hematopoietic system in mice receiving repeated injections of myleran evidently affected the survival of mice treated with DNA. During the period of observation only 20% of mice in the experimental group died.

Consequently, injection of heterologous DNA after injections of myleran increases the survival rate of the animals treated with DNA. This makred therapeutic action of DNA is evidently due to the milder development of hypoplasia in response to administration of myleran. DNA exhibited its action both during the period of injection of the cytostatic and after its discontinuation. In mice treated with DNA the number of CFU_c and nucleated cells in the bone marrow and spleen fell more slowly and the cytopenia in the peripheral blood was less marked. It is possible that the therapeutic action of DNA revealed by these experiments was due to the influence of the biopolymer on proliferative activity of hematopoietic tissue cells [3-5].

Heterologous DNA thus reduces the severity of disturbances in the hematopoietic system of mice induced by repeated administration of myleran.

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EFFECT OF VULCANIZATION ACCELERATORS ON EMBRYONIC MORTALITY IN RATS

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KEY WORDS: vulcanization accelerators; embryonic mortality; mutagens.

Vulcanization accelerators (VA) are an essential component of any rubber mix. Representatives of the chief classes of VA were chosen for study: from the thiazoles — captax and altax; from the sulfenamides — santocure and santocure-mor; and from the thiurams — thiurams D and E. Data on the effect of VA on fetal development in animals are not available, except for thiuram D [2, 4]. Depending on the aims of their investigations, different workers have used different terminologies and methods of exposure of the animals and have thus strictly speaking examined different aspects of disturbances of reproductive function. It is difficult at present to state what is the primary mechanism (mutagenic action on gametes or blastomeres at the beginning of embryogenesis, toxic action of the chemical on the embryo, or hormonal disturbances in the mother) in the process of injury to or death of the fetus. Depending on the time of exposure, the same factors may be both mutagenic and embryotoxic. There is a definite parallel in the action of these factors [1, 3, 5].

The object of this investigation was to study the level of embryonic mortality (EM) in noninbred albino rats after administration of VA.

EXPERIMENTAL METHOD

VA were administered to noninbred albino rats by two methods: I) to females before the beginning of pregnancy on the 1st and 3rd days of estrus, to males twice at an interval of 3 days (i.e., dominant lethal mutations were induced [5-7]); II) to females on the 4th and

TABLE 1. Characteristics of Groups of Experimental Animals

VA	Number in group			
	females	corpora lutea	resorption sites	living fetuses
Control	25	347	15	309
Administration before pregnancy				
Captax	11	175	14	123
Altax	11	170	33	74
Santocure	10	163	5	120
Santocure-mor	11	167	10	130
Thiuram D	11	181	8	137
Thiuram E	11	173	9	127
Administration during pregnancy				
Captax	12	183	12	146
Altax	15	238	27	148
Santocure	13	181	19	142
Santocure-mor	12	196	11	126
Thiuram D	12	177	10	130
Thiuram E	12	181	12	134

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