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Immune Reactions in the Progeny of Female Rats Treated with Paclitaxel

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Immune reactions of the progeny of female Wistar rats injected with paclitaxel a single MPD of 1 and 3 months before mating with intact males were studied. Thymic hyperplasia was detected in the progeny of female rats mated 1 month after treatment. Disorders in the antibody-specific and antibody-nonspecific mechanisms of the immune response were detected in the progeny of females mated with intact males 3 months after cytostatic treatment.

Key Words: *paclitaxel; progeny; female rats; phagocytosis; cellular and humoral immune response*

Conception in women with a history of cytostatic chemotherapy, being in a lasting complete remission was described not once [13]. High toxicity of antiblastoma agents explains the importance of evaluating the risk of delivering defective progeny [10] because of mutagenic effects of the cytostatics and their toxic effects on the maternal organism. Hematological toxicity is one of the main side effects of chemotherapy. Signs of hemopoiesis insufficiency can persist for a long time and manifest under conditions of additional strain, for example, during pregnancy [6]. Insufficiency of maternal hemopoietic tissue can cause defects of cellular and humoral immunity in the progeny [9].

We studied the reactions of the immune system in the progeny of intact male rats and female rats injected with taxane cytostatic (paclitaxel; PT). Experience gained in the clinical use of drugs of this group indicates that this treatment can led to lasting complete remissions including remissions after adjuvant chemotherapy of malignant tumors of the female reproductive system [1]. Paclitaxel is characterized by pronounced hematological toxicity. Experimental studies showed that signs of hemopoiesis suppression manifested during delayed periods after PT therapy and were paralleled by rapid age-dependent involution of the thymus [7].

MATERIALS AND METHODS

Experiments were carried out on 120 Wistar rats (200 g; age 2 months) from Laboratory of Biological Simulation of Institute of Pharmacology. The rats were kept in accordance with regulations of the European Convention for Protection of Vertebrates used for experiments and other scientific Purposes (Strasbourg, 1986). Twenty male and 40 female rats of reproductive age were mated. Experimental females (n=20) were injected with PT (mitotax; Dr. Reddy's) intravenously in a single

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MPD (4.6 mg/kg), calculated by the graphic probit analysis method. The choice of the dose was based on modern clinical use of high-dose taxane therapy. Control females (n=20) were injected with the same volume of the solvent. Since the problem of pregnancy planning in women emerges in delayed periods after chemotherapy, the animals were mated 1 and 3 months after the drug injection, which corresponded (with consideration for the length of reproductive period in rats) to 2-5 years of life in humans.

The immune status was evaluated in 60 rat pups. The progeny of female rats injected with PT (group 1) or solvent (group 2) 1 month before mating and in those injected with PT (group 3) or solvent (group 4) 3 months before mating was studied.

The parameters of hemopoietic (bone marrow, peripheral blood) and lymphoid organs were evaluated using standard hematological methods [4]. Phagocytic activity of peritoneal macrophages was evaluated by changes in optical density of lyzing solution after destruction of phagocytes, which absorbed neutral red particles. Cellular immunity was studied by delayed-type hypersensitivity (DTH) reaction [12]: the pups were subcutaneously sensitized by injection of 0.5 ml 0.5% sheep erythrocyte suspension (Virion Firm). On day 5, 50 μl 25% sheep erythrocyte suspension was injected under hind paw aponeurosis and 50 μl saline in the contralateral paw. The intensity of DTH reaction was evaluated 24 h after injection of the resolving dose of the antigen. Inflammation index was evaluated by the difference in the weights of experimental and control paws (% of control) individually for each animal.

Humoral immune response was studied by immunizing the progeny with corpuscular thymus-dependent antigen (sheep erythrocytes, washed 3 times and injected intraperitoneally in a single dose: 1 ml 15% erythrocyte suspension). The material was collected on day 7 after immunization. The content of antibody-producing cells in the spleen was evaluated by local hemolysis, the level of specific serum immunoglobulins (IgM, IgG) by hemagglutination test [8].

The results were statistically processed using nonparametric Mann—Whitney test.

RESULTS

A statistically significant increase of the thymus weight (by 29%) and absolute count of thymocytes (by 31%) was detected in group 1 progeny (Table 1). These data are in line with the results of studies revealing high percentage of fetuses with thymomegalia in female rats mated in delayed periods after single injection of PT in MPD [2].

A reduction (1.5 times) of the total splenocyte count was noted in group 3 progeny in comparison with the control (group 4; Table 1).

Parameters of humoral immunity (percent and absolute count of antibody-producing cells, IgM and IgG titers) were similar in the studied groups, except IgG level in group 3, which was significantly below the control (by 16%).

Phagocytic activity of peritoneal macrophages in group 3 rats was 67.98% of control values. The intensity of DTH reaction in groups 1 and 3 tended to increase, but the differences from the control were insignificant.

Analysis of the data indicates that the progeny of group 1 females was characterized (judging from thymus weight and absolute thymocyte count) thymic hyperplasia. Published data indicate that thymus enlargement in children is regarded as the immunodeficiency syndrome with predominant impairment of the T-cellular component [3,5] and is associated with reduced level and functional activity of T lymphocytes because of hypoproduction of thymic hormones.

Decreased phagocytic activity of macrophages and low serum level of IgG after immunization in the progeny of group 3 females presumably indicate disorders in the antigen-nonspecific and antigen-specific mechanisms of the immune response. Rapid age-associated involution of the thymus, reduced lymphocyte counts in the peripheral blood and bone marrow were previously detected in female rats treated with PT 3 months before mating

TABLE 1. Weight and Cellularity of Central and Peripheral Immune Organs in the Progeny of Rats Treated with PT (% of Control)

Parameter	Group 1	Group 3
Weight of thymus, mg	129.73*	105.16
Weight of spleen, mg	103.13	72.76
Total leukocyte count, $\times 10^9$/liter	102.59	92.67
Total karyocyte count, $\times 10^6$/femur	80.51	83.17
Splenocyte count, $\times 10^6$		
total	78.50	62.02*
percentage	74.34	83.33
Thymocyte count, $\times 10^6$		
total	131.17*	96.48
percentage	103.11	91.86

Note. *p<0.05 compared to intact animals.

[7]. We therefore have to admit that the detected shifts in the immune reaction of the progeny can result from pathological changes in the maternal hemopoietic and immune systems.

Hence, cytostatic treatment of female rats with PT during the progenesis stage leads to the development of signs of immunodeficiency in the progeny. The type of changes depends on the period between the cytostatic treatment and mating.

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