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Changes in the Thymus Gland of Rats Due to Food Intake

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Annotation: Thymocyte proliferation, differentiation and apoptosis are the main processes of thymus morphogenesis and regeneration. At the stage of antigen-independent differentiation of T cells in the thymus, their mass death occurs by apoptosis. In this regard, the study of the influence of environmental factors on the main morphogenetic processes - cell proliferation and apoptosis in the thymus - is one of the foundations of immunotoxicological research. In recent decades, endocrinologists and immunologists have actively studied the effects of low-dose endocrine disruptors on the body. The most widespread disruptor on the planet is dichlorodiphenyltrichloroethane (DDT), which is found in all ecosystems of the continents and oceans, including the Arctic and Antarctic, and can persist in soil and water for a long time [1-3].

Keywords: thymus; dichlorodiphenyltrichloroethane; DDT; thymocyte apoptosis; thymocyte proliferation.

The aim of the study was to study the processes of apoptosis and proliferation of rat thymocytes under the influence of dichlorodiphenyltrichloroethane (DDT) at low doses, provided that the maximum permissible levels of its content in food products are met.

Materials and methods. The experiment was conducted on 64 male Wistar rats receiving DDT at doses of 1.890±0.086 and 7.77±0.17 μg/kg/kg for 6 and 10 weeks. Histological examination of thymus preparations, immunohistochemical detection of the expression of proapoptotic proteins Bax and p53, assessment of the proliferative activity of thymus cells by radioisotope method, and determination of corticosterone and interleukin-2 concentrations in the blood serum of rats by enzyme immunoassay were performed.

Results. The study of the thymus of rats from the control and experimental groups 6 weeks after

the start of the experiment showed that DDT in selected doses activates the synthesis of proapoptotic proteins and induces p53-dependent apoptosis of low and high differentiated thymocytes, which leads to focal depletion of the cortex. This leads to a reactive increase in interleukin-2 production and an increase in the number of thymic cells, but this does not lead to the restoration of the thymocyte cell population. Subsequently, with the accumulation of DDT and an increase in the time of its exposure, the death of lymphocytes and reticular epithelial cells increases, despite a decrease in the secretion of glucocorticoids by the adrenal glands, the proliferative activity of thymus cells is suppressed, which is considered the main mechanism of changes in the organ.

The aim of the study is to study the processes of apoptosis and proliferation of rat thymocytes under the influence of dichlorodiphenyltrichloroethane at low doses, provided that its content in food products is within the maximum permissible levels.

Materials and methods. The experiment was conducted on 64 male Wistar rats weighing 80-100 g, which were divided into experimental and control groups. The animals were kept in a vivarium and cared for in accordance with the standards and rules for working with laboratory animals, the International Guide for the Conduct of Medical and Biological Research on Animals (1985), the Rules for Laboratory Practice in the Russian Federation (Order of the Ministry of Health of the Russian Federation dated December 19, 2007 "On the Protection of Animals") 01.12.1999. The experiment was conducted in accordance with the Rules for Working with Experimental Animals, approved by Order of the USSR Ministry of Health dated August 12, 1977 No. 577.

The animals in the experimental groups were given o,p-DDT solutions (Sigma-Aldrich, USA) with concentrations of 20 and 80 μg/l instead of water. The choice of these doses was due to the different background content of DDT in different geographical areas and different maximum permissible levels in food products (meat products - 0.1 mg/kg; dairy products - 0.05 mg/kg; cereals - 0.02 mg/kg) [4]. According to calculations, the average daily doses of DDT consumed were 1.890±0.086 and 7.77±0.17 μg/kg/day.

The animals in the control group received tap water. The rats had free access to water and food.

The first half of the animals in the control and experimental groups were removed from the experiment after 6 weeks, and the second half after 10 weeks. Thymus and blood samples were collected. After standard histological processing, thymus sections were prepared using an automated histological processor Tissue-TekVIP 5 Jr (Hygeco, France), which were stained with hematoxylin and eosin. The study of histological preparations was carried out using light microscopy using a Leica DM2500 microscope and computer morphometry using ImageScope software (Leica Microsystems Gmbh, Austria). The ratio of cortex to medulla was determined in the histological preparations of the thymus. Immunohistochemical study of the expression of proapoptotic proteins p53 and Bax was carried out using primary rabbit polyclonal antibodies (Santa Cruz Biotechnology, USA). The reaction was visualized using the UltraVision Detection System reagent kit (Thermo Scientific, USA). The preparations were stained with Mayer hematoxylin. Serum concentrations of corticosterone (IBL, Germany) and IL-2 (Bender Medsystems, Austria) were determined by solid-phase enzyme-linked immunosorbent assay using commercial kits. Ex tempore [5] thymocyte proliferation was determined using 3 H-thymidine.

Statistical analysis was performed using the Statistica software package (StatSoft Inc., USA) using parametric and nonparametric methods. Differences were considered statistically significant at $p<0.05$.

Results. The thymus of control rats 6 weeks after the start of the experiment had a typical lobular structure. The cortex accounted for an average of three-quarters of the organ parenchyma. Lymphocytes were tightly packed in the cortex. Single thymic corpuscles were found in the medulla. Expression of the p53 protein was detected in approximately 10% of thymic lymphocytes. p53-positive cells were found both in the subcapsular layer and at the cortex-

medullary boundary. Bax-positive cells were visualized only in the subcapsular layer. (Fig. 2, a).

1. The content of thymus cells expressing p53 protein in the control and experimental groups of rats that consumed DDT at doses of 1.890±0.086 and 7.77±0.17 μg/kg/day for 6 and 10 weeks. * - statistically significant difference from the values in the control group; # - from the group that consumed a low dose of DDT

Expression of Bax protein by thymus cells: a - in rats from the control group 6 weeks after the start of the experiment; Bax-positive cells are visible only in the subcapsular layer; hematoxylin staining; ×200; b - in rats from the experimental group that consumed DDT at a dose of 7.77±0.17 μg/kg per day for 6 weeks; Bax-positive cells are more common in the medulla and are almost absent in the subcapsular layer of the cortex; hematoxylin staining; ×200

After 6 weeks of DDT administration at a dose of 1,890±0.086 μg/kg/day, the boundary between the cortex and medulla in the thymus became less clear. The proportion of cells expressing the p53 protein was twice as high as in the control group (see Fig. 1). p53-positive cells were diffusely distributed throughout the cortex and were also found in the medulla. The number of Bax-positive cells in the subcapsular layer increased, and their appearance in the deep layers of the cortex was also noted (Fig. 2, b). A single Bax-positive cell was detected in the medulla. The study of the proliferative activity of mouse thymus cells showed a statistically significant increase in the ex tempore proliferation of thymocytes compared to the control group (Fig. 3). A statistically significant decrease in the concentration of corticosterone in the serum of rats was also detected compared to the values of the control group for the same study period (see table). An increase in the concentration of IL-2 was also noted, but this did not reach statistical significance.

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Rice. 3. Ex tempore proliferative activity of thymus cells of the control and experimental groups 6 and 10 weeks after the start of the experiment; * — statistically significant difference from the control group; # - from the group that consumed a low dose of DDT

yaglova-tabletsa.jpgChanges in serum IL-2 and corticosterone concentrations in rats consuming different doses of DDT for 6 and 10 weeks (M±m)

The structure of the thymus in rats that consumed DDT at a dose of 7.77 ± 0.17 μg/kg per day for 6 weeks did not have significant differences. The percentage of cells expressing the p53 protein exceeded the values in the control group, but was lower than in the group of rats that consumed DDT at a dose of 1.890 ± 0.086 μg/kg per day for a similar period of time (see Fig. 1). p53-positive cells were mainly located at the border of the cortex and medulla. Bax-positive cells were significantly more common in the medulla and were almost absent in the subcapsular layer of the cortex (see Fig. 2, b). A significant increase in proliferation was observed for more than three times both in comparison with the control group and in comparison with the previous experimental group (see Fig. 3). A decrease in the concentration of corticosterone in the blood serum compared with the control group and a decrease in the concentration of IL-2 compared with the experimental group that received a low dose of DDT were noted.

The structure of the thymus of rats in the control group did not have significant differences compared to the previous period of the study 10 weeks after the start of the experiment. The ratio of the cortex to the medulla did not change. At the same time, the number of cells expressing the p53 protein more than doubled (see Fig. 1), which is associated with the development of age-related involution. p53-positive cells were located mainly in the subcapsular space. The number of Bax-positive cells increased significantly. They were located diffusely in both the cortex and the medulla. Bax-positive cells were found among the reticular epithelial cells. After 10 weeks, the values of the thymocyte proliferation indices in the control group did not differ from the previous period of the study. Age-related dynamics also led to a decrease in the amount of IL-2 in the blood serum.

After 10 weeks of DDT consumption at a dose of 1,890±0.086 μg/kg/day, the proportion of thymic cortex in rats did not differ from the values in the control group, as well as from the group that consumed DDT at the same dose for 6 weeks. Focal reduction of the thymic cortex due to the death of lymphocytes was more pronounced than in the group that received the same dose of DDT for a shorter period. The proportion of cells expressing the p53 protein did not change compared to the control group. p53-positive cells were located diffusely in the cortex and in groups in the medulla. The number and localization of Bax-positive cells also did not change compared to the control group. A decrease in ex tempore thymocyte proliferation was noted compared to control values. A statistically significant decrease in serum corticosterone concentration was also noted compared to the value of the control group, as well as a significant increase in IL-2 concentration.

After rats were given DDT at a dose of 7.77±0.17 μg/kg/day for 10 weeks, a decrease in the size of the thymus lobules was observed. Lymphocyte death sites and cells with hyperchromatic pyknotic nuclei were observed in the cortical layer. The expression of the p53 protein by thymus cells increased compared to the control and experimental groups during the same study period. p53-positive cells were found in the cortex and medulla, and their increase was noted in the subcapsular layer. An increase in the number of Bax-positive cells in the medulla, both lymphocytes and reticular epithelial cells, was noted. However, Bax-positive lymphocytes were less common in the cortex of these animals than in the other study groups. The proliferative activity of thymocytes decreased compared to the control group and the experimental group that received a low dose for 10 weeks (see Figure 3). The concentration of corticosterone in the serum of rats in this group did not differ from the previous study period, but was significantly lower than in the control group. Serum IL-2 concentrations were increased compared to the control group, but were significantly lower than the comparison group.

Discussion: To date, at least two forms of thymocyte apoptosis are known, one of which is induced by glucocorticoids [6]. The second form of thymocyte apoptosis occurs in the presence of the p53 protein expressed by thymocytes [7, 8].

In the study of histological preparations of the thymus of rats in all experimental groups, the first thing that was observed was the death of thymocytes, manifested by the presence of areas of destruction of the cortex. To establish the mechanisms of induction of thymocyte apoptosis under the influence of low doses of DDT, the concentration of corticosterone in the blood serum, which is the main glucocorticoid in rats, was determined. In our study, a decrease in corticosterone levels was observed in all experimental groups compared with control values. This fact allows us to say that an apoptotic pathway not associated with glucocorticoids is activated in the thymus of rats under the influence of DDT.

DDT administration to rats at a dose of 1,890 ± 0.086 μg/kg/day for 6 weeks resulted in increased thymocyte apoptosis, as evidenced by a twofold increase in the percentage of cells expressing p53 protein and Bax-positive cells compared with the control group. In addition, differentiated lymphocytes located in the medulla appeared to be more sensitive to DDT, as evidenced by a "shift" of Bax expression from the subcapsular to the deeper layers of the cortex and medulla.

The group that received 7.77±0.17 μg/kg of DDT for 6 weeks showed similar lymphocyte death in the cortex as the group that received 1.890±0.086 μg/kg of DDT. The significant increase in cell apoptosis observed in the group that received the low dose of DDT during the same study period suggests that massive cell death may have occurred earlier at this high dose of DDT, leading to the appearance of cortical loss areas. Cell death led to an increase in the concentration of IL-2, a lymphocyte proliferation factor [9, 10], which in turn led to a reactive increase in thymocyte proliferation. Increased proliferation activates the p53-dependent thymocyte apoptosis pathway, but no correlation was observed between these parameters upon DDT exposure, confirming the role of low doses of the insecticide in thymocyte death. Thus, even at low doses, DDT is able to enhance cell death in the thymus primarily through the p53-dependent apoptotic pathway.

Ten weeks after the start of the experiment, differences in the thymus of rats were observed,

consistent with DDT exposure and age-related changes. In control animals, there was an increase in lymphocyte death, as evidenced by a doubling of the proportion of p53-positive cells and the appearance of areas of lymphocyte death in the cortex. These facts indicate the onset of age-related involution of the thymus in rats, which develops after puberty [11].

In contrast to the control group, where apoptosis was mainly observed in lymphoblasts of the subcapsular layer, in the group receiving DDT at a dose of 1,890±0.086 μg/kg per day for 10 weeks, apoptosis of differentiated and highly differentiated lymphocytes was observed, with an increase in cortical depletion. The fact that the percentage of p53-positive cells did not differ from the values in the control group can be explained by the significant death of thymocytes at an earlier stage. This, together with a decrease in their proliferative activity, does not allow restoring the number of this cell population, despite the increase in the synthesis of the lymphocyte growth factor IL-2.

In the thymus of rats exposed to DDT at a dose of 7.77±0.17 μg/kg/day for 10 weeks, the expression of the p53 protein by thymic cells increased compared to the control and experimental groups exposed to a lower dose of DDT during the same study period. Bax-positive cells were also more abundant among reticular epithelial cells. In our previous studies [12, 13], we found an increase in the number of thymic corpuscles in the phase of fragmentation; This fact also confirms the increased death of the reticular epithelium, which in turn is a factor contributing to the decrease in the proliferation and differentiation of thymocytes. Comparison of morphological and functional changes in the organ 10 weeks after the start of the experiment showed that their nature was the same in the control and experimental groups, but the rate of thymic cell death accelerated with DDT intake. Since age-related changes in the thymus were observed in the control group of the longer study period, the similarity of the indicators may indicate a more rapid development of involution processes in rats receiving low doses of DDT, despite a decrease in corticosteroid secretion and an increase in IL-2 synthesis.

Conclusions. Chronic exposure to dichlorodiphenyltrichloroethane at doses below the maximum permissible in food products leads to apoptotic death of thymocytes, mainly with the participation of the p53-dependent apoptotic pathway. Activation of thymocyte apoptosis leads to a reactive increase in their proliferative activity, but prolonged exposure to the insecticide suppresses the proliferative potential of cells. Increasing the dose of dichlorodiphenyltrichloroethane and the duration of exposure increase the death of lymphocytes and reticular epithelial cells, which leads to an increase in involutional changes in the organ, despite a decrease in corticosterone synthesis.

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