



IMMUNOGISTOCHEMICAL CHARACTERISTICS OF MESENTERIAL LYMPH NODES IN OFFSPRING OF RATS BORN UNDER CHRONIC EXPOSURE TO PESTICIDES

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Abstract:

The purpose of the work is to study the features of postnatal development of mesenteric lymph nodes in the offspring of female rats chronically intoxicated with pesticides. The studies were conducted on 60 sexual, non-pregnant, non-breeding white female rats weighing 160-180 g and 473 (control group - 285, experimental group - 188) rat pups born from them. Postnatal morphogenesis of mesenteric lymph nodes was studied using morphological and morphometric research methods. The results obtained showed a significant disruption of the postnatal processes of development of mesenteric lymph nodes in offspring born with chronic exposure to pesticides.

Keywords: fipronil, offspring, postnatal ontogenesis, mesenteric lymph nodes.

In the world, a number of scientific researches are being carried out in order to achieve the effectiveness of measures aimed at improving the prevention of negative complications observed in the offspring under the conditions of chronic exposure to pesticides on the mother's organism. In this regard, scientific research aimed at substantiating the features of the biological action mechanism of pesticides, determining the mechanism of their immunotoxicity, preventing the harmful effects of pesticides in the postnatal development of the fetus, and developing a set of measures to reduce their complications is of particular importance.

Certain measures are being implemented in our country aimed at developing the health care system, adapting it to the requirements of world standards, including prevention and early detection of complications arising from the deterioration of the state of reproductive health. In this regard, in accordance with the seven priorities of the development strategy of New Uzbekistan for 2022-2026, raising the level of medical services to the population to a new level, "increasing the convenience and quality of medical and socio-medical services to the population, reforming the health care sector with the aim of creating a healthy lifestyle among the population », «...improving the high-tech medical care system for reproductive age and pregnant women, children...». Based on these tasks, poisonings caused by modern pesticides, "pregnancy and

postpartum risk factors" that occurred under these conditions, revealing the mechanisms of action of pesticides on the offspring's immune system through the mother's organism, and thus preventing negative complications, early diagnosis and effective treatment methods. it is appropriate to carry out scientific research aimed at development.

THE PURPOSE OF THE STUDY is to study the morphological and morphometric characteristics of the postnatal development of mesenteric lymph nodes in female rats chronically poisoned with pesticides .

MATERIALS AND METHODS. The studies were conducted on 60 female rats of non-white breed, sexed, not previously born, with a body weight of 160-180 grams, and 473 rat pups born from them. Female rats were divided into experimental (30) and control (30) groups . Female rats in the experimental group were administered the pesticide fipronil (FPN) at a dose of 3.6 mg/kg daily for 30 days. Control rats received an equal volume of sterile saline. Female rats of both groups were mated with male rats on day 31 of the experiment. Pesticide administration was continued during pregnancy and lactation. Mesenteric lymph nodes in offspring born in the experimental and control groups were studied by morphological, morphometric, immunohistochemical methods on the 3rd, 7th, 14th, 21st and 30th days of postnatal development. All numerical data were statistically processed using a



computer software package, differences satisfying $p > 0.05$ were considered reliable.

RESULTS AND THEIR DISCUSSION.

In MLT of 3-day-old rat children, structural-functional zones were not formed, the total number of cells in the defined field of view was 1941 ± 87.6 , of which cells marked with Ki-67 were 96.2 ± 4.2 , and the proliferation index was equal to 5% it happened. The most marked cells are lymphoblasts and prolymphocytes, less marked among fibroblasts and reticular cells.

7 days after birth, the cortex and medulla differentiated in the MLT, the density of cells in the defined area in the diffuse lymphoid tissue of the cortex was 2209 ± 110 , the number of marked ones among them was 309.4 ± 15.1 , the proliferation index was equal to 14% . In the marrow, these indicators were 1544 ± 12.9 , 159.0 ± 7.3 and 10.3%, respectively.

In connection with the formation of different structural and functional zones in the MLT of 14-day-old rats, the cell density and proliferation indices were counted separately for each zone . 14-day-old control group rat pups MLT cortical plateau and lymphoid follicles were considered the most densely populated zones, the cell density in these zones was 1980 ± 166.0 and 1718.0 ± 138.0 , respectively. At the same time, the relative percentage of cells labeled with Ki-67 was the

highest in the paracortical zone and cortical plateau, and the proliferation index of cells in these areas was 12.2 and 11.3, respectively, and this indicator was slightly lower in lymphoid follicles and medullary bands. was 10.3 and 8.2, respectively. Cells labeled with Ki-67 consisted mainly of blasts, lympho- and monocytopenic cells, stromal cells were rarely labeled.

In 21-day-old rat pups, the density of cells increased in the paracortical zone by 1.3 times, in the cortical plateau and lymphoid follicles by 1.2 times, and in the medullary bands by 1.1 times, compared to the previous period, the number of marked cells decreased, and as a result, the proliferation index in these areas decreased by 1.5, 1.6, and 1.7 times, respectively. Such a decrease in the proliferative activity of cells in all structural-functional zones of MLT confirms the idea that the processes of formation of structural-functional zones of MLTs of 21-day-old rat children are completed, and a dynamic balance is established between the processes of cell proliferation, differentiation and recirculation in the organ.

By the 30th day of postnatal development, the density of cells in the structural-functional zones of the MLT, the number of marked cells and the proliferation index did not reliably differ from the analogous indicators of 21-day-old rat pups.

Dynamics of proliferative activity of cells in different structural and functional zones of the MLT of control group rat children during early postnatal ontogenesis (M ± m, %, n=8)

Zones	Indicators	Age, days after birth		
		14	21	30
Lymphoid follicle (V-Zone)	Cell density	1718.0 ± 138.0	2020.0 ± 141.6	2128.0 ± 133.8
	Number of marked cells	177 ± 12.0	129 ± 8.3	134 ± 8.0
	Proliferation index i	10, 3	6, 4	6, 3
Paracortical zone (T-Zone)	Cell density	1311 ± 102.0	1702 ± 147.0	1690 ± 145.2
	Number of marked cells	160 ± 15.0	138 ± 12.6	135 ± 9.6
	Proliferation index i	12, 2	8, 1	8.0
Bark plateau (T-Zone)	Cell density	1980 ± 166.0	2379 ± 117.0	2709 ± 133.0
	Number of marked cells	224 ± 14.0	167 ± 9.6	165 ± 7.5
	Proliferation index i	11, 3	7, 0	6, 1



Belly bands (V-Zone)	Cell density	15 39 ± 92.0	16 49 ± 103.5	180 6 ± 110.8
	Number of marked cells	12 6 ± 6.8	7 9 ± 6.4	92 ± 5.0
	P roliferation index i	8, 2	4, 8	5 , 1

Note : * – the differences are reliable compared to the indicators of the previous period, P <0.05, (structural-functional zones were not formed in MLT up to 14 days after birth).

Due to the absence of structural-functional zones in the parenchyma of MLTs in the 3-day experimental group, the number of cells labeled with Ki-67 was counted in the diffuse lymphoid tissue and the following results were obtained: the density of cells per unit area - 1869±90.4 (in the control - 1941±87.6), among them, the number of cells labeled with Ki-67 was 72.8±3.9, the proliferation index was equal to 3.9%, and it decreased by 1.3 times from the analogous indicators of the control group.

It was found that the density of cells and the number of Ki-67-marked cells in the cortex and medulla of the 7-day-old experimental group were reduced, and the proliferation index in these zones was reduced by 2 and 1.8 times, respectively, compared to the values of the control group. Cells identified in the cortex consisted of lymphoblasts, prolymphocytes, plasmablasts and monoblasts.

14 days after birth in MLT with the formation of different structural and functional zones, cell density

and proliferation index were determined separately by zones i. In animals of the experimental group, on the 14th, 21st and 30th days of postnatal development, the density of cells in all structural and functional zones of the MLT, the number of cells expressing Ki-67 and the proliferation index reliably decreased compared to the control group. A decrease in the proliferative activity of cells was significantly manifested on the 14th day of postnatal development. During this period of the experiment, the proliferation index in the MLT of rats of the experimental group reliably decreased in the T-dependent zones of the limb, and this indicator was 7.0% in the cortical plateau (11.3% in the control), 6.5% in the paracortical zone (12.2% in the control). , decreased by 35-45% from the control indicators in this period. Proliferation index in V-dependent zones (lymphoid follicle, umbilical cord) tended to decrease, but these indicators were not significantly different from those of the control group. This pattern was preserved 21 days after birth.

The dynamics of proliferative activity of cells in different structural and functional zones of the MLT of the offspring under chronic exposure to low-dose pesticides during the early postnatal ontogeny (M ± m, %, n=8)

Zon will take	Indicators	Age, days after birth		
		14	21	30
Lymphoid follicle (V-Zone)	Cell density	1598.0 ± 138.0	1918 .0 ± 141.6	2023.0 ± 133.8
	Number of marked cells	115	92	105
	P roliferation index i	7, 2	4, 8	5, 2
Paracorti cal zone (T-Zone)	Cell density	1 158.0 ± 101.5	1 391 .0 ± 99.4	1 492 .0 ± 101.8
	Number of marked cells	75	10 8	11 9
	P roliferation index i	6 , 5 *	7,8	8 , 0
Bark Plateau (T-Zone)	Cell density	1793.0 ± 139.0	2 305.0 ± 198.4	2 526 .0 ± 190.0
	Number of marked cells	12 6	13 4	13 6
	P roliferation index i	7 , 0 *	5, 8	5, 4



Belly bands (V-Zone)	Cell density	1 442 .0 ± 123.4	1 544 .0 ± 132.3	1698.0 ± 162.0
	Number of marked cells	99.4	66	68
	P roliferation index i	6.9	4.3	4.0

Note : * – differences are reliable compared to the control group , P <0.05)

Animals of the 30-day-old experimental group born from female rats chronically exposed to small doses of pesticides had low indicators of the proliferation index in the paracortical zone of the MLT, while in the rest of the structural-functional zones of the organ, these indicators were not significantly different from those of the control group.

All these, chronic exposure to pesticides during pregnancy and early postnatal periods show a significant decrease in MLT cell proliferation.

Similarly, offspring born from female rats chronically exposed to pesticides had a reduced Vcl-2

positive response of lymphoid cells in the MLT. Weak expression of Vcl-2 "+" was detected in some small and medium lymphocytes.

Quantification of the results showed that the offspring born from mother rats chronically exposed to pesticides showed a decrease in the expression of Bcl-2 -positive cells in all structural and functional zones of the MLT in all periods of early postnatal development compared to animals of the control group. The lowest levels of antiapoptotic Bcl-2 were observed at 14-21 days of postnatal development mainly in the T-dependent zones of the limb.

Indications of antiapoptotic Bcl-2 in MLT structural-functional zones during early postnatal ontogeny dynamics

Structural-functional zones		Bcl-2 expression		
		14 days	21 days	30 days
Lymphoid follicle (V-Zone)	control	2.1 ± 0.1	3.4 ± 0.17	4.2 ± 0.21
	experience	1.1 ± 0.05	1.8 ± 0.09	3.2 ± 0.16
Paracortical zone (T-Zone)	control	1.6 ± 0.08	3.8 ± 0.19	4.4 ± 0.22
	experience	0.7 ± 0.03	1.8 ± 0.09	2.8 ± 0.14
Bark plateau (T-Zone)	control	1.9 ± 0.09	4.1 ± 0.02	5.2 ± 0.26
	experience	0.8 ± 0.04	2.1 ± 0.1	4.0 ± 0.2
Belly band (V-zone)	control	1.2 ± 0.06	3.5 ± 0.17	3.9 ± 0.19
	experience	0.6 ± 0.03	1.9 ± 0.09	3.2 ± 0.16

Note: *- differences are reliable compared to control group indicators, R<0.05.

During these periods, Bcl-2 levels in the paracortical zone of the MLT in the animals of the experimental group were reliably lower by 2.4-2.1 times, respectively, compared to the control group. Among the lymphoid cells, the Bcl-2 labeled cells were mainly small and medium lymphocytes.

CONCLUSIONS

1. chronic exposure of mother rats to pesticides had a negative effect on the structural and functional formation of the MLT of the offspring born from them.

It was manifested by hypoplasia of the area of T-dependent zones, a decrease in the density of total cells in these zones, as well as the number of small lymphocytes.

2. In immunohistochemical examinations, it was found that the number of cells labeled with Ki-67 and Bcl-2 in all structural and functional zones of the organ, especially in the T-dependent zones, was significantly reduced compared to the control group.

3. The decrease in the number of lymphoid cells expressed by the Ki-67 marker in the MLT of the animals



of the experimental group under the conditions of chronic exposure to pesticides indicates a decrease in the proliferative activity of cells, and a decrease in the expression of antiapoptotic Bcl-2 indicates an increase in the process of apoptosis.

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