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MORPHOLOGICAL CHARACTERISTICS OF THE SPLEEN OF WHITE RATS IN NORMAL, CHRONIC RADIATION SICKNESS AND WHEN CORRECTED WITH A BIOSTIMULATOR

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ABSTRACT

In an experiment on 32 mongrel white rats of 3 months of age, we studied the morphological parameters of the spleen in normal, chronic radiation sickness and when correcting the biostimulator ASD-2 fraction. The study found that in response to the action of a chronic radiation factor in the spleen of white rats, there is a decrease in structural indicators. This is reflected in the morphological parameters of the organ. Improvement and approximation of these parameters to the control values shows a positive effect of the use of ASD-2 fraction during chronic irradiation.

KEYWORDS: ***immune system, spleen, lymphoid follicles, chronic radiation sickness, biostimulators.***

INTRODUCTION

In recent decades, the world is growing disease associated with damage to the immune system, which is associated with environmental pollution, leading to a violation of the protective and adaptive processes of the body [12,16].

The spleen is the largest peripheral organ of immunogenesis [3,14] and is responsible for the effectiveness of cellular and humoral immune responses of both innate and acquired immunity [9].

The effect of stress, severe pathological conditions, and ionizing radiation on the body can be expressed by changes in the function of the spleen. This is manifested by a decrease in the proliferation and differentiation of immunocompetent cells, which leads to cellular devastation, increased apoptosis and activation of macrophages [8,15].

Man and all other living beings on earth throughout their lives are continuously exposed to ionizing radiation from natural and artificial sources of ionizing radiation, by external and internal irradiation [6,13].

The increasing growth of human and animal diseases, associated with socio-economic factors, actualize the problem of obtaining biologically active substances for the correction of metabolism and immunity [5].

Currently, of great interest are tissue preparations of animal origin, which are used to prevent diseases of various types of animals, increase the natural resistance of the body and normalize metabolism [1,2,4].

Among the existing means of tissue therapy, the ASD-2 fraction occupies a special place.

ASD-2F has a stimulating effect on biochemical processes and is able to improve the metabolism of carbohydrates, lipids, and proteins [10,11].

Under the influence of the drug, pulmonary gas exchange increases, oxygen consumption by tissues and the activity of a number of enzyme systems increases, and antioxidant protection increases [7].

Purpose of research

To study the morphological parameters of the spleen in white rats of 3 months of age in normal, chronic radiation sickness and with the correction of biostimulator ASD-2F.

MATERIAL AND METHODS OF RESEARCH

The study was conducted on 32 mongrel white male rats with a weight of 90 to 130 g. they were kept in standard vivarium conditions. Laboratory animals were divided into 3 groups: control group (n=12), 1-experimental group (n=10), 2-experimental group (n=10). Irradiation of rats of both experimental groups was carried out using the device " AGAT P1 "(Baltiets plant" Narva, Estonia, 1991 year of manufacture, operation since 1994, recharge 2007) with a capacity of 25,006 SGR/ min for 20 days at a

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dose of 0.2 G. The total radiation dose for rats up to 90 days of age was 4.0 Gy. Rats of the 2-experimental group were intragastrically injected with 0.1 ml of ASD-2 fraction solution diluted with 0.4 ml of distilled water in parallel with irradiation. All experimental studies on animals were conducted in accordance with the " Rules for conducting work using experimental animals».

Animals were removed from the experiment at 90 days of age by instant decapitation under ether anesthesia.

The spleen was removed from the abdominal cavity. To conduct morphological and morphometric studies, spleen fragments were fixed in a 10% formalin solution, passed through a battery of alcohols and poured into paraffin blocks according to generally accepted methods. Paraffin sections with a thickness of 5-8 microns were stained with hematoxylin-eosin. The sections were examined morphometrically using an eyepiece-micrometer DN-107T/ Model NLCD-307B (Novel, China) measured the diameter of the periarterial lymphatic couplings, lymph nodes and their germinal centers, the width of the mantle, marginal and periarterial zones, the relative area of the white pulp and connective tissue elements of the spleen to the total area of the cut. Measurements were made in five fields of view of each histological section. The fields of view were chosen randomly.

In order to study the cytoarchitectonics of the spleen's lymphoid structures, cells were counted using a NOVELModelNLCD-307 microscope, at 10x90 magnification, under oil immersion. The cells were counted using a morphometric grid embedded in the eyepiece (10x) of a microscope.

We calculated the total number of lymphocytes, the number of large, medium and small lymphocytes per unit area of the cut in the PALM, in lymphoid nodules without a breeding center.

Mathematical processing was performed directly from the General matrix of the Microsoft Office data package "Excel 7.0" on a Pentium – IV personal computer using the capabilities of the program "STTGRAPH 5.1", the indicators of standard deviation and representativeness errors were determined

THE RESULTS OF THE STUDY AND THEIR DISCUSSION

Histological examination of the spleen of 3 month old intact rats obtained the following data:

The relative area of the white pulp varies from 19.8% to 26.2%, with an average of 22.2±0.59%. The relative area of connective tissue elements varied from 5.0% to 6.1%, on average-5.52±0.1% (to the total area of the spleen section)

The diameter of the PALC ranges from 122.6 microns to 139.6 microns, with an average of 132.14±1.56 microns. The diameter of the lymph nodes ranges from 341.8 microns to 486.05 microns, with an average of 466.05±13.27 microns. LN can be visually divided into primary and secondary, with a percentage ratio of 32% and 68%, respectively. In secondary LN, the formed germinative centers are determined. The diameter of the germinate centers ranges from 94.6 microns to 167.8 microns, with an average of 147.8±6.73 microns. They are large and often merge. The LN of the white spleen pulp is generally round, oval, and elongated.

In most cases, the LN zones are clearly distinguishable. The width of the mantle zone ranges from 39.7 microns to 49.45 microns, with an average of 45.32±0.89 microns. The width of the marginal zone ranges from 70.3 microns to 84.7 microns, with an average of 77.14±1.32 microns. The width of the periarterial zone ranges from 81.9 microns to 89.4 microns, with an average of 85.04±0.69 microns. (Fig. 1).

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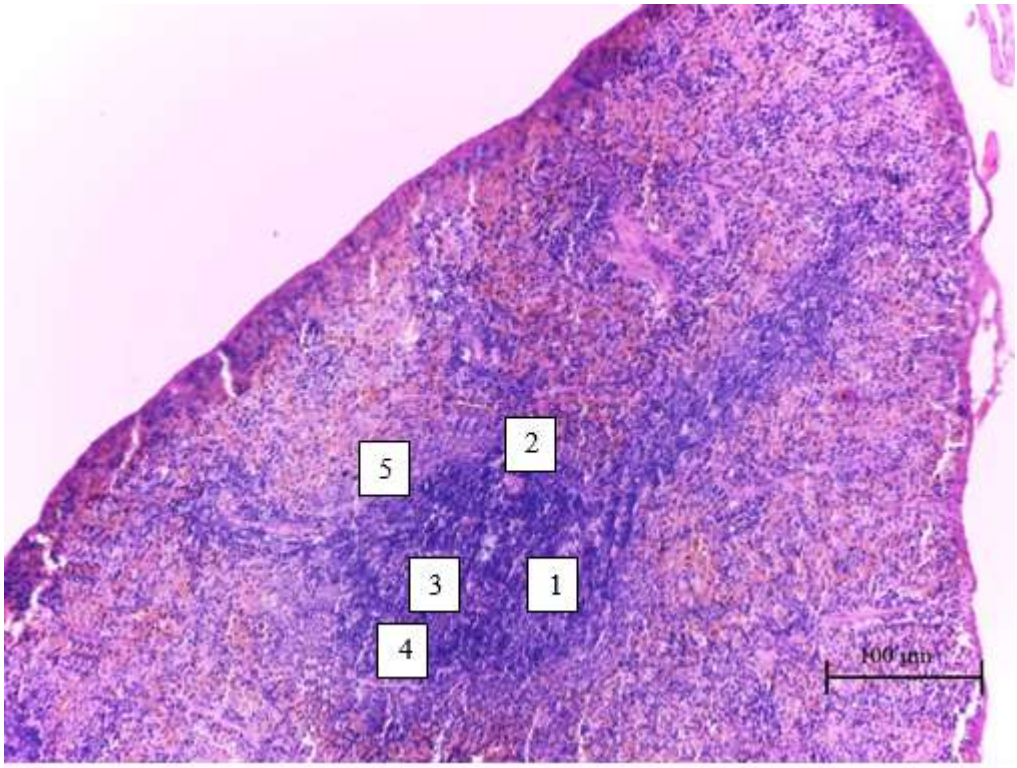

Fig.1. Spleen of a 3-month-old control rat. Painting with hematoxylin – eosin. Oc. 10 x Ob. 20. 1 - lymph node, 2 -periarterial zone, 3-germinal center, 4-mantle zone, 5-marginal zone.

It was found that the total number of lymphocytes in the LN without breeding centers is 42-53, with an average of 47.3±1.01 cells. Lymphoid nodules without centers of reproduction contain (per unit area) small lymphocytes-30-38, on average - 34.0±0.74 cells, medium lymphocytes-10-12, on average - 11.0±0.18 cells, large lymphocytes-2-3, on average-2.3±0.1 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white spleen pulp is 41-53, with an average of 47.2 ±1.1 cells. Periarterial lymphoid couplings contain (per unit area) small lymphocytes-29-37, on average-33.0±0.74 cells, medium lymphocytes-9-11, on average - 10.25±0.18 cells and large lymphocytes-3-4, on average-3.5±0.1 cells.

In histopreparations of the spleen of 3-month-old irradiated rats, the relative area of the white pulp ranges from 12.8% to 16.4%, on average-14.6±0.39%. The relative area of connective tissue elements varied from 5.2% to 6.3%, on average-5.73±0.84% (to the total area of the spleen section)

The diameter of the PALC ranges from 95.2 microns to 104.3 microns, with an average of 99.35±0.98 microns. The diameter of the lymph nodes is in the range from 160.4 microns to 240.2 microns, on average 195.81±8.62 microns in the LN there are no germinative centers. Lymphoid nodules generally take a rounded-oval, elongated (67.6%) and irregular (32.4%) shape.

In micropreparations, you can visually distinguish all the zones of LN. The width of the mantle zone ranges from 32.3 microns to 40.4 microns, with an average of 36.54±0.87 microns. The width of the marginal zone ranges from 56.8 microns to 65.7 microns, with an average of 60.26±0.96 microns. The width of the periarterial zone ranges from 57.2 microns to 65.4 microns, with an average of 59.88±0.88 microns (Fig. 2).

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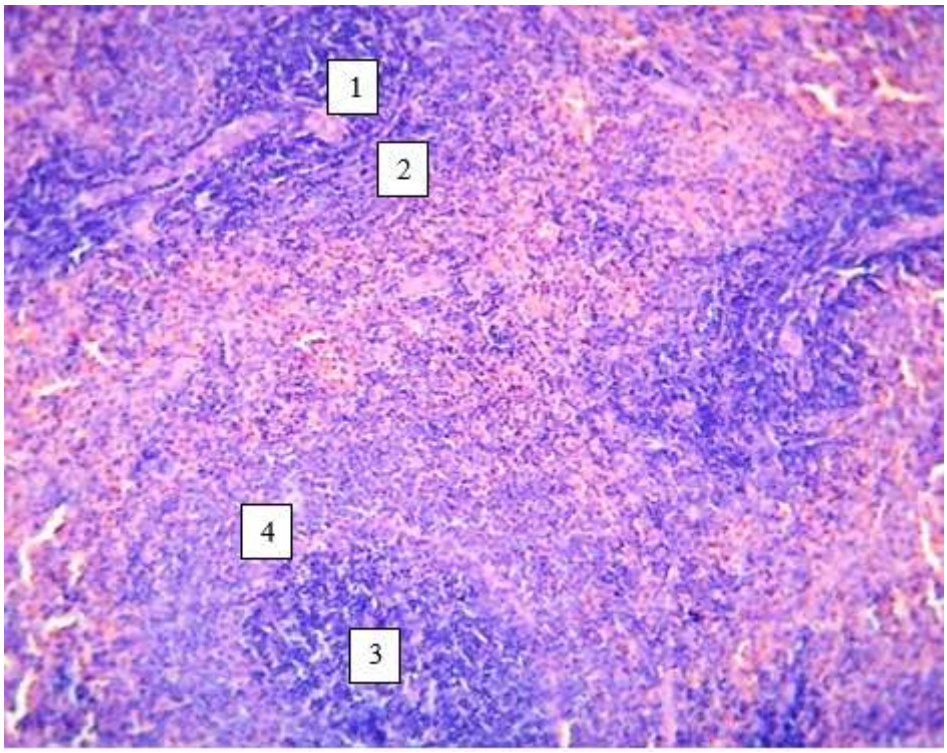

Fig.2. Spleen of a 3-month-old rat with chronic radiation sickness. Staining with hematoxylin-eosin. Oc. 10 x Ob. 20. 1-lymph node, 2-periarterial zone, 3-mantle zone, 4-marginal zone.

It was found that the total number of lymphocytes in the LN without breeding centers is 30-38, on average - 35.1±0.86 cells. In micropreparations as part of lymphoid nodules without a breeding center, large lymphocytes are not detected. Lymphoid nodules without centers of reproduction contain (per unit area) small lymphocytes-22-28 on average-26.0±0.65 cells, average lymphocytes-8-10, on average - 9.1±0.22 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white spleen pulp is 31-40, on average - 35.2 ±0.97 cells. The PALC does not contain large lymphocytes. Periarterial lymphoid couplings contain (per unit area) small lymphocytes-24-31, on average-26.8±0.76 cells, average lymphocytes-7-9, on average - 8.4±0.22 cells.

According to our data, when irradiated and simultaneously administered ASD – 2 fraction at a dose of 0.1 ml to 3-month - old rats, it was found that the relative area of the white pulp ranges from 15.6% to 19.7%, on average-17.81±0.44%. The relative area of connective tissue elements varied from 5.0% to 6.0%, on average-5.62±0.11% (to the total area of the spleen section).

The diameter of the PALC ranges from 106.4 microns to 117.3 microns, with an average of 111.98±1.17 microns. The diameter of the lymph nodes ranges from 248.7 microns to 334.6 microns, with an average of 291.28±9.28 microns. The percentage of primary and secondary LN is 44% and 56%, respectively. The diameter of the germinal centers ranges from 82.1 microns to 112.4 microns, with an average of 97.88±3.27 microns. Lymphoid nodules generally take a rounded-oval, elongated (82.3%) and irregular (17.7%) shape.

The width of the mantle zone ranges from 36.4 microns to 45.7 microns, with an average of 40.46±1.0 microns. The width of the marginal zone ranges from 60.4 microns to 71.2 microns, with an average of 66.5±1.16 microns. The width of the periarterial zone ranges from 62.3 microns to 73.6 microns, with an average of 67.49±1.22 microns (Fig. 3).

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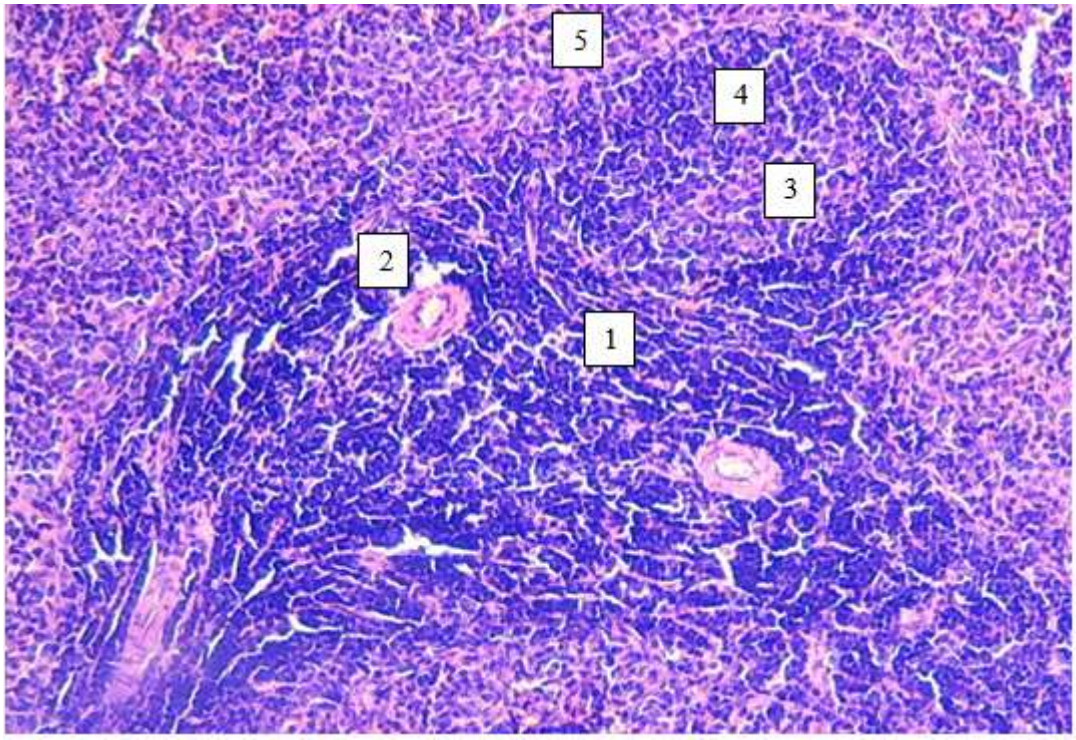

Fig. 3. Spleen of a 3-month-old rat under irradiation and simultaneous administration of ASD-2 fraction in a dose of 0.1 ml. Stained with hematoxylin-eosin. Approx. 10 x 20 vol. 1-lymph node, 2-periarterial zone, 3-germinate center, 4-mantle zone, 5- marginal zone.

It was found that the total number of lymphocytes in the LN without breeding centers is 33-43, on average - 39.3±1.08 cells. Lymphoid nodules without centers of reproduction contain (per unit area) small lymphocytes-25-32, on average - 29.2±0.76 cells, medium lymphocytes-8-10, on average-9.6±0.22 cells and large lymphocytes-0-1, on average-0.5±0.11 cells.

The total number of lymphocytes in the periarterial lymphoid couplings of the white spleen pulp is 34-45, on average - 39.5±1.18 cells. Periarterial lymphoid couplings contain (per unit area) small lymphocytes-25-33, on average-29.7±0.86 cells, medium lymphocytes-8-10, on average - 9.0±0.22 cells and large lymphocytes-1-2, on average-1.2±0.11 cells.

Thus, the relative area of the white pulp of the spleen of white rats of the intact group is on average 22.2±0.59%, and in rats with chronic radiation sickness, there is a decrease in this indicator by 1.52 times (14.6±0.39%). In rats taking ASD-2 fractions at a dose of 0.1 ml in parallel with irradiation, this indicator is 1.22 times higher than in irradiated rats and is equal to 17.81±0.44% (to the total area of the spleen section).

The diameter of the PALC and lymph nodes of the spleen in the control group of rats is on average 132.14±1.56 microns and 466.05±13.27 microns, respectively, in irradiated 99.35±0.98 and 195.81±8.62 microns, respectively, and in rats taking ASD – 2 fractions at a dose of 0.1 ml in parallel with irradiation 111.98±1.17 and 291.28±9.28 microns, respectively. The lymphoid nodules of the spleen of the healthy group were mostly rounded-oval and elongated in shape, in rats with chronic irradiation, irregular shapes were determined (32.4%), and in rats taking ASD – 2 fractions at a dose of 0.1 ml in parallel with irradiation (17.7%). The diameter of the spleen HZ in rats of the intact group is on average 147.8±6.73 microns, in rats with chronic radiation sickness, histopreparations do not reveal breeding centers. In rats taking ASD-2 fractions at a dose of 0.1 ml in parallel with irradiation, this indicator is on average 97.88±3.27 microns. The width of the mantle, marginal and periarterial zone in healthy rats is 45.32±0.89 microns, 77.14±1.32 microns and 85.04±0.69 microns, respectively, in irradiated rats 36.54±0.87 microns, 60.26±0.96 and 59.88±0.88 microns, respectively, and in rats taking ASD-2 fractions at a dose of 0.1 ml in parallel with irradiation 40.46±1.0 microns, 66.5±1.16 and 67.49±1.22 microns, respectively.

The total number of lymphocytes in the LN without breeding centers and periarterial lymphoid couplings of the spleen in control group rats is on average-47.3±1.01 and 47.2±1.1 cells, respectively,

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in rats with chronic irradiation of 35.1±0.86 and 35.2±0.97, respectively, and in rats taking ASD-2 fractions at a dose of 0.1 ml in parallel with irradiation of 39.2±1.08 and 39.5±1.18 cells, respectively.

CONCLUSIONS

1. In chronic radiation sickness in the spleen of white rats, there is a decrease in the relative area of the white pulp, the diameters of the periarterial lymphatic couplings and lymph nodes.

2. In the lymph nodes of the spleen, no centers of reproduction are detected. There are irregular forms (32.4%) of lymph nodes that are not detected in the spleen of healthy rats.

3. The width of the mantle, marginal and periarterial zone decreases, as well as the total number of lymphocytes in the lymph nodes without a breeding center and periarterial lymphatic couplings. A decrease in the morphological parameters of the spleen indicates a decrease in the level of immune protection in response to the radiation factor.

4. The use of the ASD-2 fraction biostimulator in parallel with irradiation reduces the damaging effects of radiation on the lymphoid structures of the spleen. Improvement and approximation of the morphological parameters of the spleen to the control values shows a positive effect of the use of ASD-2 fraction during chronic irradiation and leads to an earlier recovery of the morphological parameters of the spleen white pulp.

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