ISSN NO: 2770-2936
Date of Publication:04-11-2022

Pathological changes in the spleen in herbicide poisoning

Kakhkharov Navruzbek Ziyokulovich.
Tashkent tibbiyot academy and Termiz branches, assistant
Nuritov Nurpullo Razhapovich.
Tashkent tibbiyot academy and Termiz branches, associate professor

Abstract: The purpose of this work was to elucidate the structural foundations of the spleen in acute herbicide poisoning and to find ways to correct them with biocomplexes

Keywords: herbicide poisoning, spleen

Relevance: The development of industrial and household chemicals, the intensive use of various chemicals in agriculture has created a new problem for doctors and environmentalists.
Her environment. The expansion of the use of pesticides has led to environmental pollution and an increase in the number of people in contact with these drugs. This contributed to the development of various acute and chronic lesions, the manifestation of mutagenic, allergenic and other desirable pathological effects. One of the most sensitive tissues to toxic effects is the digestive tract. It clearly shows the negative effects of pesticides, which determine the value of its study in elucidating the mechanisms of action of one or another pesticide. The works devoted to the study of the pathomorphological state of the spleen in case of pesticide poisoning are few and do not allow revealing the patterns of shifts in the spleen.
The development of rational methods of prevention and pathogenetic methods for correcting the toxic effects of pesticides is one of the urgent tasks of modern medicine. Unfortunately, so far there are only a few reports on the use of drugs that have an antidote therapeutic effect in case of poisoning with certain pesticides. In that regard, the most promising are biocomplexes of various microelements with organic compounds, which have high and ionizing effects. However, the effect of these biocomplexes on the body, in particular, on the spleen in acute and chronic poisoning with organophosphorus pesticides, remains not fully understood. The foregoing indicates the relevance and timeliness of the study of the structural and functional features of the spleen in acute poisoning with herbicides and its correction with biocomplexes.

Purpose and objectives of the study:
The purpose of this work was to elucidate the structural foundations of the spleen in acute herbicide poisoning and to find ways to correct them with biocomplexes. Based on the goal, the following tasks were set:
-study of the morphofunctional features of the spleen of intact rats.
- Carrying out ultrastructural, radioautographic and morphometric analysis of cells of T- and B-dependent zones of the spleen in case of experimental acute herbicide poisoning.
Scientific novelty of the work:
In this work, for the first time, using complex general morphological, hematological, cytochemical, radioautographic and electron microscopy methods, the pathomorphological bases of the spleen reaction to acute herbicide poisoning were studied. Combined histochemical and morphometric studies determined the area of T-dependent zones of the organ, which in intact rats is 11% of the area of the white pulp. At the same time, it was found that the proliferative activity of cells and the area of B-dependent zones of the spleen significantly exceed those of the T-dependent zone, which indicates the prevalence of humoral immunity processes in the spleen of rats. With the help of complex pathomorphological research methods, the structural bases of the immune response of the spleen in the dynamics of experimental herbicide poisoning were studied and the periods of immunomorphological rearrangements of the organ were established. It was found that the most pronounced adaptive the reaction of the spleen takes place on days 3-7-15 of the experiments and is characterized by an increase in the proliferative activity of cells, an increase in the area of T- and B-dependent zones and the functional intensity of subcellular structures of immunocompetent cells. General and specific patterns of pathomorphological changes in the spleen in the dynamics of acute

ISSN NO: 2770-2936
Date of Publication:04-11-2022

intoxication with herbicides have been established. It was revealed that the most characteristic changes in the spleen during herbicide poisoning are anemia, leukocytosis with a shift to the left and thrombocytopenia of cellular and subcellular mechanisms, which constitute the inhibition of proliferation and differentiation of hematopoietic cells in the spleen, increased destructive changes in their subcellular organelles, and as a result of this, the disorganization of metabolic processes in proliferation i.e. follicle enlargement. For the first time, metal-containing biocomplexes were tested, which have a pronounced protective and therapeutic effect in acute herbicide poisoning, and the corresponding biocomplex was established and recommended for use in practice.

Scientific and practical value of the study:

The obtained data on shifts in follicular hyperplasia and cytochemical changes in neutrophilic leukocytes are additional differential diagnostic tests when examining people working with herbicides. Data on the cellular and subcellular bases of the reaction of follicles in herbicide poisoning can be used in the educational process at medical institutes for a more in-depth explanation of the body's reactions to various toxic effects. The facts established in the work on the pronounced antidote and therapeutic effect of biocomplexes make it possible to recommend these preparations for wide use in conditions of work with herbicides.

The main scientific provisions submitted for defense:

I. Pathological main reactions of follicles in acute poisoning with herbicides have common patterns and are characterized by suppression of the processes of proliferation and differentiation of spleen cells.

II. Biocomplexes have a pronounced protective and therapeutic effect, as in acute, herbicide poisoning.

Our work is experimental and performed on laboratory rats. However, there are relatively few data on the state of spleen follicles in laboratory rats, and in the vast majority of studies, only the quantitative content of certain cellular forms of the spleen is considered. In recent years, due to the intensive development of immunology and the emergence of two immune systems (T- and B-systems), ideas about immunity and organs that provide immune defense of the body have greatly expanded.

The pathomorphological and functional substrates of the immune system are immunocompetent lymphoid organs and functionally similar tissue structures, cells, biologically active substances such as prostagandins, lymphokines, and others.

Currently, it is customary to subdivide the organs of the immunocompetent system into primary, or central, and secondary, or peripheral. The central organs of immunity include the thymus (thymus gland), the bursae of fabricius in birds, and its analogues in mammals. A possible analogue of the Fabricius bag of birds in humans is red bone marrow. The peripheral organs of the immune system include lymph nodes, spleen, lymphoid formations of the gastrointestinal tract, airways, and others. In the central lymphoid organs, lymphocytes undergo a series of transformations, as a result of which they acquire the functions of cellular and humoral immunity, enter the peripheral organs of immunity, forming in them, respectively, thymus-dependent (T-dependent), as well as thymus-independent bursa-dependent, or (B-dependent) structural-functional zones.

Therefore, the immune system, including the central and peripheral lymphoid organs, as well as the energy structures with them, in unity and in interaction with each other, provides immune homeostasis of the body.

One of the main components of the system of macrophages and immunosecretory lymphoid organs of the immune system is the spleen.

It is covered on the outside with a relatively thick connective tissue capsule, very rich in elastic and collagen fibers and poor in blood vessels. The splenic capsule of some mammals contains a large amount of smooth muscle elements, while in humans, smooth muscle elements are present in a small amount and are concentrated mainly in the region of the hilum of the spleen. The thickness of the human spleen capsule is 0.10 mm, reaching 0.15 mm in the region of the hilum. Outside, the capsule is covered with peritoneum on all sides, except for the gate area. From the capsule and connective tissue of the gate area, connective tissue septa - trabeculae - penetrate into the organ. Trabeculae, being also derivatives of the capsule, in their connective tissue base contain various fibers, smooth muscles, blood vessels, and nerve fibers. Branching into the finest fibrous structures, trabeculae are closely associated with the pulp of the organ.

Thus, the capsule and the trabeculae extending from it with the blood vessels and elements of the nervous tissue embedded in them constitute the musculoskeletal apparatus of the spleen; different representatives of mammals are developed differently.

ISSN NO: 2770-2936
Date of Publication:04-11-2022

The parenchyma of the spleen is represented by white and red pulps, its stroma is made up of reticular tissue; the concept of white pulp includes reticular tissue, in the network of which clusters of various types of lymphocytes are located - lymphoid follicles or Malpighian bodies. The rest of the spleen, consisting of blood vessels, most often sinusoidal hemocapillaries and partitions located between them - pulp cords (Billroth cords), is called the red pulp.

The ratios of white and red pulps have species and age features. studying the spleens of various mammals, it was found that these ratios in different types of mammals differ from each other, which is associated with the peculiarities of the functional values of the spleen in these animals. In this regard, the authors propose to distinguish between the following two types of spleen - exchange and deposit. Spleens of the exchange type, which, by the way, include the human spleen, are characterized by greater or lesser development and the prevalence of white pulp compared to red. The spleens of the circulatory type are characterized by a predominance of red pulp. This type includes the spleens of cats, dogs, horses and other mammals.

The study of the ratios of different pulps of the spleen, depending on age, which noted that the white pulp is more developed in newborns and in children from 1 to 5 years of age and tends to increase up to 15 years. With age, the white pulp decreases, while the red pulp, on the contrary, increases. With age, there is also a gradual increase in connective tissue elements in the stroma of the organ.

Lymphoid follicles of the spleen in their structure and functional features differ sharply from similar lymph nodes. One of its main structural and functional features is the presence in the lymphoid follicles of the spleen of both thymus-dependent and thymus-independent zones, as well as elements of the system of mononuclear phagocytes, which are aimed at providing cellular cooperations necessary for immune responses.

Based on the above works, the following structural and functional zones are distinguished in the lymphoid follicles of the spleen: the light center (reproduction center, germinal center), periarterial zone, mantle and marginal zones.

Depending on the severity of these or other indicated zones, some authors propose to characterize the functional state of the white pulp of the spleen according to a three-point system, calling the 1 and 2 types of activity such a pulp, which consists of small and larger homogeneous lymphoid follicles without reproduction centers, type 3 - pulp, consisting of more voluminous follicles with distinct reproduction centers. The reticular stroma of the white pulp of the spleen is formed by reticular fibers, reticular cells, fibroblasts and cells of macrophage origin.

Biochemical and electron microscopic studies have established that reticular fibers do not differ from collagen fibers and are most likely produced by fibroblasts. At the ultrastructural level, they consist of fibrils and an interfibrillar matrix. Fibrils of retucular fibers have a diameter of 40 to 54 nm and an axial periodicity of 61 to 64 nm, some of these fibrils do not have transverse striation. The interfibrillar matrix electron microscopically has a thin fibrillar structure with a fibril thickness of 3-4 nm. It should also be noted that the reticular fibers in all mammals have a similar structure.

The issue of identification, classification, histogenesis and cytophysiology of reticular cells is one of the most controversial and confusing issues in the study of the stroma of lymphoid organs in general and the spleen in particular.

The term "reticular cell" describes a wide variety of cells that differ in their structural, functional and kinetic parameters, which explains the motley terminology and confusion of the classification of reticular cells.

Based on the work of some authors, the following three types of reticular cells can be distinguished: "phagocytic reticular cells", "non-phagocytic reticular cells" and "undifferentiated cells".

Under the name "phagocytic reticular cells" are combined cells characterized by the presence in their cytoplasm of a large number of various lysosomes and phagosomes and participation in a specific immune response.

In the light of modern data, these cells are assigned to the system of mononuclear phagocytes, which differ from other phaocytes in their origin and functional features.

The foregoing as a whole necessitates further research, which provides for the elucidation of the pathomorphological and functional features of the spleen on the action of a widespread pesticide and the possibilities of pharmacological correction of these reactions using metal-containing biological complexes.

ISSN NO: 2770-2936
Date of Publication:04-11-2022

Materials and Research Methods

The experiments were carried out in 224 outbred male rats with a body weight of 140-150 g. The animals were kept under standard vivarium conditions. Before the experiments, the animals were quarantined for two weeks to exclude various diseases.

All animals were divided into two main groups.

The first group of animals (84 rats) was divided into 4 subgroups and was used to study the state of the reaction of the blood system during acute poisoning with herbicides and in the process of its correction with biocomplexes. Three subgroups of this group received a single dose of herbicide intragastrically at a dose of 150 mg/kg. The fourth subgroup, which received an equal volume of sterile saline, served as controls. Animals of subgroup I were slaughtered by decapitation on days 1, 3, 7 and 15 after poisoning with herbicides. Animals of subgroup II, starting from the first day after poisoning, received the biocomplex "Bisaquamethylioninatesulfonium chloride" monosubstituted cobalt (II) glutamate, trihydrate, once a day subcutaneously at a dose of 10 mg/kg for 15 days. Animals of the III subgroup similarly subcutaneously injected the biocomplex "Aquamethylioninate sulfonium, doubly mixed copper (II) glutamate" with control and untreated subgroups of rats in compliance with all conditions.

Treated, control and untreated subgroups of animals in this group were slaughtered. All slaughters were carried out in the morning, on an empty stomach, by decapitation. In addition to these two groups, 20 intact rats of the same body weight were used in the work.

At the time of slaughter, the spleen was taken from all animals for the determination of pathomorphological studies.

Pieces of the spleen and the number of erythrocytes, leukocytes, hemoglobin content and serum cholinesterase activity according to the generally accepted method served as the material for research. Blood smears stained according to Romanovsky-Giemsa were used to calculate the leukocyte count and platelet count. The detection of acid and alkaline phosphatase activity, myeloperoxides and glycogen content was carried out according to the methods described in the manuals on blood cell cytochemistry (8). The results of the cytochemical reaction were evaluated by a quantitative method using a five-point system (6).

The Result of Own Research

Pathological, histochemical, and immunohistochemical features of the spleen of intact and control rats.

Before proceeding to the study, the spleen of rats is characterized by certain structural and functional specific features, in contrast to the spleen of some mammals (dogs, horses, etc.). these features are primarily due to the fact that the spleen in rats is predominantly an organ of the metabolic type, which is characterized by a relatively high specific gravity of the pulp (1:6 versus 1:10 in dogs). On the other hand, the spleen of rats is a universal hematopoietic organ, where the processes of erythro-, thrombo-, and granulocytopoiesis are carried out.

Like other mammals, the spleen of rats is surrounded on the outside by a capsule of dense fibrous connective tissue with an admixture of a small amount of smooth muscle cells. Outside, the capsule is covered with peritoneum.

The white pulp is represented by lymphoid follicles, which are most often located along the periphery of the organ and have a round or oval shape (Fig. 1). The vast majority of lymphoid follicles have light centers. On the periphery of the follicles, there are central rateries - a.a.centrales (Fig. 2). Lymphoid follicles are delimited by more or less pronounced slit-like spaces - marginal sinuses (Fig. 3).

The cellular composition of the lymphoid follicles of the spleen is not the same in different areas. In the light centers, large and medium-sized lymphocytes prevail, here lymphoblasts, macrophages and reticular cells are detected in a small amount. In the periarterial zone of lymphoid follicles, small lymphocytes with a dark nucleus mainly predominate. The reticular cells and fibroblasts of this zone are in close contact with each other, forming concentric layers.

In the marginal zone of the lymphoid follicles of the spleen of intact rats, medium and small lymphocytes are relatively evenly distributed, among which single large lymphocytes, fibroblasts, macrophages, reticular and plasma cells are detected.

The red pulp of the organ consists of a large number of pulp vessels, predominantly sinusoidal hemocapillaries and intersinusoidal tissue - pulp (Billroth) cords, where macrophages, plasma cells and a

ISSN NO: 2770-2936
Date of Publication:04-11-2022

small number of megakaryocytes are mainly localized. The presence of single erythroblastic islands, consisting of a central macrophage and surrounding erythroid cells at various stages of differentiation, is also characteristic. Relatively less often, maturing cells of graulocytopoiesis occur in the pulp cords.

In the lumen of the sinusoidal hemocapillaries of both the lymphoid follicles and the red pulp, there is always a small number of lymphocytes, neutrophils and eosinophils. In the lumen of the vessels of the red pulp, erythrocytes are predominant.

Thus, the same structural and functional zones are revealed in the spleen of intact rats as in the spleen of other mammals. At the same time, this organ in rats has certain specific features.

Pathological and histochemical features of the spleen of rats in the dynamics of experimental acute herbicide poisoning.

Our studies have shown that the experimental island of herbicide poisoning is accompanied by certain dynamics of structural and functional rearrangements in different zones of the spleen. These rearrangements, revealed using general morphological, histochemical and immunohistochemical research methods, are adaptive in nature and can be conditionally divided into the following periods.

- early changes (from 1.3 and 7 days after infection), characterized by microcirculatory disorders and a high content of antigenic products in the spleen tissue;
- pronounced immunomorphological rearrangements (from 7 to 15 days after infection), which is characterized by hypertrophy and hyperplasia of the lymphoid follicles of the spleen, a high degree of their plasmatization. At the same time, pathomorphological changes in the lymphoid tissue of the spleen reach their maximum, which ensures the immunological reactivity of the macroorganism;
- a parallel subgroup was treated with a biocomplex (Bisaquamethylmethioninatesulfonium chloride, monosubstituted cobalt II lutaminate, trihydrate at a dose of 10 mg/kg for 15 days received subcutaneous injection.

This group tends to normalize immunomorphological changes in all structural and functional areas of the spleen.

In the untreated group, in the early stages after infection, the spleen has a significant thickening, a capsule that is abundantly infiltrated with lymphocytes and granulocytes. The blood vessels of both white and red pulps are significantly dilated. A particularly pronounced deletion is observed in the sinusoidal hemocapillaries of the red pulp, which are filled with a large number of destructively altered erythrocytes and leukocytes. The blood sinuses are also dilated and filled with hemolyzed erythrocytes and destroyed lymphocytes.

Endothelial cells of the sinus wall are swollen, swelling of their cytoplasm is noted. Often visible blood cells migrate from the lumen of the sinuses through the gaps between the endothelial cells.

The expansion of the cranial vessels causes a more distinct identification of the boundaries of the lymphoid follicles of the spleen. There is some expansion of the reactive centers of the follicles, which contain mainly large lymphocytes and lymphoblasts.

The lumen of the central artery is also dilated, the extirpating endothelial cells are high, with basophilic cytoplasm.

Intersinusoidal pulp bands of the red pulp are infiltrated with blood cells, especially hemolyzed erythrocytes and granulocytes.

When setting up the reaction to RNA according to Brache, a certain increase in the number of positively reacting lymphocytes in the periarterial zones of the lymphoid follicles was observed at the specified time of the study.

After 3 days. after pesticide poisoning, there was a tendency to hypertrophy of the lymphoid follicles, which became larger. In the reactive centers of lymphoid follicles, lymphoblasts were predominant, often in a state of mitosis.

The wall of the central artery looked relatively thick due to edema and infiltration. Predominantly small lymphocytes and lymphoblasts were localized in the periarterial zone.

In all structural and functional areas of the spleen, there was a deletion of blood vessels capillary stasis. In the lumen of sinusoidal hemocapillaries, large lymphocytes, lymphoblasts, and destructively altered neutrophils were most often found.

ISSN NO: 2770-2936
Date of Publication:04-11-2022

During this period of the study, in all structural and functional zones of the white pulp of the spleen, an increase in the number of cells with a high content of RNA was noted. However, the most pronounced pyroninophilia was found primarily in large lymphocytes, lympho- and plasmablasts and plasma cells of reactive centers and periarterial zones.

Thus, experimental pesticide poisoning leads to certain structural and functional rearrangements in the spleen tissue, characterized by a certain periodicity and covering both the microvasculature and the structural components of the white and red pulps. These rearrangements, which are adaptive in nature, and the essence of which lies in the hypertrophy and hyperplasia of immunocompetent cells of the spleen, are ultimately aimed at ensuring immune homeostasis in response to antigenic exposure.

Conclusions

Conclusions. The spleen of intact rats is characterized by the following features:

The ratio of white pulp to red is 1:6, which indicates the metabolic type of this organ in rats;

The area of the T-dependent zone of the spleen is relatively small and accounts for only 11% of the area of lymphoid follicles;

Cell proliferation in the B-dependent zones of the rat spleen is 2 times higher than in the T-dependent zone.

Adaptive pathomorphological rearrangements of the spleen during experimental herbicite poisoning, covering both white and red pulps, are characterized by certain dynamics, including periods of early changes, pronounced immunomorphological rearrangements and convalescence.

In the early period (1, 3 days of the experiment), microcirculatory disorders are detected, a decrease in the area of T-dependent zones of lymphoid follicles and a decrease in the proliferative activity of cells in these zones.

The period of pronounced immunomorphological rearrangements (3, 7 days of the study) is characterized by:

- Increased cell proliferation in both T- and B-dependent zones, moreover, the rate of increase in the number of proliferating cells in T-dependent zones significantly exceeds the same rates in the B-dependent zone;
- An increase in the area of white and red pulps of the spleen as a whole, as well as their individual structural and functional zones;
- Functional tension of subcellular structures of immunocompetent cells (lymphocytes, plasma cells, macrophages), manifested as hyperplasia of mitochondria of the granular endoplasmic reticulum and lysosomes.

The period of convalescence (from 15 to 21 days of the experiment) is characterized by a tendency to decrease in the areas of both T- and B-dependent zones of the lymphoid follicles of the spleen, a decrease in the proliferation of their cells, which is associated with the elimination of the antigen from the organ. However, the tension of the subcellular structure cells of the T- and B-dependent zones of the spleen is preserved.

The antigenic effect, which was modeled with an experimental herbicide, made it possible to reveal the cellular and subcellular mechanisms of immunomorphological rearrangements of the spleen, covering both the microcirculatory bed and T- and B-dependent zones of the organ. This gives reason to recommend this model for solving a number of issues of adaptive responses of the immune system organs.

Literature

1. Afanasiev Yu.I. Organs of hematopoiesis and immunological protection. - In the book: Histology (under the editorship of Eliseev V.G., Afanasiev Yu.I., Yurina N.A.) - M., Medicine, 1983, p.345-370.
2. Ataev M.A. Influence of the pesticide magnesium chlorate on the coagulating activity of blood: Abstract of the thesis. ... c.m.s. - Ashgabat, 1980, 25 p.
3. State catalog of pesticides and agrochemicals permitted for use on the territory of the Russian Federation. Part I. Pesticides. M., 2018. 877 p.
4. European Convention for the Protection of Vertebrate Animals used for Experimental or Other Scientific Purposes (ETS N 123), together with the Guidelines for the Housing and Care of Animals,

ISSN NO: 2770-2936
Date of Publication:04-11-2022

Statistical Tables and Explanatory Notes (Strasbourg 03/18/1986) (as amended on 06/22/1998) URL: http://base.garant. ru/4090914/ (date of access: 10.10.2018).

5. Zakharenko V. A. Pesticide market in Russia and prospects for its development // Protection and quarantine of plants. 2014. No. 11. P. 3–6.
6. Bespamyatnykh E. N., Donnik I. M., Isaev M. L., Mikheev M. N., Shilova E. N. Optimization of environmental pollution control of dairy farms in the zone of intensive technogenic emissions // Agrarian Bulletin of the Urals . 2007. No. 6 (42). pp. 38–40.
7. Pamuzhak N. G. Improving the range of pesticides constant attention // Protection and quarantine of plants. 2007. No. 1. S. 7–11.
8. Paptsov A. G., Popova A. G. World market of chemical plant protection products and trends in its development // Agro-food policy of Russia. 2013. No. 11 (23). pp. 104–107.