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**ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ
ТБИЛИСИ - НЬЮ-ЙОРК**

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MICROSCOPIC FEATURES OF THE SPLEEN UNDER THE INFLUENCE OF LAPROXIDES

Avilova O., Marakushin D., Nakonechna O., Gargin V.

Kharkiv National Medical University, Ukraine

Pollution of the environment and its influence on the body is a very urgent problem for medicine, as it is accompanied with increase in the incidence among the population [4,15]. A variety of environmental factors determines a wide range of morphological and functional changes in humans and animals. Diverse xenobiotics have an immunosuppressive effect and, therefore, the organism becomes responsive to viral, bacterial and parasitic diseases [11,14].

Chronic exposure to some well-absorbed but slowly eliminated xenobiotics can lead to their bioaccumulation in living organisms [2]. Exposure to environmental agents can compromise numerous immunological functions with immunotoxicological focuses on the evaluation of the potential adverse effects of xenobiotics on immune mechanisms that can lead to harmful changes in host responses such as: increased susceptibility to infectious diseases and tumorigenesis; the induction of hypersensitivity reactions; or an increased incidence of autoimmune disease [6].

One of xenobiotics type is class of polyethers belonging to the group called "Laproxides", which are used in various sectors of the economy for the obtaining plastics, epoxy resins, lacquers, enamels, adhesives, etc. For the present research widely used polyether – tryglycidyl ether of polyoxypropylenetriol (TEPPT) [13] with molecular weight 303 (L-303) was chosen. Manufactures based on polyethers are used in machine-building, radio engineering, pharmaceutical, chemical, aviation, automotive and other branches of the national economy. The choice of this group of substances was performed due to large volumes of production, extensive contact with the population, the lack of prognostic characteristics of their potential danger for humans and warm-blooded animals, and the need to justify pathological mechanisms of structural and metabolic disorders under prolonged intake of subtoxic doses

Immunotoxicology is the study of undesired modulation of the immune system by extrinsic factors. Toxicological assessments have demonstrated that the immune system is a target following exposure to a diverse group of xenobiotics including ultraviolet radiation, chemical pollutants, therapeutics, and recreational drugs [10,16]. The immune system plays a crucial role in maintaining health; however, accumulating evidence indicates that this system can be the target for immunotoxic effects caused by a variety of chemicals including the environmental pollutants [6]. The organs of the lymphoid system take participation in maintaining the constancy of homeostasis, among the first responding to exogenous influences and have the high potential to ensure the adaptation processes while the adverse impact of chemicals. According to the scientific literature, detergents have found wide enough tests on laboratory animals; a number of experimental studies demonstrated

that they meet the requirements for substances which are constantly in contact with the humans [3]. Spleen is one of the recommended organs to evaluate for enhanced histopathology of the immune system [3] as it is the largest secondary lymphoid organ and it is considered the draining site for compounds that are administered intravenously, and, therefore, serves as an important organ to evaluate for treatment-related lesions. However, nowadays, there are a small number of research papers regarding this course. In connection with the above, the purpose of our work was detection of microscopic peculiarities of the spleen under the influence of laproxides.

Material and methods. Experimental work is a part of the research topic of the human anatomy department of the Kharkiv National Medical University «Morphological features of the organs and systems of the human body at the stages of ontogenesis», (number of the state registration 0114U003388). The study was performed on 72 outbreed WAG male matured rats with the weight 200 ± 10 g. The control and experimental series consisted of animals of the same age. Animals were divided into 2 series. The first series - control animals (3 groups, 6 animals in each), were fed a regular diet and received an appropriate amount of water. The second series was experimental animals. They were randomly divided into 3 groups 6 in each depending on the dose of induced polyether and length of administration: 7 days, 15 days and 30 days. All laboratory animals were maintained in the conventional environment of Kharkiv National Medical University vivarium in a controlled-temperature room $t 20 \pm 2^\circ\text{C}$, humidity $65 \pm 10\%$. All rats were treated via gastric gavage during 7, 15, 30 days by aqueous solutions of TEPPT in the doses $1/10 \text{ LD}_{50}$ in conversion to 5.75 g/kg. At the end of the investigation, changes were observed. Food intake and body weight were measured every 2 days. All rodents were deduced from the experiment by immediate cervical dislocation according to European Convention for the Protection of Vertebrate Animals (Strasbourg, 18.03.1986), principles of Ukrainian law №3447-IV about the protection of animals from cruel treatment.

Obtained material was fixed in 10% neutral buffered formalin for 24 hours, were subjected to standard proceeding and embedded in paraffin. From the prepared blocks made serial sections thick 5×10^{-6} m. Slides were stained with hematoxylin and eosin [1]. Histological examination of removed spleens was performed according to accepted guidelines with microscope «Olympus BX41» followed by morphometric study using "Olympus DP-soft 3.12" program. Staining was scored independently by two observers and a high level of concordance (90%) was achieved. All slides were independently reviewed twice and intra-observer

disagreements (<10%) were reviewed a third time followed by a conclusive judgment. Evaluation of expression was performed using a quantitative scale. All values are expressed as means, standard deviation and standard error of the mean for statistical analysis. Statistical comparison was performed using Mann-Whitney test for statistical analysis [12]. The accepted level of significance was $p \leq 0.05$.

Results and their discussion. The available evidence suggests, that spleen weight reflects the functional activity of the organ and dynamic balance of immune system. Therefore there are no significant changes in body weight of any of the TEPPPT-treated rats' groups in comparison to the control group. The animals that received TEPPPT in dose 1/10LD₅₀ are characterized by the spleen's weight increasing from 438.33±33.20mg to 545±22.02mg during the period of observation. The spleen weight due to 1/10LD₅₀ administration reduced on 18.3% (7th day), on 13.71% (on the 15th day), on 11.86% (on 30st day) in comparison to the control group (Table 1). All of the indexes were considered to be statistically significant ($p < 0.05$).

Linear dimensions of the spleen are changed also. In the 1 seria of animals that received TEPPPT in dose 1/10LD₅₀ the spleens length increased from

32.57±1.21mm to 36.33±1.44mm which is less than control groups on 12.71% ($p=0.0076$), on 9.67% ($p=0.0094$), and on 8.75% ($p=0.0467$) on 7th, 15th, 30st day respectively. The spleens breadth during period of observation increased from 7.10±0.12mm to 7.33±0.13mm which is on 8.27% ($p=0.0173$), on 11.81% ($p=0.0240$), on 10.41% ($p=0.0417$) lower than the showings of intact animals on 7th, 15th and 30th day. The height of the spleen during the experiment has changed from 3.19±0.15mm to 3.98±0.19mm which is lower than the control group indicators on 17.58% ($p=0.0216$) on 7th day, on 5.84% ($p=0.404$) on 15th day, on 3.4% ($p=0.0652$) on 30th day. Generally, the changes in linear dimensions of the spleen under this dose of xenobiotic were characterized by statistically significant indicators decrease except the height alterations on 15th and 30th day.

The study of histological preparations of the spleen of rats of control groups is characterized by the presence of a pronounced connective tissue capsule and trabeculae containing blood vessels. The parenchyma of the organ is represented by red and white pulp. The composition of the red pulp includes sinusoidal capillaries and splenic cords. White pulp consists of numerous lymphatic follicles.

Table 1. Influence of the 1/10 LD₅₀ of xenobiotic on the rats' spleen linear dimensions and weight in different periods of observation ($M \pm m$, $n=36$)

Dose of polyether 1/10 LD ₅₀	Control group №1	7 days	Control group №2	15 days	Control group №3	30 days
Length	37.31±0.75	32.57±1.21*	37.54±0.81	33.91±0.79*	39.81±0.53	36.33±1.44*
Breadth	7.74±0.19	7.10±0.12*	7.96±0.24	7.02±0.26*	8.18±0.34	7.33±0.13*
Height	3.87±0.20	3.19±0.15*	3.77±0.26	3.55±0.15	4.12±0.25	3.98±0.19
Weight	536.66±27.40	438.33±33.20*	571.66±11.94	493.33±28.24*	618.33±20.56	545±22.02*

note: * - statistically significant differences with the control group ($p < 0.05$)

Table 2. Influence of the 1/10 LD₅₀ of xenobiotic on the histological dimensions in different periods of observation ($M \pm m$, $n=36$)

Dose of polyether 1/10 LD ₅₀	Control group №1	7 days	Control group №2	15 days	Control group №3	30 days
The area of the white pulp (%)	17.87±1.04	23.47±1.14*	18.21±1.13	24.64±1.54*	18.84±1.14	27.37±1.71*
Diameter of lymphatic follicles (µm)	426.59±11.18	396.21±10.27*	421.72±12.82	387.13±10.42*	427.54±12.98	382.31±11.73*
The width of the mantle zone (µm)	45.73±1.08	39.19±1.15*	43.37±3.22	38.56±2.17	44.42±2.25	37.18±2.29*
Width of the marginal zone (µm)	81.32±1.79	76.86±2.24	80.34±1.29	75.93±2.14	81.87±1.07	74.63±2.08*
The width of the periarterial zone (µm)	88.73±2.69	94.82±2.76	89.64±2.07	95.82±2.03	90.46±1.97	97.24±2.61*

note: * - statistically significant differences with the control group ($p < 0.05$)

In rats after the administration of TEPP, the spleen retains a typical structure. White pulp is represented by periarterial lymphoid follicles occasionally containing germinal centers. The diameter of the lymph follicles is statistically significantly different with the control data from 7th day (Table 2), in later observation periods the indices become smaller than in the control groups. The germinal centers of the lymph nodes in the early periods of observation are visualized only in single lymphatic follicles. Their diameter is smaller than in the corresponding groups of control animals. The parameters of the width of the mantle and marginal zones of lymphatic follicles are also reduced in comparison with the control. The revealed structural changes in the spleen of animals indicate the hypoplasia of white pulp, which some authors attribute to the increased incidence of apoptosis and a decrease in the level of cell proliferation in response to the effect of an unfavorable factor. The central arteries of lymphatic follicles have thicker walls due to the development of sclerotic changes. Trabecular connective tissue is well defined, its thickness is increased.

It has now been recognised that the immune system as a whole can be the target for xenobiotic induced toxicity. The discipline of immunotoxicology encompasses non specific direct immunotoxicity and immunostimulation, and specific responses like hypersensitivity and autoimmunity [4]. Immunosuppression, hypersensitivity and autoimmunity may occur due to changed immune activity. Evaluation of the immunotoxic potency of agents as part of risk assessment is currently established in vivo with animal models and in vitro with cell lines or primary cells [17]. Immunotoxicology can be defined as the study of the adverse effects of environmental chemicals, certain therapeutics, and biologicals on the immune system [9].

Measure of correction are continue to be created for immunopathological conditions [8] and immune organs are reacted for different influence. So, spleen as the largest secondary lymphoid organ is changed under influence of xenobiotics that was detected even in macroscopic dimensions. But microscopic changes of spleen due to the presence of B and T lymphocytes, the immunotoxic effects of xenobiotics or their metabolites on these cell populations may be reflected in the spleen even more significantly. Spleen is one of the recommended organs to evaluate for enhanced histopathology of the immune system [5]. The two major functional zones of the spleen are the hematogenous red pulp and the lymphoid white pulp (periarteriolar sheaths, follicles and marginal zones). For enhanced histopathology, these splenic compartments should be evaluated separately for changes in size and cellularity, and descriptive rather than interpretive terminology should be used to characterize any changes [5,7].

Our obtained data can be sign of specific decreased cellularity of the B cell-rich marginal zone, as it could be similar with treatment-related effect due to possible increasingly encountered with the development of novel immunomodulatory drugs. Such a change may be suggestive of

deficits in T-independent humoral immune responses. For example some chemicals, such as N,N-dimethyl-*p*-toluidine and AZT/Methadone HCL will cause an overall decrease in the cellularity and area of the white pulp [5].

The red pulp should also be evaluated for changes in lymphocyte numbers. Reactive extramedullary hematopoiesis may be seen in conjunction with conditions that target the destruction of lymphocytes [5]. It is also possible to identify decreased lymphocyte cellularity of the red pulp in those cases where systemic lymphocyte numbers are significantly decreased. Although the red pulp contains lymphocytes and macrophages, it is predominantly composed of red blood cells [5]. The induction of 1/10LD₅₀ is characterized severe impact that is apparently explained by the dose, and, hence during investigation was noticed that this dose has statistically significant impact almost on all indicators of weight and linear dimensions where the higher numbers of alterations are observed on 7th day. On 30th day the morphometric indexes lower down what can be caused by their normalization, which indicates the ability of spleen to adjust.

In our subsequent investigation we are aiming to distinguish the features of the histological alterations of the spleen following exposure to the different doses of tryglycidyl ether of polyoxypropilene triol.

Conclusions. Thereceived and analyzed data demonstrate the morphological changes of the spleen, specifically changes of the linear dimensions and weight of the spleen due to the influence of the TEPP. The spleen is very sensitive to the effects of xenobiotics, in this case TEPP that is even reflected in its grossly (weight and linear dimensions) and histological features (reliable changes of the of the white pulp area of the spleen from 17.87±1.04% to 27.37±1.71%, diameter of lymphatic follicles from 426.59±11.18 µm to 382.31±11.73 µm, width of the mantle zone from 45.73±1.08 µm to 37.18±2.29 µm, width of the marginal zone from 81.32±1.79 µm to 74.63±2.08 µm, width of the periarterial zone from 88.73±2.69 µm to 97.24±2.61 µm).

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SUMMARY

MICROSCOPIC FEATURES OF THE SPLEEN UNDER THE INFLUENCE OF LAPROXIDES

Avilova O., Marakushin D., Nakonechna O., Gargin V.

Kharkiv National Medical University, Ukraine

Rapid technology growth and its implementation in all spheres of the people's lives dictates the necessity for thorough study of the influence of different chemicals on human's health. This study was undertaken to elucidate the structural changes that occur in the matured rats' spleen experimentally induced by selected xenobiotic, so, purpose of our work was detection of microscopic peculiarities of the spleen under the influence of laproxides. In subacute

experiment were uncovered organometric alterations of the matured male rat's spleen after the administration of 1/10 LD₅₀ of polyether-triglycidyl ether of polyoxypropylene triol (TEPPT). The study was performed on 72 outbreed WAG male matured rats with the weight 200±10g. Histological slides were studied with performing morphometric and statistical methods. We revealed changes of morphologic data in comparison to control data which shows reactivity of the spleen in response to the induced xenobiotic. The received and analyzed data demonstrate the morphological changes of the spleen, specifically changes of the linear dimensions and weight of the spleen due to the influence of the TEPPT. The spleen is very sensitive to the effects of xenobiotics, in particular, TEPPT that is even reflected in its grossly (weight and linear dimensions) and histological features (reliable changes of the of the white pulp area of the spleen from 17.87±1.04% to 27.37±1.71%, diameter of lymphatic follicles from 426.59±11.18 μm to 382.31±11.73 μm, width of the mantle zone from 45.73±1.08 μm to 37.18±2.29 μm, width of the marginal zone from 81.32±1.79 μm to 74.63±2.08 μm, width of the periarterial zone from 88.73±2.69 μm to 97.24±2.61 μm).

Keywords: spleen, histology, xenobiotics, polyesters

РЕЗЮМЕ

МИКРОСКОПИЧЕСКИЕ ОСОБЕННОСТИ СЕЛЕЗЕНКИ ПОД ВОЗДЕЙСТВИЕМ ЛАПРОКСИДОВ

Авилова О.В., Маракушин Д.И., Наконечная О.А., Гаргин В.В.

Харьковский национальный медицинский университет, Украина

Быстрое развитие технологий и их реализация во всех сферах жизни человека диктуют необходимость тщательного изучения влияния различных химических веществ на здоровье. Данное исследование посвящено установлению структурных изменений, происходящих в селезенке зрелых крыс, экспериментально индуцированных ксенобиотиком.

Целью исследования явилось определение микроскопических особенностей селезенки под воздействием лапроксидов. В подостром эксперименте изучались органометрические изменения селезенки после введения 1/10 LD₅₀ простого полиэфира-триглицидилового эфира полиоксипропилентриола. Исследование проведено на 72 зрелых крысах-самцах линии WAG весом 200±10 гр. Гистологические микропрепараты изучались посредством морфометрических и статистических исследований. В сравнении с контрольными животными выявлены морфологические изменения, которые указывают на реактивность селезенки в ответ на индуцированный ксенобиотик. Анализ полученных

данных демонстрирует морфологические изменения селезенки, в частности ее линейных размеров и веса под воздействием лапроксида. Селезенка чувствительна к воздействию ксенобиотиков, в данном случае лапроксидов, что отражается в ее макроскопических (вес и линейные размеры) и гистологических особенностях (достоверные изменения площади белой пульпы с $17,87 \pm 1,04\%$ до $27,37 \pm 1,71\%$, диаметра лимфоидных фолликулов с $426,59 \pm 11,18$ мкм до $382,31 \pm 11,73$ мкм, ширины мантийной зоны с $45,73 \pm 1,08$ мкм до $37,18 \pm 2,29$ мкм, ширины краевой зоны с $81,32 \pm 1,79$ мкм до $74,63 \pm 2,08$ мкм, ширины периаартериальной зоны с $88,73 \pm 2,69$ мкм до $97,24 \pm 2,61$ мкм).

რეზიუმე

ელენთის მიკროსკოპული თავისებურებანი ლაპროქსიდების ზემოქმედების პირობებში

ო. ავილოვა, დ. მარაკუშინი, ო. ნაკონენაია,
ვ. გარგინი

ხარკოვის ეროვნული სამედიცინო უნივერსიტეტი,
უკრაინა

ტექნოლოგიების სწრაფი განვითარება და მათი რეალიზება ადამიანის ცხოვრების ყველა სფეროში განაპირობებს ჯანმრთელობაზე სხვადასხვა ქიმიური ნივთიერების ზემოქმედების გულდასმით შესწავლის აუცილებლობას.

წინამდებარე კვლევა ჩატარდა სტრუქტურული ცვლილებების დასადგენად, რომელიც ვითარდება

ქსენობიოტიკებით ექსპერიმენტულად ინდუცირებული ვირთაგვების ელენთაში.

კვლევის მიზანს შეადგენდა ელენთის მიკროსკოპული თავისებურებების განსაზღვრა ლაპროქსიდების ზემოქმედების პირობებში. ქვემწვავე ექსპერიმენტში შესწავლილია ორგანომეტრიული ცვლილებები ზრდასრული ვირთაგვების ელენთაში $1/10 LD_{50}$ პოლიოქსიპროპილენტრიოლის მარტივი პოლიეთერ-ტრიგლიციდილური ეთერის შეყვანის შემდეგ. ჰისტოლოგიური მიკროპრეპარატები შესწავლილია მორფომეტრიული და სტატისტიკური კვლევებით. ცხოველთა საკონტროლო ჯგუფებთან შედარებით, გამოვლენილია მორფოლოგიური ცვლილებები, რომელიც მიუთითებს ელენთის რეაქტიულობაზე ქსენობიოტიკის ინდუცირების საპასუხოდ. მიღებული და გაანალიზებული მონაცემები ავლენს მორფოლოგიურ ცვლილებებს ელენთაში, კერძოდ – ელენთის ხაზოვანი ზომებისა და წონის ცვლილებას ლაპროქსიდის გავლენით. ელენთა მგრძობიარეა ქსენობიოტიკების, სახელობრ – ლაპროქსიდის ზემოქმედების მიმართ, რაც აისახება მის მაკროსკოპულ (წონა და ხაზოვანი ზომები) და ჰისტოლოგიურ თავისებურებებზე (სარწმუნო ცვლილებები: თეთრი პულპის ფართობისა - $17,87 \pm 1,04\%$ -დან $27,37 \pm 1,71\%$ - მდე, ლიმფოციტური ფოლიკულების დიამეტრისა - $426,59 \pm 11,18$ მკმ-დან $382,31 \pm 11,73$ მკმ-მდე, მანტიის ზონის სიგანისა - $45,73 \pm 1,08$ მკმ-დან $37,18 \pm 2,29$ მკმ-მდე, განაპირა ზონის სიგანისა - $81,32 \pm 1,79$ მკმ-დან $74,63 \pm 2,08$ მკმ-მდე, პერიარტერიული ზონის სიგანისა - $88,73 \pm 2,69$ მკმ-დან $97,24 \pm 2,61$ მკმ-მდე).

MORPHOLOGICAL CHARACTERISTICS OF ANGIOGENESIS IN ADENOCARCINOMA OF COLON

¹Gvamichava T., ^{1,2}Janberidze E., ¹Machavariani T., ³Mdivani N., ⁴Latsabidze I.

¹I. Javakhishvili Tbilisi State University, Al. Natishvili Institute of Morphology; ²Georgian National Association for Palliative Care, Tbilisi; ³Georgian State Teaching University of Physical Education and Sport; ⁴“EnMedic” Clinic, Tbilisi, Georgia

The formation of microcirculation bed in tumors and its specific characteristics differentiating it from the original tissue has a great significance [1,2]. The specificities of the microcirculation bed itself greatly defines the course of malignant process and its relationship with the whole body [11-13]. The subject concerning the characteristics of perfusion of malignant tumors, in present time, not only loses its actuality, but on contrary, gains more and more importance in relation with the necessity of the development of new approaches to treatment. The study of angiogenesis process

and microcirculation considering the modern capabilities of morphological science, taking into account the type and maturity of tumor, its location, has to be accepted as the issue of specific importance and value.

The definition of the stages of angiogenesis, as the key factors of histogenesis, is also important. They refer to the general issues needed to understand the blastomatic growth [3,4-6,10,14]. The study in this aspect will give highly useful information in terms of assessment of characteristics of perfusion in tumors. All abovementioned defines the