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SPLEEN MORPHOMETRY UNDER THE IM- PACT OF LAPROXIDES IN EXPERIMENT

Experimental work is a part of the research topic «Morphological features of the organs and systems of the human body at the stages of ontogenesis» (number of the state registration 0114U003388).

Abstract. In subacute experiment were uncovered organometric alterations of the matured male rat's spleen after the administration of 1/10, 1/100 LD₅₀ of polyether – tryglycidyl ether of polyoxypropylene triol. Rapid technology growth and its implementation in all spheres of the people's lives dictates the need 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, exactly the linear dimensions and the weight of the rodents spleen. Were used morphometric, mathematical and statistical methods. Revealed changes of morphologic data in comparison to control data shows high reactivity of the spleen in response to the induced xenobiotic. These data are useful for understanding the toxicological effects induced by investigated polyether and provide evidence for the mechanism of toxicity of the agent and how it impacts the immune system.

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Авілова О.В. Морфометрія селезінки під впливом лапроксидів в експерименті.

Реферат. Були виявлені органометричні зміни селезінки зрілих самців-щурів після введення 1/10, 1/100 ДЛ₅₀ поліефіру – тригліцидилового ефіру поліоксипропілентріолу в підгострому експерименті. Швидкий розвиток технологій та їх впровадження у всіх сферах життєдіяльності людей свідчать про необхідність ретельного вивчення впливу різних хімікатів на здоров'я людини. Це дослідження було проведено для з'ясування структурних змін, а саме лінійних розмірів та ваги селезінки гризунів, що відбуваються в селезінці тварин експериментально індукованих обраним ксенобіотиком. Були використані морфометричні, математичні та статистичні методи. Виявлені зміни морфологічних даних у порівнянні з контрольними даними свідчать про високу реакційну здатність селезінки у відповідь на діючу речовину. Ці дані корисні для розуміння токсикологічної дії цього агента і його впливу на імунну систему.

Ключові слова: селезінка, органометрія, ксенобіотики.

Citation:

Avilova O. Spleen morphometry under the impact of laproxides in experiment. *Morphologia*. 2017;11(4):18-22.

Introduction

A variety of environmental factors determines a wide range of morphological and functional changes in humans and animals. The chemicals that are commonly considered the term "xenobiotics" have specific effects on organs, tissues, cells and subcellular components. These chemicals include products of human activities, household chemical substances and most of the medicines. Toxicants of different chemical nature to some extent have damaging properties and, under certain conditions, permeate into the body, making a harmful impact on health, including the immune system [1].

As the material for the study was used xenobiotic, which has a marketable name "Laproxides"

with regulated physico-chemical property: tryglycidyl ether of polyoxypropylene triol (TEPPT) with molecular weight 303 (L-303). The choice of this group of substances was made on grounds of large volumes of production, extensive contact with the population, the lack of prognostic characteristics of their potential danger for humans and warm-blooded animals [2, 3], and the need to justify pathophysiological mechanisms of structural and metabolic disorders under prolonged intake of subtoxic doses. The organs of the lymphoid system participating 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 [1,4]. Spleen is one of the recommended organs to evaluate for enhanced histopathology of the immune system [5] 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.

TEPPT is a typical polyether that has characteristics enabling wide use in the production of many materials such as drugs prolongators, ointment ingredients, additives to the household products, coatings, and adhesives. Potential toxic effects attracting wide attention and only a few studies have assessed the effects of TEPPT on the immune system [3, 5, 7-12]. These studies have shown that TEP is a class of environmental pollutant with versatile toxicities.

Material and Methods

The study was conducted on 72 outbred 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 seria - control animals (3 groups, 6 animals in each), were fed a regular diet and received an appropriate amount of water. The second seria 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 KhNMU 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 and 1/100 LD₅₀ 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 killed by immediate cervical dislocation at the end of the experiment 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. Methods: morphometrical, mathematical, statistical.

Results

The available evidence suggests, that spleen weight reflects the functional activity of the organ and dynamic balance of immune system [6,13]. No significant changes were found in body weight of any of the TEPPT-treated rats' groups in comparison to the control group. In the 1 seria of the animals that received TEPPT in dose 1/10LD₅₀ the spleen's weight during the period of observation increased from 438.33 ± 33.20 mg to 545 ± 22.02 mg. However, 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 30th day) in comparison to the control group (Table 1). All of the indexes were considered to be statistically significant ($p < 0.05$). In the 2 seria of animals that received TEPPT in dose 1/100LD₅₀ the spleen's weight during the period of observation increased from 528.3 ± 28.81 mg to 561.66 ± 22.27 mg. Therefore, an impact of 1/100LD₅₀ was less noticeable when on 7th day of experiment the spleen weight reduced on 2.17%, on 15th day on 2.82% and on 30th day – on 13.82% (Table2). Statistically significant change was observed only on 30th day ($p < 0.05$). Particular attention was drawn to the fact that the biggest alterations occurred on 7th day with the 1/10LD₅₀ dose and on the 30th day with the dose 1/100LD₅₀. In the 1 seria abrupt weight reduction on 18.3% in early time observation (7 days) while in 2 seria gradual spleen weight reduction with the highest number on 30th day (on 13.82%). Thus, the most noticeable changes are found on 7th and 30th day of the experiment. The gross appearance and size of the spleen are variable, depending on the species and the degree of distension, nonetheless, spleen weights can be important in its evaluation [1, 7, 14].



Fig. 1. Linear measurements of the spleen of the 1 seria experimental group by digital caliper. 1-impact of the TEPPT in dose 1/10 LD₅₀ on 7th day of the experiment; 2- impact on the 15th day, 3 – impact on the 30th day, 4 and 5 – spleens of the control group.

Different doses of the TEPPT had also an impact on linear dimensions of the spleen. In the 1 seria of animals that received TEPPT in dose 1/10LD₅₀ the spleens length increased from 32.57 ± 1.21 mm to 36.33 ± 1.44 mm 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, 30th day respectively. The spleens breadth during period of observation increased from 7.10 ± 0.12 mm to 7.33 ± 0.13 mm 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 ± 0.15 mm to 3.98 ± 0.19 mm 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 (Fig.1). 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 (Table 1).

Table 1
Influence of the 1/10 LD₅₀ of xenobiotic on the rats' spleen linear dimensions and weight in different periods of observation (M±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)

However, the alterations of spleen's linear dimensions under the impact of TEPPT in dose 1/100LD₅₀ (2 seria) were slightly different. The spleens length increased from 37.22±0.53mm to 37.94±1.21 mm which is less than control groups on 0.11% (p=0.9581), on 1.83% (p=0.4170), and on 4.7% (p=0.1750) on 7th, 15th, 30th day respectively. The spleens breadth during the period of observation increased from 7.46±0.32 mm to 7.81±0.41 mm which is on 1.72% (p=0.7306), on 2.19% (p=0.7872), and on 1.89% (p=0.8148) lower than the showings of intact animals on 7th, 15th and 30th day. The height of the spleen during the experiment has changed from 3.98±0.26 mm to 3.95±0.34 mm where on 7th day the height increased on 5% (p=0.6232) compared to control group, on 15th day the indexes were lower on 3.3% (p=0.7646), on 30th day lower on 1.76% (p=0.9440) (Fig.2). Generally, the changes in linear dimensions of the spleen under this dose of xenobiotic were characterized by statistically insignificant indexes decrease except the

height alterations on 7th day where the indicators increased on 5% (Table 2).



Fig. 2. Identification of the linear dimensions of the spleen of the 2 experimental seria – the impact of the TEPPT in dose 1/100 LD₅₀

Table 2
Influence of the 1/100 LD₅₀ of xenobiotic on the rats' spleen linear dimensions and weight in different periods of observation (M±m, n=36)

Dose of polyether 1/100 LD ₅₀	Control group №1	7 days	Control group №2	15 days	Control group №3	30 days
Length	37,26±0,52	37,22±0,53	38,44±0,44	37,74±0,70	39,81±0,42	37,94±1,21
Breadth	7,59±0,18	7,46±0,32	7,77±0,29	7,60±0,54	7,96±0,47	7,81±0,41
Height	3,79±0,27	3,98±0,26	3,73±0,15	3,61±0,36	3,98±0,24	3,95±0,34
Weight	540±25,94	528,3±28,81	591,66±28,25	575±23,21	651,66±23,44	561,66±22,27*

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

Conclusion

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 different doses of the TEPPT.

Revealed changes suggest:

1. The spleen is very sensitive to the effects of xenobiotics, in this case, polyesters, in particular, TEPPT that is even reflected in its morphological features, like weight and linear dimensions. However, changes in the rats' weight have not been regis-

tered.

2. The spleen actively responds to the administration of above mentioned polyether. Its 1/10LD₅₀ and 1/100LD₅₀ induction causes a reduction in weight and linear dimensions of the spleen compared to intact animals, especially pronounced in the early stages of the surveillance, which may be related to its immunosuppressive effect due to its toxicity and can be associated with immunological sensitivity.

3. However, the induction of 1/10LD₅₀ has more severe impact rather than 1/100LD₅₀, 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 organometric indexes lower down what can

be caused by their normalization, which indicates the ability of spleen to adjust.

4. The impact of 1/100LD₅₀ characterized by slight statistically insignificant changes where the higher number of organometric indexes alterations were observed on 30th day which may be approved by the ability of spleen to accumulate. Thus, alteration in spleen weight and linear dimensions are the reliable indicators in toxicity assessment. Weight and gross morphology are the first parameters studied in toxicity assessment and a response to injury is often expressed as a change in tissue size and weight parameters.

Perspectives of the further research

In the following publications we plan to highlight the features of the cells spleen changes on histological level under the influence of tryglycidyl ether of polyoxypropylene triol in different doses.

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Авилова О.В. Морфометрия селезёнки под влиянием лапроксидов в эксперименте.

Реферат. Были выявлены органомерические изменения селезёнки взрослых крыс-самцов после введения 1/10, 1/100 ДЛ₅₀ простого полиэфира-триглицидилового эфира полиоксипропиленгликоля в подостром эксперименте. Реализация технологического прогресса во всех сферах жизни людей обуславливают необходимость тщательного изучения влияния различных химических веществ на здоровье человека. Это исследование было проведено для выяснения структурных изменений, а именно линейных размеров и веса селезёнки грызунов, происходящих в селезенке зрелых крыс, экспериментально индуцированных выбранным ксенобиотиком. Использовались морфометрические, математические и статистические методы. Выявленные изменения морфологических данных по сравнению с контрольными данными показывают высокую реакционную способность селезёнки в ответ на вводимое вещество. Эти данные полезны для понимания механизма токсичности агента и его влияния на иммунную систему.

Ключевые слова: селезёнка, органомерия, ксенобиотики.