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MORPHOMETRIC FEATURES OF THE THYMUS STRUCTURE DUE TO THE INFLUENCE OF XENOBIOTICS

Denis Shyian, Olga Avilova

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Nowadays scientific achievements in various areas of lives have caused the creation of more and more «foreign body substances» known as xenobiotics. Different chemicals have the detrimental effect on the body systems and, thus, all humanity. The purpose of this work is to determine the alterations of the white rats' organometric indexes of the thymus after xenobiotic induction in 1/10 and 1/100 LD₅₀ doses with the average lethal dose in 5,75 g/kg of the body. For the study was chosen commonly used polyether that belongs to a group of «Laproxides» - tryglycidyl ether of polyoxypropylene triol. The research presents a theoretical generalization of the experiment results and establishes the mechanism of action of this xenobiotic. Were used morphometric, mathematical and statistical methods. The morphometric investigation revealed the size reduction of the thymus after chemical induction on 7th, 15th, 30th day with the high degree of authenticity that was proved by the changed morphometric parameters of the organ – weight, length, breadth, thickness. The analysis of the obtained results allows to state that this substance, in conditions of the oral administration to the body in the above mentioned doses, can lead to atrophy and thymus dysfunction

Keywords: thymus, organometry, xenobiotics, polyesters

Introduction. Diverse xenobiotics have an immunosuppressive effect and, therefore, the organism becomes responsive to viral, bacterial and parasitic diseases [5]. The immune system reacts sensitively to a concentration of chemical substances that are not yet toxic to other systems of the organism. A huge attention has been paid lately to study of the impact of extraneous substances from the external environment on humans and animals [6]. The impact is mainly due to the high contamination of the environment by chemical substances used in industry and agriculture.

Our planet and organisms living on it are influenced by the pharmaceutical substances, industrial pollution, pesticides and household chemicals products, food additives and preservatives. Not an exception the 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 polyoxypropylene triol (TEPPT) with molecular weight 303 (L-303) was chosen [4,11]. Manufactures based on polyethers are used in machine-building, radio engineering, pharmaceutical, chemical, aviation, automotive and other branches of the national economy.

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. The thymus is a primary lymphoid organ that manifests dynamic physiological changes as animal age in addition to being exquisitely sensitive to stress and toxic

insult [1,2,7]. It is typically the first lymphoid tissue to respond to immunotoxic xenobiotics. According to the National Toxicology Program [4] decrease in thymus weight is often the first indicator of toxic action of a xenobiotic on the immune system.

In concordance with scientists, xenobiotics have found wide enough experimental works on animal, wide range of tests demonstrate that they meet the requirements to study the influence of substances which are continuously in contact with the humans [9,10]. However, nowadays, there is a small number of research papers regarding this course. [3,4,8]. Pursuant to the investigated literature, there is a complete absence of the data about morphofunctional changes in the immune system organs makes it vital to study the problem in a deeper aspect.

Connection to scientific topics and plans. The given experimental work has been done in accordance with the plan of scientific research of the Human anatomy department of the Kharkiv National Medical University on 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).

The aim of the research This study was undertaken to elucidate the structural changes that occur in the thymus experimentally induced by TEPPT, exactly the linear dimensions and the weight of the rodents' thymus.

Material and Methods The investigation was conducted on 72 outbred WAG male matured rats weighing 200±10g. Animals were randomly divided into 2 groups according to the dose intake, and each of two groups was subdivided into two subgroups. The first subgroup was

control animals (3 groups with 6 animals in each) and the second one was experimental animals (3 groups with 6 animals in each). The control and experimental groups consisted of the animals of the same age. The intact group was fed with a regular diet and received an appropriate amount of water. 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 $65 \pm 10\%$. The groups of the experimental animals were formed according to the dose of induced polyether, 1/10 and 1/100 LD_{50} respectively, and the length of administration: 7 days, 15 days and 30 days. Aqueous solutions of TEPPT at the above mentioned doses in conversion to average lethal dose to 5.75 g/kg of the animal body were administered endogastrically with a metal feeding tube on an empty stomach in the morning. Food intake and body weight were measured every 2 days. At the end of the investigation, thymus was extracted and the changes were observed. Firstly, the linear dimensions were thoroughly measured by digital caliper without detachment of the adipose tissue in order to save the proper anatomical structure of the fragile organ. Afterwards primary lymphoid organ was scrupulously cleaned from adipose tissue to be weighted on the analytical balance. Obtained results were noted down and statistically processed. All rodents were killed 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. Taking into account the rhythm of immune system functioning, material intake has been done at the same time of the day. Used methods: morphometric, mathematical, statistical.

Results. The primary lymphoid organ – thymus in the rats is located in the thoracic cavity, in the upper part of the anterior mediastinum, right behind the breastbone, approximately in the shape of a triangle facing by the apex the gorge, consists of two asymmetrical, flattened in anterior-posterior parts, divided by a thin layer of the connective tissue.

It was established that during all course of the experiment in the male-mature WAG rats occurs reduction of the absolute indices of weight, length, breadth, and thickness of the thymus, which were estimated in comparison to the intact group. The severity reduction of the thymus weight and linear dimensions after each period of investigation of the different doses of TEPPT in comparison to the control indexes wasn't alike. (Table1, Table2).

Thus, the thymus weight of the control animals in the first group of the rodents that received TEPPT in a dose 1/10 LD_{50} decreased from $298.5 \pm 7.16\text{mg}$ to 219.33 ± 4.60 , corresponds to the accidental involution of the thymus at this age of the rats. Nevertheless, the thymus weight due to the 1/10 LD_{50} TEPPT administration reduced by 34.03%, 27.81%, and 20.68% on the 7th, 15th, 30th days respectively in comparison to the control group (Table1). All the indexes were considered to be extremely statistically significant ($p < 0.001$). Thymus weight of the control animal during the period of observation has decreased from 286.83 ± 7.36 to

266.5 ± 4.08 in the second experimental group that received TEPPT in a dose 1/100 LD_{50} . In comparison to the control group, changes in the experimental group occurred in the weight which were shown as a reduction by 13.19% (on 7th day), 11.08 (on 15th day), 10.74% (on 30th day). All the indexes were considered to be extremely statistically significant ($p < 0.001$), even though the impact of the 1/100 dose was less detrimental than 1/10 dose. In both groups of the TEPPT influence can be distinguished abrupt reduction of the weight on the first period of experiment termination – the 7th day and then diminution gradually continues.

The analysis of the thymus organometric indexes has shown that at all terms of the experiment the length, breadth, and thickness of the organ had a tendency to diminution. The reduction severity in the thymus linear dimensions was not the same at different times of observation. Regarding the abrupt reduction of weight on the 7th day the same was happening with the linear dimensions, where more changes had appeared in the thickness of the thymus as it's the smallest organometric index in the control animals.

The 1/10 LD_{50} dose of TEPPT had a more severe impact than 1/100 LD_{50} dose (Table1, Table2). In the 1st group, the length decreased by 14%, 9.36%, 8.51% on the 7th, 15th and 30th days of observation respectively in comparison to control animals. The breadth of the thymus had decreased by 22.8%, 17.76% and 18.22% at the same periods of observation comparing to control group. The highest number of reduction was noted in the thickness of the thymus which is 30.03%, 20.13% and 18.22% in comparison to intact animals (Fig1). By conventional criteria, this difference is considered to be extremely statistically significant ($p < 0.001$).

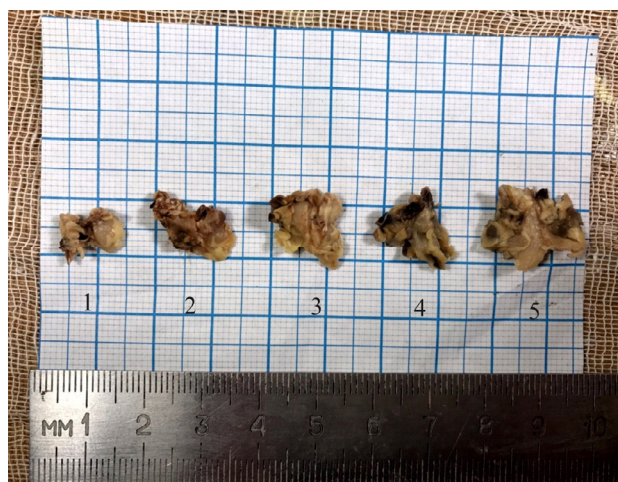


Fig1. Linear measurements of the thymuses of the 1st experimental group by digital caliper. 1 – Thymus on the 7th day of the TRPPT impact in 1/10 LD_{50} ; 2 – Thymus on the 15th day of the TRPPT impact in 1/10 LD_{50} ; 3 – Thymus on the 30th day of the TRPPT impact in 1/10 LD_{50} ; 4 and 5 – thymuses of the control group of the animals.

Within the course of the experiment after the exogenous factor influence of the 1/100 LD_{50} TEPPT length of the animal thymus tended to reduce by 8.05%, 6.69%, 6.2%, breadth decreased by 10.92% on the 7th day of the

investigation, by 8.02% on the 15th and by 7.75% on the last 30th day. Occurred changes in the thickness, where it decreases by 14.37%, 9.04% and 8.14% on the 7th, 15th, 30th days respectively (Table2, Fig2). All received results are in comparison to the intact animals considered to be extremely statistically significant ($p < 0.001$).

The greatest reduction in the morphological parameters such as weight, length, breadth, thickness during the study of impact of widely used polyether in different doses is observed on the 7th day (1 period of experiment termination) and then the indexes approximately stabilize at the same level, which probably proves the formation of adaptive reactions of the organism to this period.

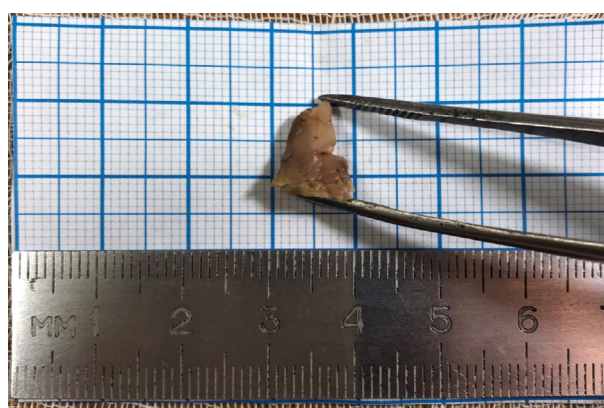


Fig2. Thymus cleaned from the adipose tissue of the 2nd experimental group – the impact of the TEPPT in the dose 1/100 LD₅₀

Conclusion

· In response to the administration of investigated polyether, there is an intensive alteration in the thymus morphometric parameters, which indicates its active response to exogenous effects. However, the effect of 1/100 LD₅₀ is less detrimental than 1/10 LD₅₀ according to the revealed changes, even though the results are statistically significant.

· Induced TEPPT caused not only weight reduction of the organ but also decrease of length, breadth, and thickness, especially in the earlier stages of the experiment the changes are severely pronounced.

· Among all organometric parameters the most alterable was revealed is the thickness of the organ, apparently, it is due to the fact that this index is the smallest in the control group.

· Particular attention was drawn to the fact that on the 15th and 30th days the changed indexes are almost the same, compared to the control group which can indicate the possibility of the thymus to adjust.

· To sum up the results of the morphometric study of the rats' thymus, under the impact of the exogenous factor in the different doses it should be emphasized that subacute retention of rats under this conditions leads to an abrupt reliable decrease of all the investigated organometric parameters. The mass deficiency of this primary lymphoid organ in the experimental animals of this series is especially pronounced. This may be a prerequisite for the formation of a secondary immunodeficiency state in a population coming in contact with this group of chemicals in different branches of national economy. Due to provided experiment can be assumed that thymus is susceptible to this particular xenobiotic

Table 1.

Dynamics of changes in the organometric parameters of the mature rats' thymus after the effect of the 1/10 LD₅₀ dose of xenobiotic in different periods of observation (M±m, n=36)

Groups of the animals	Weight (gr)	Length (mm)	Breadth (mm)	Thickness (mm)
Control group №1	298.5±7.16	16.00±0.03	9.52±0.02	3.93±0.03
7 th day of experiment	196.83±13.00*	13.76±0.21*	7.35±0.02*	2.75±0.04*
Control group №2	293.83±4.66	15.92±0.02	9.46±0.11	3.18±0.15
15 th day of experiment	211.83±14.64	14.43±0.14*	7.78±0.02*	2.54±0.03
Control group №3	276.5±3.52	15.88±0.10	9.38±0.06	3.02±0.07
30 th day of experiment	219.33±4.60*	14.53±0.12*	7.80±0.03*	2.47±0.04*

Note:* - $p < 0.0001$ in comparison to the control group

Table2.

Dynamics of changes in the organometric parameters of the mature rats' thymus after the effect of the 1/100 LD₅₀ dose of xenobiotic in different periods of observation (M±m, n=36)

Groups of the animals	Weight (gr)	Length (mm)	Breadth (mm)	Thickness (mm)
Control group №1	286.83±7.36	16.03±0.30	9.44±0.02	3.55±0.01
7 th day of experiment	249±3.98	14.74±0.02	8.41±0.02*	3.04±0.03*
Control group №2	274±4.81	15.95±0.02	9.36±0.01	3.10±0.03
15 th day of experiment	243.66±5.42	14.90±0.06*	8.61±0.02*	2.82±0.02*
Control group №3	268.66±4.52	15.81±0.02	9.30±0.01	2.95±0.02
30 th day of experiment	266.5±4.08	14.83±0.02*	8.58±0.03*	2.71±0.02*

Note:* - $p < 0.0001$ in comparison to the control group

Perspectives of further research: In our subsequent investigation we are aiming to distinguish the features of the histological alterations of the thymus following exposure to the different doses of tryglycidyl ether of polyoxypropylene triol.

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МОРФОМЕТРИЧНІ ОСОБЛИВОСТІ БУДОВИ ТИМУСА ЗА УМОВ ДІЇ КСЕНОБІОТИКІВ

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Створення і використання в різних сферах життя людини «чужорідних речовин» більш відомих як ксенобіотики в наш час швидкого технологічного прогресу має пагубний вплив на системи організму і, отже, на все людство. Мета роботи - визначення ефекту впливу ксенобіотиків на морфометричні показники тимуса в підгострому експерименті. У дослідженні представлено теоретичне узагальнення результатів експериментального дослідження та встановлено механізм дії тригліцидилового ефіру поліоксипропилентріола з морфологічної точки зору. В експериментальній роботі використовувалися морфометричні, математичні і статистичні методи. У результаті введення дорослим щурам лінії Вістар простого поліефіру із групи «Лапроксида» в дозі 1/10 і 1/100 ДЛ₅₀ при розрахунку середньо смертельної дози в 5,75 г/кг тіла тварин на протязі 7, 15 і 30 днів виявлене статистично значиме зниження розмірів тимусу, що було підтверджено змінами не тільки органометричних параметрів органу - довжини, ширини, товщини, але і маси тимуса. Аналіз отриманих результатів дослідження дає змогу стверджувати, що дана речовина в умовах перорального надходження до організму у запропонованих дозах здатна призводити до атрофії та дисфункції тимусу.

Ключові слова: тимус, органометрія, ксенобіотики, поліефіри

МОРФОМЕТРИЧЕСКИЕ ОСОБЕННОСТИ СТРОЕНИЯ ТИМУСА ПРИ ВОЗДЕЙСТВИИ КСЕНОБИОТИКОВ

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Создание и использование в различных сферах жизни человека «чужеродных веществ» более известных как ксенобиотики в наше время быстрого технологического прогресса имеет пагубное воздействие на системы организма и, следовательно, на все человечество. Цель работы - определение эффекта влияния ксенобиотика на морфометрические показатели тимуса в подостром эксперименте. В исследовании представлено теоретическое обобщение результатов экспериментального исследования и установлен механизм действия триглицидилового эфира полиоксипропилентриола с морфологической точки зрения. В экспериментальной работе использовались морфометрические, математические и статистические методы. В результате введения взрослым крысам линии Вистар простого полиефира из группы «Лапроксида» в дозе 1/10 и 1/100 ДЛ₅₀ при расчёте средней смертельной дозы в 5,75 г/кг тела животных на протяжении 7, 15 и 30 дней отмечено снижение размеров тимуса с высокой степенью достоверности, что было подтверждено изменениями не только органометрических параметров органа - длины, ширины, толщины, но и массы тимуса. Анализ полученных результатов исследования позволяет утверждать, что данное вещество в условиях перорального поступления в организм в предложенных дозах способно приводить к атрофии и дисфункции тимуса.

Ключевые слова: тимус, органометрия, ксенобиотики, полиефиры