ORIGINAL ARTICLE

THE PECULIARITIES OF MORPOLOGICAL CHANGES OF RATS' OVARY AND BIOCHEMICAL STATE UNDER THE DAMAGE WITH DIFFERENT DOSES OF LEAD ACETATE

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ABSTRACT

The aim of the study was to study the effect of low and high doses of lead acetate on biochemical parameters and morphological status of rat ovaries in the experiment.

Materials and methods: The study was performed on 36 nonlinear female rats weighing 180-210 g, aged 4 months, divided into 3 experimental groups: I - control (C), II - rats, which were given 30 days to drink a solution of lead acetate with at the rate of 0,05 mg / kg of animal weight, group III - rats, which were given for 30 days to drink a solution of lead acetate at the rate of 60 mg/kg of animal weight. Biochemical research methods were included determination of diene conjugate concentration in animals' blood, concentration of TBA-active products, study of oxidative modification of proteins in blood plasma, determination of superoxide dismutase and catalase activities. Endogenous intoxication was assessed by the definition of medium-mass molecules, the content was expressed in units of extinction. The material for light microscopy investigation from the ovary was performed according to the generally accepted method.

Results: Lead acetate causes activation of peroxidation of lipids and proteins in the body of female rats, which is directly dependent on the dose of lead. In response to the activation of free radical oxidation there are changes in the antioxidant system, which depend on the dose of lead acetate: at a dose of 0.05 mg / kg superoxide dismutase and catalase activity increase, at a dose of 60 mg / kg superoxide dismutase and catalase activity. Small doses of lead do not cause endogenous intoxication. Lead acetate causes the development of endogenous intoxication in animals only in large doses: increases the formation of toxic compounds, cell apoptosis, decreased excretory function of the kidneys, which is associated with multiorgan disorders. As a result of the action of lead acetate, morphological changes of the ovaries were observed, which increased with increasing dose of lead acetate. There was a dose-dependent decrease in massometric parameters, the number of follicles and changes in the thickness of the surface structures of the ovary, which is more pronounced at 60 mg/kg.

Conclusions: Under the influence of small and large doses of lead acetate on biochemical changes in blood and morphological changes in the ovaries in male rats the oxidative stress is developed. Under the influence of small doses, the changes are adaptive, and under the influence of large doses - damaging.

KEY WORDS: ovary, female rats, plumbum, lead intoxication, morphological changes, biochemical changes in blood

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INTRODUCTION

According to the WHO, plumbum is one of the most global and dangerous pollutants, because this metal has a wide sphere of using in various sectors of the economy and household, as well as large volumes of production and world trade, which leads to a regular supply of lead to the environment, where it spreads over considerable distances from sources of pollution [1, 2].

Plumbum as an environmental pollutant and a classic toxicant continues to be the focus of attention not only of ecologists, toxicologists and hygienists, but also of morphologists and clinicians representing various fields of medicine and biology. This is due to the fact that in a relatively short period of time the content of this potentially toxic chemical substance in the environment has increased tens or even hundreds of times, and most importantly, acquired a global character [2, 3].

Poisoning by lead compounds is accompanied by dysfunction of the nervous, cardiovascular, digestive and other systems and organs, lesions of hematopoiesis, reproductive system [4, 5, 6], so the study of structural and functional aspects of lead intoxication is an actual task.

Of particular interest is the effect of plumbum on the reproductive system, which leads to various disorders in fetal development, as confirmed by experimental and clinical studies [2, 7, 8].

THE AIM

The purpose of the research**:** to study the effect of low and high doses of lead acetate on biochemical parameters and morphological status of rat ovaries in the experiment.

MATERIALS AND METHODS

The work was doing at the Central Research Laboratory of I. Horbachevsky Ternopil National Medical University.

All experiments were performed in the morning in a specially designated room at a temperature of 18-22°C,

Table I. Groups of experimental animals

Group	Characteristics of the experimental model group	Number of animals
I	Intact white rats (control)	12
II	Lead acetate (0.05 mg/kg)	12
III	Lead acetate (60 mg/kg)	12

relative humidity of 40-60% and illumination of 250 lux. Animals were kept and experiments on them in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for research and other scientific purposes [9].

The study was performed on 36 nonlinear female rats weighing 180-210 g, aged 4 months, divided into 3 experimental groups (Table I): I - control (C), II - rats, which were given 30 days to drink a solution of lead acetate with at the rate of 0,05 mg / kg of animal weight [10], group III - rats, which were given for 30 days to drink a solution of lead acetate at the rate of 60 mg / kg of animal weight [8]. Each rat was kept in a separate cage to reduce the risk of uneven consumption of the toxicant with drinking water. Rats were kept in standard vivarium conditions with free access to food and water, which was given only after the animal drank lead acetate solution.

Euthanasia of rats was performed by total bloodletting from the heart after previous thiopental-sodium anesthesia (60 mg kg^{-1} body weight intraperitoneally). After removing the animals from the experiment, blood was taken for biochemical examination and ovary was taken for histological examination. The fence was carried out at the same time of day from 11^{00} to 15^{00} hours indoors at an air temperature of 18-20 °C.

Biochemical research methods were included determination of diene conjugate (DC) concentration in animals' blood, concentration of TBA-active products, study of oxidative modification of proteins in blood plasma, determination of superoxide dismutase and catalase activities. Endogenous intoxication was assessed by the definition of medium-mass molecules, the content was expressed in units of extinction [11, 12, 13].

The material for light microscopy investigation was performed according to the generally accepted method [14]. After removing the ovary, it was weighed and cut from the middle part of the organ pieces. The material was fixed for 2-3 weeks in a 10% solution of neutral formalin with three changes of fixative, then dehydrated in alcohols of increasing concentration, and then poured into paraffin blocks. Microtome sections 5 μm thick were stained with hematoxylin-eosin. Microscopic examination of the specimens was performed using the world microscope «Nicon Eclipse Ci» (made in Japan), using lenses x 4, 10, 20 and eyepiece x 10. Photographed histological specimens with a Sigeta camera (made in Japan).

Statistical processing of digital data was performed using Excel software (Microsoft, USA) and STATISTICA 7.0 (Statsoft, USA) using parametric and non-parametric methods of data evaluation. The values of the arithmetic mean (M), its variance and the error of the mean (m) were calculated for all indicators. The reliability of the difference between the values between the independent quantitative values was determined at the normal distribution by the Mann-Whitney test.

RESULTS

The analysis of lipid peroxidation (LPO) in animals of group II, which were given low-dose levels of lead that did not exceed the general toxic effect, showed an increase in DC by 46,4% ($p<0,001$) compared with animals of the control group, and TB-active products - by 36,8% ($p<0,001$), respectively.

Animals of group III, which were given large doses of lead, causing intoxication in the body, showed an increase in DC in 6,3 times ($p<0,001$), compared with animals in the control group, and TBA-active products – 3,7 times ($p<0,001$), respectively.

When comparing the degree of toxic effects of lead on LPO processes, it was found that in group III animals compared to group II, DCs were 4,3 times higher ($p<0,001$), TBA-active products were found 2,7 times more ($p<0,001$).

Thus, lead acetate causes dose-dependent activation of lipid peroxidation processes in rats.

The analysis of oxidatively modified proteins (OMP) in animals of group II, which were given low-dose levels of lead acetate, not exceeding the general toxic effect, revealed a decrease in redox proteins, in particular the value of OMP_{370}, decreased by 2,95% compared to animals of the control group, OMP_{430}, by 3,41% ($p<0,001$), respectively.

In animals III, which were given large doses of lead, causing intoxication in the body, found an increase in OMP_{370} 1,23 times ($p<0,001$) compared with animals in the control group, OMP_{430}, 1,41 times ($p<0,001$).

When comparing the degree of toxic effects of lead, it was found that in group III animals, compared with II, OMP were higher RP_{370} 1,24 times and OMP_{430} 1,45 times, respectively ($p<0,001$).

Thus, lead acetate causes dose-dependent activation of lipid peroxidation processes in rats.

Serum of rats of group II, which were given low-dose levels of lead that did not exceed the general toxic effect, showed an increase in SOD by 54% ($p<0,001$) compared with animals of the control group, and catalase activity increased 2,5 times.

In animals III, a decrease in SOD by 43% ($p<0,001$) compared with animals in the control group, and catalase activity - by 66,3% ($p<0,001$), respectively.

When comparing the degree of toxic effects of lead on the enzyme system of antioxidant protection of female rats, it

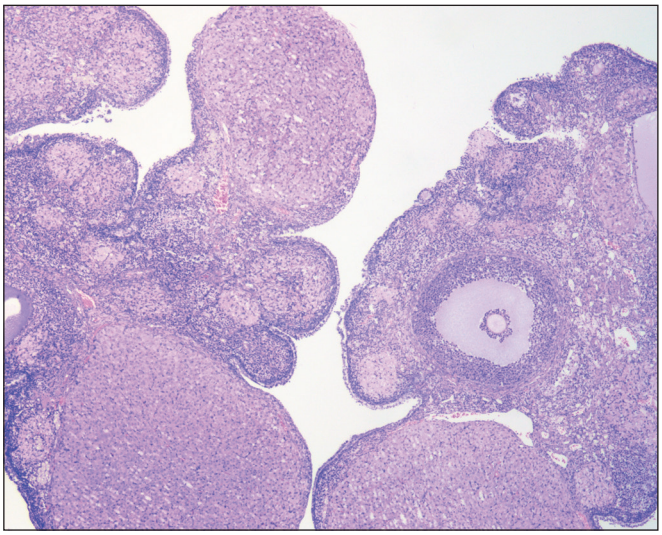

Fig. 1. Structural organization of the ovary of the control animals group. Follicles at different stages of development in the cortex. Stained with hematoxylin-eosin x 40.

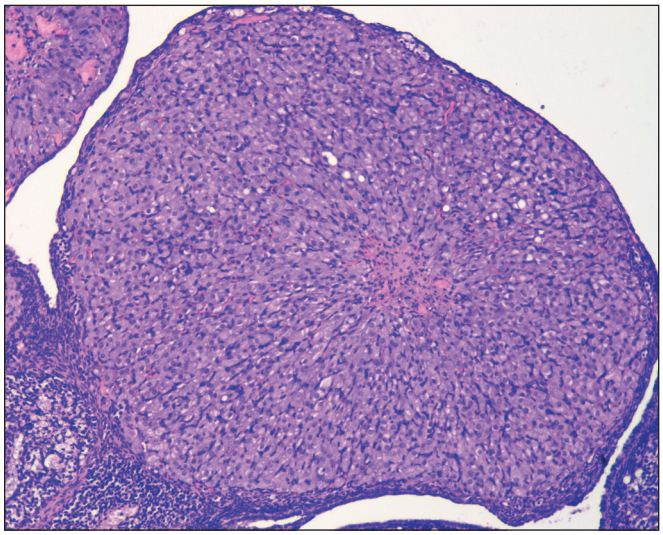

Fig. 2. The structure of the corpus luteum in the cortex of the ovary of the animal under the influence of small doses of lead acetate. Luteocytes in the corpus luteum. Stained with hematoxylin-eosin x 100.

was found that in group III animals, compared with group II, SOD was lower by 63% (p<0,001), catalase activity - by 86,6% (p<0,001).

Thus, lead acetate activates antioxidants only in small doses, and large doses cause inhibition of antioxidant activity.

Since the experiments showed an increase in oxidative processes, it was advisable to study the content of molecules of medium weight (MMW).

It was found that in the second group of animals there were no significant changes in the MMW of any of the studied fractions.

In the third group of animals, compared with the control, there was also an increase in all fractions of MMW: MMW_{238} increased by 67,1% (p<0,001), MMW_{254} – 2,2 times (p<0,001), MMW_{260} – 2,1 times (p<0.001), MMW_{280} – 2,6 times (p<0,001).

It was found that all fractions of MMW increase with increasing dose of lead acetate. Comparing groups II and III, the latter showed higher indicators of MMW_{238} by 60,8% (p<0,001), MMW_{254} – 2 times (p<0,001), MMW_{260} – 2,2 times (p<0.001), MMW_{280} – in 2,4 times (p<0,001).

Therefore, only large doses of lead acetate cause an increase of molecules of medium weight.

Light microscopy has shown that the ovaries are a parenchymal gland with endocrine function, which is realized through the production of sex hormones by theca cells, granulosa follicle cells and the corpus luteum.

Morphological studies of the ovaries in animals of the control group showed that the organ is covered mainly by a single layer of cuboidal epithelium, but there were areas of prismatic and squamous epithelium, on the apical surface of which microvilli are visualized. The results of light microscopy of the ovaries showed that under the epithelium is a tunica albuginea, which consists mainly of collagen and elastic fibers, as well as a small number of smooth myocytes.

The structural organization of the organ is clearly visualized external cortex and internal medulla (Fig. 1).

The cortex of the ovaries of group I surrounds the medulla in the form of a horseshoe. The stroma is formed by connective tissue containing collagen and a small amount of elastic fibers, as well as a large number of fibroblasts (interstitial cells). The parenchyma of the ovaries of rats of group I is represented by follicles of different stages of their maturity (primordial, primary, secondary, tertiary follicles), which were mainly go to physiological atresia, corpus luteum and blood vessels. Primordial follicles were located under the tunica albuginea in the cortex. The medulla is represented by a connective tissue stroma, which contains a large number of elastic fibers, many blood vessels, nerve fibers and nerve endings.

Histological examination of the ovaries of group II rats found that the structural organization of the ovary of experimental animals is almost no different from the control. During this period of the experiment in the cortex and medulla of the ovaries there is a dense network of collagen fibers in tunica albuginea. Light microscopy reveals single primordial follicles that contain an oocyte surrounded by a single layer of squamous epithelial cells. Primary follicles are surrounded by a single layer of cuboidal epithelium. Secondary follicles were characterized by a well-defined transparent shell, the oocyte is surrounded by a multilayered epithelium of cuboidal shape. Also a characteristic feature of secondary follicles is the appearance of a layer of granulosa cells around it, which provides a separate blood supply to the follicle.

Tertiary follicles are also well visualized, which are characterized by the formation of a cavity in the thickness of follicular cells, which is filled with follicular fluid. A mature (preovulatory) follicle has a fully formed cavity with follicular fluid in which the egg floats freely.

However, it was found that in the cortex of the ovaries of rats of group II increases the relative area occupied by

corpus luteum than in control animals. In all animals, the yellow bodies are externally covered with a connective tissue capsule, have a rounded shape. In the thickness of the corpus luteum, connective tissue layers are visualized, in which there are blood and lymphatic vessels. The corpus luteum is based on luteocytes - cells of irregular shape, with basophilic stained nuclei in the center of the cell and eosinophilic cytoplasm, which indicates active steroidogenesis. Luteocytes differ in size depending on the location in the corpus luteum - the periphery is dominated by small, star-shaped cells with a large oval nucleus (Fig. 2).

Morphological studies of ovaries in animals of group III, which were administered high doses of lead acetate, showed that compared with the control, macroscopically there is a decrease in ovarian size, hyperemia, reduction in surface epithelial thickness and tunica albuginea thickness.

Light microscopy examination of ovaries of rats of group III found that the number of follicles in the cortex at all stages of their development and maturation: almost no primary and secondary and tertiary follicles, there is a small number of primordial follicles located on the periphery of the ovarian cortex and single corpus luteum, which are chaotically localized in the parenchyma of the organ.

Thus, with increasing dose of lead acetate, morphological changes in both cortex and medulla of rat ovaries deepen, leading to changes in the thickness of ovarian surface structures and a decrease in the number of follicles, indicating impaired growth and maturation.

DISCUSSION

Harmfulness of plumbum for humans is determined by its significant toxicity and high cumulative capacity [2, 6]. It has pronounced membrane-toxic properties, changes the activity of enzymes and the course of biochemical processes, is capable of material and functional accumulation and causes long-term exposure to long-term negative bioeffects [2, 15]. Lead is a poison with a polytropic mechanism of action, which manifests itself in specific toxic effects on hematopoietic organs, lesions of the central and peripheral nervous system, gastrointestinal tract, cardiovascular and immune systems. It has a detrimental effect on the liver, kidneys, disrupts metabolic processes, including protein synthesis, has gonadal and embryotoxic effects [7, 10, 16].

Studies have shown the damaging effect of lead on the sex and urinary systems, its direct gonadotoxicity and adverse effects on reproductive function [2]. In 49% there is a decrease in the average concentration of sperm, changes in shape and motility at high doses, increases the number of chromosomal aberrations, decreases testosterone in blood plasma, there are violations of sperm structure [4].

The experiment proved the ability of lead to cross the placental barrier at 12-14 weeks of pregnancy. At high blood concentrations, lead has an abortive effect. At low levels of lead in the mother's body, it accumulates in the tissues of the fetus, causing premature birth, low fetal weight. There is evidence of neurological disorders in children born to women whose blood levels were more than 10 mg / dL [4, 7, 8].

At any influences on an organism there is a nonspecific reaction of an organism, processes of free radical oxidation of lipids and proteins are activated. In our experiments we see the growth of LPO products. Such changes were dose-dependent, with high doses of lead acetate there was a significantly greater increase in DC and TBA-active products compared to low doses. The obtained results indicate damage to the lipid layer of cell membranes. Changes in OMP are multidirectional: at a dose of 0.05 mg / kg, their content decreases, at 60 mg / kg - increases.

In our experiments in females with low doses of lead acetate, the MMW decreases, which indicates an adaptive mechanism aimed at preserving cell membrane proteins. This variant of MMW changes is the most appropriate, which prevents DNA damage, as obtained by scientists who did not find abnormalities in fetal development when using small doses of lead acetate. Obviously, at low doses, the reverse primary carbonylation of proteins occurs, and at large - irreversible. Therefore, at high doses, fetal malformations may occur.

Also an important role in reducing the products of WMD play an increase in the activity and content of antioxidants. Therefore, the next step was to analyze the activity of antioxidants. Lead is known to reduce the potency of the glutathione system because it binds to proteins, primarily due to free SH groups [17]. Since the glutathione system under the action of lead acetate has already been well studied, we investigated superoxide dismutase and catalase activity. It was found that at low doses of lead acetate enzyme activity increases. It is possible to think that there is an activation of processes of peroxidation of lipids, but at the same time there is a protection against free radical damage of proteins, and also lipids. The correctness of this conclusion is evidenced by changes in the ovaries, where there is an increase in the number of corpora lutea. These changes can occur under any stress of low and medium intensity, under the influence of any substances, the action of ionizing radiation in small doses. That is, such a reaction is nonspecific. However, they can lead to diseases of adaptation, premature aging of the body. Indeed, histological specimens show an increase in the number of connective tissue fibers, thickening of the ovarian capsule, signs of steroidogenesis. Such changes indicate that the possible development of secondary infertility (on the one hand, the number of corpora lutea increased, which contributes to multiple pregnancies, and on the other - thickening the capsule reduces the possibility of sperm penetration) with prolonged exposure to lead acetate [18, 19]. Given the fact that the sexual cycle in female rats lasts 5 days, and lead acetate was used for 30 days, in terms of human life expectancy - it is six months, which is quite short-lived. Signs of steroidogenesis can be regarded as an imbalance in the work not only of sex steroids, but also in the work of mineral and glucocorticoids, which may indicate a stress response of the body, leading to an imbalance of steroid hormones.

When exposed to large doses of lead acetate, antioxidant enzymes are reduced, which contributes to the activation

of prooxidants and damage to the body. Indeed, the ovaries in rats decreased in size, which may be due to a decrease in hormonal activity. They were hyperemic, indicating the development of inflammation. Signs of dystrophy were noted (the thickness of the germinal epithelium and tunica albuginea decreased). Such changes indicate the development of infertility. At light microscopic examination almost all follicles are absent, there are single corpus luteum which are chaotically localized in an organ parenchyma. Therefore, biochemical changes are confirmed by morphological ones. Given the signs of inflammation in which the cytokine cascade is activated, one can think of the possibility of rapid tumor development, which requires more research.

Thus, the effect of small and large doses of lead acetate on biochemical changes in blood and morphological changes in the ovaries in male rats was studied. The development of oxidative stress, which causes damage, has been established. Under the influence of small doses, the changes are adaptive, and under the influence of large doses - damaging.

CONCLUSIONS

1. Lead acetate causes activation of peroxidation of lipids and proteins in the body of female rats, which is directly dependent on the dose of lead.
2. In response to the activation of free radical oxidation there are changes in the antioxidant system, which depend on the dose of lead acetate: at a dose of 0.05 mg / kg superoxide dismutase and catalase activity increase, at a dose of 60 mg / kg superoxide dismutase and catalase activity.
3. Small doses of lead do not cause endogenous intoxication. Lead acetate causes the development of endogenous intoxication in animals only in large doses: increases the formation of toxic compounds, cell apoptosis, decreased excretory function of the kidneys, which is associated with multiorgan disorders.
4. As a result of the action of lead acetate, morphological changes of the ovaries were observed, which increased with increasing dose of lead acetate. There was a dose-dependent decrease in massometric parameters, the number of follicles and changes in the thickness of the surface structures of the ovary, which is more pronounced at 60 mg/kg.

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The Authors declare no conflict of interest

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