



The level of lipid peroxidation products in the rats blood under prolonged cadmium and lead loading

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Abstract

Lipid peroxidation is a form of tissues respiration. This process is characteristic of normal tissues and occurs, as a rule, after the construction of lipid membrane structures, their updates and during the biosynthesis of many hormones. However, free radical oxidation can be activated in an unfavorable environmental situation, since in our case it happened under the action of Cadmium and Lead. The purpose of the work was to investigate the Cadmium and Lead effects on the lipid peroxidation processes intensity in rats. The experiments were carried out on 200 – 220 g male “Wistar” rats, from which 4 groups of animals were formed: 1) control group – animals were administered drinking water through a metal probe in bulk, which is equivalent to the volume of aqueous salt solution Cd^{2+} i Pb^{2+} ; 2) experimental group 1 – animals were administered 0.029 % an aqueous solution of cadmium chloride in a dose 4.0 mg/kg; 3) experimental group 2 – animals were administered 16.6 % aqueous lead acetate solution at a dose 200 mg/kg; 4) experimental group 3 – animals were administered 16.6 % aqueous lead acetate solution at a dose 100 mg/kg and 0.029 % an aqueous solution of cadmium chloride in a dose 2.0 mg/kg. Throughout the experiment, rats were kept in a balanced diet containing all the necessary components, the animals were given drinking water without restrictions from 0.2 liter glass bowls. Based on our research, we detected activation of lipid peroxidation (LPO) products in the blood of rats under lead-cadmium loading, as indicated by the growth of intermediate and final products in comparison with the group of intact animals. Probable level increase of LPO products was observed from the first day of the experiment. For the 7th day of the experiment, the level of diene conjugates in the blood of the third experimental group increased by 88.9 %, and the level of Thiobarbituric acid reactive substances (TBARS) increased by 31.8 %. At 14 and 21 days of the experiment, the level of of LPO products in the rats blood under the lead-cadmium load was the highest. These changes in the LPO products level indicate an increase in the intensity of radical formation processes. Peroxide oxidation forms, at almost all stages of its course, a number of products that result from the interaction of free radicals with each other and with biological macromolecules.

Key words: toxicology, cadmium, lead, rats, diene conjugates, TBARS-active products.

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1. Introduction

The problem of environmental pollution by Cadmium and Lead, which is one of the consequences of the intensification of industrial and agrarian production, has become especially relevant now (Ali et al., 1986; Myslyva, 2013; Hutyi, 2015; Gutyj et al., 2018; 2019). The growth of these metals content in Ukrainian soils and other countries over the last decades has been accompanied by the accumulation of Cd^{2+} and Pb^{2+} in agricultural products and feed, thus increases the threat to human and animal health (Myslyva, 2013; Lavryshyn & Gutyj, 2019; Kuras & Ersteniuk, 2019).

The results of many important experimental studies indicate that Cadmium and Lead have a toxic effect on a number of organs and systems in the body of mammals (Marushko et al., 2010; Petrynych et al., 2017; Ostapyuk & Gutyj, 2018; 2019). Lead and Cadmium are easily associated with

thiol groups of apoenzymes when released into the body of animals, and thus switch the activity of the antioxidant protection, microsomal and other enzymatic systems, affect the exchange of macro and microelements, have a carcinogenic effect (Al-Azemi et al., 2010; Stepanchuk, 2014; Lavryshyn et al., 2018; 2019).

Although there are many works in the literature on the mechanisms of Lead and Cadmium negative effects on the organism of laboratory and farm animals, however the question of functional changes and the state of the antioxidant system in liver cells under the combined effects of these metals remains relevant.

The purpose of the work was to investigate the Cadmium and Lead impact on the intensity of lipid peroxidation processes in rats.

2. Materials and methods

The experiments were carried out in 200–220 g male “Wistar” rats, from which 4 groups of animals were formed:

1 – control group – animals were administered drinking water through a metal probe in bulk, which is equivalent to the volume of aqueous salt solution Cd^{2+} i Pb^{2+} ;

2) – experimental group 1 – animals were administered 0,029 % an aqueous solution of cadmium chloride in a dose 4.0 mg/kg;

3) – experimental group 2 – animals were administered 16.6% aqueous lead acetate solution at a dose 200 mg/kg;

4) – experimental group 3 – animals were administered 16.6% aqueous lead acetate solution at a dose 100 mg/kg and 0.029 % an aqueous solution of cadmium chloride in a dose 2.0 mg/kg.

Throughout the experiment, rats were kept in a balanced diet containing all the necessary components, and were given drinking water without restrictions from 0.2 liter glass bowls.

All animal manipulations were carried out in accordance with the European Convention on the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1986).

Blood for biochemical studies was collected under ether anesthesia from the jugular vein on 1, 7, 14, 21 and 28 days of the experiment. The level of diene conjugates and TBARS active products was studied by the method (Vlizlo, 2012).

The Statistica 6.0 software package was performed for analysis of the research. The reliability of difference between the groups was assessed by Student's t-test. The results were considered plausible at $P \leq 0.05$.

3. Results and discussion

Lipid peroxidation (LPO) is one of the most important oxidation processes in the animal body. Actually LPO is considered to be one of the major causes of cell damage and

death due to the action of reactive oxygen species (ROS) and free radicals. It is known that any sufficiently powerful environmental impact on the animal body can initiate processes of LPO. It is established that the LPO processes in the rats body under cadmium loading enhanced, this is indicated by an increase in the level of intermediates and end LOPs, specifically diene conjugates and TBARS active products (Table 1).

Credible increase of diene conjugates was noted on the first day of the experiment in the blood of the first experimental group, in which this investigated indicator raised up by 20.6 % compared to the control group. Subsequently, the level of DC continued to grow and accordingly grew up by 85.5 % on the 7th day, 2.1 times on the 14 th day in the rats of this group. And for the 21st day of the experiment, the level of the investigated indicator increased to 1.015 ± 0.023 c/u./ml.

Similar changes are observed in the study of the TBARS-active products level, which also increased for 1 day of the experiment. At the 7th day of the experiment, the level of the final product of the LPO credible raised by 30.4 %, and by the 14th day – by 56.8 % relative to the control. The level of TBARS-active products increased to 11.12 ± 0.22 $\mu\text{mol/l}$ at the 21st day of the experiment, whereas in the control this indicator was equal to 6.41 ± 0.10 $\mu\text{mol/l}$.

The study of the effect of lead on the rats of the second experimental group showed an increase in the level of diene conjugates and TBARS-active products in comparison with the control group for 1 day of the experiment by 19.0 and 11.7 % (Table 2). At the 7th day of the research, the intermediate and final products of LPO were credible raised by 76.1 and 23.6 %, respectively. The level of diene conjugates and TBARS-active products in the blood rats of the second experimental group increased to 0.986 ± 0.020 c/u/ml and 10.41 ± 0.20 $\mu\text{mol/l}$ for 21 days of experience. For the 28th day of the experiment, the level of LPO products remained high.

Table 1

The level of LPO products in the rats serum under cadmium loading ($M \pm m$, $n = 6$)

Indicators	Groups					
	Control	1 experimental; experiment day				
		1	7	14	21	28
DC, c.u/ml	0.461 ± 0.011	0.556 ± 0.013 ***	0.855 ± 0.020 ***	0.970 ± 0.019 ***	1.015 ± 0.023 ***	0.779 ± 0.016 ***
TBARS-active products, $\mu\text{mol/l}$	6.41 ± 0.10	7.30 ± 0.12 ***	8.36 ± 0.17 ***	10.05 ± 0.24 ***	11.12 ± 0.22 ***	8.68 ± 0.19 ***

Table 2

The level of LPO products in the rats serum under lead loading ($M \pm m$, $n = 6$)

Indicators	Groups					
	Control	2 experimental; experiment day				
		1	7	14	21	28
DC, c.u./ml	0.461 ± 0.011	0.549 ± 0.011 ***	0.812 ± 0.019 ***	0.943 ± 0.017 ***	0.986 ± 0.020 ***	0.732 ± 0.016 ***
TBARS-active products, $\mu\text{mol/l}$	6.41 ± 0.10	7.16 ± 0.14 **	7.92 ± 0.15 ***	9.75 ± 0.24 ***	10.41 ± 0.20 ***	8.13 ± 0.14 ***

The highest levels of diene conjugates and TBARS-active products, were established with the combined use of Cadmium and Lead compared to the first and second study groups (Table 3). Credible growth in the level of LPO products has been observed since the first day of the experiment. The level of diene conjugates in the blood of the third experimental group grew up by 88.9 %, and the level of TBARS-

active products – by 31.8 % for 7th day of the research. At the 14th and 21st day of the experiment, the level of LPO products in the blood of rats under lead-cadmium load was the highest and fluctuated, respectively, within the diene conjugates of 0.986 ± 0.011 and 1.026 ± 0.021 c.u./ml and TBARS-active products – 10.23 ± 0.24 and 11.29 ± 0.20 $\mu\text{mol/l}$.

Table 3

The level of LPO products in the rats serum under lead-cadmium lead loading ($M \pm m$, $n = 6$)

Indicators	Groups					
	Control	3 experimental; experiment day				
		1	7	14	21	28
DC, c.u./ml	0.461 ± 0.011	$0.549 \pm 0,019$ ***	$0.871 \pm 0,022$ ***	0.986 ± 0.011 ***	1.026 ± 0.021 ***	0.812 ± 0.015 ***
TBARS-active products, $\mu\text{mol/l}$	6.41 ± 0.10	7.36 ± 0.10 ***	8.45 ± 0.18 ***	10.23 ± 0.24 ***	11.29 ± 0.20 ***	8.87 ± 0.16 ***

At the 28th day of the research, the level of LPO intermediate products in the blood of rats of the third experimental group increased by 76.1 %, and the level of TBK-active products – by 38.4 % relative to the control group.

Accordingly, introduction of Cadmium and Lead salts into the body leads to the accumulation of both intermediate and final LPO products. These changes in the level of LPO products indicate an increase in the intensity of radical formation processes. Peroxide oxidation forms, at almost all stages of its course, a number of products which result from the interaction of free radicals with each other and with biological macromolecules.

Lipid peroxidation is a form of tissue respiration. This process is characteristic of normal tissues and occurs, as a rule, after the construction of lipid membrane structures, their updates and during the biosynthesis of many hormones (Martyshuk et al., 2016). However, free radical oxidation can be activated in an unfavorable environmental situation, since in our case it happened under the action of Cadmium and Lead.

It is known that free radical lipid peroxidation is an important stage of the body's damage by heavy metals, and the newly formed products of this process (hydroperoxides and peroxides of organic substrates) cause a pronounced damaging effect on cell membranes and activate lipid peroxidation in them (Lavryshyn et al., 2016). This leads to disruption of the structure and integrity of the membranes, and also causes changes in the system of acid-base status of the blood with the subsequent activation of lipid peroxidation, which is accompanied by changes in the functioning of ion-transport systems.

Excessive activation of LPO processes leads to disruption of the structure of membranes, lipid metabolism, has a toxic effect on tissues, promotes oxidation of sulfhydryl groups of proteins, enhances lysis, leads to the development of structural changes after diseases of the cardiovascular, respiratory and digestive systems (Zhukova et al., 2016; Martyshuk et al., 2016).

Based on our research, we detected the activation of LPO in the blood of rats under lead-cadmium loading, which indicates an increase in intermediate and final products in comparison with the group of intact animals (Lavryshyn et al., 2016). Possibly, such changes may be due to the fact that in the mechanisms of toxic action of cadmi-

um and lead an important role is played by stimulating the formation of free radicals and active forms of oxygen, resulting in a balance between the content of oxidants and antioxidants (Hutyi, 2015).

The increase in LPO processes intensity found in our experiments is apparently related to the development of hypoxia, which is dominated by anaerobic processes of metabolism in tissues.

4. Conclusions

A series of experiments allowed to establish a significant disturbance of the oxidation-antioxidant equilibrium in animals under the conditions of lead-cadmium loading, which is characterized, first of all, by the activation of the processes of free radical oxidation of lipids with excessive accumulation of both intermediate and final floor products contents. Therefore, it was found that the modeling of lead-cadmium toxicosis in rats of the third experimental group leads to a significant increase in the content of diene conjugates and TBARS-active products in the blood of animals in 2.23 and 1.59 times compared with intact animals.

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