MODELING THE EFFECTS OF LEAD POLLUTION ON LACTATE DEHYDROGENASE ACTIVITY

Olena Konovalova
PhD, V. N. Karazin Kharkiv National University,
e-mail: o.o.konovalova@karazin.ua, http://orcid.org/0000-0002-6681-4088, Ukraine

Abstract. The changes of activity of LDH and its isoenzymatic fractions under load with lead acetate are described. The introduction of a subacute dose of Pb^{2+} led to a decrease in the LDH activity of the rat liver and an increase in the heart. Preliminary introduction of small doses of Pb^{2+} partially prevents increased LDH activity in the heart and completely inhibits its increase in the liver. The proportion of urea-stable fraction in the heart and blood serum is significantly increased, indicating an increase in the aerobic direction of energy metabolism. The obtained results are the experimental substantiation of further investigations of influence mechanism of heavy metals on the organism, as well as the motivation to prevent environmental pollution by man-made xenobiotics.

Keywords: heavy metals, lead, manganese, lactate dehydrogenase.

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Introduction

Technogenic pollution of the environment is becoming a global problem of the present, requiring urgent measures. Most industrial emissions are a mixture of toxic substances, in particular heavy metals.

Currently 80% of Ukraine's population lives in dangerous or potentially dangerous areas, causing them a number of diseases that are related to environmental pathologies, in particular caused by such environmental factors as high content of heavy metals and radionuclides in the environment. Chemical pollution of nature negatively affects the vital functions of living organisms and, above all, human health. Knowledge of the causes and patterns of this influence allows to prevent possible negative processes that cause disease of the organism. Regions of residence, where the excess of maximum permissible concentrations of Pb is observed, are simultaneously characterized by deterioration of the health status of the population, an increase in the morbidity index.

The analysis of the composition of waters and soils in the Kharkiv region carried out by us in previous studies has shown that the soil from surveyed settlements has an excess of lead and zinc in drinking water, in addition, the manganese content significantly exceeds the MPC. Thus, in areas of the Kharkiv region, lead and manganese are the predominant pollutants of drinking water *(Konovalova, Andreiko, 2018)*.

The negative effects of these heavy metals on health, from the embryonic stage of development, and the linkage of environmental degradation, are being studied by scientists from different countries *(Sanders et al., 2014; Ashley-Martin et al., 2018)*. At the same time, the mechanisms of both toxic and physiological action of lead to the present time are studied rather weakly and revealed much worse than for other trace elements.

The disclosure of the mechanisms of the influence of xenobiotics, in particular lead, on the living organism remains an actual problem of the present. Therefore, it is necessary to

study the mechanism of adaptation rearrangements in the body under the influence of heavy metals in order to develop promising methods for the further prevention of negative effects on the health of technogenic pollution of the environment. Such studies are carried out using model animal objects and the patterns of loading of these organisms by heavy metals *(Lu et al., 2018).*

One of the recognized models used for diagnosis of health by G. L. Apanasenko is the assessment of health by the level of bioenergy. At the same time, the level of health is considered to be higher in the conditions of implementation of the energy link of metabolism to the aerobic way. One of the important enzymes for energy metabolism is lactate dehydrogenase (LDH, EC 1.1.1.27), which is why this enzyme was chosen as a marker for health assessment at the molecular level. This topic in our time is very relevant, since numerous studies devoted to the analysis of the action of lead on the body do not take into account its influence on this enzyme of energy metabolism. Also, the study of the influence of lead on the activity of enzymes will not only deepen knowledge about the mechanism of its action, but also will allow to predict the changes caused by it in the state of the organism.

Model study of LDH activity in serum of blood, liver and heart of experimental animals in conditions of lead load modeling

Experimental animals were used to simulate the influence of the leading ecological factors of the eastern regions of Ukraine. The selection of animals for the construction of the model is due to the need to take into account the physiological and biochemical peculiarities of the organism of the experimental animals, as well as the nature of the effect of the investigated element. The work was performed on 24 male Wistar male rats, weighing 192-276 g, 3 months old, who were in the standard vivarium of V. N. Karazin Kharkiv National University with free access to water. Experimental studies were conducted in the spring (March - April), the impact was carried out at the same time - 9 - 10 hours in the morning. Two models of lead load were used. Animals were divided into 3 groups. First group (7 individuals) - intact. The second (9 individuals) - the animals received a single intra-muscle solution of lead acetate (Pb $(CH_3COO)_2 \times 3H_2O$) with an equivalent amount of lead 62.5 mg per 1 kg of the weight of the rat (62.5 mg / kg). The third (8 individuals) - a group of double load PbAc - was injected on the first day of a solution of lime acetate with an equivalent amount of metal ions of 15.5 mg / kg, and on the seventh day, the solution was re-injected with an equivalent amount of metal ions 62.5 mg / kg. At the 8th day, the animals were decapitated under ethereal anesthesia. All procedures were performed in compliance with the bioethical principles of working with laboratory animals in accordance with the World Medical Association Declaration of Helsink (Ethical Principles for Medical Research) after decapitation, blood was collected from the rats, then serum was isolated from it by a 10-minute centrifugation at 1500 rpm to detect the activity of enzymes. To determine LDH activity, homogenates of liver and heart samples were also used in phosphate buffer pH 7.4. Determination of LDH activity was carried out using the colomotor dinitrophenylhydrazine method for Sewell and Tovarek using reagents from the firm "Filisit-diagnostika" (Sevela, Tovarek., 1959). The concentration of total protein in serum and liver homogenates was carried out by the method of O. Lowry in the modification of G. L. Miller *(Miller, 1959)*.

All obtained laboratory and instrumental studies were entered into a computer database created in the Microsoft Exel program. The results of the study were processed using a personal computer using the SPSS 15.0 "for Windows" static software package. The methods

of descriptive statistics determined the numerical characteristics of the indicators (mean, mean square deviation, mean error and correlation coefficients, etc.). The calculation of the main statistical indicators was carried out according to the direct quantitative data obtained as a result of the research (arithmetic mean value - M, standard error of the arithmetic mean - m). Statistical analysis was performed using Student's criterion (t). For work, a computer based on Intel Core 2 Duo operating system Windows XP (Microsoft, USA) was used. The construction of graphs and statistical processing of data were carried out using SPSS 15.0 for Windows and Microsoft Excel (2003). Likely differences were found in which the probability of statistical error is P <0.05.

The obtained data on the action of various doses of lead acetate on the activity of lactate dehydrogenase and its urea-stable fraction in organs and tissues of experimental rats are shown in tables 1 - 3.

Table 1

Activity of LDH in blood serum of rats under the action of lead acetate, μmol / h × l (M ± m)

Group	Total LDH	Urea-stable LDH fraction	% urea-stable fraction from the total
Intact	0,153±0,043, n=6	0,090±0,034, n=4	58.8
One dose	0,154±0,033*, n=8	0,050±0,014, n=4	32.5
Two dose	0,153±0,04*, n=7	0,136±0,025#, n=5	88.9

Note: * - statistically significant difference of Student t - criterion at the level $p \leq 0.05$; # - there is a tendency towards changes in the intact group at the level $p \leq 0.1$; ¤ - there is a tendency to change relative to another dose at the level $p \leq 0.1$.

As can be seen from Table 1, in the study of LDH activity in serum, no significant changes were observed between the studied groups. Perhaps this is a compensatory mechanism for ensuring the energy needs of the organism. A similar picture occurs when studying the activity of LDH and its urea-stable fraction in the liver of rats at the administration of a double dose of Pb^{2+}, possibly for the same reason.

The total activity of LDH in the serum of blood, liver and heart of the experimental animals was at the same level, however, as shown in Tables 1 to 3, the proportion of urea-stable fraction in serum was 58.8%, liver 26.7%, and heart 65%, which corresponds to literary data and shows that urea-stable LDH5 is inherent in the heart.

It is known that the activity of the urea-stable fraction best reflects the state of the heart. Increasing activity of LDH occurs mainly due to increased LDH1 and LDH2 content. This may indicate a toxic effect of lead acetate on the myocardium, especially since the urea-stable LDH fraction is a marker of energy metabolism of the myocardium. Yes, it is the activity of urea-stable fraction of LDH increased in acute myocardial infarction.

The activity of LDH in serum also increases with anemia, malignant neoplasms, hepatitis, obstructive jaundice, liver cirrhosis, various diseases of the kidneys, musculoskeletal system, myocardial infarction, lung lesions, as well as any damage to cells resulting in cytolysis and loss of the cytoplasm (this enzyme easily passes into the blood plasma in non-fibrotic processes in cells).

The introduction of a single dose of lead acetate to rats did not cause changes in the total activity of LDH in serum, but led to a decrease in the activity of urea-stained fraction of LDH by 44.4% compared with control, a tendency to decrease is observed and relatively acute dose. It is difficult to interpret this fact clearly, but it is possible to assume the toxic effect of lead on the one hand and the priority of synthesis of ATP in tissues, and not in serum from another. That is, the body works on "economical mode" under the conditions of intoxication.

n the case of double administration of lead acetate, there was no such reduction in the activity of urea-stable fraction of LDH, on the contrary, there was a tendency to increase the activity of intact group by 51.1%, which may indicate an adaptation of enzyme systems of the organism under conditions of chronic intoxication. This may also be due to the launch of the mechanisms of hormoses - the previous introduction of heavy metal animals in a small dose forms resistance to these animals to a further large, even lethal dose.

The proportion of urea-stable LDH fraction from the total was 58.8% in the intact group, 32.5% in the one-time lead acetate lead management and 88.9% in the group of two-time administration of Pb^{2+}. Thus, the proportion of urea-stable fraction of LDH in serum after administration of lead significantly increases, which indicates an increase in aerobic direction of energy metabolism.

Table 2

Activity of LDH in the liver of rats following the action of lead acetate, nmol/h/mg of protein (M ± m)

Group	Total LDH	Urea-stable LDH fraction	% urea-stable fraction from the total
Intact	0,015±0,0044, n=5	0,0040±0,00204, n=4	26.7
One dose	0,011±0,0054# ¤, n=7	0,0031±0,0011#, n=6	28.2
Two dose	0,016±0,0076# ¤, n=7	0,0036±0,00103#, n=5	22.5

Note: * - statistically significant difference of Student t - criterion at the level $p \leq 0.05$; # - there is a tendency towards changes in the intact group at the level $p \leq 0.1$; ¤ - there is a tendency to change relative to another dose at the level $p \leq 0.1$.

All metals, regardless of their biological significance, for excessive intake of the animal and humans cause toxic effects that manifest themselves in disturbing the biochemical processes and physiological functions in the body. It is known that the liver is the main organ that performs the detoxification function in the body, and the accumulation of lead in it can be caused by its binding to metallothioneins, a significant amount of which is found in the liver.

With single administration of lead acetate in rats, there is a tendency to decrease the activity of total LDH in the liver relative to the intact group by 26.6% compared with control. In case of double administration, changes in the activity of the total LDH in the liver relative to the intact group are not observed.

This may indicate a toxic effect of lead on the enzymatic system, which is a natural barrier to toxic substances and the inhibitory effect of heavy metals on enzymes.

A single injection of lead acetate to rats reduced the activity of urea-stable fraction of LDH in the liver by 22.5% compared with control, a decrease in the trend was observed for two-way administration, albeit to a lesser extent. The percentage of urea-stable fraction of

LDH from the total was in the intact group - 26.7, in the group of one-time lead acetate treatment - 28.2 and in the group of two-time administration of Pb^{2+} -22.5, that is, the introduction of lead did not change the isoenzyme spectrum in the liver of rats.

Table 3

LDH activity in the rat heart following the action of lead acetate, nmol/h/mg protein (M ± m)

Intact	Total LDH	Urea-stable LDH fraction	% urea-stable fraction from the total
One dose	0,12±0,038, n=7	0,078±0,011, n=5	65
Two dose	0,21±0,072, n=9	0,143±0,036#, n=7	68.1
Intact	0,151±0,045, n=8	0,143±0,032#, n=6	94.7

Note: * - statistically significant difference of Student t - criterion at the level $p \leq 0.05$; # - there is a tendency towards changes in the intact group at the level $p \leq 0.1$; ¤ - there is a tendency to change relative to another dose at the level $p \leq 0.1$.

One-time administration of single-dose lead acetate to rats has led to an increase in the activity of total LDH in the heart by 75% compared to control. Two-fold injection of lead partly prevented this increase - there was an increase in the activity of the general LDH relative to the intact group by 25.8%.

The growth of LDH activity in the heart of rats for action of lead may indicate a shift in the equilibrium of the reaction towards the formation of pyruvate, which contributes to the transformation of the latter in the cycle of tricarboxylic acids.

The activity of urea-stable fraction of LDH in the heart, both in single and double cases, when administered with lead acetate, is increased by 80 compared with control.

The proportion of urea-stable fraction of LDH from the total was in the intact group - 65%, in the group of one-time lead acetate-68.1%, and in the group of two-time administration of Pb2 + - 94.7%. The proportion of urea-stable fraction in the heart increases significantly, which indicates an increase in the aerobic direction of energy metabolism.

The proportion of urea-stable fraction in intact animals in the blood serum was 58.8%, the liver 26.7%, and the heart - 65%, which corresponds to literary data and suggests that urea-stable LDH5 is inherent in the heart.

Analyzing results from Tables 1 -3 it can be noted that the whole enzyme system of the body of rats changes its activity under the influence of lead acetate, indicating its toxic effects.

The obtained results are an experimental substantiation of further investigations of the negative influence of heavy metals on the organism and the motivation to prevent environmental contamination by man-made xenobiotics.

Conclusions and suggestions

1. The total activity of LDH in the serum of blood, liver and heart of the experimental animals was at the same level, however, the proportion of urea-stable fraction in serum was 58.8%, the liver 26.7%, and the heart - 65%, which corresponds to the literary data and indicates , that the urea-stable LDH5 is inherent to the heart.

2. The introduction of a subcutaneous dose of Pb2 + led to a decrease in the LDH activity of the liver of rats and an increase in the heart, indicating a restructuring of the metabolism to the aerobic in adaptation to lead loading. The proportion of urea-stable fraction remained unchanged in serum of blood and heart but decreased in the liver, indicating a decrease in the release of lactate dehydrogenase from the heart.

3. Pre-administration of small doses of Pb2 + partially prevents the increase of LDH activity in the heart and completely inhibits its elevation in the liver, indicating an improvement in adaptation in animals that received a low dose of lead to the substrate. The proportion of urea-stable fraction in the heart and blood serum is significantly increased, indicating an increase in the aerobic direction of energy metabolism. The obtained results are the experimental substantiation of further studies to prevent the negative influence of heavy metals on the body.

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