THE ACTIVITIES OF THE ASPARTATE AMINOTRANSFERASE, ALANINE AMINOTRANSFERASE AND ALKALINE PHOSPHATASE ENZYMES IN THE BLOOD SERUM OF RATS IN CONDITIONS OF CHRONIC LEAD POISONING

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Abstract

Aim: The objective of this study was to analyze the activities of the Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) enzymes in the blood serum of rats in conditions of chronic lead poisoning.

Material and Methods: Research included 40 female rats of the strain, weighing about 150-200 g, and 4-5 months of age and 10 young offspring of the above females, 1.5 months old and about 80-100 g of weight. Lead in the form of lead – acetate Pb $(CH_3COO)_2$ was given to female rats orally, by means of water.

Results: In conditions of chronic lead poisoning of female rats and their offspring a significant increase in the activities of AST, ALT and ALP enzymes in the blood sera of experimental groups in relation to the control ones was determined. A higher dose of lead resulted in a more significant increase of ALP activity in the blood sera of female rats.

Conclusion: The activities of AST, ALT and ALP enzymes in the blood serum of young rats were significantly increased in conditions of chronic lead poisoning of their mothers during periods of pregnancy and lactation, but dependence on the dose of lead being received by their mothers was not established.

Keywords: Lead, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), chronic lead poisoning.

Introduction

Lead is a toxic metal that can be found everywhere in the environment. Overexposure to lead continues to be an important worldwide problem. Food is an important source of lead and determination of lead in food can be used for the estimation of lead exposure. The level of lead in the Earth's crust is about 20 μg/g. In areas where leaded gasoline is banned, the major exposure pathways of nonsmoking adults are from food and water. Several studies were done to determine the concentration of lead in foods [1, 2] and to study its dangerous effects. Recently, the USA, all European countries and many developing countries have outlawed or strictly regulated the use of leaded petrol. In such countries, levels of lead in food and drinking water are closely monitored. Lead may reach and contaminate plants, vegetables, fruits and canned food through air, water and soil during cultivation and also during industrial processing and packaging. Fruits and vegetables grown in polluted soils may become contaminated as a result of plant uptake of lead from soils or direct deposition of leaded dust onto plant surfaces. Therefore, through these diverse mechanisms, lead deposited in soil becomes a persistent and long-term source of lead exposure for humans.

Lead is ubiquitous in the environment, persists indefinitely, and can be found at low levels in almost all living organisms [3]. Sources of lead contamination of air, water, and soil include internal combustion engines, oil burners, smelters, lead pipes, glass and alloy processing plants, incinerators, industrial effluents, and smokestack fallout [4]. Lead is found in the soil, plants and grains grown on contaminated soil, and tissues of animals that eat contaminated plants and feed grains [5]. Because of widespread environmental exposure, low levels of lead can be demonstrated in tissues of clinically normal birds and animals [3] Lead toxicosis occurs when an animal or a bird inhales or ingests a concentrated source of lead. Concentrated lead sources include lead-based paint, lead arsenate crop sprays, lead plates in automotive batteries, fishing sinkers, lead shotgun pellets, drapery weights, sewage sludge, and lead mine tailings [6].

Toxicity of lead is expressed *inter alia* by changes in the activities of certainenzymes. Namely, lead can directly destroy the enzyme structure by inter-reactingwith bioelements built in the

metalloenzymes as in the case of aminolevulinic acid dehydratase (ALAD) which enables the process of HEM biosynthesis and subsequently results in the occurrence of anemia [7, 8, 9, 10]. In addition to this, when exposed to increased lead concentrations, the activities of certain enzymes can be disturbedas a direct consequence of damages to the tissues and organ cells [1, 9, 11, 12].

This study investigates the effects of chronic lead poisoning on the activitiesof alanine and aspartate aminotransferases (ALT and AST) and alkalinephosphatase (ALP) in the blood sera of female rats and their off spring.

The aim of this study was to analyze the activities of the AST, ALT and ALP enzymes in the blood sera of rats in conditions of chronic lead poisoning. Theabove aim was fulfilled by defining the activities of the above enzymes in the bloodsera of female rats and their offspring in conditions of chronic lead intoxicationand in relation to the control group, the period of intoxicationandleaddose.

Aspartate aminotransferase (AST) is found in high concentrations in liver, heart, skeletal muscle and kidney. AST is present in both cytoplasm and mitochondria of cells. In cases involving mild tissue injury, the predominant form of AST is that from the cytoplasm. Severe tissue damage results in more of the mitochondrial enzyme being released. High levels of AST can be found in cases such as myocardial infarction, acute liver cell damage, viral hepatitis and carbon tetrachloride poisoning. Slight to moderate elevation of AST is seen in muscular dystrophy, dermatomyositis, acute pancreatitis and crushed muscle injuries.

Alkaline phosphatase (ALP) is present in a number of tissues including liver, bone, intestine, and placenta. Serum ALP is of interest in the diagnosis of 2 main groups of conditions-hepatobiliary disease and bone disease associated with increased osteoblastic activity.

Materials and Methods

The survey used a total of 50 female rats of which 40 weighed 150-200 g at the age of 4-5 months and 10 young female rats weighing 80-100 grams and about 1.5 months old. The room where theexperimental rats were kept wasconstantly monitored for temperature, humidity and brightness. In terms of nutrition, the rules applying to feeding and breeding laboratory animals were respected.

Lead intoxication of the animals was performed using lead acetate Pb $(CH_3COO)_2$, orally, by adding it to drinking water. Each female was kept in a cage and the amount of water that was drunk was measured every day by changing the amount of water in the flask. Thus a specific concentration of lead acetate was received through drinking water. The selection of the lead acetate for chronic lead intoxication of experimental animals was made because of its good solubility and thus better absorption in the digestive tract in comparison to other species. The procedure of oral receptionof lead was chosen because of its simplicity, as, besides breathing, a natural way of intoxicationwith lead from the external environment is digestion of polluted water and food.Lead in the form of lead acetate was givenin the concentration of 30 and 100 mg/kg body weight daily over a period of 60 days. These amounts of lead acetate are considered sub toxic doses. Regardless of the fact that they were being increased, they caused no toxic effects on experimental animals.

The experiment was conducted during a period of 60 days. Female rats were treated with lead acetate ten days before mating, seven days during mating, 21 days during pregnancy, and 21 days during breastfeeding. In addition to the determination of the activity of enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in the blood serum, the survey was designed to determine lead content in some tissues and organs as a result of absorption and deposition of lead.Young experimental rats were separated from their mothers after childbirth and put in separate cages. They have not been treated with lead acetate because in them the lead content that they received from their mothers was determined during breastfeeding through milk and during pregnancy through the placenta.

Blood was taken from adult female experimental rats to determine the activity of enzymes - AST, ALT and ALP. The activity of the enzymes AST and ALT was measured by the spectrophotometric method (UV test), while the activity of ALP was determined by automated spectrophotometric analyzer - Hitachi 911.

The results obtained were statistically processed by the mean value (X), standard deviation, standard error, students’t-test, and variance analysis (ANOVA).

Results

Table 1 shows the results of the activity of AST, ALT and ALP in the blood serum of female rats that were chronically intoxicated daily with lead acetate with a concentration of 100 mg / kg BM per day. Compared to the control group, the value of the three enzymes was increased.

The statistical processing of the results showed that the concentration of the administered dose of lead strongly affected ALP activity in the blood serum of female rats. Namely, when female rats were treated with a higher concentration of lead acetate (100 mg / kg BM daily) it resulted in a higher increase in ALP activity in the blood serum of female rats in relation to lower dose (30 mg / kg, BM daily) at equal intervals (20, 30, 40, 50 and 60 days) (Table 1 and Table 2.)

Table 1. Activity of AST, ALT and ALP (mean value ± SD U/L) in blood serum of female rats treated with lead acetate (100 mg/kg BM per day) in relation to the controlgroupand the period of intoxication

	AST	ALT	ALP
Controlgroup	35.89	39.98	89.86
10 days	112.45	55.98	115.53
20 days	124.21	62.34	143.22
30 days	130.62	86.93	167.52
40 days	145.72	99.37	186.19
50 days	152.22	124.66	201.13
60 days	167.12	129.42	227.15

Table 2 shows average values of AST, ALT and ALP in the blood serum of female rats that were treated with a lower concentration of lead acetate (30 mg / kg BM per day). Compared to the control group, there is significant increase in the activity of examined enzymes (AST, ALT and ALP), but these results are lower compared to the results that were obtained in the treatment of female rats with higher concentrations of lead acetate (100 mg / kg BM per day). Compared to the control group which was not treated with lead acetate, in experimental groups there was an increase in the activity of AST, ALT and ALP from 2 to 5 times. This fact indicates that there is a positive correlation between the level of the applied concentration of lead acetate in relation to the measured values of the activity of AST, ALT and ALP.

Table 2. The activity of AST, ALT and ALP (mean value ± SD U/L) in the blood serum of female rats treated with lead acetate (30 mg / kg BM per day) compared to the control group and the period of intoxication

	AST	ALT	ALP
Controlgroup	35.89	39.98	89.86
10 days	92.21	42.12	92.83
20 days	110.68	49.90	118.45
30 days	115.98	57.44	134.22
40 days	124.27	71.10	157.78
50 days	129.35	95.58	172.12
60 days	134.32	110.13	198.59

One of the aims of the experiment was to examine the activity of enzymes in the blood serum of those young rats whose mothers were exposed to chronic lead poisoning (100 mg / kg BM per day and 30 mg / kg, BM per day) during the periods of pregnancy and lactation. The results showed that the activity of the enzymes tested was significantly higher in the blood serum of young experimental groups compared to the control group, and the mentioned differences are statistically significant ($p > 0.05$) (Table 3 and Table 4).

Table 3. Activity of AST, ALT, and ALP in the blood serum of young rats (mean value U/L) whose mothers had been treated with lead acetate (100 mg/kg BM per day) during pregnancy and lactation compared to the control group

	AST	ALT	ALP
Control group	35.12	37.43	156.29
First analysis	75.88	66.59	328.14
Second analysis	83.21	83.90	355.15

Table 4. Activity of AST, ALT, and ALP in the blood serum of young rats (mean value U/L), whose mothers had been treated with lead acetate (30 mg/kg, BM per day) during pregnancy and lactation compared to the control group

	AST	ALT	ALP
Control group	35.12	37.43	156.29
First analysis	83.12	72.30	370.96
Second analysis	85.18	75.29	381.25

Discussion

In conditions of chronic lead poisoning of female rats and their descendants a significant increase in the activities of AST, ALT and ALP enzymes in the blood serum of experimental groups was established compared to control groups. No dependence on the lead dose for AST and ALT enzymes was established. However, for ALP it was found that the dose of lead significantly affected the level of increase in the ALP in the blood serum of female rats, but it was not the case with their descendants.Namely, the higher dose of lead resulted in a significant increase in ALP activity in the blood serum of female rats. The dependence on the period of intoxication was established with the extension of the period of exposure to lead influence. The activities of the aforementioned enzymes in blood serums linearly increased.The increase in the activity of the enzyme AST was more significant compared to ALT enzyme, i.e. Ritis coefficient - which shows the relationship between AST/ALT activities). It was higher than 1. (The **AST/ALT ratio** is the ratio between the concentrations of the AST and ALT in the blood of a human or animal. It is measured with a blood test and is sometimes useful in medical diagnosis to differentiate between causes of liver damage, or hepatotoxicity.

The increase of the activities of AST and ALT in blood serum was probably a consequence of the hepatotoxic effects of lead, i.e. the appearance of toxic hepatitis. The above is supported by the fact that De Ritis ratio was higher than 1. It is true that the liver, among its many other vital functions, also has a role in the accumulation and detoxification of foreign substances, and therefore of toxic metals. The lead that enters the body by ingestion is transferred to the liver via the vena portae hepatis, where most of it remains stored.Only a smaller portion of this toxic metal "crosses the barrier of the liver" and enters the blood. Lead accumulated in the liver can act by directly damaging hepatocytes, primarilyby destroying the permeability of the cell membrane.

The influence of chronic lead poisoning on the activity of some enzymes in serum can be explained by the increased permeability of the cell membrane of hepatocytes, which results in an increased release of cytosol enzymes, that is AST and ALT in blood. The exact mechanism of hepatotoxicity of lead remains unknown, although several researchers indicate that there is a tendency of lead to be linked particularly to mitochondriamembranes.Furthermore, lead toxicity, at molecular level, is explained with interaction between lead and calcium. Lead reacts with calcium based on very similar ionic properties.These interactions are observed at the level of Ca^{2+} entering the cell, at the level of Ca^{2+} - binding proteins and receptors, and at the level of maintaining stability of the cell membrane with calcium. Osteoblasts, neurons, capillary endothelial cells and hepatocytes accumulate the excess of Ca^{2+} in mitochondria in the presence of lead.Interaction of lead and calcium is observed

at the transport system level of plasma membranes, such as Ca^{2+} - channel and Ca^{2+} - pump, during which lead disturbs the homeostasis of the intracellular Ca^{2+} and reacts with numerous Ca^{2+} - dependent mechanisms, such as: calmodulin (CaM), protein kinase C, etc.The most striking negative effect of lead is the impaired calcium homeostasis, which results in an increased intake of calcium into the cell. Ca^{2+} ions are very reactive and lead to the inhibition of the mitochondria function.When this is damaged, the release of energy in the form of ATP is disrupted, and this is the most important factor for the maintenance of the normal function of plasma membranes. The result of this is the destruction of the integrity and permeability of the hepatocytes'membranes, which has the consequence of increased release of cytosol enzymes from the cell, as is the case with AST and ALT. It is well known that enzymes found in the cell cytosol are likely to be released into the extracellular space even with minimal damage to the cells or their membranes.While the process of damaging the cell and its membrane extends and develops, the amount of enzymes that are released into the circulation increases.The results of this research have proven this because with the extension of the lead intoxication period the activities of AST and ALT in blood serum of female rats were linearly increased. On the other hand, the factor which contributes to the rapid release of enzymes from liver cells is the high permeability of the capillaries in the tissue.Another possible mechanism for hepatotoxicity of lead is indirect - interference within metabolicpaths. Lead impairs liver function by inhibiting enzymes involved in protein synthesis [13, 14]. Regarding the dose of lead, some differences are observed in terms AST and ALT activities in the blood serum of female rats. The higher dose of lead resulted in slightly higher activities of AST and ALT, but those differences did not show statistical significance. This can be explained by the fact that the enzymes AST and ALT do not belong to the "fine" indicators of liver damage at all levels, but only at the level of plasma membranes. It has already been pointed out that the dissolved enzymes in the cytosol are released easily and quickly from damaged cells, even in case of minimal damage to the plasma membrane (e.g. edema without disturbing the integrity of the membrane).

Similar results for young rats were also obtained with mothers that were intoxicated with lead. Higher or lower doses were noticed during periods of pregnancy and lactation. A significant increase in the activity of AST and ALT in the blood serum of experimental groups of young mice compared to the control groupwas found, but no significant differences were observed in terms of the dose of lead.Because of this, it is likely that the lead that entered the young organism through placenta and milk has hepatotoxic effect. The results obtained in this study are consistent with the results of other studies, but the review of available literature showed no data on the effects of lead on young experimental animals.A significant increase in the activities of ALT and AST in conditions of chronic lead poisoning in humans and in experimental animals is also established. These studies explain the increase in the activities of these enzymes with the hepatotoxic effects of lead. However, other studies have emphasized the significant reduction in the activities of AST and ALT under the influence of lead.

The authors explain this with the possible inhibition of the synthesis of AST and ALT enzymes affected by this toxic metal [2, 1, 15]. A possible explanation for such diverse research results is that thestudies were quite different in terms of their experimental design and the applied doses of lead, length of exposure, and manner in which lead entered the body of animals.

Regarding ALP, this enzyme may be an indicator of liver and bone damage that are associated with the increased activity of osteoblasts. Besides lead deposited in the liver, it can also be deposited in the bones.By increasing the time of lead intoxication it linearly increases its concentration in bones. It has been observed on the culture of bone cells that this toxic metal reduces the synthesis of proteins and collagen fibers of type I. Reduced synthesis of osteonectin, the protein that binds calcium, was discovered. Interaction between lead and calcium at osteoblasts level was observed. Namely, these cells accumulate the excess of calcium in mitochondria in the presence of lead and it is found that lead reacts with proteins and hormonal signals that normally regulate the status of intra- and extra-cellular calcium.As a result of the interaction between lead and calcium a significant reduction in the concentration of calcium in the bones in conditions of poisoning by this elementmay appear, as some studies have already shown. This is probably about heteroionic replacement of calcium from the crystalline hydroxyapatite - (group of phosphate minerals) Ca_{10} (PO_4) 6 $(OH)_{2)}$ with the lead from the blood, due to their very similar electronic configurations.However, despite the stated negative effects that lead can cause when it is found in

bones, most researchers still think that the bones are not the primary place where the toxicity of lead is shown.The fact is that pregnancy may affect the activity of ALP in terms of increasing its activity because through the placenta lead enters the young organism during pregnancy.However, the control groups of pregnant female rats in the same stage of pregnancy that were not treated with lead denied this. In the light of the above mentioned facts, the noticed increase of ALP activity in the serum experimental animals under the influence of lead in this research cannot be attributed to the increased release of this enzyme from the bones or from the placenta, but from the liver and within the already mentioned hepatotoxicity. ALP is an enzyme known as a biochemical marker of intrahepatic cholestasis that occurs in some forms of toxic hepatitis. While the pathological process develops over time, as the level of intrahepatic cholestasis increases, so the ALP activity increases in the blood serum, as shown by the results of this study.

ALP activity in the blood serum of female rats basically depended on the applied dosage of lead, which was not the case with AST and ALT enzymes. Namely, a higher dose of lead resulted in a significantly increased activity of ALP in the blood serum. This can be explained by the assumption that a higher dose of lead causes a higher level of intrahepatic cholestasis.The results obtained for the young rats were identical to the results obtained for their mothers. Through the placenta and milk lead enters the young rats and deposits in the liver and possibly causes toxic hepatitis followed by intrahepatic cholestasis, resulting in increased activity of ALP in the blood serum.Some previous studies provided results similar to those obtained in this study concerning the activity of ALP and lead poisoning, but some other studies also gave different results - decreased activity of ALP. The authors explain such results with interactions between lead and zinc and/or magnesium in the active center of the metalloenzyme ALP which resulted in the fall in enzyme activity [16, 17]. The available literature does not give any information about the impact of lead on young rats during periods of pregnancy and lactation. As for the impact of lead on the activity of ALP, various studies again provided different results which can be the consequence of different experimental designs, as in the case of AST and ALT.

Conclusions

1. The activities of AST, ALT, and ALP in the blood serum were significantly increased in conditions of chronic intoxication of female rats with lead. These activities were closely dependent on the period of intoxication. By increasing the period of intoxication, the activity of the above enzymes also linearly increased. The activities of AST and ALT were not dependent on the administered dose of lead, and in the case of ALP, the increase in activity was basically dependent on the dose of lead.

2. The activities of AST, ALT and ALP enzymes in the blood serum of young rats were significantly higher in conditions of chronic lead poisoning of their mothers during periods of pregnancy and lactation, but dependence on the dose of lead obtained from their mothers is not established.

3. The increase in the activities of AST, ALT, and ALP in the blood serum of experimental animals is the result of the hepatotoxic effect of lead followed by intrahepatic cholestasis.

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