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Lead and Zinc Influence on Antioxidant Enzyme Activity and Malondialdehyde Concentrations

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Abstract

The influence of zinc on the activity of antioxidant enzymes in the blood of rats intoxicated with lead was studied. Exposure to lead at the concentration of 500 mg/dm^3 for 6 weeks caused a decrease in the activities of SOD, GSH-P_X and GR as well as an increase in MDA concentrations in the serum. Zinc at the concentration of 60 mg/dm^3 administered for a period of 2 weeks after exposure to cadmium reduced the effects of toxic action of lead leading to a normalization of the activities of SOD and GR in the blood as well as MDA concentration in the serum.

Keywords: lead; zinc; blood; antioxidant enzymes

Introduction

Lead toxicity is a well-known problem. Many studies show that the toxicity of this heavy metal depends on its chemical form, the rout of its administration, and dose, time and intensity of exposure.

An increase in blood lead concentration is an especially important problem because of the fact that this metal has a very strong ability to accumulate in the body. The accumulation of lead is not indifferent to intracellular metabolism. The toxicity of lead is mainly connected with its influence on the enzymatic systems of cells, which leads to many biochemical disorders.

Lead interacts with trace metals (especially zinc) at the stage of their intestinal absorption and distribution in tissues as well as with their biological functions. Lead can substitute ions of other metals in many metaloenzymes, including delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase leading to their inhibition.

It has been shown that increased dietary intake of zinc reduces the accumulation and toxicity of lead, probably by decreasing its intestinal absorption [1]. A decrease in lead absorption from the gastrointestinal tract as a result of pre-administration of zinc was noted. On the other hand, lead, even in low concentrations, reduces uptake of zinc and copper into the intestinal epithelial cells [2].

It has been suggested that lead takes part in the formation of free oxygen radicals [3].

The aim of this work was to examine to what extent zinc influences the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase) in the blood as well as the concentration of malondialdehyde in the serum of rats exposed to lead.

Materials and Methods

The study was performed on 40 male Wistar rats, of initial body weight of 180 ± 20 g. The animals were randomly divided into 5 experimental groups of 8 rats each.

Group 1 - the control group; the animals received redistilled water for drinking,

Group 2 - was exposed to lead at the concentration of

500 mg/dm^3 in the form of water solution of lead acetate (for drinking), for a period of 6 weeks (Pb),

Group 3 - received a water solution of zinc chloride at the concentration of 60 mg Zn/dm^3 for a period of 2 weeks (Zn),

Group 4 - after exposure to lead (500 mg/dm^3 for 6 weeks) the animals received zinc (60 mg/dm^3) for 2 weeks (Pb + Zn),

Group 5 - after exposure to lead (500 mg/dm^3 for 6 weeks) the rats received redistilled water for a period of 2 weeks (Pb + H_2O).

The animals were fed the standard LSM diet. The rats consumed on average 25 g of the diet per day. The mean concentration of lead in food was 17 µg/g. After calculations, the mean intake of lead in the food was 425 µg/24 h/rat.

Twenty-four hours before the end of the respective periods of the exposure the animals were placed in metabolic cages for 24-hour urine collection.

After exposure was completed the rats were euthanized and the blood from the heart was collected.

In the blood the following parameters were determined:

- the activity of superoxide dismutase (SOD), glutathione peroxidase (GSH-P_X) and glutathione re ductase (GR) using tests by RANDOX,
- the activity of catalase (CAT) according to Aebi [4],
- the concentration of malondialdehyde according to Buege and Aust [5],
- the concentration of lead by means of atomic absorp tion spectrophotometry (AAS) with electrothermical atomization in a graphite cuvette.

In the blood and urine the concentration of -SH groups was assessed according to Ellman [6].

The concentrations of zinc and copper in the serum (after its dilution with redistilled water 1:1) were measured by means of flame AAS method [7]. The analyses were performed on an atomic absorption spectrometer AAS 30 produced by the Zeiss Jena Company.

For each series of the serum samples the working standard solutions of lead, zinc and copper in respective concentrations were prepared.

The obtained results (mean values and standard deviations) are presented in tables. The data were statistically analyzed according to the t-Student's test using the variance analysis. The level of $p < 0.05$ was considered as statistically significant.

Results

The influence of lead on the activity of antioxidant enzymes in the blood and MDA concentration in the serum of rats is presented in Table 1.

Exposure to 500 mg Pb/dm^3 caused a decrease in the activity of SOD, GSH-P_X and GR in the blood as well as an increase in the serum MDA concentation. The activity of GSH-P_X was decreased by 40%, SOD and GR by about 20%. However, the concentration of MDA was increased by 37% in comparison to the control group.

The administration of redistilled water for the period of 2 weeks after the completed exposure to lead (Pb + H_2O) led to a further decrease in the activity of GSH-P_X and GR in comparison to the control group as well as the group exposed to lead (Pb). The concentration of MDA in the serum and the activity of SOD in the blood of those animals (Pb + H_2O) were at the same level as in the rats receiving lead for 6 weeks. Moreover, at the same time the activity of CAT in the blood was increased in comparison to the control group and the group exposed to lead (Pb).

The administration of zinc at the concentration of 60 mg/dm^3 for the period of 2 weeks (Zn) had no influence on the activity of the assessed antioxidant enzymes (SOD, CAT, GSH-P_X, GR) in the blood and on MDA concentration in the serum.

The administation of zinc after the exposure to lead (Pb + Zn) restored the activity of SOD and GR in the blood and MDA concentration in the serum to the control values. The activity of CAT in the blood remained at the same level as in the group exposed to lead. The activity of GSH-P_X in the blood was increased by 15% in comparison to the group exposed to lead, but did not reach the control value.

The influence of lead and/or zinc on the concentration of -SH groups in the blood and urine of rats is presented in Table 2.

Table 1. The influence of lead and/or zinc on the activity of antioxidant enzymes in the blood and the concentration of MDA in the serum of rats.

Group	SOD [U/ml]	CAT [mmol H_2/min]	GSH-P_x [U/l]	GR [U/l]	MDA [mmol/l]
Control	178.00 ± 17.20	152.60 ± 26.00	786.00 ± 100.50	28.70 ± 7.60	4.30 ± 0.70
Pb	143.00 ± 11.30^{a}	190.70 ± 51.00	474.00 ± 72.60^{a}	22.40 ± 2.10^{a}	6.70 ± 1.10^{a}
Zn	165.00 ± 9.00^{b}	170.00 ± 24.00	668.00 ± 98.00^{b}	24.60 ± 3.80	4.10 ± 0.40^{b}
Pb + Zn	180.00 ± 36.00^{b}	202.00 ± 42.00^{a}	544.00 ± 75.00^{a,b,c}	27.60 ± 8.50	4.50 ± 1.00^{b,d}
Pb + H_2O	152.00 ± 15.00^{a}	249.00 ± 20.00^{a,b,c,d}	367.00 ± 38.00^{a,b,d}	9.00 ± 1.70^{a,b,c,d}	6.30 ± 0.80^{a,c,d}

Values are means ± SD

Statistical significance $p < 0.05$: ^{a} - to the control group; ^{b} - to the Pb group; ^{c} - to the Zn group; ^{d} - to the Pb + Zn group.

Table 2. The concentration of -SH groups in the blood and urine of rats.

Group	-SH groups [mmol/l]	
	blood	urine
Control	0.700 ± 0.160	0.050 ± 0.006
Pb	0.370 ± 0.050[a]	0.039 ± 0.005[a]
Zn	0.210 ± 0.080[a,b]	0.040 ± 0.009
Pb + Zn	0.680 ± 0.150[b,c]	0.044 ± 0.009
Pb + H_2O	0.404 ± 0.110[a,c,d]	0.038 ± 0.003[a]

Values are means ± SD
Statistical significance $p < 0.05$: [a] - to the control group; [b] - to the Pb group;[c] - to the Zn group; [d] - to the Pb + Zn group.

The administration of lead as well as zinc led to a decrease in -SH groups concentration in the blood and urine. After the 6-week exposure to lead the concentration of -SH groups was decreased by 48 and 22% in the blood and urine, respectively, in comparison to the control group. The administration of zinc decreased the concentration of -SH groups by 70% in the blood and by 20% in the urine as compared to the control group.

The administration of zinc to the rats previously intoxicated with lead (Pb + Zn) caused an increase in -SH groups concentration in the blood and urine to the control value.

Concentrations of lead in the blood as well as zinc and copper in the serum of rats are presented in Table 3.

In the animals exposed to lead the concentration of this metal in the blood was 7 times higher than in the control group. The increase of the blood lead concentration was accompanied by a 17% increase in zinc concentration and a 27% reduction of copper concentration in the serum.

Table 3. The concentration of lead in the blood as well as zinc and copper in the serum of rats.

Group	Pb [µg/dl]	Zn [µg/dl]	Cu [µg/dl]
Control	8.30 ± 2.00	120.00 ± 13.70	136.00 ± 14.00
Pb	62.00 ± 12.20[a]	140.00 ± 11.60[a]	107.00 ± 20.00[a]
Zn	17.50 ± 3.70[a,b]	244.00 ± 22.00[a,b]	95.00 ± 16.00[a]
Pb + Zn	34.20 ± 1.80[a,b]	194.00 ± 18.00[a,b]	102.00 ± 14.00[a]
Pb + H_2O	41.40 ± 9.10[a,b,c]	152.00 ± 16.50[a,c,d]	101.00 ± 21.00[a]

Values are means ± SD
Statistical significance $p < 0.05$: [a] - to the control group; [b] - to the Pb group;[c] - to the Zn group; [d] - to the Pb + Zn group.

The administration of redistilled water after lead exposure (Pb + H_2O) led to a decrease in the blood lead concentration in comparison to the group receiving lead for 6 weeks, but the concentration was still higher than that in the control group. The concentrations of zinc and copper in the serum were unchanged by zinc and remained at the level of the group exposed to lead.

The administration of zinc caused a 2-fold increase in the concentrations of zinc in the serum and lead in the blood in comparison to the control group. Simultaneously, a 30% decrease in the concentration of copper in the serum was observed.

Zinc used after exposure to lead (Pb + Zn) led to a 50% decrease in the blood lead concentration in comparison to the group exposed to lead, but had no influence on the concentration of copper in the serum.

Discussion

Although lead does not directly take part in the transport of electrons, the catalysis of peroxidative reactions by this metal probably is a major contributor to its toxic action.

In the conducted experiment we have described a 7-fold increase in the blood lead and above 20% decrease in the serum copper concentration as a result of exposure to lead. Simultaneously, the changes in the activity of the antioxidant enzymes in the blood and MDA concentration in the serum as well as the concentration of -SH groups in the blood and urine were noted.

The rats intoxicated with lead showed a decrease in SOD activity.

The mechanism of reactions catalyzed by SOD consists in the reduction and oxidation of metal ions which are present in the active centre of this enzyme (Zn, Cu). As a result of these reactions hydrogen peroxide (H_2O_2) is produced. The biosynthesis of SOD is under close control. Unfortunately, so far this process has been described only for bacterial cells [8]. But it is known that the inductor of the synthesis is a product of molecular oxygen (O_2) reduction and that this process is regulated by iron ions. It has been noted that the level of iron ions in the serum increases in conditions of exposure to lead. Even a small increase in the concentration of iron ions leads to a sudden production of reactive oxygen species (ROS), for example in the Fenton-like reaction [9].

The mechanism of catalysis influenced by SOD suggests that this enzyme is an incomplete antioxidant which protects from the action of one free oxygen radical O_2. Biologically the action of SOD through H_2O_2 is connected with the action of CAT. The function of CAT is removal of H_2O_2 formed as a result of the action of the oxygen dehydrogenases. Available literature data have shown that H_2O_2 inhibits the activity of SOD, while O_2 inhibits the action of CAT [10].

The decrease in SOD activity in rats intoxicated with lead can be a result of the decrease in copper concentration in the serum noted in these animals. Copper shows a dualistic character in its action. In the form of free ions (Cu^{2+}) copper is a strong peroxidative factor. On the other hand, this metal is a component of important enzymes such as SOD and ceruloplasmin, which protect against peroxidative processes. The proper ratio of Cu/Zn is necessary for the maintenance of the oxido-reductive balance in the organism [11].

Pigeolet et al. [12] have shown that enzymes such as SOD, CAT and GSH-P_X can also be inactivated by the products of their own reactions in the presence of high concentrations of H_2O_2 or OH (hydroxyl radical).

The enzyme responsible for the decomposition of lipid peroxides is GSH-P_X. This enzyme protects cellular membranes from peroxidative damage. The presence of the free -SH groups is necessary for the proper action of this antioxidant enzyme. In this study we have noted a decrease in the concentration of the free -SH groups in the blood as well as in the urine of the rats exposed to lead [13]. The decrease in the activity of GSH-P_X in the blood and the increase in MDA concentration in the serum of the rats exposed to lead can be a result of this heavy metal-induced depletion in the free -SH groups noted in these animals.

We have also noted that the activity of another antioxidant enzyme - GR decreases in conditions of exposure to lead. This enzyme is responsible for the transformation of the oxidized glutathione into its reduced form (GSH). The diminution in the concentration of GSH leads to fast accumulation of lipid peroxides in a cell [14].

Our results are in accordance with the data of other authors who have noted an increase in the concentration of MDA in liver and serum and a reduction in the activity of antioxidant enzymes such as SOD, GSH-P_X and GR in rats intoxicated with lead.

The administration of redistilled water for a period of 2 weeks after exposure to lead caused a reduction in the blood lead concentration in comparison to the animals receiving lead alone but did not diminish this metal concentration to the level of the control group. The cessation of the exposure had no influence on the concentration of -SH groups in blood and urine. The concentrations of -SH groups in these animals remained at the levels noted in the group exposed to lead. The activities of GSH-P_X and GR were further decreased, while the activity of CAT was considerably increased. Furthermore, the concentration of MDA in the serum and the activity of SOD in the blood still remained at the level of the lead exposed group. It can be supposed that the changes in the studied parameters noted 2 weeks after the completed exposure to lead are of reoxidative character. The reoxidative damage is characterized as the damage of a tissue which occurs at the time of bringing the proper oxygen metabolism back after the period of anoxia. Intensively conducted studies appear to confirm the hypothesis that ROS are responsible for the appearance of the disturbances at this time. Reintake of oxygen to the sites of anoxia is characterized by a decrease in the concentrations of ATP and intracellular antioxidants as well as the presence of transition metals. It leads to an increase in the production of H_2O_2, OH and O_2 [15].

Zinc administred after exposure to lead caused a 50% decrease in the blood lead concentration in comparison to the group exposed to lead. The administration of zinc resulted also in normalization (returned to the control value) of the activities of SOD and GSH in the blood and MDA concentation in the serum as well as the concentration of -SH groups in the blood and urine.

For a long time it was thought that the role of zinc and copper in the maintenance of the balance between the pro- and antioxidative processes depends on the presence of these metals in the molecules of antoxidant enzymes.

Bettgev and O'Dell [16] have shown that zinc is an essential element for the maintenance of the proper structure and function of cellular membranes. These data and results of later conducted studies [17] prove the fact that zinc shows antioxidative action independently on the kind of enzymes in which it is present. But the mechanism of the action of zinc is still unknown. These authors have suggested that zinc shows antioxidative action only in high concentrations. The antioxidant role of zinc (only extracellular) probably consists in the protection of -SH groups against oxidation and inhibition of the formation of ROS in whose transition metals take part.

Increased lead consumption causes, among other things, a decrease in copper absorption from the gastrointestinal tract. The proper concentration of copper in the body has an influence on the activity of SOD [18].

Lead does not induce directly the peroxidation of lipids. This metal however, shows peroxidative action in facilitateing the formation of free oxygen radicals. The action of lead is probably a result of the impoverishment of cells in GSH and reduction of the total pool of -SH groups bound with proteins.

Conclusions

1. Lead caused a decrease in the activities of SOD, GSH-P_X and GR in the blood. Simultaneausly, the con centration of MDA in the serum was increased.
2. Zinc had no influence on the activities of the studied antioxidant enzymes.
3. Zinc administered after the completed exposure to lead reduced the effects of toxic action of lead, leading to a normalization of the activities of SOD and GR in the blood as well as the concentration of MDA in the serum.

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