The effects of lead and zinc ion exposure on the antioxidant status of mice liver

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The present study was conducted to investigate the effect of lead (Pb) and/or zinc (Zn) ions on the content of metallothionein (MT), reduced glutathione (GSH) and malondialdehyde (MDA) in mouse liver.

Two weeks of mice intraperitoneal treatment with ZnSO₄ and Pb(CH₃COO)₂ solutions, increased the content of MT by 25% and 55%, respectively. Mice pre-treatment with ZnSO₄ for 20 min before Pb(CH₃COO)₂ injections, attenuated the effect of Pb²⁺ and partly reduced (by 30%) the increase of MT content in mice liver. Two weeks of administration with Pb(CH₃COO)₂ solution, induced the decrease of GSH content by 22% comparing to the control. Treatment with ZnSO₄ didn't have any effect on the content of GSH. Pre-treatment with ZnSO₄ for 20 min before Pb(CH₃COO)₂ injections decreased GSH content in mice liver by 48% as compared to the control group of mice. Neither Pb²⁺ nor Zn²⁻ caused any remarkable alterations on MDA content in liver of mice.

Key words: lead, zinc, metallothionein, reduced glutathione, malondialdehyde, oxidative stress

INTRODUCTION

Lead (Pb) is a common environmental and industrial pollutant that has been detected in all phases of environmental and biological systems. It has been found to produce a wide range of toxic-biochemical effects, besides a behavioral dysfunction in man and in experimental animals [1]. Poisoning by Pb is a potential factor in brain damage [2], mental impairment and severe behavioral problems, as well as anemia, kidney insufficiency, neuromuscular weakness, and coma [3]. Pb is known to cause damage to critical biomolecules, such as lipids, proteins and DNA [4]. Recent studies have indicated that reactive oxygen species (ROS) play an important role in the pathophysiology of Pb poisoning [5]. Overexposure to Pb can damage blood-forming, nervous, urinary and reproductive systems. Pb can also substitute for Zn in several proteins that function as transcriptional regulators [6].

Zn is an essential trace mineral nutrient required for growth and reproduction in man and other living organisms, but toxic when accumulated to excess [7]. It is an integral component of numerous metalloenzymes, structural proteins and transcription factors and contributes to physiological processes including neurotransmission, hormone secretion, DNA synthesis and gene expression [8]. Zn is not only a structural component of proteins and a co-factor for enzymes, but it has a cell signalling function which regulates the cellular resistance to oxidative stress. Deleterious effects of Zn²⁺ may be caused by its inhibitory action on the pathways of RNA and protein synthesis [9].

Metallothionein (MT) is a cysteine-rich, metal-binding protein of low molecular mass. It is synthesized by cells in response to various stimulants, including Pb. MT has the capacity to bind both the physiological and xenobiotic heavy metals through the thiol group of its cysteine resi-
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MATERIALS AND METHODS

The subject of research. Experiments were done on 4–6 weeks old white laboratory outbred mice weighing 20–25 g. All experiments were performed according to the Law of the Republic of Lithuania on the Care, Keeping and Use of animals (License of State Veterinary Service for working with laboratory animals No. 0200).

Mice were randomly assigned into four groups: three experimental and one control. Each group included 8–12 mice. Mice were intraperitoneally injected for 14 days (once in a day) with metal salts, dissolved in saline. Mice of the first experimental group were intraperitoneally injected with Pb(CH\textsubscript{3}COO\textsubscript{2}) solution in deionised water (10 mg Pb kg of body mass). Mice of the second experimental group received intraperitoneal injection of ZnSO\textsubscript{4} solution at a dose level 1.56 mg Zn kg of body mass. Mice of the third experimental group were injected with ZnSO\textsubscript{4} solution and after 20 min — with Pb(CH\textsubscript{3}COO\textsubscript{2}) solution in aforementioned dose. Control animals (the fourth group) received injection of the same volume of saline.

MT content assay in mice liver. MT was assayed in mice liver according to the method of Peixoto N. C. [12]. To the aliquots of 1 ml of supernatant were added 1.05 ml of cold (–20 °C) absolute ethanol and 80 μl of chloroform; then the samples were centrifuged at 6000 × g for 10 min. The supernatant was combined with 3 volumes of cold ethanol (–20 °C), kept at –20 °C for 1 h and centrifuged at 6000 × g for 10 min. The MT-containing pellets were then rinsed with 87% ethanol and 1% chloroform and centrifuged at 6000 × g for 10 min. The MT content in the pellet was evaluated using the colorimetric method with Ellman’s reagent. The pellet was resuspended in 150 μl 0.25 M NaCl and subsequently 150 μl 1 N HCl containing 4 mM EDTA (ethylenediaminetetraacetic acid) was added to the sample. A volume of 4.2 ml 2 M NaCl containing 0.43 mM DTNB buffered with 0.2 M Na-phosphate, pH 8.0 was then added to the sample at the room temperature. The sample was finally centrifuged at 3000 × g for 5 min; the supernatant absorbance was evaluated at wave 412 nm and MT concentration was expressed as μg / g of wet weight of kidney.

Measurement of GSH in mice liver. GSH was measured by reaction with DTNB to give a compound that absorbs at wave length 412 nm. Each sample cuvette contained 2 ml 0.6 mM DTNB in 0.2 M sodium phosphate, pH 8.0, 0.2 ml supernatant fraction and 0.8 ml 0.2 M phosphate buffer to the final volume of 3 ml. The reference cuvette contained 0.2 ml 5% trichloracetic acid instead of the sample. GSH concentration was expressed as μg / g of wet weight of kidney.

Determination of MDA in mice liver. Lipid peroxides were determined as MDA formed after reaction with thiobarbituric acid (TBA) and expressed as μg / g of wet weight. The liver was removed and homogenised with 9 volumes (as compared with liver weight) with cold 1.15% KCl to make a 10% homogenate. To 0.5 ml of 10% homogenate there were added 3 ml 1% H\textsubscript{3}PO\textsubscript{4} and 1 ml 0.6% TBA aqueous solution. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and supernatant absorbance was determined at 535 and 520 nm.

Statistical analysis. The results were expressed as the mean ± standard error of the mean. Statistical significance was set at p < 0.05. The analysis was performed using a statistical software package (Statistica 6.0).

RESULTS AND DISCUSSION

The present study was conducted to investigate the effect of Pb\textsuperscript{2+} and/or Zn\textsuperscript{2+} on the content of MT, GSH and MDA in mouse liver. Recent studies suggest oxidative stress as one of the important mechanisms of toxic effects in lead [13]. Pb toxicity leads to free radical damage via two separate, although related, pathways: the generation of ROS including hydroperoxides, singlet oxygen, and hydrogen peroxide, and the direct depletion of antioxidant reserves [14]. Several studies reported alterations in antioxidant enzyme activities such as Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx), and changes in the concentrations of some antioxidant molecules, such as GSH in Pb-exposed...
animals and workers [15]. The competition between Zn and Pb might decrease the absorption of Pb, thus reducing Pb toxicity. Zn, on the other hand, was reported to be able to prevent and treat Pb intoxication in rats, either alone and/or in combination with methionine, ascorbic acid or thiamine [15, 16]. As a result, Zn appears to have a mitigating effect on Pb toxicity. When Pb-exposed rats were given Zn, previously depressed levels of SOD returned to normal and Delta-aminolevulinic acid dehydratase (ALAD) inhibition was reversed [14]. Deficiency of Zn usually seems to be associated with higher than normal levels of tissue oxidative damage, including increased lipid, protein and DNA oxidation. Several experiments on animals confirmed that chronic or long-term deprivation of Zn makes an organism more susceptible to oxidative stress-induced injury. Zn deficiency effects, linked with formation of ROS, have been documented by MDA formation and induced lipid peroxidation in liver of rats [17].

Our obtained data showed (Fig. 1), that in mice liver treated with ZnSO4 and Pb(CH3COO)2 solutions, MT content was increased by 25% and 55%, respectively (p < 0.05). Reported data state that a marked increase in the total sulfhydryl group was observed in rat liver with blood lead level (BLL) higher than 80 μg/dl. This increase may be attributed to the possible induction of MT by Pb and enhanced per-oxidative damage to the membrane [18]. It has also been reported that chronic exposure of an organism to Zn on a long-term basis results in enhanced synthesis of metallothioneins [17, 19].

Mice pre-treatment with ZnSO4 for 20 min before Pb(CH3COO)2 injections attenuated effect of Pb ions and partly reduced (by 30%) the increase of MT content in mice liver (p < 0.05) (Fig. 1). Zn and Pb compete for similar binding sites on the MT-like transport protein in the gastrointestinal tract [20]. Some scientists have shown that in Zn-supplemented animals, Pb concentration was significantly reduced in kidney, bone, liver, spleen, testis, and blood compared to animals treated only with Pb. The concentration of Pb in these organs was reduced 30 to 50% by Zn co-administration [21]. It may explain why the appeared increase of synthesis of MT was reduced after Zn co-administration.

After 14 days of mice treatment with Pb(CH3COO)2 solution, GSH content was decreased by 22% (p < 0.05) as compared to the control group of mice (Fig. 2).

Many scientists have reported that Pb has a very high affinity for thiol groups and, therefore, decreases GSH levels [15, 22, 23]. According to scientific sources, Pb treatment caused significant decreases, both in liver and brain GSH levels, for young and adult Pb-exposed groups when compared with their control ones. The levels of oxidized glutathione (GSSG) in all samples (liver, brain, and erythrocytes) were higher in Pb-exposed groups as compared with their controls [24–26]. Under oxidative stress, GSSG is reduced to GSH by glutathione reductase (GR), an indirect component of the antioxidant defense system. It was suggested that lead inhibits GR by attacking the disulfide group on the active site of this enzyme [18]. These reasons might explain why the decrease of GSH was determined.

![Fig. 1. Hepatic concentration of MT of mice after 14 days of exposure to 10 mg/kg of Pb and/or 1.56 mg/kg of Zn. The content of MT in the liver of control mice was set at 100% (27.15 μg / g wet weight). * – p < 0.05 as compared to the control mice; # – p < 0.05 as compared to the group of Pb-treated mice. Data represent results of 8–12 separate experiments](image-url)
According to our results, mice treatment with ZnSO₄ didn’t have any effect on the content of GSH. Pre-treatment with ZnSO₄, 20 min before Pb(CH₃COO)₂ injections, decreased GSH content in mice liver by 48% as compared to the control group (p < 0.05) (Fig. 2). There are some results stating that Zn itself, depending on the dose, can cause depletion of GSH [27]. Our results showed that antioxidant Zn did not suppress this effect of Pb. Hepatic GSH depletion might appear because of the enhanced effect of two metals or it could also be associated with protection against endogenous ROS and the synthesis of MT. Although MT synthesis requires cysteine, which may be derived from the breakdown of GSH, there is no evidence that GSH itself has an additional function in MT formation.

According to our results, the treatment of mice with ZnSO₄ and Pb(CH₃COO)₂ solutions, didn’t have any effect on MDA content in liver of mice (Fig. 3). Unlike literature references state, we couldn’t find statistical evidence that lead might cause liver lipid peroxidation process.
CONCLUSIONS

Mice exposure to Pb or Zn ions during 14 days induced synthesis of MT, Zn pre-treatment attenuated effects of Pb and reduced the content of MT. Pb ions decreased the content of GSH, however, mice pre-treatment with antioxidant Zn could not suppress this effect, induced by lead. MDA concentrations in all three metal treated groups were at the control level.

References

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**ŠVINO IR CINKO JONŲ POVEIKIS PELIŲ KEPENŲ ANTIOKSIDACINĖS SISTEMOS KOMPONENTAMS**

**Santrauka**
Šio darbo tikslas buvo įvertinti švino ir / arba cinko jonų 14-os dienų įžeidimo poveikį pelių antioksidacinės sistemos kūnimui — metalotioneinai (MT), redukuoto glutationo (GSH) bei lipidų peroksidacijos galutinio produkto malondialdehido (MDA) — koncentracijoms pelių kepenyse. Po 14-os dienų ZnSO₄ ir Pb(CH₃COO)₂ tirpalų įpildymo MT koncentracija padidėjo atitinkamai 25 % ir 55 %, lyginant su kontrolė. Sušvirštę ZnSO₄ tirpalą likus 20 min. iki Pb(CH₃COO)₂ tirpalo įpildymo, nustatėme, kad Zn²⁺ tik iš dalies sumažino (30 %) MT koncentracijos padidėjimą kepenyse. Keturiolikos dienų Pb²⁺ įpildymo kursas GSH koncentraciją sumažino 22 %, tuo tarpu Zn²⁺ paveikė GSH koncentracijos pelių kepenyse, lyginant su kontrolės grupės. Sušvirštę ZnSO₄ tirpalą likus 20 min. iki Pb(CH₃COO)₂ tirpalo įpildymo ne tik neapsaugojo GSH sintezės sistemos nuo slopinančio Pb²⁺ poveikio pelių kepenyse, bet ir slopino GSH sintezės 48 %, lyginant su kontrolės grupės. Mūsų eksperimentų duomenimis nei Zn²⁺, nei Pb²⁺ jonių nebuvo statistiškai patikimai poveikio MDA koncentracijai pelių kepenyse.

**Raktažodžiai:** švinas, cinkas, metalotioneinai, redukuotas glutationas, malondialdehidas, oksidacinis stresas