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EFFECT OF ZINC SUPPLEMENTATION ON THE DISTRIBUTION OF LEAD IN TISSUES OF RATS INTOXICATED BY LEAD COMPOUNDS

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Abstract

The permissible threshold level of lead in blood (Pb-B) is currently established at 5 μg dL^{-1}, but evidence suggests that it is impossible to determine the safety threshold for lead (Pb) and any exposure, especially in children, must be considered as potentially harmful. Methods used to reduce the concentration of Pb in blood (e.g. EDTA, penicillamine) are not always effective and are associated with serious side effects. One of the proposed dietary supplements in the case of exposure to Pb and low blood Pb concentrations is zinc (Zn), but the published literature on its effectiveness is limited. Therefore, the aim of this study was to clarify whether Zn supplementation may help reduce the concentration of Pb in the blood and tissues of rats, at the Pb-B level previously recognized as safe. Tests were performed on 6-8 week old male Wistar rats. Rats were divided into control and experimental groups: Group C – rats receiving drinking water *ad libitum* for 4 weeks; Group Pb – rats receiving Pb acetate 0.1% (PbAc) in drinking water *ad libitum* for 4 weeks; Group Zn – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks; Group Pb+Zn – rats receiving PbAc in drinking water *ad libitum* plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks. The applied dose of 300 mg of $ZnCO_3$ kg^{-1} diet results in a high but non-toxic Zn level. The concentrations of Pb and Zn in blood, plasma, liver and bone were determined by emission spectrometry in inductively coupled argon plasma (ICP OES). Incidental exposure of adult rats to Pb at doses resulting in the level of Pb in blood below the previously recognized as safe one caused: (i) increased Pb concentration in the bones and plasma and its reduction in the whole blood and liver (ii) simultaneous supplementation of rats exposed to Pb with a high but non-toxic dose of zinc did not result in the reduction of the Pb concentration in the blood and tissues of rats, nor did it induce any changes

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in the distribution of Pb in the examined tissues (iii) supplementation of diets with a high but non-toxic dose of Zn is not an effective method of reducing the concentration of Pb in blood at Pb-B previously recognized as safe. However, the therapy consisting of zinc supplementation to support the action of chelators could be crucial for the elimination of Pb from the body.

Key words: lead, zinc, lead tissue distribution, zinc supplementation, lead threshold level.

INTRODUCTION

Despite the implementation of many regulations governing amounts of lead (Pb) in the environment, the risk of exposure to Pb in different periods of human life remains high and the toxicity of Pb is still one of the major health problems resulting from both environmental and occupational exposure (CDC 2004, 2005, 2007, Abadin et al. 2007, WHO 2010, Hrubá et al. 2012). In developed countries, the increasing awareness of harmful effects of a lead-contaminated environment on the human body has eliminated Pb from production of fuels, paints, batteries and many consumer products such as cups, plastic toys and jewelry (EP 2005, EU 2008, EP 2009, WHO 2010). However, exposure to Pb, especially in children, continues to be mainly due to contact with soil and house dust contaminated with Pb or tap water contaminated by leaching Pb pipes. There is considerable aerial Pb emission from smelters, ore mining and processing, Pb acid battery manufacturing and coal combustion activities such as electricity generation (CDC 2007, 2012). Pb exposure particularly affects children, due to behavioral factors such as frequent hand-to-mouth activities *(pica),* greater gastrointestinal absorption and an immature blood/brain barrier (Lidsky, Schneider 2003).

The permissible threshold level of Pb in blood (Pb-B) is currently established at 5 μg dL^{-1} (CDC 2012), but evidence suggests that it is impossible to determine the safety threshold for Pb, hence any exposure, especially in children, must be considered potentially harmful (Baranowska-Bosiacka et al. 2012, 2013). It should also be noted that Pb is subject to cumulative effects, and because it does not break down nor is it biodegradable, any assessment of the environmental threat should take into account the effect of chronic exposure. The half-life of Pb in the body ranges from 30 days in the fast exchange systems (blood, soft tissue) to as much as 27 years in slow exchange systems (bone) (Riedt et al. 2009). Constant exposure to Pb at low concentrations leads to its accumulation in the body, where it is distributed to organs (liver, kidney, lung, pancreas, spleen, brain) and accumulated in bones (Gulson et al. 2002, Nash et al. 2004, Chełchowska et al. 2012) – Figure 1.

At the same time. there are no effective and safe methods of detoxification. Techniques used to reduce the concentration of Pb in blood (e.g. EDTA, penicillamine) are not always effective and are associated with serious side effects. One of the proposed dietary supplements in the case of exposure to Pb and low blood Pb concentrations is zinc (Zn), but the published literature

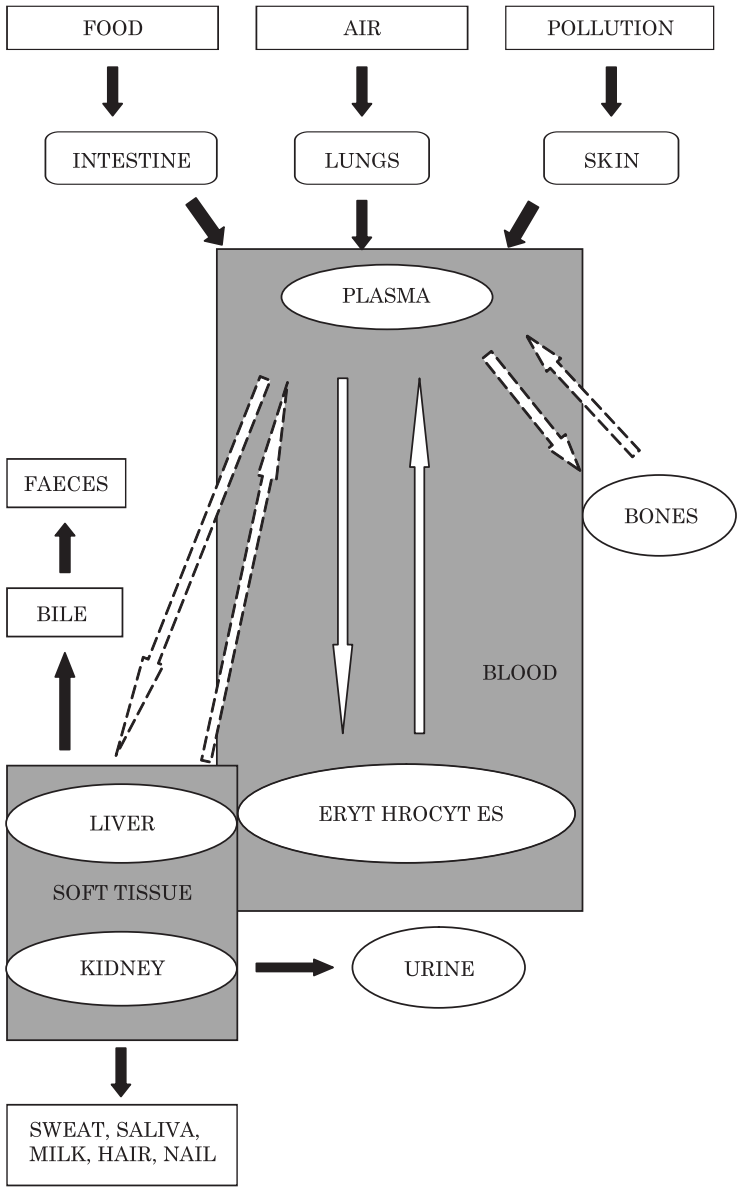


Fig. 1. Diagram illustrating the absorption, distribution and elimination of Pb from the body: bold arrows – routes of absorption, thin arrows – excretion pathways, dashed arrows – movement inside the body; the main locations of Pb in the body (on the basis of Giel-Pietraszuk et al. 2012)

on its effectiveness is limited. Therefore, the aim of this study was to clarify whether Zn supplementation may help reduce the concentration of Pb in the blood and tissues of rats, at the Pb-B level previously recognized as safe.

MATERIAL AND METHODS

Animals

The study was performed according to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals and the European Community Council Directive for Care and Use of Laboratory Animals, and was approved by a local ethics committee (The Pomeranian Medical University in Szczecin Committee on the Use and Care of Animals).

Toxicity procedure

Tests were performed on 6-8 week old male rats, inbred Wistar strain (n = 32). The mean body weight of rats at the start of experiment was about 105 g (21.30±SD). Clinically healthy animals were received from farms of the J. Nofer Institute of Occupational Medicine in Łódź (Poland). During the experiments, the rats were kept in cages under standard conditions. Animals were fed with Murigran feed (Agropol Motycz, Poland) and had free access to food and water. According to the manufacturer, the feed contained grains, products from cereal grains, products from genetically modified legume seeds, fish meal, by-products of oilseeds, feed fats and mineral supplements, enzymes, amino acids and a mineral-vitamin mixture. The detailed composition of the feed according to the manufacturer was (the content is given as % of the volume of feed): crude protein 23%, crude fat 3%, ash 8%, P 0.6% at 0.2%, Ca 1.1%, crude fiber 5.0% max., and dietary additives such as L-lysine (1.3%), dl-methionine (0.7%), L-threonine (0.7%), vit. A (E672) 500,000 IU g^{-1}. vit. D3 (E671) 500,000 IU g^{-1}. The manufacturer does not provide information about the content of Zn or Pb in feed (leaflet of Murigran, Agropol).

Rats were divided into four experimental groups. The study was conducted according to the following pattern:

Group C – rats receiving drinking water ad libitum for 4 weeks (n = 8);

Group Pb – rats receiving Pb acetate 0.1% (PbAc) in drinking water ad libitum for 4 weeks (n = 8);

Group Zn – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks (n = 8);

Group Pb+Zn – rats receiving PbAc in drinking water ad libitum plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks (n = 8).

The solution of PbAc was prepared daily in disposable plastic bags (hydropac, Anilab. Poland) from solid reagent directly in the desired concentration; the solution was not acidified. The volume of intaken liquids did not differ significantly between the experimental and control rats.

Previous studies (Baranowska-Bosiacka et al. 2012) have shown that the applied dose resulted in the concentration of Pb in the whole blood of animals (Pb-B) below the limit permissible in the blood in humans (<5 μg dL^{-1}). The applied dose of 300 mg of $ZnCO_3$ kg^{-1} diet caused a high but non-toxic Zn level (Jamieson et al. 2006).

After the completion of the experiment, the animals were anesthetized with pentobarbital sodium given intraperitoneally at a dose of 200 mg kg^{-1} body weight, and then exsanguinated by cutting the apex of the heart. Blood (5 mL) was collected into vacutainer tubes intended for the determination of heavy metals with a heparin containing needle (250 IU) used as anticoagulant. Organ samples were collected (liver and bones) and immediately frozen in liquid nitrogen for further analysis at -80°C.

Emission spectrometry in inductively coupled argon plasma (ICP OES) Pb, Zn determination

The concentrations of Pb and Zn in the material were determined by emission spectrometry in inductively coupled argon plasma (ICP OES) using the camera Optima 2000 DV (Perkin Elmer), after prior digestion in a microwave oven Microwave (Anton Paar).

Test portions of the liver and bone (0.6 g) were transferred to a quartz pressure vessel, to which then 5.0 mL of 65% HNO_3 (Suprapur. Merck) and 0.5 mL of 30% H_2O_2 (Suprapur. Merck) were added. After closing, the vessels with whole blood were placed in a microwave oven equipped with a temperature control system and a constant pressure in each of the quartz vessel. Mineralization parameters were: 0-5 min; a linear gradient from 100 to under 600 W, 6-10 min - 600 (const.), 11-20 min - 1000 W or less after reaching the limit value (75 MPa or 300°C), from 21 to 35 min - cooling the vessel.

Pb and Zn were determined directly in solutions prepared as explained above or diluted 100 times or 1000 times in order to obtain an optimal concentration range for ICP (of the order of several mg L^{-1}). A certified multi -element standard called Merck ICP Multielement Standard IV served as a reference. Solution standards were supplemented with the acid used for digestion added at the level found in the mineralized samples. In order to further minimize the potential of possible interference of sample into the plasma and other types of physical disorders in the plasma of argon, the analysis was performed using an internal standard, hence yttrium (Y) was introduced to the solutions of samples and standards at a concentration of 0.5 mg L^{-1} Y. All measurements of the emitted radiation intensity were performed by selecting a longer optical path of the axial (along the plasma) spectrometer. The measurement parameters: RF power generator – 1300; spray temperature – 30°C, the injection of the sample into the nebulizer – 1.5 mL min^{-1}; gas – argon 5.0, the flow of argon plasma – 15 L min^{-1} flow auxiliary argon – 0.5 L min^{-1}; the transfer of argon nebulizer – 0.8 L min^{-1}; wavelength of 206.200 nm, Zn, Pb wavelength – 220.353 nm.

Statistical analysis

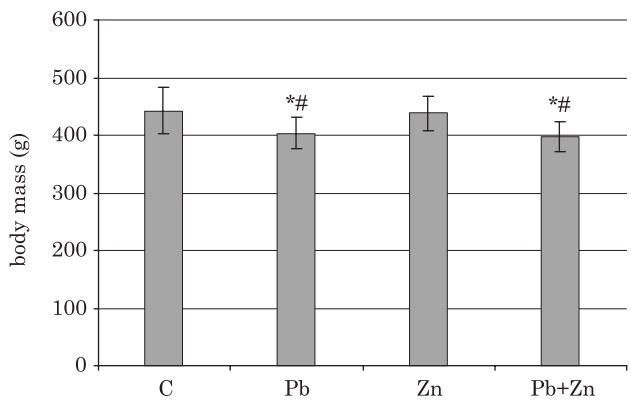
The results were analyzed statistically using Statistica 6.1 software. Arithmetical means and Standard Deviations (SD) were calculated for each of the studied parameters. The distribution of results for individual variables

was obtained by the Shapiro-Wilk test. As most of the distributions deviated from a normal distribution, non-parametric tests were used for further analyses. Correlations between the parameters were examined by the Spearman's rank correlation coefficient (Rs). In order to assess differences between the studied groups, the non-parametric Mann-Whitney *U*-tests or Kruskal-Wallis tests were used, and *p*-values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Body mass

The mean body weight of rats exposed to lead (Pb group) was significantly lower compared to the control group (C group), by 9% (p = 0.049). Significantly lower than the control group (11%, $p = 0.049$) was also the body weight of rats that received Pb and were supplemented with zinc (Zn+Pb group). Also, the reduction of the body weight of rats treated with Pb compared to the group treated with Zn alone (Zn group) was statistically significant: 8% ($p = 0.05$). The 9% decrease ($p = 0.04$) observed in the group of rats that received both Pb and Zn, compared with the group supplemented with Zn (Zn group), proved to be statistically significant as well (Figure 2).



C group – rats receiving drinking water *ad libitum* for 4 weeks ($n = 8$);
Pb group – rats receiving Pb acetate 0.1% (PbAc) in the drinking water *ad libitum* for 4 weeks ($n = 8$);
Zn group – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks ($n = 8$);
Pb+Zn group – rats receiving PbAc in drinking water *ad libitum* plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks ($n = 8$);
* – difference statistically significant vs C group (control) $p < 0.05$;
# – difference statistically significant vs Zn group $p < 0.05$;

Fig. 2. Body mass of rats subjected to 4-week exposure to: Pb (Pb group), lead and zinc supplementation (Pb+Zn group), zinc supplementation (Zn group) and control group (C group)

Lead concentration in the whole blood

The average concentration of Pb in whole blood of rats in the group exposed to lead (Pb group) was approximately 160-times higher (significantly at $p = 0.00015$) than in the control group (C group). Also, the Pb concentration in the blood of rats which received Pb and Zn (Pb+Zn group) ($p = 0.00015$) was significantly higher than in the control (by 94%). A statistically significant increase in the Pb concentration (by 94%) also occurred in the Pb compared to Zn group (Zn group) ($p = 0.00031$). An increase in the concentration was also observed in the group administered lead and zinc (Zn+Pb group) compared with the one supplemented with zinc (Zn group), being about 160-fold higher ($p = 0.00031$) – Table 1.

Lead concentration in plasma

The average concentration of Pb in the plasma of rats exposed to lead (Pb group) was significantly higher in comparison with the control group (C group), by about 171-fold ($p = 0.00031$). Significantly higher, by 99% as compared to the control group, was also the concentration in plasma of rats that received the Pb and Zn (Pb+Zn group, $p = 0.00031$). A statistically significant increase was observed in the Pb concentration (by 99%, $p = 0.00031$) in the group receiving lead (Pb group) compared with the one supplemented with zinc (Zn group). An increased concentration (about 130-fold, $p = 0.00031$) occurred in the group receiving Pb and Zn (Zn+Pb group) compared with the one supplemented with Zn (Zn group) – Table 1.

Table 1

The concentration of Pb in the whole blood (Pb-B) and plasma (Pb-P) of rats after 4 weeks of exposure to Pb (Pb group), lead and zinc supplementation (Pb+Zn group), zinc supplementation (Zn group) and the control group (C group)

Group	**C** ($n = 8$)	**Zn** ($n = 8$)	**Pb** ($n = 8$)	**Pb+Zn** ($n = 8$)
	Pb-B (µg dL^{-1})			
	0.183±0.014	0.188±0.012	3.1317±0.333$^{***###}$	3.270±0.395$^{***###}$
	Pb-P (µg dL^{-1})			
	0.003±0.0007	0.003±0.0002	0.410±0.0627$^{***###}$	0.400±0.0346$^{***###}$

C group – rats receiving drinking water *ad libitum* for 4 weeks ($n = 8$);
Pb group – rats receiving Pb acetate 0.1% (PbAc) in the drinking water *ad libitum* for 4 weeks ($n = 8$);
Zn group – rats receiving $ZnCO_3$ 300 mg kg^{-1} diet for 4 weeks ($n = 8$);
Pb+Zn group – rats receiving PbAc in drinking water *ad libitum* plus 300 mg $ZnCO_3$ kg^{-1} diet for 4 weeks ($n = 8$).
The results are expressed as means ±standard deviation.
*** – difference statistically significant vs C group, $p < 0.001$ (Mann-Whitney test);
– difference statistically significant vs Zn group, $p < 0.001$ (Mann-Whitney test).

Lead concentration in liver

The average concentration of Pb in the liver of rats exposed to lead (Pb group) was significantly higher (by about 400 times) compared to the control group (C group), $p = 0.000155$. Also, the concentration of Pb in the liver of

rats that received Pb and Zn (Pb+Zn group) was significantly higher than in the control (by 97%, $p = 0.00015$). A statistically significantly higher concentration of lead in the liver (96%, $p = 0.00031$) was found as well in animals treated with Pb (Pb group) compared to the group treated with Zn alone (Zn group). Over a 250-fold increase ($p = 0.00031$) was observed in the group of rats that received both lead and zinc (Zn+Pb group) versus the group supplemented with zinc (Gr Zn), which was a statistically significant difference, too (Table 2).

Table 2

The concentration of Pb in the liver and bones of rats subjected to 4-week exposure to Pb (Pb group), Pb and zinc supplementation (Pb+Zn group) zinc supplementation (Zn group) and control group Pb group (C group)

Group	**C** (n = 8)	**Zn** (n = 8)	**Pb** (n = 8)	**Pb+Zn** (n = 8)
	Pb liver (µg g^{-1} d.m.)			
	1.751±1.026	2.297±1.001	7.212±2.150$^{***\#\#\#}$	6.063±1.053$^{***\#\#\#}$
	Pb bones (µg g^{-1} d.m.)			
	0.333±0.145	0.427±0.106	28.744±18.197$^{***\#\#\#}$	27.166±8.256$^{***\#\#\#}$

Key: under Table 1.

Lead concentration in bones

The average concentration of Pb in the bones of rats exposed to lead (Pb group) was significantly higher (by about 850 times) than in the control group (C group), $p = 0.000155$. Significantly higher (by 98%, $p = 0.000155$) than the control was also the Pb concentration in the bones of rats that received Pb and were supplemented with zinc (Zn+Pb group). A statistically significant increase (by 98%) was observed in the bone Pb concentration among the group receiving Pb (Pb group) compared with the group supplemented with Zn (Zn group), $p = 0.00031$. An increase in the concentration (about 600-fold, $p = 0.0003108$) was likewise observed in the group receiving Pb and Zn (Zn+Pb group) in comparison with rats receiving Zn alone (Zn group) – Table 2.

Zinc concentration in plasma

The average concentration of Zn in the plasma of rats exposed to zinc (Zn group) was significantly higher (by 63%) than in the control group ($p = 0.00031$). Also, the concentration in plasma of rats that received Pb and Zn (Pb+Zn group) was significantly higher (42%, $p = 0.000155$) than the control (C group). A decrease in plasma Zn in the group receiving Pb (Pb group) was statistically significant compared with the group supplemented with Zn (Zn group), at 72% ($p = 0.00031$). An increase in the Zn concentration (by 81%, $p = 0.00031$) was also observed in the group receiving Pb (Pb group) versus the one receiving Pb and Zn (Zn+Pb group) – Table 3.

Zinc concentration in liver

The average concentration of Zn in the liver of rats exposed to lead (Pb group) was significantly lower, by 32% (p = 0.049), in comparison with rats supplemented only with Zn (Zn group) – Table 3.

Zinc concentration in bones

The average concentration of Zn in the plasma of rats exposed to lead (Pb group) was significantly lower (by 17%) than in the control group (C group), p = 0.0018. Also, a decrease (by 12%) in the concentration of plasma Zn in the group receiving Pb (Pb group) compared with the group supplemented with Zn (Zn group, p = 0.0029) was statistically significant. A decrease in the Zn concentration by 10% (p = 0.0028) was also observed in the group administered Pb (Pb group) compared to the group receiving Pb and Zn (Zn+Pb group) – Table 3.

Table 3

The zinc concentration in plasma (Zn-P), liver, and bone of rats after 4 weeks of exposure to Pb (Pb group), Pb and zinc supplementation (Pb+Zn group) zinc supplementation (Zn group) and control groups (C group)

Group	**C** (n = 8)	**Zn** (n = 8)	**Pb** (n = 8)	**Pb+Zn** (n = 8)
	Zn-P (mg L^{-1})			
	1.376±0.204	2.242±0.158***	1.3041±0.235###&&&	2.358±0.160***
	Zn liver (mg kg^{-1} d.m.)			
	3.919±0.945	4.290±0.887	3.245±0.921#	3.191±1.354
	Zn bones (mg kg^{-1} d.m.)			
	232.722±28.952	216.748±19.787	193.163±14.636***#&	213.304±13.226

Key to the groups: under Table 1
The results are expressed as means ± standard deviation.
*** – difference statistically significant vs C group; $p < 0.001$ (Mann-Whitney test);
# – difference statistically significant vs Zn group; $p < 0.05$ (Mann-Whitney test);
– difference statistically significant vs Zn group; $p < 0.001$ (Mann-Whitney test);
& – difference statistically significant vs Zn+Pb group; $p < 0.05$;
&&& – difference statistically significant vs Zn+Pb group; $p < 0.001$.

Distribution of lead in the tissues examined

In the control group (C group), almost half of the average Pb level was found in the bones of rats (49%), the remainder being almost equally divided between whole blood and liver (about1/4). An average percentage of lead in blood plasma was 0.4% (Figure 2).

In the group of rats subjected to 4-week exposure to lead (Pb group), the average percentage of lead in the bones increased compared to the control group (C group) by nearly a quarter, and was over 70%. However, Pb levels in the liver and blood were reduced by 7% and 18%, while the content in plasma significantly increased in comparison to the control group and amounted to 1% of the average amount in the tissues examined (Figure 2).

In the group of rats given Zn supplementation (Zn group), the average percentage of lead in bone was similar to the control group (C group). Distribution of Pb in the whole blood and plasma was at the same level as in the control group. There was a significant positive correlation between the concentration of Zn and Pb in the liver (Rs = +0.89) in this group (Figure 2).

In the group of rats subjected to 4-week exposure to lead and zinc (Pb+Zn group), the percentage distribution of Pb in bones was similar to the group of rats treated with lead (Pb group). The percentages of Pb in the whole blood, plasma and liver were also similar to the ones in the group of rats treated with lead, in which there was a significant negative correlation between the serum Zn concentrations and Pb content in the liver (Rs = -0.71) and bone (Rs = -0.73) – Figure 3, Table 4.

Lead

The four-week exposure of rats to 0.1% PbAc in drinking water caused an increase in the concentration of Pb in all examined tissues. The available literature informs that more than 90% of lead contained in the blood is absorbed by red blood cells, while 10% remains in the plasma, of which from 40% to 75% binds to plasma proteins, mainly albumin. The rest of Pb in plasma binds to low molecular weight ligands, while only 0.01% of the total pool of Pb is in the free state (Giel-Pietraszuk et al. 2012). In the present study, a 16-fold increase was shown in the Pb concentration in the blood as compared to the control group, 87% of Pb was found in erythrocytes, while only 13% remained in the plasma, thereby confirming the strong affinity of the metal to erythrocytes.

The bones are considered to be the main reservoir of Pb in the body – over 90% Pb has been shown to accumulate in the tissue, where it is far less metabolically active than in soft tissues. In the view of the fact that bones belong to slow changing tissues, Pb detoxification in bones takes a very long time and can last from a dozen to several dozens of years. In our study, more than a 90-fold increase in the concentration of Pb compared with the control group was observed in the bones of the tested animals, and the content of Pb in the tissue was 10 times higher than in the whole blood, thereby confirming the cumulative capacity of the tissue with regard to lead. However, according to many recent studies, even the bone lead pool, previously regarded as a deposit site for this element and also a form of its detoxification, can be mobilized in various physiological states such as pregnancy, osteoporosis and hormonal disorders (Gulson et al. 2002, Nash et al. 2004, Riedt et al. 2009). Under these conditions, lead deposited in bones may be released into the bloodstream and therefore to other tissues/organs (Giel-Pietraszuk et al. 2012).

Kulikowska et. al (1994) also observed increased concentrations of Pb in all examined tissues of rats exposed to 500 ppm of PbAc for 6 weeks. The concentration of Pb in the liver was four times higher (1.15 μg g^{-1} dry mass), and in the whole blood several times higher (62 μg dL^{-1}) than in the control

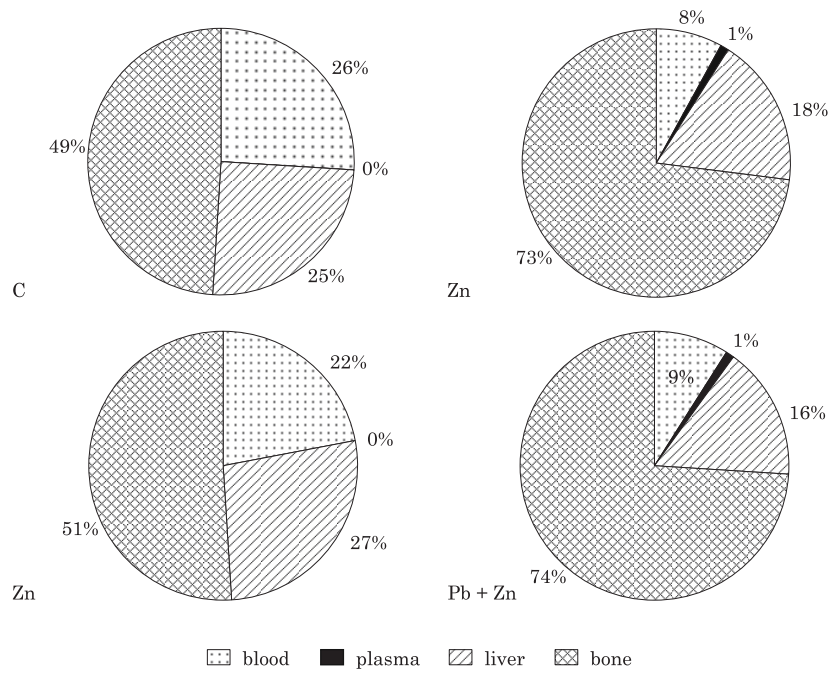


Fig. 3. Average per cent distribution of Pb in the control group (C group), rats subjected to 4-week exposure to Pb (Pb group), zinc supplementation (Zn group), lead and zinc supplementation (Pb+Zn group)

Table 4

The correlation between the concentration of zinc in blood plasma (Zn-P) and the concentration of Pb in the tissues tested

Group	Correlation	Spearman rank correlation coefficient	Significance of difference (p)
C	Zn-P – Pb plasma	0.19	0.6514
	Zn-P – Pb liver	-0.05	0.9108
	Zn-P – Pb bone	+0.38	0.3518
Pb	Zn-P – Pb plasma	+0.04	0.9394
	Zn-P – Pb liver	+0.25	0.5887
	Zn-P – Pb bone	-0.17	0.7016
Zn	Zn-P – Pb plasma	0.04	0.9394
	Zn-P – Pb liver	**+0.89**	**0.0068*****
	Zn-P – Pb bone	-0.53	0.2152
Zn+Pb	Zn-P – Pb plasma	-0.35	0.4316
	Zn-P – Pb liver	**-0.71**	**0.0503***
	Zn-P – Pb bone	**-0.73**	**0.0503***

group. The lead concentration in the bones of the examined rats reached a value of more than 90-fold higher than in the control and was approximately 94 μg g^{-1} d.m.

Ademuyiwa et al. (2010) investigated the effect of various concentrations of Pb on its distribution in the blood and other organs such as the liver, kidney, spleen and heart. For 12 weeks, rats were given 200, 300 and 400 ppm Pb. In all examined tissues, there was an increase in the concentration of the toxic element. In the whole blood of rats treated successively with 200, 300 and 400 ppm Pb doses, the Pb concentration increased 6, 9 and 5 times in comparison to the control. Similarly, in the liver there was a 10-, 3.5- and 2.5-fold increase in the concentration of this element in relation to the control group. The authors concluded that an increase in the concentration of Pb in blood is proportional to the administered dose. In our study, the spleen was the only organ which showed such dose dependency.

Batra et. al (2001) studied the effect of different doses of testicular and epididymal Pb in rats treated sequentially with 10, 50 and 200 mg Pb kg^{-1}. The increase in the concentration of Pb in the testes of rats was proportional to the administered dose, but there was no such relation in the case of the epididymis, confirming the data obtained by Ademuyiwa et al. (2010).

Kozielec et. al. (1994) conducted research on children to demonstrate the relationship between the age and Pb level in hair. They examined 135 children aged 5 to 14 years. The experiment did not take into account the body weight and sex of the children. The experimental results showed that the highest average Pb content in hair appeared in the youngest group of respondents, i.e. children from 5 to 6 years old, where it averaged 2.8 μg g^{-1} d.m. and 2.68 μg g^{-1} d.m. The level of Pb in hair of older children was significantly lower. This relationship may indicate a better ability to uptake Pb by the youngest children.

Zinc

The four-week supplementation of rats with zinc carbonate at a dose of 300 mg kg^{-1} diet did not cause significant changes in the body weight compared with the control group. Also, the amount of food intake was similar to the control animals. There was a significant, 63% increase in plasma Zn compared with the control group, while in the liver and bone there was no significant increase in the concentration of Zn. A significant increase in plasma Zn indicates its actual absorption from the gastrointestinal tract. The duration of the experiment may have been insufficiently long for Zn contained in the plasma to be transferred to organs. It cannot be excluded that extension of the experimental period would have increased the concentration of Zn in all examined tissues. This argument is supported by the work of Mazurek-Machol et al. (2005), who analyzed the content of Zn in tissues in a 12-week experiment. Rats were given two different doses of Zn in water: the lower dose was 1 mg kg^{-1} while the higher one reached 3 mg

kg^{-1}. Same as in the present study, the supplementation had no effect on the body weight and mobility of rats in comparison with the control group. The Zn concentration in serum was about 18% higher versus the control in both supplemented. groups The Zn concentration in urine was approximately 32% higher in the group receiving the higher dose and slightly lower in the group supplemented with the lower dose Zn. In the group treated with the higher Zn dose, there was also a significant, 24% increase in the concentration of this element in bones. In both supplemented groups, there was an increase in bone mass caused by zinc supplementation, although it was not statistically significant. It was shown that the degree of absorption of Zn was proportional to the dose.

In our study, rats treated with Zn supplementation and simultaneously receiving PbAc in drinking water had a lower concentration of Zn in plasma. This could be due to some antagonism between Zn and Pb (Pasternak et al. 2000), since both elements have been shown to possess a similar mechanism of absorption in the intestine, hence competing with each other in the process of intestinal transport and intracellular transport (Morawiec 1991). In addition, Pb also induces the inhibition of intestinal absorption of other microelements (Hornowska et al. 1996), and act antagonistically towards Zn in cells (e.g. binding by proteins). Presumably, this was the case in our study, where rats fed Pb in drinking water had a reduced Zn concentration in plasma. Slight decreases in the content of Zn during Pb administration compared with the control group were observed in blood and the liver, although the differences were not statistically significant. However, there was a significant decrease in the bone Zn concentration (about 17% compared to the control group). It can be assumed that exposure to Pb had a negative effect on the concentration of Zn in bone probably also due to an increased elimination of zinc from the body in response to the increased supply of Pb. It is also conceivable that longer exposure or a higher dose of Pb could be additionally reflected by decreased concentrations of Zn in other examined tissues.

Pasternak et al. (2000) examined the effect of different concentrations of Pb on the Zn content in the skin of rats. The animals received 100 or 300 mg L^{-1} PbAc in drinking water. After six weeks of the experiment, a decrease was observed in the concentration of Zn compared with the control group in both groups. Under exposure to a higher concentration of Pb, the Zn concentration in the skin was 43.6 μg g^{-1} of tissue, which was about 17% less than in the control group, where the concentration of Zn was 52.1 μg g^{-1} of tissue. At a Pb concentration of 100 mg L^{-1}, a 5% decrease in the concentration of Zn in the skin was also observed, although it was not statistically significant. It was found that the Pb administered in a diet lowered the concentration of Zn in the skin of rats, and this relationship was proportional to the dose of Pb. A slightly different result was obtained by Ademuyiwa et al. (2010), who investigated the relationship between the administration of various concentrations of Pb on the Zn content in the blood, heart, liver, spleen, and kidneys. The animals were divided into 4 groups, which received 200,

300 or 400 ppm Pb. Their results confirmed the negative impact of Pb on the Zn concentration in the blood, and the lowering of the blood Zn content was directly proportional to the dose, same as reported by Pasternak et al. (2000). However, such a dose-related dependence was not found in other organs, i.e. the kidney, liver and spleen.

Kozielec et al. (1994) studied the Pb content in the blood of children of different ages and its impact on the level of other microelements in hair. It was shown that 7.4% of patients had low levels of zinc in their hair. Those children also had the highest blood Pb (from 3.5 μg dL^{-1} - 8.7 μg dL^{-1}), which would confirm the mutual rivalry of both elements.

Zinc supplementation

Numerous studies have concluded that Pb affects the metabolism of many divalent elements (Skoczyńska 2006). It has been shown that both the excess and deficiency of individual elements acts on other micronutrients primarily by affecting their absorption from the gastrointestinal tract and/or cellular transport. The mechanism of this influence may be used to eliminate or reduce the absorption of Pb from the body, because it has been shown that it competes for binding of ligands to such elements as Zn, Cu or Ca (Hornowska et al. 1996). Another important factor is the aforementioned antagonism with other elements, such as Ca, Fe and Zn in absorption from the gastrointestinal tract (Pasternak et al. 2000).

Our study on rats treated for four weeks with PbAc in drinking water and rats which, in addition to PbAc, were supplemented with 300 mg kg^{-1} $ZnCO_3$ diet, showed a Zn-induced reduction in Pb in all examined tissues except blood. A decrease in the concentration of Pb was the highest in the liver (by 16%) compared to a 5% decline in the bones, while only 2% in plasma. Differences in concentrations were not statistically significant although it is possible that longer supplementation time would have resulted in a significant decrease in the concentration of Pb, as evidenced by the observed significant strong negative correlation between the concentration of Zn and Pb in the liver (Rs = -0.71, $p = 0.05$) and bone Rs = -0.73, $p = 0.05$).

In the experiment of Batra et al. (2001), the effect of 3-month zinc supplementation on the concentration of Pb was investigated in the testes and epididymis of rats treated with lead. One group of animals received 50 mg Pb kg^{-1}, and the other one was given 50 mg Pb kg^{-1} and supplemented with 1 mg Zn kg^{-1}. The concentration of Pb in the testes of rats that had received only Pb was 1586 ng g^{-1} tissue while in rats also supplemented with Zn the Pb concentration decreased by as much as 33% and was 1064 ng g^{-1} tissue. Similarly, Pb concentration in the epididymis decreased from 477 ng g^{-1} tissue to 218 ng g^{-1} tissue, a 55% decline. According to the researchers, the main cause of the protective effect of Zn was the limited accessibility of binding sites for Pb in the intestine. Kulikowska et. al. (1994) during a six-week-long experiment gave 500 ppm Pb to rats, of wihch one

group received Zn concentration of 240 ppm for two weeks. There was a 30% decrease in the bone Pb content and a 50% decrease in blood Pb. In other organs such as the liver, kidney and spleen, despite Zn supplementation, Pb levels were maintained at a level similar to the group exposed to lead.

In treatment of children with elevated blood Pb levels above the currently regarded as safe (5 μg dL^{-1}), chelation therapy is recommended. As it is known, chelates are complexes in which the so-called central ion (in most cases this a divalent or trivalent metal) is bound to organic ligands. Due to the excess of Pb or the deficiency of micronutrients with a competitive activity in the body (Ca, Zn, Fe, Cu), these elements may be displaced and replaced by lead. Compounds which can prevent this in chelation therapy are EDTA (ethylene-diamine-tetraacetic acid) or DMSA (2,3-dimercaptosuccinic acid). The latter has been used in the removal of Pb from the soft tissues and bones since in the 1990s, when it was introduced in the United States in order to reduce Pb in children (Sapota, Ligocka 1996). The answer to the question whether these compounds are able to eliminate the already established effects of exposure to Pb, however, requires further research, but they certainly cause a reduction in Pb concentration in blood. A study by Tandon et al. (1994) was expected to answer to the question whether chelators with an additional methionine and Zn supplementation cope better with the removal of Pb from tissues of rats than chelators without additives. It turned out that the addition of zinc and methionine to EDTA or DMSA significantly improves the removal of Pb from tissues and excretion in the urine.

Honey-based preparation called Propolis was tested as an alternative to the previously used DMSA. Sapota, Ligocka (1996) in an experiment carried out on 4 groups of rats studied the effect of various concentrations of Propolis and DMSA on the content of Pb in tissues. Each treatment group received Pb for 6 days at a dose of 5 mg kg^{-1} per day. During the experiment, the first group was supplemented with DMSA preparation, while the other was given Propolis at a lower concentration of 15/2, and the third one received Propolis at a higher concentration of 25/3. The control group consisted of rats that received only lead. A significant decrease was shown in the concentration of Pb in rats treated with DMSA. The reduction of this element was observed in almost all examined tissues. The effect of Propolis was less spectacular, with the concentrations of Pb decreasing in almost all examined tissues (from 2-36%) *albeit* in a statistically insignificant manner. The comparison of the two Propolis concentrations showed a higher Pb reduction and better elimination of this element after the more diluted preparation had been administered.

The use of chelators such as EDTA and DMSA is the most effective method to remove Pb from cells. It should be borne in mind that both chelators have a number of side effects, for example non-selective binding to all divalent metals, including the essential ones, meaning that a long-term therapy of Pb poisoning causes acute deficiencies of other elements; thus indications for the use of chelating agents remain controversial. Chelation

has eliminated the actual mortality in Pb poisoning, but there are no reports indicating that chelation is effective in relieving neurological effects of Pb poisoning. In addition, the use of chelating preparations such as EDTA or BAL requires a painful and expensive parenteral therapy with the concomitant risk of nephrotoxicity. Currently used chelating agents such as d-penicillamine (hydrocracking, Cuprimine) are effective as oral chelating agents at lower concentrations of Pb in the whole blood. The side effects, however, include allergy, nausea, haematuria, and a drop in the counts of blood platelets and white blood cells. Undoubtedly, an important role is also played by proper nutrition and satisfying the demand for vitamins, ions and minerals, especially in the most vulnerable patients such as small children. It must be remembered that the shortage of elements such as Cu, Zn, Fe and Mg increases the harmful effects of Pb (Hornowska et al. 1996). Therefore, therapy consisting of Zn supplementation only or Zn supplementation supporting the action of chelators already in use may be crucial for the elimination of Pb from the body.

CONCLUSIONS

Incidental exposure of adult rats to Pb at doses resulting in the level of Pb in blood below the previously recognized as safe resulted in: (i) increased Pb in the bones and plasma and the reduction in the whole blood and liver (ii) simultaneous supplementation of rats exposed to Pb with high but non-toxic dose of zinc: – did not result in the reduction of the concentration of Pb in the blood and tissues of rats, – did not result in changes in the distribution of Pb in the tissues examined (iii) supplementation of diets with high but non-toxic dose of zinc is not an effective method for reducing the concentration of Pb in the blood at Pb-B previously recognized as safe. However, the therapy consisting of zinc supplementation to support the action of chelators could be crucial for the elimination of Pb from the body.

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