

Exposure to lead affects male biothiols metabolism

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Abstract

The most important biothiols include glutathione, homocysteine (HCY), cysteine and proteins. The aim of the presented study was to evaluate the influence of lead on the biothiol turnover – the concentration of HCY and protein sulfhydryl groups (P-SH) in the serum and reduced glutathione (G-SH) in erythrocytes – in individuals (employees of metal works) exposed to lead and to evaluate its probable oxidative disorders, measured as the carbonyl protein (CP) concentration in serum. The exposed workers were divided into 2 subgroups: 1) low lead exposure (LPb), with a lead concentration in the blood (PbB) of 20–45 µg dl⁻¹ (n= 102), and 2) high lead exposure (HPb), with PbB = 45–60 µg dl⁻¹ (n= 81). The control group consisted of 72 office workers or other healthy subjects with no history of occupational exposure to lead. All the controls had normal PbB (<10 µg dl⁻¹) and ZPP (<2.5 µg dl⁻¹) levels. The concentration of HCY was higher in the LPb group by 11% and in the HPb group by 26%, compared with the control group (n=72). The CP concentration in these 2 groups was more than twice as high as that of the control group, with 108% and 125% increases for the LPb and HPb groups, respectively; G-SH was lower by 6.6% and 7.4% for the LPb and HPb groups, respectively; P-SH was lower by 8.2% and 13% for the LPb and HPb groups, respectively. Lead decreases levels of glutathione and protein thiol groups. Lead-induced oxidative stress contributes to the observed elevation of protein carbonyl groups. Besides, lead poisoning seems to be associated with hyperhomocysteinaemia, which may promote the development of atherosclerosis.

Key words

lead poisoning, biothiols, homocysteine, glutathione, oxidative stress

INTRODUCTION

With the development of industry and technological progress, human exposure to many harmful metals, including lead (Pb), has significantly increased. Apart from the increased content of this element in the soil, water and air, an increased exposure to lead is also observed in workers in the metallurgical and chemical industries [1].

The deleterious effect of lead depends on the exposure period, received dose, route of absorption (through the respiratory system, digestive system or skin), the presence of other xenobiotics, age, gender and genetic factors [2]. After absorption by inhalation, lead enters directly into the circulatory system. In contrast, after entry via the gastrointestinal tract, lead is absorbed in the stomach and small intestine, whence it is transported first to the liver and then into the general circulation. However, regardless of the route of entry into the body, its distribution and accumulation always show a similar pattern [3].

Lead toxicity entails the functional impairment of many tissues and organs, such as the nervous, digestive, skeletal, genitourinary, haematologic, cardiovascular, and immune systems [4]. Exposure to this element may also contribute to the development of hypertension [5] and the formation of atherosclerotic plaques [6]. The action mechanism of lead

in the living organism is not entirely known. It is believed to involve an interaction with other metals (typically with a similar electron shell structure), bonding with various molecules in a cell, or a change in the oxido-reductive potential inside the cell that is associated with an increased intensity of the oxidative processes [7, 8]. Oxidative stress caused by lead may result from directly generating reactive oxygen species (ROS), modifying the antioxidant system activity or both mechanisms simultaneously [9].

The antioxidant system of the body, apart from antioxidant enzymes (such as superoxide dismutase, glutathione peroxidase and catalase) and vitamins (A, E and C), also includes thiol compounds (R-SH) which are compounds with a sulfhydryl group (-SH). The most important thiols in the body (called biothiols) are glutathione, homocysteine (HCY), cysteine, coenzyme Q, lipoic acid, ergothioneine, phosphopantetheine and proteins, e.g., albumin. These compounds are chelators of the metal ions that generate ROS, and they are a component of thiol-disulfide redox buffers [10]. In reactions with ROS, single-electron oxidation of thiols and the formation of the thiyl radical (RS[•]) occurs [11]. The newly formed thiyl radical undergoes reduction and inactivation, and the biothiols regain their ability to scavenge harmful free radicals [10]. Therefore, biothiols are compounds that largely determine the effectiveness of antioxidant defence in the body.

The association between lead-exposure and biothiols metabolism is poorly understood. Therefore, the presented study evaluates the turnover of biothiols with respect to the

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degree of exposure to lead and the possible effects of probable disorders (protein oxidation).

MATERIAL AND METHODS

The study examined male employees of metal work plants in the southern region of Poland who had been exposed to lead. To determine the degree of exposure to lead compounds, the concentrations of lead (PbB) and zinc protoporphyrin (ZPP) in the blood samples were recorded. The study was based on the current concentrations of PbB and ZPP (PbB_{last} and ZPP_{last}) and on their average values (PbB_{mean} and ZPP_{mean}) from the last 2 years, measured every 3 months. The exposed workers were divided into 2 subgroups based on the values of PbB_{mean} : low lead exposure (LPb), with a PbB_{mean} between $20 - 45 \mu g dl^{-1}$ ($n=102$) and high lead exposure (HPb), with a PbB_{mean} over $45 \mu g dl^{-1}$ (maximum value $59.8 \mu g dl^{-1}$, $n=81$). Workers with malignant tumors or serious liver, kidney or heart diseases were excluded. The control group consisted of 72 office workers or other healthy subjects with no history of occupational exposure to lead. All the controls had normal PbB ($<10 \mu g dl^{-1}$) and ZPP ($<2.5 \mu g g^{-1} Hb$) levels.

Blood was collected to obtain serum and erythrocytes with ethylenediamine-tetraacetic acid (EDTA) solution used as an anticoagulant. The PbB and ZPP levels were determined in the whole blood.

The determination of PbB was performed by graphite furnace atomic absorption spectrophotometry, using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z Graphite Furnaces. The data were reported in $\mu g dl^{-1}$. ZPP was measured using an Aviv Biomedical Haematofluorometer, Model 206. The results were expressed as micrograms per gram of haemoglobin ($\mu g g^{-1} Hb$).

The remaining blood was centrifuged. The erythrocyte pellet was rinsed using 0.9% NaCl. Next, the erythrocytes were hemolyzed with de-ionized water. In 10% haemolysate, the concentration of haemoglobin was indicated using Drabkin reagent, and that of reduced glutathione (G-SH) was determined as described by Pawelski [12] with minor modifications. This procedure involved a reaction with DTNB (5,5'-dithiobis(2-nitrobenzoic acid)), which undergoes reduction by G-SH, yielding the anion derivative 5-thio-2-nitrobenzoate, which has a yellow colour. The absorbance was measured at 412 nm, and the samples tested in relation to a control sample containing double-distilled water instead of haemolysate. The G-SH concentrations were expressed in $\mu mol/g$ of haemoglobin.

Protein sulfhydryl groups (P-SH) concentration was determined as described by Koster et al. [13], using DTNB, which undergoes reduction by compounds containing sulfhydryl groups, yielding the yellow anion derivative 5-thio-2-nitrobenzoate, which absorbs at a wavelength of 412 nm. The concentrations were presented in $\mu mol/g$ of serum proteins.

The concentration of homocysteine (HCY) in the serum was determined by an enzyme-linked immunosorbent assay, using a pre-made kit by Axis-Shield Diagnostics Ltd. (Dundee, Scotland, UK). The HCY in the serum, whether bound to proteins or existing as a mixed disulfide after reduction to the free form (by dithiothreitol), is converted to S-adenosyl-L-homocysteine (SAH) in the presence of a suitable hydrolase. After the reaction of SAH with monoclonal anti-SAH

antibodies and then with antibodies containing peroxidase, the substrate for peroxidase was introduced into the solution, and the absorbance was read at 450 nm. The concentrations were presented in $\mu mol/l$.

For determination of the carbonyl protein (CP) group in the serum, the method of Reznick and Packer [14] was used, applying the reaction with dinitrophenylhydrazine. The resultant precipitate was washed with trichloroacetic acid and a mixture of ethanol/ethyl acetate, then a solution of guanidine was added. The absorbance was read at a wavelength of 360 nm. The concentrations were presented in $nmol/g$ of serum proteins.

The experimental set-up was approved by the Bioethics Committee of the Medical University of Silesia in Katowice.

Statistical analysis was performed using Statistic 8.0 PL software and included mean, standard deviation (SD) and standard error of the mean (SEM). Shapiro-Wilk's test was used to verify normality and Levene's test to verify homogeneity of variances. An analysis of variance or Kruskal-Wallis ANOVA test was used for multiple comparisons of data. Additional statistical comparisons were made by t-test, t-test with separate variance estimates or Mann-Whitney U test. Spearman non-parametric correlation was calculated. To assess the qualitative variables, the Chi-square statistics were used. A value of $p < 0.05$ was considered to be significant.

RESULTS

The groups exposed to lead (LPb and HPb) did not differ in age, weight or frequency of smoking (Tab. 1), but the indicators of exposure to lead (PbB and ZPP) were significantly higher in both groups than in the controls. The PbB values were nearly 5–6 times higher in the LPb group and nearly 6–7 times higher in the HPb group, compared to the control group, and ZPP exceeded approximately 3 times the allowable limits in both lead-exposed groups. The HCY concentration was higher in the LPb group than in the control by 11%, although this difference was not statistically significant, and in the HPb group by 26% ($p=0.003$) in comparison to the control group, while statistically significantly lower concentration relative to the control were observed for G-SH (by 6.6% and 7.4% for the LPb and HPb groups, respectively) and P-SH (by 8.2% and 13% for the LPb and HPb groups, respectively) (Tab. 1). The concentration of CP, however, was more than twice as high as in the control group, with an increase of 108% (LPb) and 125% (HPb), respectively.

Analysis of correlation (Tab. 2) showed a positive relationship between the concentrations of HCY and CP and the indicators of exposure to lead ($R = 0.26 - 0.31$ and $R = 0.28 - 0.37$, $p < 0.05$, respectively), negative for G-SH (R between -0.25 and -0.31 , $p < 0.05$) and negative between P-SH and PbB (R between -0.26 and -0.28 , $p < 0.05$). Moreover, a strongly positive correlation between the duration of exposure to Pb (years of lead exposure) and CP ($R = 0.38$, $p < 0.001$), stronger than that for age ($R = 0.26$, $p = 0.003$), was determined.

DISCUSSION

In experimental and clinical studies, it has been reported that the presence of lead promotes an increased production of ROS [15], which may react with proteins, lipids and DNA.

Table 1. Epidemiologic and toxicology parameters, concentration of homocysteine (HCY), protein sulfhydryl groups (P-SH), carbonyl protein (CP) in serum, and reduced glutathione (G-SH) in erythrocytes in the study population (*P* value – when compare to control group).

	Control group n=73		L-Pb group PbB=20–45 n=102			H-Pb group PbB>45 n=81		ANOVA	
	Mean	SD	Mean	SD	<i>p</i> value	Mean	SD	<i>p</i> value	<i>p</i> value
Age (years)	41.55	9.23	40.5	10.42	0.482	43.9	7.44	0.084	0.058
Years of lead exposure			15.9	10.01		20.1	9.86		0.006
Weight (kg)	80.9	10.41	81.2	11.38	0.870	83.1	12.18	0.228	0.400
Smoking habit (%)	47%		50%		0.657	54%		0.340	0.630
PbB _{mean} (µg dl ⁻¹)	6.45	2.49	38.4	5.44	<0.001	49.2	4.01	<0.001	<0.001
PbB _{last} (µg dl ⁻¹)	6.39	2.47	43.3	9.22	<0.001	50.7	6.09	<0.001	<0.001
ZPP _{mean} (µg g ⁻¹ Hb)	1.93	0.47	7.56	3.91	<0.001	8.50	2.99	<0.001	<0.001
ZPP _{last} (µg g ⁻¹ Hb)	1.96	0.51	8.28	4.77	<0.001	8.11	3.16	<0.001	<0.001
HCY (µmol l ⁻¹)	13.3	3.79	14.8	4.55	0.126	16.8	5.24	0.003	0.009
P-SH (µmol g ⁻¹ protein)	6.55	1.31	6.01	3.21	0.012	5.68	1.96	0.005	0.010
G-SH (µmol g ⁻¹ Hb)	14.3	1.96	13.3	1.64	<0.001	13.2	1.61	<0.001	<0.001
CP (nmol g ⁻¹ protein)	467	157	970	424	<0.001	1052	443	<0.001	<0.001

Table 2. Correlation between studied parameters (Spearman R values, *p*<0.05, NS – non significant).

	HCY	G-SH	P-SH	CP
Age	NS	NS	-0.32	0.26
Years of lead exposure	NS	NS	NS	0.38
Weight	NS	NS	NS	NS
PbB _{mean}	0.31	-0.31	-0.26	0.28
PbB _{last}	NS	-0.28	-0.28	0.37
ZPP _{mean}	0.27	-0.26	NS	0.34
ZPP _{last}	0.26	-0.25	NS	0.34

In the reaction of ROS with sulfhydryl groups (-SH) of the amino acid residues of proteins (P), thiyl radicals of proteins (PS*) are created, followed by peroxy radicals with the participation of oxygen. In the reaction of the thiyl radical with another protein, the peroxide protein is produced (P-SOOH), as is another protein – thiyl radical.

The basic effect of free radical reactions in proteins is the formation of peroxides of proteins and the oxidation of sulfhydryl groups. In addition, the enhanced hydroxylation and nitration of amino acid residues and the conversion of certain amino acid residues to their carbonyl derivatives are observed [16]. Increased protein oxidation under the influence of lead has been confirmed by the results of tests on animals [17] and clinical tests [18]. Our studies conducted on individuals exposed to lead showed that their concentrations of sulfhydryl groups were significantly reduced relative to controls, whereas their concentrations of carbonyl protein were elevated, which may indirectly indicate the enhanced peroxidation of proteins. Both indicators are considered to be markers of the oxidative damage to proteins [19]. It can be concluded that, among the tested individuals, changes occur in the structure and function of proteins and in the accumulation of modified protein products. These effects may result in significant disturbances in the total biothiyl pool and in a reduction of the efficiency of the antioxidant defence system.

One of the most important antioxidants of body fluids is glutathione. In the body, glutathione oxidation occurs enzymatically, which is greatly accelerated by the enzyme

glutathione peroxidase in the presence of oxidizing agents, such as hydrogen peroxide and organic peroxides. The thiol group of glutathione also reacts with the hydroxyl radical and organic radicals present in the aqueous phase. In subjects in both lead-exposed groups in the presented study, the concentration of G-SH was significantly lower than that in the control group, being inversely proportional to the degree of exposure to harmful conditions. The decreased concentration of the reduced form of glutathione may indicate not only the increased production of free radicals, but also a direct effect of lead on glutathione metabolism. This effect is confirmed by other authors of papers on the toxicity of lead [20].

Glutathione has 6 sites to which lead may potentially be bound, 2 peptide bonds and 4 free groups: the carboxylic group of glutamic acid and the glycine, amino and sulfhydryl groups. Lead shows the greatest affinity to the sulfhydryl groups. As a result, lead mercaptides (G-S-Pb-S-G) are formed spontaneously without the participation of enzymes. The thiol compound participating in the formation of lead mercaptides is primarily glutathione (G-SH), but potentially may also be protein (P-SH), cysteine, or lipoic acid [21]. The generation of the lead mercaptides of glutathione decreases the pool of reduced glutathione and may partially explain the presented results.

The produced mercaptide of glutathione (G-S-Pb-S-G) may replace one of the attached ligands (G-S-) in contact with other thiol compounds on the new ligand [21] through the production of the glutathione thiyl radical (G-S). In consequence, increased amounts of oxidized glutathione (G-S-S-G) and mixed disulfides (G-S-S-B) are produced. This mechanism explains the increased S-glutathionylation of proteins reported under the influence of lead [22]. Besides, increased concentrations of glutathione disulfide (G-S-S-G) under the influence of lead have been reported in the liver, kidney and brain of animals exposed to Pb [23]. This effect has been confirmed also by studies on cell cultures [24].

Thiyl radicals of proteins and glutathione, which may be overproduced in lead poisoning, demonstrate their ability to receive hydrogen atoms from other compounds and can themselves generate ROS. After the combination of thiyl radicals with oxygen, peroxy radicals are created (R-SOO),

and after their combination with glutathione, the radical of glutathione disulfide (G-S-S-G) is formed, from which G-S-S-G and the superoxide anion ($O_2^{\cdot-}$) are created. The thiyl radical also reacts with polyunsaturated fatty acids participating in peroxidative processes [25]. Therefore, the thiyl radical may simultaneously explain the disturbances in the thiol turnover of the cell and intensification of the free radical processes induced by lead. However, thus far, there has been no research documenting the formation of the thiyl radical under the influence of lead.

The decline in the concentration of the reduced form of glutathione may also be caused by an insufficient supply of methionine. In mammalian cells, methionine is delivered in the food, and HCY is one of its metabolites. In the body, HCY is metabolized by transsulfuration or remethylation. The choice of the route of these changes is determined by the concentration of S-adenosylmethionine, which is formed from methionine. In normal conditions, approximately 50% of HCY is catabolized via the transsulfuration pathway. Thus, HCY reacts with serine, and as a result of this process, cystathionine is formed. This compound, in turn, breaks down into cysteine and α -ketobutyrate. The remainder of the HCY undergoes remethylation, yielding methionine [26].

In the human plasma, approximately 65% of the HCY is associated with proteins, approximately 30% is HCY in free asymmetric disulfides (mainly disulfides with cysteine), and 1.5–4% exists as reduced HCY. The fractions of HCY are components of the general redox status of all the biothiols in the plasma. With the increase in the total concentration of HCY, the concentration of reduced HCY increases, which significantly changes the redox status of other thiol compounds. As a result, there is a change in the availability of thiol residues in proteins and enzymes as a result of the thiol-disulfide exchange. If the amount of resulting HCY exceeds the amount of metabolized HCY, then the metabolic capacity of the cell will be exceeded, and HCY will be exported to the extracellular space, mainly to the blood [10].

The total concentration of HCY in the plasma normally amounts to 10–15 mM. It increases, among other factors, with age and in smokers. In the presented study, the age and percentage of smokers among the control and examined groups were similar. Despite this similarity, the concentration of HCY was significantly higher in the subjects exposed to higher doses of lead. Consistently, a significant association between blood lead and HCY among Vietnamese factory workers was reported after controlling for age and gender [27]. In another study, a linear association between blood lead and HCY in older adults after controlling for age, gender, race/ethnicity, alcohol intake, cigarette smoking, educational level and BMI was reported [28]. Besides, Yakub and Iqbal [29] confirmed the existence of an association between PbB and HCY in Pakistani civilians. Therefore, it is reasonable to expect that lead must have an influence on HCY metabolism. Elevated HCY levels in lead poisoning may result in many adverse health effects. First, the increased concentration of HCY is a risk factor for venous thrombosis. Second, hyperhomocysteinaemia induces increased free radical processes, as HCY generates the superoxide anion ($O_2^{\cdot-}$) and hydrogen peroxide (H_2O_2) in the presence of copper ions or ceruloplasmin. Both ROS are also produced in the presence of lead ions. This effect leads to the oxidation of lipoproteins and may promote the cellular absorption of modified LDL and impair the relaxation of the blood vessels. Therefore, this

condition is conducive to the development of atherosclerosis, coronary heart disease and stroke [26].

One of the hypotheses concerning the toxicity of homocysteine suggests that it results from the conversion of HCY into homocysteine thiolactone. This compound, upon forming adducts with proteins, modifies the structures of the proteins and negatively affects their functions [30]. All of these effects of increased concentrations of HCY are similar to the changes in the cardiovascular system found in lead poisoning [31].

Many experimental studies document the beneficial influence of various thiol compounds in lead poisoning. In tests on animals, methionine [32], N-acetylcysteine [33], S-adenosyl-L-methionine [34], cysteine [35] and selenocysteine [24] were applied. Their application after exposure to lead may have a positive impact on the turnover of biothiols in humans, but this issue requires further study.

CONCLUSIONS

Lead decreases levels of glutathione and protein thiol groups. Lead-induced oxidative stress contributes to the observed elevation of protein carbonyl groups. Besides, lead poisoning seems to be associated with hyperhomocysteinaemia, which may promote the development of atherosclerosis.

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