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LEAD-ELEVATED ACTIVITY OF XANTHINE OXIDASE IN LEAD-EXPOSED WORKERS

INDUKCJA AKTYWNOŚCI OKSYDAZY KSANTYNOWEJ PRZEZ OŁÓW
U ZAWODOWO NARAŻONYCH PRACOWNIKÓW

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ABSTRACT

Background: The aim of the present study was to explore the connection between lead toxicity and the activity of xanthine oxidase (XO). In addition, we indicated the uric acid (UA) and creatinine levels and concentration of erythrocyte malondialdehyde (MDA) to estimate oxidative stress intensity. **Materials and Methods:** The examined group consisted of 125 healthy male employees of zinc and lead works. The examined group was divided into tertiles according to blood lead levels. In the collected blood samples, concentrations of lead-exposure indices, UA, creatinine, and MDA as well as activity of XO were measured concomitantly. The control group consisted of 32 healthy male administrative workers who were exposed to lead only environmentally. **Results:** XO activity and MDA level were significantly elevated in all tertiles compared to the control group. Creatinine level was significantly elevated in the medium and high tertiles. However, the level of UA was significantly elevated in the high tertile, while in the low and medium tertile only a tendency toward higher values was observed. **Conclusions:** Occupational exposure to lead induces activity of XO. This induction may contribute to the observed simultaneously increased oxidative stress, measured as MDA level, and the increased level of UA. *Med Pr* 2013;64(2):175–180

Key words: lead poisoning, xanthine oxidase, uric acid, creatinine, oxidative stress

STRESZCZENIE

Wstęp: Celem pracy była analiza wpływu narażenia na ołów na aktywność oksydazy ksantynowej (xanthine oxidase – XO). Dodatkowo wyznaczono stężenia kwasu moczowego (uric acid – UA) i kreatyniny. Natężenie stresu oksydacyjnego oszacowano na podstawie stężenia dialdehydu malonowego (malondialdehyde – MDA). **Materiał i metody:** Grupę badaną stanowiło 125 zdrowych pracowników huty cynku i ołowiu. Stopień narażenia na ołów oceniano na podstawie stężenia ołowiu i cynkopropofiryny we krwi, a także kwasu delta-aminolewulinowego w moczu. Na podstawie stężenia ołowiu we krwi grupa badana została podzielona na tercyle. W próbkach krwi uzyskanych od uczestników badania dokonano analizy wyżej wymienionych parametrów biochemicznych. Grupę kontrolną stanowiło 32 zdrowych pracowników administracji nienarażonych na ołów. **Wyniki:** Aktywność XO i stężenie MDA były znacząco wyższe we wszystkich tercylach w porównaniu z grupą kontrolną. Stężenie kreatyniny osiągnęło także znacząco wyższe wartości, lecz tylko w środkowym i górnym tercylu. Z kolei stężenie UA było znacząco wyższe wyłącznie w górnym tercylu. Jednocześnie zaobserwowano tendencję do wyższych wartości jego stężenia w dwóch pozostałych tercylach.

Wnioski: Zawodowe narażenie na ołów indukuje wzrost aktywności XO, który może przyczyniać się do nasilenia stresu oksydacyjnego, mierzonego jako stężenie MDA, i powodować wzrost stężenia UA. Med. Pr. 2013;64(2):175–180

Słowa kluczowe: zatrucie ołowiem, oksydaza ksantynowa, kwas moczowy, kreatynina, stres oksydacyjny

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INTRODUCTION

Lead is a pleiotropic toxicant. Health effects at high blood lead levels are demonstrable, while the effects at lower blood levels of lead remain unclear (1). Despite the fact that there is no safe level of exposure to lead, it has been widely used in industry due to its malleability, resistance to corrosion, and low melting point. Lead accumulates in bones, liver, kidneys, and other organs one hour after intestinal absorption. Exposure to lead results in many adverse health effects, including behavioral disorders or the dysfunction of liver, kidneys and many systems of the human body, such as the hematological, the immunological, and the nervous system (2).

The mechanisms involved in lead toxicity are poorly understood, nevertheless, it is well-documented that one of the most important toxic effects of lead is oxidative stress. Lead generates reactive oxygen species (ROS), such as superoxide radicals, hydrogen peroxide, or hydroxyl radicals, and weakens antioxidant defenses. Lead does not only deplete glutathione (GSH) content but also alters the expression and activities of antioxidant enzymes, such as superoxide dismutase (SOD) or glutathione peroxidase (GPx). In consequence, elevated levels of lipid peroxidation products, including malondialdehyde (MDA), have been reported in lead poisoning (3).

Lead influences activities of enzymes via interactions with sulfhydryl groups and metal cofactors. An inhibitory effect of lead on delta-aminolevulinic acid dehydratase (ALAD) and ferrochelatase is well-known. As a result, lead impairs the chain reaction that leads to the formation of heme and causes anemia. Due to the fact that the accumulation of delta-aminolevulinic acid (ALA) and zinc protoporphyrin (ZPP) occurs simultaneously, levels of these compounds are used as human lead-exposure indices (4).

Lead-exposure has been associated also with increased serum uric acid (UA) level. However, this association remains unclear, especially among individuals

without chronic kidney disease. Hyperuricemia associated with lead poisoning may be due to the increased production or decreased excretion of UA. The decreased excretion of UA may occur in lead-induced nephropathy and be a result of the isolated proximal tubular defects (1,5). On the other hand, the production of UA depends on the activity of xanthine oxidase (XO) (6). Ariza et al. (7) demonstrated that lead ions elevate XO activity in AS52 cells. Xanthine oxidase does not only catalyse the formation of UA but also generates ROS. Therefore, the elevated activity of XO could hypothetically explain the association between lead poisoning and both the increased ROS production and hyperuricemia. To our knowledge, in the available literature, there is no study on this topic conducted on humans. In the light of this, the aim of the present study was to explore the connection between lead toxicity and the activity of XO. In addition, we indicated the UA and creatinine levels and the concentration of erythrocyte MDA to estimate oxidative stress intensity.

MATERIALS AND METHODS

Study population

The examined group consisted of 125 male employees of zinc and lead works localized in Miasteczko Śląskie. Their age ranged between 23 and 59 years. They were exposed to lead from 1 to 38 years. Workers suffering from chronic diseases and receiving any drugs were excluded.

Blood lead levels (PbB) and concentrations of ZPP in the blood and ALA in the urine served as the biomarkers of lead-exposure. All of these indices had been determined, on average, every three months during two years of observation and afterwards mean values of them were calculated (PbB_{mean} , ZPP_{mean} , ALA_{mean}). The examined group was divided into tertiles according to the PbB_{mean} levels (low tertile – $PbB_{mean} = 20.0\text{--}31.6 \mu\text{g/dl}$, medium tertile – $PbB_{mean} = 31.7\text{--}40.0 \mu\text{g/dl}$, high tertile – $PbB_{mean} = 40.1\text{--}56.2 \mu\text{g/dl}$).

In the last collected blood samples, concentrations of PbB, ZPP, UA, and creatinine as well as activity of XO were measured concomitantly. To obtain erythrocytes for MDA concentration, ethylenediaminetetraacetic disodium acid solution as anticoagulant was used. ALA levels were determined in the urine samples.

Control group consisted of 32 healthy male administrative workers who were exposed to lead only environmentally and had no history of occupational exposure to lead. Their age ranged between 28 and 57 years. Every individual in this group had the levels of PbB or ZPP lower than the normal levels which were 10 µg/dl and 2.5 µg/g Hb, respectively.

Laboratory procedures

Whole blood was used for the analysis of PbB and ZPP. The concentration of PbB was measured by graphite furnace atomic absorption spectrophotometry. Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z Graphite Furnaces were used. Data was shown in µg/dl. The concentration of ZPP was measured directly using the Aviv Biomedical hematofluorometer model 206. The instrument measured the ratio of fluorescent substance (ZPP) to the absorption of light in the sample (hemoglobin). Results were displayed as µg ZPP per gram of hemoglobin (µg/g Hb).

The concentration of ALA was measured in the urine samples by Grabecki et al. (8). In this method, ALA reacted with acetylacetone and formed a pyrrole substance which reacted with dimethylaminobenzoic aldehyde. The colored complex was measured spectrophotometrically. Results were expressed as mg/dl.

The activity of XO was measured in serum according to Majkić-Singh et al. (9). In this method, chromogen 2,2'-azino-di(3-ethylbenzthiazoline-6-sulfonate) (ABTS) was oxidized in the system of coupled reactions catalyzed by XO, uricase, and peroxidase. The absorbance of oxidized ABTS was directly proportional to the XO activity. Results were expressed as U/l.

The concentration of UA was measured using the A25 biochemical analyzer (BioSystems, Spain) according to the manufacturer's instructions. Results were expressed as µmol/l.

The concentration of MDA in hemolysate of erythrocytes was determined by assaying the thiobarbituric acid reactive substance (TBARS) according to the method of Ohkawa et al. (10) using spectrofluorometer LS45 (Perkin Elmer). To improve the specificity of the method, we used sodium sulfate and butylated hy-

droxytoluene (BHT). Results were expressed as µmoles per dl of erythrocytes (µmol/dl of erythrocytes).

The concentration of creatinine was measured by the method with picric acid. Results were expressed as mg/dl.

Statistical analysis

Statistica 9.1 PL software was used to perform the statistical analysis. Statistical methods included the mean and standard deviation. Levene's test was used to verify the homogeneity of variances. Shapiro-Wilk test was used to verify normality. Statistical comparisons between the examined groups and the control group were made by a t-test, t-test with a separate variance estimates, or a Mann-Whitney U test. The Spearman non-parametric correlation was also calculated. The value of $p < 0.05$ was considered to be significant.

RESULTS

There were no significant differences in the mean age, body mass index (BMI), and smoking habits between the examined population and the control group.

The biomarkers of lead-exposure were significantly higher in the exposed group compared to the controls.

Xanthine oxidase activity was significantly elevated in all tertiles compared to the control group. The level of UA was significantly elevated in the high tertile, while in the low and the medium tertile only a tendency toward higher values was observed. The concentration of creatinine was significantly elevated in the medium and high tertiles, while erythrocyte MDA level was significantly elevated in all tertiles compared to the control group (Table 1).

The Spearman correlation showed that there are positive correlations between lead-exposure markers and MDA level. Besides, XO activity correlates positively with PbB, ALA, and MDA levels (Table 2).

DISCUSSION

Purine oxidation is catalyzed by xanthine oxidoreductase (XOR) that catabolizes hypoxanthine to xanthine and then to UA by hydroxylation (11). Xanthine oxidoreductase is a molybdenum iron-sulfur flavin hydroxylase and it is present in various organs, such as the liver, gut, lungs, kidneys, heart, brain, and plasma. Xanthine oxidoreductase exists in two inter-convertible forms: XO (EC 1.1.3.22) and xanthine dehydrogenase (XDH) (EC 1.1.7.1.4) (12). The xanthine dehydrogenase form of the enzyme uses NAD⁺ as the preferred electron

Table 1. The epidemiologic parameters, the levels of lead in the blood (PbB), the levels of zinc protoporphyrin in blood (ZPP), the levels of delta-aminolevulinic acid in the urine (ALA), the activity of xanthine oxidase (XO), and the levels of uric acid (UA), creatinine, and malondialdehyde (MDA) in the study population

Tabela 1. Dane epidemiologiczne, stężenie ołowiu (PbB) we krwi, stężenie cynkoprotoporfiryny (ZPP) we krwi, stężenie kwasu delta-aminolewulinowego (ALA) w moczu, aktywność oksydazy ksantynowej (XO) oraz stężenie kwasu moczowego (UA), kreatyniny i dialdehydu malonowego (MDA) w badanej populacji

Parameter Parametr	Control group Grupa kontrolna (N = 32)		Study population Badani									ANOVA p
			low tertile dolny tercyl (N = 42)			medium tertile środkowy tercyl (N = 41)			high tertile górnny tercyl (N = 42)			
	M	SD	M	SD	p	M	SD	p	M	SD	p	
Age / Wiek [w latach]	43.3	8.29	42.00	10.40	0.563	41.60	9.03	0.412	42.30	9.13	0.639	0.887
Seniority [years] / Staż pracy [w latach]	-	-	18.20	11.50	-	17.80	10.00	-	17.60	10.30	-	0.860
Weight / Masa ciała [kg]	80.90	9.94	80.20	12.20	0.795	81.60	12.90	0.806	81.20	12.20	0.900	0.960
BMI	26.60	2.74	26.30	3.38	0.660	26.80	3.15	0.837	27.30	4.20	0.444	0.625
Smokers / Palący [%]	50.00	-	55.00	-	0.689	46.00	-	0.760	52.00	-	0.842	0.890
PbB _{mean} / PbB _{sr.} [µg/dl]	8.03	2.47	29.80	6.40	< 0.001	37.90	6.29	< 0.001	44.60	5.10	< 0.001	< 0.001
PbB [µg/dl]	7.88	2.44	26.10	4.52	< 0.001	36.20	2.60	< 0.001	45.60	4.08	< 0.001	< 0.001
ZPP _{mean} / ZPP _{sr.} [µg/g Hb]	1.91	0.69	3.87	3.03	0.001	4.81	2.55	< 0.001	6.36	3.77	< 0.001	< 0.001
ZPP [µg/g Hb]	1.93	0.72	4.28	2.90	< 0.001	5.41	3.26	< 0.001	7.87	5.33	< 0.001	< 0.001
ALA _{mean} / ALA _{sr.} [mg/l]	2.28	0.85	3.45	1.03	< 0.001	3.80	0.92	< 0.001	4.21	1.13	< 0.001	< 0.001
ALA [mg/l]	2.18	0.84	3.10	1.07	< 0.001	3.00	1.31	0.003	3.55	1.51	< 0.001	< 0.001
UA [µmol/l]	4.52	0.80	4.87	0.97	0.104	4.94	1.25	0.107	5.25	1.36	0.008	0.050
XO activity [U/l]	0.54	0.21	0.93	0.43	< 0.001	1.03	0.70	< 0.001	0.86	0.37	< 0.001	< 0.001
Creatinine / Kreatynina [mg/dl]	0.95	0.14	1.00	0.10	0.060	1.03	0.13	0.012	1.010	0.14	0.050	0.048
MDA [µmol/dl erythrocytes/ / erytrocytów]	15.80	4.04	19.60	3.84	< 0.001	20.10	3.91	< 0.001	20.90	2.90	< 0.001	0.005

Low tertile / dolny tercyl: PbB = 20.0–31.6 µg/dl; medium tertile / środkowy tercyl: PbB = 31.7–40.0 µg/dl; high tertile / górnny tercyl: PbB = 40.1–56.2 µg/dl.

BMI – body mass index / wskaźnik masy ciała.

M – mean / średnia.

SD – standard deviation / odchylenie standardowe.

acceptor, while the XO form uses oxygen as the electron acceptor, producing superoxide anions and hydrogen peroxide. Xanthine oxidoreductase originally exists in the XDH form, but could be converted to XO either reversibly by oxidation of cysteine residues to form disulfide bridges or irreversibly by proteolysis (6,12,13).

The results of the present study support the findings of Ariza et al. (7). Consistently, Kilikdar et al. (14) reported the increased activity of XO in rats administered with lead acetate in the dose of 15 mg/kg body weight. However, Prasanthi et al. (15) observed the decreased activity of XO in the brains of developing and adult mice exposed to lead. According to the authors of this study, the decrease of XO activity may be due to the binding

of lead to the sulfhydryl groups of the enzyme. On the other hand, the interactions between lead and the sulfhydryl groups under other conditions may contribute to the increased conversion of XDH to XO. Besides, lead could theoretically induce structural changes in the enzyme by replacing essential metals (7). The hypothetical ability of lead to induce the conversion of XDH to XO supports our results and may also explain why exposure to lead induces oxidative stress and elevates the UA level. Due to the fact that lead-exposure alters the levels of IL-1 and TNF-α (4), which have been shown to up-regulate the transcription of XOR (12), the second possible explanation for our results may be associated with lead-induced changes in the immunological response.

Table 2. Correlations (Spearman R values) between the analyzed parameters**Tabela 2.** Korelacje Spearmana

Parameter Parametr	Correlations (Spearman R values) Współczynnik R korelacji			
	creatinine kreatynina	UA	XO	MDA
PbB _{mean} / PbB _{sr.}	0.16	0.18	0.29	0.31
PbB	0.17	0.20	0.26	0.30
ZPP _{mean} / ZPP _{sr.}	ns	0.19	0.16	0.23
ZPP	ns	0.17	0.17	0.31
ALA _{mean} / ALA _{sr.}	0.20	0.20	0.28	0.23
ALA	ns	ns	ns	ns
UA	0.23		ns	ns
XO	0.21	ns		0.29

p < 0.05.

ns – non-significant / nieistotne statystycznie.

Other abbreviations as in Table 1 / Inne objaśnienia jak w tabeli 1.

Purine metabolism in humans leads to the formation of UA that is present intracellularly and in all body fluids and excreted in the urine. Uric acid has been proposed to be one of the most important low-molecular-mass antioxidants in the human biological fluids. It is believed that UA does not only act as a radical scavenger, but also chelates metal ions and converts them to poorly reactive forms unable to catalyse free-radical reactions. On the other hand, some studies indicate that UA has pro-inflammatory properties (16) and can cause endothelial dysfunction through the stimulation of vascular smooth muscle proliferation. Besides, the elevated levels of UA are known to inhibit the release of nitric oxide within the vasculature of kidneys resulting in reduced renal blood flow and glomerular filtration rate (5).

The association between lead-exposure and the elevation of UA has been investigated in various studies in both occupationally exposed and general population. It has been reported that much lower, than it was previously thought, lead doses cause the increase in serum uric acid level (5). However, in the present study, significantly elevated UA level was observed only in the high tertile. Slightly but significantly elevated levels of creatinine in the medium and high tertile were simultaneously observed. Despite not so strong correlation between the levels of UA and creatinine ($r = 0.23$), the obtained results indicate that UA elevation in the examined population may be due not only to XO induction but also to lead-induced nephropathy. Other studies

rather support our research. Alasia et al. (5) reported elevated UA and creatinine levels in lead-exposed workers ($PbB = 50.4 \pm 24.6 \mu\text{g/dl}$). In this study, UA level correlated positively with serum creatinine level ($r = 0.134$) and negatively with creatinine clearance ($r = -0.151$). After the adjustment for age, weight and height, Ehrlih et al. (17) also found positive exposure response relations between lead-exposure indices and serum creatinine and UA concentrations in exposed workers ($PbB = 53.5 \mu\text{g/dl}$). Consistently, Khan et al. (18) reported elevated UA level and positive correlations between PbB and serum creatinine ($r = 0.51$) and UA ($r = 0.29$) levels in lead-exposed workers ($PbB = 29.1 \mu\text{g/dl}$). On the other hand, Omae et al. (19) and Roels et al. (20) investigated workers exposed to lead and concluded that exposure up to $70 \mu\text{g/dl}$ of PbB may not cause adverse effects on renal function. However, when examining aboriginals and non-aboriginals living in Taiwan, Lai et al. (21) reported that people with PbB exceeding even $7.5 \mu\text{g/dl}$ are at a higher risk of renal dysfunction and hyperuricemia. Despite the discrepancies between the above-mentioned results, it is possible to state that there is a dose-effect relationship between blood lead and the UA level.

Lipids are the principal targets of oxidative stress because they easily undergo oxidation. Malondialdehyde is the most studied product of polyunsaturated fatty acid peroxidation and it is able to impair several physiological mechanisms of human body through its reactivity with DNA and proteins (22). The elevated concentration of erythrocyte MDA observed in the present study confirms the potency of lead to induce oxidative stress and may be partially caused by increased XO activity. A positive correlation between the MDA level and XO activity ($r = 0.29$), observed in the present study, supports this hypothesis. Positive correlations between MDA level and indices of lead-exposure were shown as well. Consistently, elevated MDA levels were reported in many studies conducted on both lead-exposed animals and humans. Our previous reports also showed increased MDA levels in workers exposed to lead (23–25).

CONCLUSIONS

Occupational exposure to lead induces activity of XO. This induction may be due to the increased conversion of XOR to XO. Elevated XO activity may have contributed to the observed simultaneously increased oxidative stress, measured as MDA level, and the increased level of UA.

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