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SERUM VASOACTIVE AGENTS IN LEAD-TREATED RATS

ANNA SKOCZYŃSKA[1], EWA STOJEK[1], HELENA GÓRECKA[2] and ANNA WOJAKOWSKA[1]

[1] Department of Internal Medicine, Occupational Diseases and Hypertension
Wrocław Medical University
[2] Institute of Inorganic Technology and Mineral Manure
Wrocław University of Technology
Wrocław, Poland

Abstract
Background: It is still unknown whether mechanisms of the hypertensinogenic effect of lead include changes in synthesis or release of vasoactive agents. This question is essential with regard to lead dissemination in the human environment as well as to frequent occurrence of arterial hypertension. **Objectives:** The aim of the study was to evaluate the effect of chronic exposure to lead on the vasoactive agents in blood in relation to redox system activity in vessel walls and to disturbances in homeostasis of essential metals. Using lead in double small, hypertensive doses we tried to estimate whether this effect dependents on the degree of lead exposure. **Methods:** The study was performed on the male Buffalo rats which were given lead in drinking water, 50 or 100 ppm (lead acetate dissolved in distillate water) for 12 weeks. Control rats were given distillate water. Rats were fasted starting the night before the experiment, and the next day were anesthetized intramuscularly with ketaminum at a dose of 300 mg/kg body weight. The abdomen was opened and the aorta was isolated. Blood samples were drawn from heart, abdominal and thoracic aorta and then kidneys were excised. Serum nitric oxide and prostaglandin $PGF_{2\alpha}$ concentrations were measured using R&D systems, and the plasma endothelin-1 level with enzymoimmunoassay; 5% homogenates of aorta were prepared from thoracic fragment in saccharose buffer. Lipid peroxides in homogenates were determined colorimetrically and glutathione was measured using colorimetric assay BIOXYTECH GSH-400. The concentration of metals (lead, copper and zinc) in blood and aorta were determined with a plasma spectrometer. **Results:** The study shows different changes in toxicological and biochemical status, depending on the dose of metal. Mean serum nitric oxide concentration was higher in rats treated with lead in a dose of 50 ppm ($p < 0.01$) or 100 ppm ($p < 0.001$) than in the control group. The plasma endothelin-1 level was lower in rats given lead in a dose of 50 ppm ($p < 0.05$) than in controls, whereas serum prostaglandin $PGF_{2\alpha}$ concentration was similar in all animals. Glutathione concentration in aorta was higher in both groups of rats treated with lead ($p < 0.001$) in comparison to controls. There were positive linear dependencies between: (a) blood lead and serum nitric oxide; (b) aorta glutathione and serum nitric oxide; (c) copper in aorta and glutathione in aorta; (d) serum zinc and plasma endothelin-1 concentrations. **Conclusions:** It was concluded, that lead in small doses increases synthesis and/or releases nitric oxide and its concentration in serum. This effect of lead is probably connected with the augmented production of glutathione in vessel walls. Additionally, lead in a dose of 50 ppm provokes the decrease in the level of plasma endothelin-1, probably through the decreased level of serum zinc. We suppose that the mechanisms responsible for the vascular effect of lead differ even within the range of hypertensive doses.

Key words:
Nitric oxide, Endothelin, Lipid peroxidation, Lead, Rats

INTRODUCTION

Lead, present in the human natural environment and given in small doses to experimental animals induces arterial hypertension [1,2]. The mechanisms responsible for the hypertensinogenic effect of lead differ and involve nervous, hormone and intracellular signaling pathways. Lead induces disturbances in the regulation of arterial blood pressure by the central and peripheral nervous sys-

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Address reprint requests to A. Skoczyńska, MD, PhD, Department of Internal Medicine, Occupational Diseases and Hypertension, Wrocław Medical University, Pasteura 4, 50-367 Wroław, Poland (e-mail: annaskoc@ak.am.wroc.pl).

tem, changes reactivity of the cardiovascular system to catecholamines, and influences the kallikrein-kinin and renin-angiotensin-aldosterone systems [3–6]. Simultaneously, lead increases serum norepinephrine concentration and reduces the amount of beta-adrenoreceptors in vessel walls and cAMP levels in plasma and aorta [2,7]. Intracellular effect of lead involves perturbations in the calcium ions homeostasis due to altered ATP-ases, protein kinase C and cyclic guanyl cyclase activities [8–10].

However, it is still unknown, if the mechanisms of the hypertensinogenic effect of lead embrace changes in the synthesis or release of vasoactive agents, such as nitric oxide (NO) or endothelin. This question is essential with regard to lead dissemination in the environment and the incidence of arterial hypertension. Epidemiological studies provide data that indicate the involvement of lead in the development of spontaneous arterial hypertension [11]. In patients with genetic predisposition to develop hypertension even a short-term exposure to small doses of lead causes a persistent increase in blood pressure [12]. However, it is not easy to find out whether the relationship between exposure to lead and its level in the body and the incidence of hypertension does really exist in view of the presence of numerous confounding factors**.** These factors are alcohol drinking and smoking, which considerably increase the blood lead concentration [13,14].

In experimental animal studies, lead in small doses raises the arterial blood pressure, depending on an absorbed dose, exposure duration, and way of its administration, as well as on the animal species, age and metabolic activity. It is supposed that a possible mechanism of pathogenic influence of lead on blood vessels is associated with the synthesis and release of vasoactive factors. The relationship between the occurrence of lead-induced hypertension and changes in nitric oxide metabolism confirm the results of experiments performed on rats treated with lead. Lead given in hypertensive doses decreased the amount of NO metabolites eliminated from urine, and increased nitrotirosine concentration in kidneys, heart, liver and cerebral tissue [15,16]. Both the antioxidants and NO precursor (L-arginine) protected from lowering the synthesis of NO and made blood pressure normal in lead-poisoned rats [17,18]. The experiments performed *in vitro* on isolated and perfused mesenteric superior artery of lead-poisoned rats showed that lead induced changes in vascular reactivity to NO synthase inhibitor [19] and endothelial vasodilator activity [20].

Thus the results of many studies show that lead-induced hypertension could be associated with the decreased synthesis, and/or release, and/or bio-availability of nitric oxide in vessel walls. On the other hand, in hypertensive lead-poisoned rats, the paradoxical growth of expression of nitric oxide synthase, as well as the inducible and/or endothelial form in blood vessels and renal tissue were observed [18,21]. Similarly, the results of measurements of endothelin in serum of lead-poisoned rats were inconsistent; the increased and unchanged levels of endothelin-1 (ET-1) were shown [18,22].

The aim of this study was to evaluate the impact of chronic treatment with lead on the vasoactive agents, such as nitric oxide, endothelin-1 and prostaglandin $F_{2\alpha}$ in blood. It is known that the concentration of this agent in blood represents the value resulted from the balance between its synthesis, release, distribution and elimination. However, the comparison of blood levels of the main vasoconstrictor and vasodilator (endotelin-1 vs nitric oxide) could be useful in the estimation of vascular effect of lead.

The relation of the observed changes to the redox system activity in vessel walls and to disturbances in homeostasis of essential metals should explain the mechanism of lead effect on vasoactive agents. Through administration of lead in double small or hypertensive doses, we tried to find out whether this effect depends on the degree of lead exposure.

MATERIALS AND METHODS

Study material

The study design was approved by the Local Ethics Committee to the Experimental Studies in Wrocław.

The study protocol was performed according to the International Convention on Animal Experimentation. Buffalo rats, aged 6–7 weeks (155 to 185 g) were kept in screen floored cages in a vivarium at 20°C and relative humidity

of 50%. The animals had free access to LSM chow and water. The experimental rats were given lead in drinking water, 50 or 100 ppm (lead acetate dissolved in distillate water) for 12 weeks. The control rats were given distillate water.

Rats were fasted starting the night before the experiment, and the next day were anesthetized intramuscularly with ketaminum at a dose of 300 mg/kg body weight. The abdomen was opened through a midline incision, and the aorta was isolated. Blood samples were taken from heart, abdominal and thoracic aorta. Kidneys were excised and washed in 0.9% NaCl solution. Blood was collected using EDTA as an anticoagulant. Serum was obtained after centrifugation (10 min at 1000 • g). Whole blood, serum, abdominal aorta and kidneys, collected to determine concentration of metals, were stored at temperature -20°C. Lead was measured in whole blood and organs, copper and zinc in serum and organs. Biological samples were digested in microwave system MILESTONE, Italy. Multielemental analyses were carried out with use of inductively coupled plasma mass spectrometry (ICP-MS) – VARIAN UltraMass 700, Australia:

Reagents

Chemicals were of analytical ultrapure grade. Deionized water was in each solution. Multielemental standard solution prepared according to the Johnson-Mattey ICP standard solutions. Certified reference materials: whole blood, MI 1256, Seronorm™ trace elements obtained from the Scandinavian blood bank; bovine muscle, CRM 184 and pig kidney, CRM 186, Commission of the European Communities, Community Bureau of Reference.

A sample of 0.5 g exactly weighed was mixed with 5 cm^3 of nitric acid (65%) in teflon microwave digestion vessels. Complete digestion was carried out under the following conditions: 600 W for 5 min under total pressure of 100 atm. in the microwave system. After digestion, a sample solution was filled up to the volume of 50 ml with deionized water. Blank sample solution was prepared in the same way. The same digestion method was used for preparing reference materials. Samples and standards were spiked with 100 μg/dm^3 as an internal standards for all elements. Reference material and samples were introduced for the analysis and diluted in the manner that 5 million counts per sec were not exceeded.

The following instrumental conditions were used for plasma spectrometry ICP-MS UltraMass 700 from Varian: plasma flow – 16.0 dm^3/min, auxiliary flow – 1.25 dm^3/min, nebulizer flow – 1.0 dm^3/min, sampling depth – 1.0 dm^3/min, power – 1.35 kW, dwell time – 10 ms, scans replicate – 10. Calibration curves of the analyzed elements were performed in the concentration range of 0.1, 10, and 100 ppb. Multielemental matrices, as well as certified reference material underwent the procedure of mineralization, identical with that used in samples.

Determination limit for analyzed elements in blood: Pb – 0.002 ppm, Cu – 0.001 ppm, Zn – 0.004 ppm, and in muscle: Pb – 0.030 ppm, Cu – 0.015 ppm, Zn – 0.043 ppm.

For the quantitative determination of total nitric oxide in serum, NO_2^-/NO_3^- assay of R&D systems was used. This assay involves the conversion of nitrate to nitrite by the enzyme nitrate reductase. The detection of nitrite is than determined as a colored azo-dye product of the Griess reaction that absorbs visible light at 540 nm. The concentration of NO is indirectly measured by determining both nitrate and nitrite levels in the sample. The relative levels of nitrate and nitrite can vary substantially, depending on ambient conditions and redox state of the given biological fluid. Therefore, the most accurate determination of total nitric oxide production requires quantification of both nitrate and nitrite.

Prostaglandin $F_{2\alpha}$ in serum was determined using R&D systems for $PGF_{2\alpha}$ immunoassay. If samples were not assayed immediately, a prostaglandin synthase inhibitor (ketonal) was added to all samples at approximately 10 μg/ml before storage at temperature -20°C. Endothelin-1 was measured in plasma using BIOMEDICA, Wien Enzymoimmunoassay. Freshly collected EDTA-plasma was put on ice immediately and centrifuged within one hour. The samples were stored at temperature -20°C no longer than two weeks.

Aorta 5% homogenates were prepared from thoracic fragment in saccharose buffer. Tissue samples were homogenized using homogenizer MPW 309 and centrifuged at

10 000 in temperature of 4°C for ten min. Determination of lipid peroxides (LPO) in homogenates was performed colorimetrically, according to the Satoh method [23], glutathione (GSH) was measured using colorimetric assay BIOXYTECH GSH-400.

Statistical analysis

The values are presented as the means ± standard deviation (SD). One-way analysis of variance for repeated measurements was applied. The significance of differences between mean values was determined by *post-hoc* comparison performed by Newman-Keuls test. P-values below 0.05 were considered statistically significant. Each mean value was derived from nine to ten rats. Statistical analysis was performed with STATISTICA 5.0 software.

RESULTS

Lead present in drinking water in concentration of 50 or 100 ppm increased the level of lead in abdominal parts of aorta in poisoned rats (Table 1). There was a linear correlation between blood lead level and aorta lead concentration ($r = 0.66$; $p < 0.01$) and between blood lead level and kidney lead concentration ($r = 0.80$; $p < 0.001$). The analysis of the results also showed the linear dependence between lead concentrations in aorta and in kidneys ($r = 0.72$; $p < 0.001$).

In rats treated with lead in a dose of 50 ppm the increase in blood lead level was associated with the decreased serum zinc concentration ($p < 0.01$), whereas in rats given lead in a dose of 100 ppm, the serum zinc level was similar to that in controls (Table 2). The change in zinc concentration in kidneys was also observed in rats treated with lead in a dose of 50 ppm only (the increase statistically significantly higher than in controls). In addition, the increase in the copper level in aorta was observed in the study rats (Table 2).

Rats poisoned with lead in a dose of 100 ppm did not display any significant disturbances in homeostasis of essential metals.

An analysis of linear regression showed the linear correlation only between lead and zinc levels in the kidney. Correlation of linear regression coefficients is presented in Table 3.

The mean serum NO_2^-/NO_3^- concentration was higher in rats treated with lead in a dose of 50 ($p < 0.01$) or 100 ppm ($p < 0.001$) than in the control animals. The plasma endo-

Table 1. Lead concentrations in blood (μg/l), kidney and aorta (mg/kg) of rats poisoned with lead in doses of 50 ppm or 100 ppm and in controls

Lead concentration	Groups		
	Pb 50 ppm (n = 9)	Pb 100 ppm (n = 10)	Controls n = 10
Blood (μg/l)	112.3 ± 42.3**	173.1 ± 59.3**	1.61 ± 3.4
Kidney (mg/kg)	1.25 ± 0.19**	1.74 ± 0.67**	0.08 ± 0.04
Aorta (mg/kg)	0.24 ± 0.07*	0.63 ± 0.18**	0.08 ± 0.07

Each value represents the mean ± SD.
n – number of rats.
* $p < 0.01$; ** $p < 0.001$ vs corresponding vehicle-treated group.

Table 2. Concentrations of copper and zinc in serum (μg/l), kidney and aorta (mg/kg) of rats poisoned with lead in doses of 50 ppm or 100 ppm and in controls.

Copper and zinc concentrations		Groups		
		Pb 50 ppm (n = 9)	Pb 100 ppm (n = 10)	Controls (n = 10)
Serum (μg/l)	Cu	273.4 ± 33.5	250.8 ± 62.5	255.6 ± 35.8
	Zn	132.0 ± 10.1**	167.0 ± 17.5	163.6 ± 24.7
Kidney (mg/kg)	Cu	10.4 ± 1.24	11.0 ± 1.8	9.9 ± 1.7
	Zn	19.2 ± 3.5*	17.3 ± 2.3	15.9 ± 1.5
Aorta (mg/kg)	Cu	1.4 ± 0.37*	1.2 ± 0.2	1.1 ± 0.2
	Zn	34.3 ± 9.55	38.2 ± 7.8	36.9 ± 10.2

Each value represents the mean ± SD.
n – number of rats.
* $p < 0.05$; ** $p < 0.01$ vs corresponding vehicle-treated group.

Table 3. Coefficients of linear regression between concentrations of lead, copper and zinc in serum, kidney and aorta of rats (n = 29)

Correlation coefficient (r)	Pb in blood	Pb in kidney	Pb in aorta
Cu in serum	0.1970	-0.0468	-0.0494
Cu in kidney	0.1296	0.2086	0.2423
Cu in aorta	0.2167	0.1522	0.0049
Zn in serum	-0.0564	-0.2565	0.0953
Zn in kidney	0.3039	0.3752*	-0.0287
Zn in aorta	0.0720	-0.1017	0.1696

* Statistical significance of coefficient r; $p < 0.05$.

thelin-1 level was changed only in rats given lead in a dose of 50 ppm; it was lower in comparison with controls ($p < 0.05$), whereas the mean serum $PGF_{2\alpha}$ level was similar in all groups of rats (Table 4).

The results obtained in lead-treated rats and in controls taken jointly, showed the linear correlations between blood concentration and vasoactive agents. There were positive correlations between serum nitric oxide concentration and the lead level in blood ($p < 0.001$), or kidney ($p < 0.001$), or aorta ($p < 0.05$). The opposite correlations appeared between zinc content in serum (positive) or kidney (negative) and plasma endothelin-1 level ($p < 0.05$ and $p < 0.01$, respectively). The serum level of copper was positively correlated with prostaglandin $F_{2\alpha}$ ($p < 0.05$). The concentration of copper in kidney was connected with the serum concentration of ET-1 in plasma (Table 5).

Rats given lead in a dose of 50 ppm displayed the increased, in comparison to controls, content of lipid peroxides ($p < 0.05$) and glutathione ($p < 0.001$) in aorta. Homogenates of aorta obtained from rats treated with lead in a dose of 100 ppm showed the increased amounts of LPO and glutathione, although the difference between mean concentration of LPO in aorta of these rats and controls was not statistically significant (Table 4). The glutathione level in aorta was linearly dependent on blood (kidney) lead concentration, aorta copper and aorta lipid peroxides. There was also a positive linear correlation between glutathione in aorta and NO in serum (Table 5).

Table 4. Concentrations of nitric oxide, endothelin-1 and prostaglandin $F_{2\alpha}$ in blood and lipid peroxides and glutathione in aorta of rats poisoned with lead in doses of 50 ppm or 100 ppm and in controls

Vasoactive agent concentration	Groups		
	Pb 50 ppm (n = 9)	Pb 100 ppm n = 10	Control n = 10
Serum NO (μmol/l)	56.4 ± 10.1**	60.2 ± 4.9***	43.8 ± 9.7
Plasma ET-1 (fmol/ml)	1.1 ± 0.3*	1.5 ± 0.5	1.7 ± 0.6
Serum $PGF_{2\alpha}$(pg/ml)	7.2 ± 2.9	6.1 ± 2.4	7.3 ± 3.9
Lipid peroxides and glutathione in aorta			
LPO (nmol/ml)	3.9 ± 3.2*	2.3 ± 1.6	1.2 ± 0.8
GSH (μmol/l)	14.8 ± 2.8***	13.6 ± 1.6***	9.5 ±1.7

Each value represents the mean ±SD.
n – number of rats.
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs corresponding vehicle-treated group.

Table 5. Coefficients of linear regression between concentrations of lead, or copper, or zinc in the studied samples and serum concentration of nitric oxide (NO) or prostaglandin $PGF_{2\alpha}$ and plasma endothelin-1 (ET-1) levels in rats (n = 29)

	NO in serum	$PGF_{2\alpha}$ in serum	ET-1 in plasma	GSH in aorta	LPO in aorta
NO in serum	1.000	-0.1483	0.0538	0.3898*	0.3464
$PGF_{2\alpha}$ in serum	-0.1483	1.000	0.2557	-0.2071	-0.1974
ET-1 in plasma	0.0538	0.2557	1.000	-0.1779	-0.3617
GSH in aorta	0.3898*	-0.2071	-0.1779	1.000	0.5000**
LPO in aorta	0.3464	-0.1974	-0.3617	0.5000**	1.000
Pb in blood	0.5996***	0.0077	-0.1293	0.4569*	0.1036
Pb in kidney	0.6287***	-0.1570	-0.1251	0.5764***	0.2695
Pb in aorta	0.4637*	-0.2510	0.0979	0.3350	0.1138
Cu in serum	-0.0523	0.3979*	0.1148	0.1438	-0.0890
Cu in kidney	0.0784	-0.0838	-0.4446*	0.1110	0.2713
Cu in aorta	0.0331	0.3226	0.1146	0.5291**	0.2523
Zn in serum	-0.1219	0.1122	0.4510*	-0.2619	-0.3796
Zn in kidney	0.1617	-0.0668	-0.5365**	0.3726	0.3954*
Zn in aorta	-0.2095	0.2576	0.2107	-0.0288	-0.0996

Statistical significance of coefficient r; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

DISCUSSION

Results of recent studies have showed that accumulation of lead in vessel walls depends on the species and caliber of vessels [24]. In this study, poisoning with lead given to rats in drinking water in a dose of 50 or 100 ppm for three months increased lead concentration in abdominal part of aorta. In rats poisoned with lead in a dose of 50 ppm, this increase, contrary to the enhanced lead level in blood and kidney, was not associated with significant changes in zinc concentration. This probably results from a relatively small production of metallothionein binding zinc in vessel walls. The elevation of zinc content in kidney, in which lead stimulates synthesis of this protein, could be the result of zinc removal from blood. However, the decrease in serum zinc level could also result from the known antagonism between lead and zinc in the intestinal absorption of these metals [25]. Interestingly, the zinc level was changed only in rats poisoned with a smaller dose of lead. Similarly, only rats poisoned with lead in a dose of 50 ppm revealed the increased copper level in aorta as compared to controls. Animals poisoned with lead in higher doses showed no changes in zinc or copper homeostasis. These dose-dependent differences indicate that the mechanisms regulating interactions between toxic and essential metals are very precise.

It is known that subchronic poisoning with lead given in concentration of 50 or 100 ppm in drinking water for three month induces persistent increase in arterial blood pressure in rats of different strains [6,16,17,22,26,27]. This study shows that the impact of lead on vasoactive agents in blood could differ, depending on the dose of metal, even in the range of hypertensive doses (50 or 100 ppm).

The mean blood lead concentration in rats poisoned with lead in a dose of 50 ppm (112 ± 42 μg/l) was approximately close to the upper limit of standard for the population non-exposed occupationally to lead, whereas in rats poisoned with lead in a dose of 100 ppm (173 ± 59 μg/d) it was higher. However, both values were comparable to the concentration of this metal in blood of people with lead-induced hypertension (below 400 μg/l) [28,29]. The changes in vasoactive agents in blood of rats treated with lead in this study, the increase in serum nitric oxide in all rats and the decrease in plasma endothelin-1 in rats given lead in a dose of 50 ppm, remain in contrast to hypertensinogenic effect of lead.

The increase in NO level in blood of lead-poisoned rats was also described by Vaziri and Ding [30], however, in other studies the decreased concentration of NO was also observed [15,27]. Lead can also exert a different effect on NO in vessel walls. The augmented NO degradation and decreased functional pool of NO, as well as unchanged content of vascular NO were shown [9,16]. The positive linear correlation between lead level in blood (also in kidney or aorta) and serum NO concentration in this study could be explained by a stimulating effect of lead on the activity of endothelial NO synthase. It is shown that lead in small doses can directly stimulate the eNOS gene transcription or expression [16], however, in cultured endothelial cells, lymphocytes and macrophages, lead inhibited eNOS and decreased NO concentration [24,31].

The decrease in ET-1 concentration in plasma of rats poisoned with 50 ppm of lead was unexpected. ET-1 is the one of the most vasoconstrictor factor that directly affects vessel walls and indirectly via stimulation of adrenergic transmission. ET-1, participating in regulation of vessel wall tone, is important in pathogenesis of arterial hypertension [32]. It is also known that ET-1 interacts with endogenous vasodilators, such as prostaglandins PGE_2 and PGI_2. Vasodilator action of these prostaglandins probably results from inhibition of ET-1 secretion or ET-1 gene translation or expression in endothelial cells [33]. The stable metabolite of PGE_2 and PGI_2 in blood is vasoactive $PGF_{2\alpha}$.

Since in rats poisoned with lead in a dose of 50 ppm, the decreased plasma ET-1 level was associated with unchanged, as compared to controls, serum concentration of $PGF_{2\alpha}$, we suppose that lead in a dose of 50 ppm decreased ET-1 in blood through other pathways than those associated with PG metabolism disturbances. This mechanism could include the inhibition synthesis of ET-1 by NO [32]. Although the serum NO level was also increased in rats poisoned with lead in a dose of 100 ppm, the mean ET-1 in these animals was similar to ET-1 obtained in the control group.

The effect of lead on ET-1 level probably results from the effect of lead on homeostasis of zinc. Bound to endothelin converting enzyme (ECE), zinc ions regulate activity of this enzyme and effectiveness of hydrolysis of Tryp21 Val22 bond in big ET-1. In this way zinc can modulate the velocity of ET-1 synthesis and concentration of its active form [34]. In our study, the existence of zinc-endothelin relation was confirmed by the linear correlation between zinc concentration in serum (or kidney) and ET-1 in plasma. Our hypothesis seems to confirm also the co-existence of decreased zinc and ET-1 levels only in rats poisoned with lead in a dose of 50 ppm and unchanged significantly zinc and ET-1 levels in rats poisoned with lead in a dose of 100 ppm.

The recent studies have shown that lead, increasing free oxygen radicals in vasculature, can affect the functional pool of nitric oxide [9,16]. Therefore, these differences in the effect of lead on the vasoactive agents in blood in the relation to the impact of lead on the activity of redox system in vessel walls were analyzed.

Lead given in hypertensive doses to rats augmented the lipid peroxidation in vessel walls. The increased lipid peroxide level associated with the increased glutathione concentration in aorta could result from the defense/adaptive response to toxic effect of lead. This response was observed by other authors in organs of rats poisoned with small doses of lead [35]. A higher content of NO in blood, connected with a higher level of glutathione in vessel walls of rats poisoned with lead (there was positive linear correlation between these parameters), might also point out to an adaptive reaction. It is quite possible that lipid peroxidation stimulated by lead induces adaptive response in vessel walls manifested as the increased glutathione and nitric oxide syntheses.

On the other hand, the increased level of glutathione in aorta may indicate not an adaptive response, but an augmented generation of free oxygen radicals. At the end of the 1990s it was shown that the enhanced production of reduced glutathione and its precursors could be a symptom of pro-oxidative action of glutathione [36]. The latter process is copper-dependent and results from the formation of a copper-glutathione complex, in which copper catalyzes glutathione autooxidation [37]. The positive linear correlation between copper concentration and glutathione level in aorta ($p < 0.01$) observed in this study seems to support this hypothesis.

To sum up, this study shows different, dependent on the metal dose, changes in toxicological and biochemical status in lead poisoned rats. The differences appeared although both doses of lead, 50 and 100 ppm, are those that induce arterial hypertension.

Lead in a dose of 50 ppm, acting pro-oxidatively, increased the concentration of lipid peroxides in aorta. The increased glutathione level in vessel walls was probably a symptom of defensive response to toxic effect of lead. It was associated with increased synthesis or release of NO and its increased concentration in serum. Simultaneously, lead in a dose of 50 ppm, probably attenuating serum zinc content, provoked the decrease in endothelin-1 level in plasma.

Rats poisoned with lead in a dose of 100 ppm did not display significant changes in homeostasis of essential metals (copper or zinc). The changes in the lipid peroxide and endothelin-1 levels were lower than those found in rats poisoned with lead in a smaller dose, whereas the glutathione level in vessel walls and nitric oxide in serum were similar as compared to animals poisoned with lead in a dose of 50 ppm. In conclusion, lead in small doses, known as hypertensive, increases synthesis and/or release of NO and its concentration in serum. This effect of lead is probably connected with the augmented production of glutathione in vessel walls. The participation of ET-1 mediated mechanisms in vascular action of lead differs, depending on the dose of lead. Thus lead in doses of 50 or 100 ppm can induce arterial hypertension through mechanisms other than those mediated by NO or endothelin-1. The increased vascular reactivity to endogenous catecholamines could be one of such mechanisms.

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