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BLOOD CONCENTRATION OF ESSENTIAL TRACE ELEMENTS AND HEAVY METALS IN WORKERS EXPOSED TO LEAD AND CADMIUM

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Abstract. The aim of the study was to determine blood concentration of essential trace elements (Se, Zn, Cu) and toxic metals (Pb, Cd), markers of antioxidant (activities of glutathione peroxidase (GPx), superoxidase dismutase and ceruloplasmin) and prooxidant processes (thiobarbituric acid reactive substances (TBARS)) in workers exposed to Pb and Cd. Forty three male workers of the lead-acid batteries department, aged 25–52 years, and twenty two workers, including 15 women, aged 36–51 years, exposed to Cd in the alkaline batteries department were examined. The reference group consisted of 52 healthy inhabitants of the same region. It was found that Se concentration and GPx activity in both erythrocytes and plasma of Cd exposed workers were significantly lower ($p < 0.001$) than in the reference group. We found an inverse linear correlation between blood Se and Cd concentrations in the workers exposed to Cd ($r = -0.449$; $p < 0.01$). Moreover, the activity of erythrocyte and plasma GPx was shown to be significantly lower in the study group of workers ($p < 0.001$). It was observed that TBARS concentration in plasma was significantly higher ($p < 0.05$) in the lead exposed workers than in the group without contact with Pb. Our results indicate that exposure to Pb and Cd affects the antioxidant potential of blood in workers exposed to heavy metals.

Key words:
Trace elements, Heavy metals, Workers, Antioxidants, Free radicals

INTRODUCTION

The air in the work environment usually contains a number of chemicals, which inhaled and absorbed by the body, pose a potential risk for workers' health. In the recent years, evidence has accumulated that interactions between air pollutants and living tissues may cause disturbance of pro- and antioxidant balance of the body. Highly reactive oxygen species (ROS) are formed as a result of partial reduction of oxygen and may cause cell damage by destruction of proteins, degradation of nucleic acids or lipid peroxidation [1]. Peroxidation has been implicated in various pathological conditions, and in toxicity induced by chemicals, including certain metals [2,3,4]. Oxidative attack of essential cell components by ROS is a mechanism generally recognized as that responsible for the pathogenesis of several human diseases [5,6]**.** Oxidative stress is a process in which physiological balance between the concentration/activity of prooxidants and antioxidants is disturbed in favor of the former. The increase in various biological indicators of oxidative stress has been demonstrated in such

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situations, and the modifications could sometimes be correlated with markers of the pathological processes [7]. In this context, much interest has been devoted to the determination of thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation in body fluids. The body is protected against oxidant injury by enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase, as well as by other antioxidants, including glutathione, serum/plasma trace elements and proteins such as ceruloplasmin [8]. Some of the trace elements – selenium (Se), zinc (Zn) and copper (Cu) are cofactors or structural components of antioxidant enzymes. Interaction of cadmium (Cd) and lead (Pb) with essential trace elements has been studied extensively in animals [9,10]. However, in many cases the doses and character of exposure differed from those observed in human exposure. The main purpose of this work was to investigate blood levels of trace elements (Se, Zn, Cu) and TBARS, as well as activities of antioxidant enzymes in workers exposed to lead and cadmium, and to find out whether any association between the levels of toxic and essential trace elements does really exist.

MATERIALS AND METHODS

Two groups, one exposed to Pb, of 43 male workers employed in the lead-acid batteries department, aged 25–52 yrs (mean 39.4 ± 6.5 yrs), and the other made up of 22 workers, including 15 women, aged 36–51 yrs (mean 44.5 ± 5.2 yrs), exposed to Cd in the alkaline batteries department were examined. The reference group consisted of 52 healthy inhabitants, including 17 women, of the same region, employed in industry (students, administration workers). The protocol of the study was approved by the Regional Ethical Committee for Scientific Research.

Blood samples were collected into heparinized tubes (free of trace elements) by cubital venipuncture during the work-shift. Methods for obtaining and preparing the samples were described previously [11].

The concentrations of Se in plasma and Pb and Cd in whole blood were determined using graphite furnace atomic absorption spectrometry (AAS) (Unicam 989 QZ) [12–14] while Zn and Cu levels were established using flame AAS (Pye Unicam SP9 800) [15]. Erythrocyte and plasma GPx activities were assayed according to the method of Paglia and Valentine [16], as modified by Hopkins and Tudhope [17]. Ceruloplasmin was determined colorimetrically, according to Sunderman and Nomoto [18]. TBARS concentration was determined fluorometrically, according to Wąsowicz et al. [19].

Statistical analysis

The data were expressed as means ± SD and were subjected to statistical analysis using the non-parametric Mann-Whitney U-test, analysis of variance, and calculation of the correlation coefficient. The statistical significance was set at $p < 0.05$.

RESULTS

The study demonstrated that in comparison with the control group, the workers exposed to cadmium showed significantly higher Cd levels in whole blood, significantly lower plasma Se concentration (50.0 ± 8.6 µg/l, $p < 0.001$ vs. 63.0 ± 13.5 µg/l), and significantly lower GPx activity in red blood cells and plasma (12.9 ± 3.7 U/g Hb, vs. 19.1 ± 3.7 U/g Hb and 122 ± 20 U/l vs.199 ± 44 U/l, respectively) (Table 1). We found an inverse linear correlation between Se and Cd concentrations ($r = -0.449$; $p < 0.01$). No statistically significant differences in the mean values were observed between men and women or smokers and non-smokers (data not shown).

The analysis of the results in the group of workers (men only) exposed to high Pb concentrations involved the comparison of the mean values with the results obtained in the reference group (43 men). The determinations in the workers indicated significantly lower plasma Zn concentration (0.82 ± 0.16 mg/l; $p < 0.01$ vs 0.95 ± 0.15 mg/l) and GPx activity in erythrocytes and plasma ($p < 0.001$) than in the reference group. It was observed that TBARS concentration in blood plasma was significantly higher ($p < 0.05$) in workers exposed to lead than in those non-exposed (Table 2). Statistically significant linear correla-

Table 1. Concentrations/activities of determined parameters in blood of cadmium-exposed workers and in controls

Parameters	Exposure group (Cd), n = 22	Controls n = 52	Significance
Se, µg/l	50.0 ± 8.6* 35.3 – 70.0**	63.0 ± 13.5 38.4 – 99.2	p < 0.0001
Zn, mg/l	0.5 ± 0.15 0.62 – 1.08	0.91 ± 0.15 0.65 – 1.39	N.S.
Cu, mg/l	1.22 ± 0.12 1.02 – 1.49	1.27 ± 0.17 0.99 – 1.61	N.S.
GPx RBC U/g Hb	12.9 ± 3.7 7.6 ± 19.8	19.1 ± 4.0 10.1 – 28.2	p < 0.0001
GPx plasma u/l	122 ± 20 76 – 153	199 ± 44 68 – 310	p < 0.0001
SOD U/g Hb	7.73 ± 2.26 4.76 – 11.84	7.58 ± 1.47 1.73 – 13.51	N.S.
Cp u/l	0.543 ± 0.078 0.413 – 0.730	0.518 ± 0.097 0.340 – 0.934	N.S.
TBARS µmol/l	1.48 ± 0.30 0.93 – 2.04	1.45 ± 0.36, n = 28 0.67 –1.99	N.S.
Cd, µg/l	15.2 ± 7.7 5.4 – 30.8	1.66 ± 1.83 0.2 – 9	p < 0.0001

* Mean ± standard deviation; ** range; N.S. – not significant.

Table 2. Concentrations/activities of determined parameters in blood of lead-exposed workers and in controls

Parameters	Exposed group (Pb), n = 43	Controls n = 33	Significance
Se, µg/l	59.5 ± 8.6* 41.3 – 78.8**	62.9 ± 13.7 41.6 – 99.2	N.S.
Zn, mg/l	0.82 ± 0.16 0.53 – 1.19	0.95 ± 0.15 0.65 – 1.39	p < 0.01
Cu, mg/l	1.19 ± 1.17 0.72 – 1.53	1.22 ± 0.15 0.99 – 1.53	N.S.
GPx RBC U/g Hb	15.7 ± 3.4 9.5 – 23.8	19.1 ± 3.6 13.6 – 27.3	p < 0.001
GPx plasma u/l	172 ± 51 107 – 296	190 ± 36 68 – 267	p < 0.001
SOD U/g Hb	8.16 ± 3.22 1.15 – 16.74	6.94 ± 1.27 4.67 – 10.0	N.S.
Cp u/l	0.502 ± 0.073 0.353 – 0.647	0.495 ± 0.79 0.340 – 0.666	N.S.
TBARS µmol/l	2.08 ± 1.18 0.93 – 5.97	1.58 ± 0.23 1.15 –1.89	p < 0.05
Pb, µg/l	504.4 ± 92.1 282.0 – 752.0	62.0 ± 25.7 37.0 – 180.0	p < 0.001

* Mean ± standard deviation; ** range; N.S. – not significant.

Table 3. Coefficient of correlation (r) between determined parameters in blood of lead-exposed workers (n = 43)

Parameter	GPx plasma	Cp	TBARS
Se	0.498*		
Cu		0.545*	
GPx RBC			-0.315*

* Statistical significance at $p < 0.05$.

tions were found between some of the parameters determined in the blood samples from the workers of the acid battery department (Table 3). Similarly, there were no statistically significant differences between smokers and non-smokers or between individuals working in the department for different periods of time (data not shown). The changes in Cd and Se levels are presented in Fig.1.

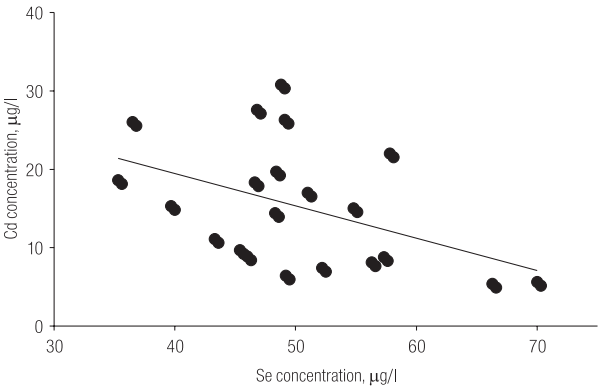

Fig. 1. Inverse linear correlation between selenium and cadmium concentrations in the blood of cadmium-exposed workers ($y = -0.411x + 35.9$; $r = -0.449$; $p < 0.01$).

DISCUSSION

The workers included in the study were exposed to high Cd or Pb concentrations, depending on the department in which they were employed. Cadmium is absorbed by the body through the lungs and the digestive tract. In the work environment, absorption through the lungs is of essential importance [20]. The absorption of any element from the inhaled air varies from 7 to 10%, while absorption from the alimentary tract is lower and amounts to 3–7%. It has been documented that in the deficiency of calcium, iron or proteins in the body, absorption from the alimentary tract may increase up to 20% [20]. This route of absorption plays an important role in the case of bad hygienic habits such as not washing contaminated hands, ingestion of contaminated food or smoking during occupational exposure [21]. Under industrial conditions, inhalatory intoxication is most common, while in long-term environmental exposure, the effects include disturbances in the resorption in the proximal tubules, lung diseases and the skeletal system disorders [20]. Recent animal studies have demonstrated a rapid increase in peroxidation processes related primarily to lipids, associated with increased activity of aminotransferases in rabbits exposed to high Cd concentrations, which is probably due to the liver damage [22].

It may be assumed that under long-term exposure to cadmium in the work environment, disturbances in the parameters of antioxidant barrier might occur. In the workers of the alkaline battery department who participated in our investigation both Se concentration and erythrocyte and plasma GPx activity were found to be significantly lower than in the group of the non-exposed in the workplace. In this group of workers, lipid peroxidation, measured as TBARS concentration, was not found to be increased. The mechanism of this effect is not clear and impossible to explain at the present stage. The small number of the subjects examined does not allow to draw explicit conclusions.

In their studies of the changes in the antioxidant system in erythrocytes, measured as GPx, SOD, glutathione reductase and ceruloplasmin activity in the serum of welders exposed to welding fumes, Mongiat et al. [23] did not find differences between the examined parameters in the study and the reference groups. The study also took account of cigarette smoking but, like our investigation, did not show associations between smoking and the values of the parameters, except for the concentration of ceruloplasmin which was significantly elevated in smokers [23]. It should be stressed that tobacco smoke is a strong oxidant. Oxidative effect of smoke increases the level of reactive oxygen species released by macrophages of smokers [24]. Our findings are in agreement with the results of Greening et al. [25] and Pierre et al. [26], who did not show changes in the smoking-related concentration of

ceruloplasmin, but are contrary to the data of Galdstone et al. [27] and Pacht and Davis [28] who reported a higher concentration of this protein.

Ceruloplasmin is an important antioxidant not only in serum/plasma, but also in the broncho-alveolar lining, so it is considered to be a significant pulmonary antioxidant, and the concentration of this protein in serum/plasma may reflect the condition of the lungs [23].

A few of the published studies report changes in the blood levels of essential trace elements in people exposed to heavy metals either in the work or living environment. Ellingsen et al. [29] demonstrated the influence of cadmium on the decrease of blood Se levels in the Norwegian population without finding a relation to smoking. There was an inverse and statistically significant correlation ($r = -0.80$; $p < 0.001$) between Se concentration in whole blood and Cd concentration. Although in our study Se concentration was not determined in whole blood but in plasma, the correlation coefficient was $r = -0.449$ ($p < 0.01$) despite the fact, frequently stressed, that a small group of population was studied.

The Belgian authors [30] investigated serum Zn concentration in a large population (1977 individuals) exposed to different concentrations of cadmium in the environment (four areas of the country were included in the investigation: two with high and two with low exposure to cadmium), and found, as we did in our study, similar Zn concentration in smokers and in the reference group. They demonstrated a weak, but statistically significant negative ($r = -0.12$, $p < 0.001$) linear correlation between serum Zn and urinary Cd concentrations. This may be explained by the fact that in relatively high exposure to Cd, it is accumulated primarily in kidneys and liver. The main Cd (but also Zn) binding protein is metallothionein, whose synthesis is stimulated by cadmium. Therefore, if Cd concentration is high, Zn may be redistributed from plasma/serum to kidneys and liver.

Our investigation also brought about an interesting observation that the linear correlation coefficient between blood Zn and Cd concentrations in the workers exposed to cadmium was $r = -0.137$ (not shown in the tables), but as the number of subjects was small, the relationship was not statistically significant, and thus any firm conclusion could be drawn. Thijs et al. [30] obtained the coefficient $r = -0.12$, which, with a large number of study cases, reached a statistically significant value.

Lead has been used for centuries. The high occupational exposure-related hazard occurs in the processes of lead ore smelting, welding and cutting of metal constructions covered by lead-containing paints, casting of non-ferrous metals, production of batteries, production of lead containing pigments and in many other processes [20]. Occupational Pb exposure may result in chronic poisoning. It mostly affects the haematopoietic, central and peripheral nervous systems.

Like in the case of Cd, there are only few reports concerning human antioxidant barrier under occupational exposure to lead. Our investigation showed that Zn concentration was significantly lower in the workers exposed to lead than in the reference group (Table 2). We also observed an increased lipid peroxidation measured as TBARS concentration in plasma, however, we did not find any relationship between TBARS concentration and blood Pb. This is in contrary to the results of Jiun and Hsien of Taiwan [31] who found significant correlation (although the correlation coefficient was low – $r = 0.361$; $n = 130$) between the degree of lipid peroxidation and Pb level in individuals exposed to Pb concentration lower than 350 μg/l. The correlation coefficient increases when Pb concentration is higher than 350 μg/l ($r = 0.848$), and reaches the maximum value when the concentration exceeds 400 μg/l [31]. But, the authors determined the concentration of malonyl dialdehyde by the chromatographic method which allowed selective determination of compounds. The method used in our study was simpler and allowed fast screening, although it was less selective. [19]. This may account for the lack of similar findings.

Although we did not observe lower Se concentration, the activity of the selenoenzyme was found to be decreased in both erythrocytes and plasma (Table 2). In this group, like in the group exposed to high concentrations of cadmium, smoking habit and the number of years of occupational exposure did not influence the concentrations/activities of the parameters.

In conclusion, it should be pointed out that in occupational exposure to cadmium or lead, antioxidant potential of the body is disturbed, which is manifested by changes in both the concentration of trace elements and the activity of some enzymes. The most evident changes occur in the activity of glutathione peroxidase. Regression analysis demonstrates a relationship not only between single elements of the antioxidant barrier but also between the toxic and essential elements, which after further thorough study, may prove to have scientific as well as practical values.

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