ACTIVITY OF SUPEROXIDE DISMUTASE AND CATALASE IN PEOPLE PROTRACTEDLY EXPOSED TO LEAD COMPOUNDS

Sławomir Kasperczyk, Ewa Birkner, Aleksandra Kasperczyk, Jolanta Zalejska-Fiolka

Department of Biochemistry, Medical University of Silesia, Katowice, Poland

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Abstract: Lead can modify pro/antioxidant status by influencing antioxidant enzymes. As the results of experimental researches are divergent, the purpose of this research was to evaluate the activity of enzymes that play a vital role in the defence against ROS in blood of people protractedly exposed to lead compounds. The study population included 172 healthy employees of zinc and lead steelworks. Workers exposed to lead (L) were divided into 2 groups: the first included workers with mean lead concentration (PbB) from 25–35 µl/dl (LL group), and the second group of high exposure (HL group) – with PbB over 35 µl/dl. The administration workers were the control group. There were no significant changes in activity of catalase and mitochondrial SOD in the study population. The activity of ZnCu-SOD significantly increased, both in plasma and erythrocytes, but first in plasma in the LL subgroup by about 42% (p=0.044), and then in erythrocytes in the HL subgroup by about 23% (p=0.012) when compared to the control group. Concentration of TBARS-MDA increased both in serum and erythrocytes. In people protractedly exposed to lead (mean 15 ± 10 years), there is observed an increased activity of SOD in blood, which seems to be an adoptive mechanism against the raised amount of production of reactive oxygen species (ROS) caused by lead.

INTRODUCTION

Despite the fact that pollution of the natural environment with lead (Pb) has decreased lately, it is more and more commonly known that this element may be toxic even in low doses. Most usual is the binding activity of lead compounds with oxidative stress, and with the generation of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide ion ($O_2^{\cdot-}$), singlet oxygen and hydroxyl radical ($OH^\cdot$) membrane lipids peroxidation, interaction with different metals and toxic activity of delta-aminolevulinic acid (ALA) [3, 8, 9, 38]. Antioxidant enzymes and glutathione play an important role in the defence against ROS. Most experimental research has indicated that after exposure of animals to lead there is an increased amount of oxidized glutathione (GSSG) with a parallel drop of reduced glutathione (GSH) in different organs (for example in liver or kidneys) [18, 33]. Similarly, incubation of human blood in the presence of lead compounds (concentration 100-400 µg/dl) causes a drop of GSH concentration [22]. Glutathione is necessary for proper the functioning of glutathione peroxidase and glutathione transferase – enzymes taking part in the elimination of noxious compounds and ROS. Much research has shown that the above-mentioned enzymes, together with glutathione reductase, may be modified under the influence of lead [17, 22, 37].

Besides, those enzymes, enzymes not bound with glutathione also play a vital role in the defence against ROS: superoxide dismutase (SOD) and catalase (CAT). Superoxide dismutase occurs in organisms using oxygen in metabolism. Two isoenzymes are known: cytoplazmatic isoenzyme (extracellular) which is built up from 2 subunits, each containing 1 Zn and 1 Cu (ZnCu-SOD), whereas the mitochondrial enzyme contains manganese (Mn-SOD). High activity of SOD is noticed in cells, especially in places where processes which need oxygen may be found – in liver, kidneys and erythrocytes. SOD activity may also be observed in skeletal muscles, endothelium and blood plasma [12].

There are both SOD isoenzymes mitochondrial and cytosol in blood plasma, whereas cytosol isoenzyme is found only in mature erythrocytes (lacking mitochondria). The biological role of superoxide dismutase is to dismute superoxide ion; hydrogen peroxide (H_2O_2) produced in this reaction is eliminated by catalase, one of the most active enzymes in the human organism. Catalase consists of 4 protein subunits, each of them containing hem group with iron (Fe), connected to its active centre. The highest activity of catalase in the human body may be found in the liver and red blood cells [2, 13].

Because most research about lead influence on SOD and CAT activity has mainly been experimental, and the results often divergent, the purpose of this research was to evaluate the activity of these enzymes and concentration of products of reactive oxygen species in the blood of people protractedly exposed to lead compounds.

MATERIALS AND METHODS

The study population included healthy male employees of zinc and lead steelworks in the south of Poland. The lead exposed group (L) consisted of 137 workers divided into 2 subgroups: low exposure to lead (LL) and high exposure to lead (HL). The criterion of the division was mean concentration of lead in blood, calculated from the previous 6-12 indications of lead concentration in blood in the last 3 years of work. Workers with mean lead concentration (PbB) from 25–35 µl/dl were the low exposure group, and the second group, high exposure – with concentration of lead over 35 µl/dl. Workers were exposed to zinc insignificantly. Administration workers (n=35), with no history of exposure, who had normal levels of PbB and zinc protoporphyrin in the blood (ZPP), were the control group. Blood was collected by venipuncture into 10-ml evacuated tubes containing ethylenediamine-tetraacetic disodium acid (EDTA) solution as anticoagulant to obtain erythrocytes.

Analysis of lead in blood (PbB) was carried out by graphite furnace atomic absorption spectrophotometry, using Unicam 929 and 939OZ Atomic Absorption Spectrometers with GF90 and GF90Z Graphite Furnaces. Data are shown in µg/dl. Concentration of zinc protoporphyrin in the blood (ZPP) was measured directly using Aviv Biomedical hematofluorometr model 206. The light was filtered by means of an interference filter transmitting at 415 nm. The excitation light focused on a drop of blood. The emitted light passed through a narrow band interference filter which transmitted at 596 nm. The instrument measured the ratio of fluorescent substance (ZPP) to the absorption of the light in the sample (hemoglobin) displayed as µg ZPP/g hemoglobin (µg/g Hb). Urine was collected at the same time. Delta-amino-levulinic acid (ALA) was estimated in urine samples by the method of Grabecki [15]. Delta-aminolevulinic acid reacted with acetylacetone and formed pyrrole substance which reacted with dimethylaminobenzoese aldehyde. The coloured complex was measured spectrophotometrically at 553 nm. The results were expressed as mg/dl.

The activity of superoxide dismutase (SOD) was indicated by the Oyanagui [29] method. Superoxide anion radical, produced in the reaction of xantine with O_2 catalysed by xantine oxydase, reacts with hydroxylamine producing nitric ion. Nitric ion combines with naphthalene diamine and sulfaniline acid producing coloured product; the concentration of this mixture is proportional to the amount of superoxide anion radical produced. Enzymatic activity was expressed in nitric unit (NU) in each ml of blood plasma or g of haemoglobin [Hb]. 1 nitric unit (NU) means 50% of inhibition by SOD of nitric ion production in method conditions. SOD activity was indicated in blood plasma and erythrocytes. Whole blood was centrifuged. Sediment of erythrocytes were rinsed 3 times using 0.9% NaCl. Then, erythrocytes were haemolysed with deionised water. In 10% haemolysate activity of SOD and concentration of haemoglobin by Drabkin reagent were indicated. In blood plasma, SOD isoenzymes were also indicated – Mn-SOD and ZnCu-SOD, using KCN as the inhibitor of the ZnCu-SOD isoenzyme by the Oyanagui [29] method.

Catalase was indicated by the Aebi [2] kinetic method. Whole blood was centrifuged. Sediment of erythrocytes were rinsed 3 times using 0.9% NaCl. Rinsed erythrocytes were lysed in 3 volumes of cold, redestillated water and left in ice for 30 minutes. Because catalase is bound with erythrocytic membranes, the samples were not centrifugalised. Before catalase was marked, the haemolysate was diluted 100 times with TRIS/HCl buffer, pH 7.4. Kinetic designation was carried out in a quartz tank. 2.5 ml of substrate was mixed, consisting of 50mM TRIS/HCl buffer with pH=7.4 and perhydrol with 50 µl of haemolysate. After 10 seconds, absorbance was measured with λ=240nm and the kinetic changes of absorbance were marked every 30 seconds for 2 minutes. Enzymatic activity was expressed in IU/mg Hb.

Lipid peroxidation was measured fluorometrically as 2-thiobarbituric acid-reactive substance (TBARS) in blood plasma, and in erythrocytes by the Ohkawa [27]. Samples (plasma or erythrocytes) were mixed with 8.1% sodium dodecyl sulfate, 20% acetic acid and 0.8% 2-thiobarbituric acid. After vortexing, samples were incubated for 1 hour in 95ºC and butanol-pirydine 15:1 (v/v) was added. The mixture was shaken for 10 min. and then centrifuged.
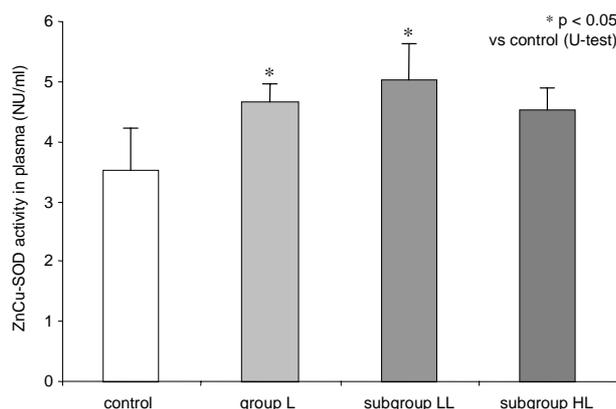
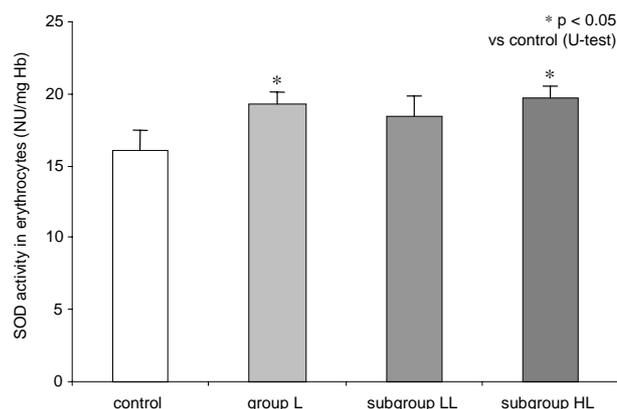**Figure 1.** Activity of Zn,Cu-SOD in plasma in all groups (mean ± SEM).

Figure 2. Activity of SOD (Zn,Cu-SOD) in erythrocytes in all groups (mean ± SEM).

Butanol-pirydine layer was measured fluorometrically at 552 nm (515 nm excitation). TBARS values were expressed as malondialdehyde (MDA) equivalents. Tetraethoxypropane was used as the standard. Data are shown as µmol/l plasma or µmol/l erythrocytes.

Statistical analysis was performed with Statistic 6.0 PL software. Statistical methods included mean, standard deviation (SD), and standard error of mean (SEM). Shapiro-Wilk's test was used to verify normality and Levene's test to verify homogeneity of variances. Statistical comparisons were made by t-test, t-test with separate variance estimates or Mann-Whitney U test. Spearman non-parametric correlation was calculated. A value of p<0.05 was considered to be significant.

RESULTS

There were no differences in age and years of work at the lead steelworks (time of exposure to lead) in all groups (Tab. 1). Table 2 shows lead concentration in

blood (PbB) and zinc protoporphyrin (ZPP), and urine ALA level. The blood lead level was 325% significantly higher in the lead exposed group (L) and 229% higher in the low lead exposed subgroup (LL), and 368% in the high lead exposed subgroup (HL) than in control group; such ZPP increased by about 273%, by about 165% and 322% respectively. ALA significantly increased by about 85% in the L group, by about 62% in LL subgroup, and by about 96% in the HL subgroup when compared to the control.

The activity of Zn,Cu-SOD in plasma significantly increased by about 32% in the L group (p=0.040), the increase was greater (42%) in the LL subgroup (p=0.044) and there was a tendency of increase in the HL subgroup (p=0.073) when compared to the control group (Fig. 1). Simultaneously, the activity of ZnCu-SOD in erythrocytes increased by about 20% in the L group (p=0.020); the increase was greater (23%) in the HL subgroup (p=0.012) when compared to the control group (Fig. 2). There were no significant changes in the activity of CAT in the study

Table 1. Age and years of work in lead steelworks (time of exposure to lead) in all groups.

	control group (C)	lead exposure group (L)	p level (t-test)	low lead exposure subgroup (LL)	p level (t-test)	high lead exposure subgroup (HL)	p level (t-test)
number	35	137		43		94	
age in years (mean ± SD)	39.4 ± 10.8	41.0 ± 9.85	0.376	40.6 ± 10.1	0.584	41.3 ± 9.76	0.345
years of work (mean ± SD)	15.9 ± 11.2	14.8 ± 9.97	0.517	15.1 ± 10.4	0.805	14.6 ± 9.83	0.439

Table 2. Blood lead level (PbB), zinc protoporphyrin in the blood (ZPP) and urine δ-aminolevulinic acid (ALA) concentration in all groups.

	control group (C)	lead exposure group (L)	p level (U-test)	low lead exposure subgroup (LL)	p level (U-test)	high lead exposure subgroup (HL)	p level (U-test)
	mean ± SD	mean ± SD		mean ± SD		mean ± SD	
blood lead concentration (PbB) µg/dl	9.21 ± 2.71	39.1 ± 7.5	<0.001	30.3 ± 2.96	<0.001	43.1 ± 5.21	<0.001
zinc protoporphyrin in blood (ZPP) µg/g Hb	1.31 ± 0.30	4.90 ± 3.00	<0.001	3.49 ± 2.42	<0.001	5.55 ± 3.02	<0.001
uric 5-aminolevulinic acid (ALA) mg/l	1.94 ± 1.23	3.60 ± 2.09	<0.001	3.16 ± 1.82	0.001	3.80 ± 2.18	<0.001

Figure 3. Concentration of malondialdehyde (TBARS-MDA) in plasma in all groups (mean ± SEM).
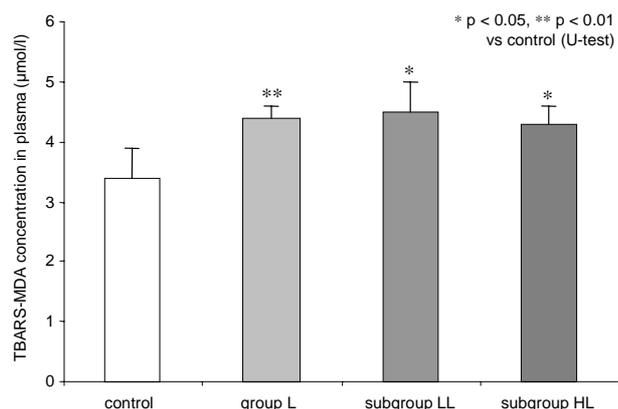

Figure 4. Concentration of malondialdehyde (TBARS-MDA) in erytrocytes in all groups (mean ± SEM).

population (Tab. 3). Concentration of TBARS-MDA in serum increased in the L group (p=0.009), LL subgroup (p=0.022) and HL group (p=0.017), and in erythrocytes in the L group (p=0.005), LL subgroup (p=0.052) and HL group (p=0.004) when compared to the control group (Fig. 3 and 4).

Spearman correlation showed a positive correlation between the activity of SOD in erythrocytes and PbB (R=0.18 p=0.022). There was no significant correlation between PbB, ZPP, ALA and SOD in plasma (total and both isoenzymes) as well as CAT in erythrocytes.

DISCUSSION

As almost all research has discovered that lead increased concentration lipid peroxidation in blood, both in experimental or clinical studies [1, 4, 7, 9, 17, 23, 24, 32, 35, 37, 39], the results of research on lead influence on superoxide dismutase activity are divergent. In *in vitro* research on erythrocytes, a decreased SOD activity under lead influence was noted [22, 23, 35], while 0.1-1 mol Pb^{2+}/l doses activated ZnCu-SOD. In studies on animals with high exposure to this element (PbB 50-250 µg/dl), a decreased SOD activity in erythrocytes was often noted, whereas in lower doses (PbB=40 µg/dl) there were no changes in activity [23, 25]. Adonaylo and Oteiza [1] did not notice any changes in SOD activity in the brain, whereas Sandhir and co-workers [31] indicated a drop in
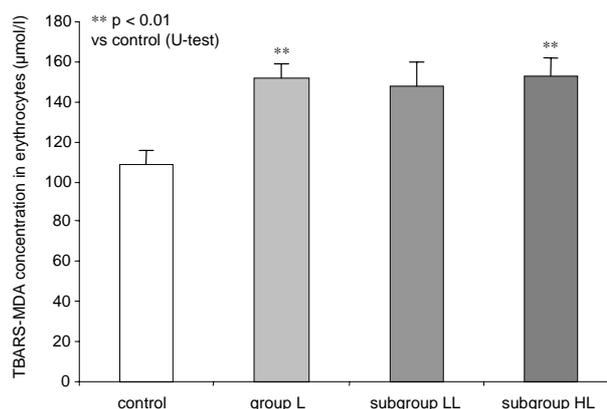
the activity of this enzyme. In liver, generally decreased activities of superoxide dismutase were noted [5, 10, 32]. For the proper functioning of SOD cytosol isoenzyme, some metals are necessary: copper, which takes part in oxidation and reduction reactions, and zinc, which stabilizes the enzyme [16]. Decreased activity of ZnCu-SOD, observed by different authors, may be caused by interaction between Pb and copper (Cu). Lower activities of ceruloplasmine in blood plasma of children with protracted saturnism have been described and it is known that ceruloplasmine may forward copper ions for SOD [20]. Myroie and co-workers, on the basis of studies on rats receiving lead acetate (100-500 ppm) in water, stated that a drop of SOD activity in rat erythrocytes was caused by a drop of copper concentration in blood and erythrocytes, and not by the direct action of lead (Pb) [25]. They also noted a strong, negative correlation between lead and copper concentrations in blood (r=-0.75 p<0.001) [25].

Clinical research has divergent results. Ito *et al.* [23] observed a drop of SOD activity in blood of people with PbB=30-40 µg/dl, whereas SOD activity in blood plasma was unchanged. Sugawara *et al.* [35] noticed a lower activity of erythrocyte SOD of 57% (with PbB=57.1 µg/dl), but simultaneously noted a raised activity of blood plasma SOD of 80%. This state was explained by the direct blocking action of this metal on SH groups of erythrocyte SOD. However, Wąsowicz *et al.* [37], in

Table 3. Total activity of superoxide dismutase (SOD) and mitochodrial isoenzyme (Mn-SOD) in plasma and catalase (CAT) in erythrocytes in all groups.

	control group (C)	lead exposure group (L)	p level (t-test)	low lead exposure subgroup (LL)	p level (t-test)	high lead exposure subgroup (HL)	p level (t-test)
	mean ± SD	mean ± SD		mean ± SD		mean ± SD	
total activity of SOD in plasma (NU/ml)	10.4 ± 4.65	11.5 ± 5.39	0.275	12.6 ± 4.87	0.052	11.1 ± 5.57	0.567
activity of Mn-SOD in plasma (NU/ml)	6.96 ± 3.93	6.93 ± 5.53	0.529	7.66 ± 5.60	0.849	6.60 ± 5.50	0.328
activity of catalase in erythrocytes (IU/mgHb)	254 ± 43.1	265 ± 58.5	0.351	269 ± 63.2	0.253	263 ± 56.4	0.447

group of people with similar exposure: PbB=50.4 µg/dl, did not observe any changes in SOD activity in erythrocytes, compared to the control group. Monteiro *et al.* [24] indicated that in 3 populations exposed to lead, SOD activity was raised from 63–105% was noticed. Similarly, Costa *et al.* [7] observed over 3 times higher activity of SOD in blood of people with PbB=53.4 µg/dl. At the same time, they noted a strong, positive correlation between SOD activity and PbB. Also, Ye *et al.* [39] observed higher activity of SOD in blood of people with PbB, over 37 µg/dl, with simultaneous increase in MDA.

In our research, the activity of manganese SOD isoenzyme in blood plasma was unchanged. Activity of SOD in erythrocytes (ZnCu-SOD) was increased in the subgroup of high exposure, and in blood plasma there was increased activity of the zinc-copper isoenzyme, especially in the subgroup of low exposure. Simultaneously, a positive correlation between erythrocyte SOD and PbB was observed. Received results are compatible with the research by Monteiro [24], Costa [7] and Ye [39]. Raised SOD activity is probably a response to increased production of superoxide anion radicals caused by lead [30]. Although the exposure to zinc in the experimental group was low, it could also be possible that zinc can up-regulate ZnCu-SOD that contains Zn^{2+} as a result of increased zinc in the tissue.

It seems that production of superoxide ions ($O_2^{\bullet-}$) decreases most of all in blood plasma (the reason for increased SOD activity in blood plasma) and may be bound with the peroxidative influence of ALA. Lead, even in low doses, inhibits activity of delta-aminolevulinic acid dehydratase, which increases ALA in blood plasma and urine. Much research, both experimental and on cell breeding, confirms that ALA may generate reactive oxygen species (ROS) in biological systems [7, 21, 26, 28].

Research concerning lead influence on catalase activity, as with SOD, gave divergent results. In organs of animals exposed to this element, raised activity in kidney was observed [18] or no changes at all [19], decrease in liver activity [5, 10, 32], or no changes at all [11, 19], and in brain: a decrease in activity [31], no changes [14] or increased activity [6, 34, 36]. In lymphocytes, a several-times higher activity was indicated [11], similarly as in erythrocytes [17, 18]. In people exposed to lead, a decrease in activity of catalase was observed, accompanied by increased MDA [35]. In our research, no influence of exposure to examined metal on activity of catalase was indicated. This may be caused by increased synthesis of this enzyme in the erythroblastic system, and simultaneously by inhibited activity under lead ion influence. Catalase contains hem as the prosthetic group, the biosynthesis of which is inhibited by lead. This element may also inhibit iron absorption from the digestive tract [31]. On the other hand, lead ions have the ability to active chelation on the surface of insoluble lead salts, which may activate ROS [4]. Furthermore, increased production of H_2O_2 by SOD, with no changes in catalase activity, may damage cell structures or production of the hydroxyl radical ($OH^{\bullet}$).

Most research on lead toxicity concerns animals, in which acute poisonings are often observed because of high doses in a short period time. In the examined population of people, exposure to this metal was significantly lower, and entered to the organism by the respiratory tract (and not with drinking water or intraperitoneally, as in experimental research), and the time of exposure was a few or several years, which allowed the organism to adopt and activate a number of detoxicating mechanisms, for example, by increasing the activity of enzymes.

CONCLUSION

In people protractedly exposed to lead, an increased activity of superoxide dismutase in blood was observed, which seems to be an adoptive mechanism against the raised amount of production of reactive oxygen species (ROS) caused by lead.

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