INHIBITION OF THIOL-CONTAINING ENZYMES IN ERYTHROCYTES OF WORKERS EXPOSED TO LEAD

THEREZA LUCIANO TROMBINI, EVANDRO OLIVEIRA, DAIANE BOLZAN BERLESE, RENATO MINOZZO, TÁSSIA DE DEUS, CRISTINA DEUNER MULLER, RAFAEL LINDEN, VANDRÉ CASAGRANDE FIGUEIREDO, CLÓVIS MILTON DUVAL WANNMACHER, GREICY MICHELLE MARAFIA CONTERATO and LUCIANE ROSA FEKSA

SUMMARY

Lead (Pb^{2+}) is thought to enter erythrocytes through anion exchange and to remain in the cell by binding to thiol groups. Creatine kinase (CK) and adenylate kinase (AK) are two thiol-containing enzymes that exert a key role for cellular energy homeostasis in erythrocyte. δ-aminolevulinic acid dehydratase (δ-ALAD) is the second enzyme in the heme biosynthetic pathway and plays a role in the pathogenesis of lead poisoning. Our objective was to study the effect of Pb^{2+} on the activity of the thiol-enzymes δ-ALAD, CK and AK in erythrocytes from lead-exposed workers. The study sample comprised 22 male lead-exposed workers and 21 normal volunteers. The lead-exposed workers were employed in manufacturing and recycling of automotive batteries. Basic red-cell parameters were assayed and total white blood cell counts were performed. CK, AK and δ-ALAD activities and blood lead concentrations (BPb) were determined in all subjects. Lead-exposed individuals had significantly higher blood lead levels than controls. Both CK and δ-ALAD activity levels were significantly lower in lead-exposed individuals than in controls. Lead had no effect on AK activity. Lead-exposed individuals had lower values than the controls for several red cell parameters. These results indicate an apparent dose-effect relationship between CK, δ-ALAD activity and BPb and that lead inhibits δ-ALAD and CK activity by interacting with their thiol groups. It is therefore possible that lead disrupts energy homeostasis and may be linked with a reduction in energetic metabolism contributing to the cell dysfunction observed in these in lead-exposed individuals.

KEYWORDS / Adenylate Kinase / Blood Lead / Creatine Kinase / Dehydratase / Δ-Aminolevulinic Acid / Gluthatione / Lead Toxicity /

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Thereza Luciano Trombini. Graduate in Biomedicine, Universidade Feevale (UFeevele), Brazil. Biomedical Specialist, UFeevale, Brazil.

Evandro Oliveira. Graduate in Biomedicine, UFeevale, Brazil. Biomedical Specialist, UFeevale, Brazil.

Daiane Bolzan Berlese. Doctor in Toxicological Biochemistry, Universidade Federal de Santa Maria (UFSM), Brazil. Professor, UFeevale, Brazil. Address: Grupo de Pesquisa em Bioanálise, Instituto de Ciências da Saúde, Universidade Feevale, Novo Hamburgo, RS, Brazil. e-mail: daianeb@feevale.br

Renato Minozzo. Doctor in Genetics and Applied Toxicology, Universidade Luterana do Brasil. Professor, UFeevale, Brazil.

Tássia de Deus. Graduate in Biomedicine, UFeevale, Brazil. Biomedical Specialist, UFeevale, Brazil.

Cristina Deuner Muller. Graduate in Biomedicine, UFeevale, Brazil. Biomedical Specialist, UFeevale, Brazil.

Rafael Linden. Doctor in Cell and Molecular Biology, Pontifícia Universidade Católica do Rio Grande do Sul, Brazil. Professor, UFeevale, Brazil.

Vandré Casagrande Figueiredo. Master in Biology, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. Doctoral student, University of Auckland, Nova Zealanda.

Clóvis Milton Duval Wannmacher. Doctor in Biology, UFRGS, Brazil. Professor, UFRGS, Brazil.

Greicy Michelle Marafiga Conterato. Doctor in Toxicological Biochemistry, UFSM, Brazil. Professor, Universidade Federal de Santa Catarina, Brazil.

Luciane Rosa Feksa. Doctor in Biology, UFRGS, Brasil. Professor, UFeevale, Brazil.

Heavy metals are among the most widespread potential chemical contaminants in the environment and are transferable to man and animals through diet and other routes (Pace and Lannucci, 1994). Lead is a naturally occurring heavy metal used in the manufacture of consumer products including batteries, paints, metal products, ammunition, petrol, cable covering and ceramic glaze. Lead exists in three forms: metallic lead, inorganic lead (or lead salts) and organic lead (containing carbon). The widespread use of lead in manufacturing results in continuous occupational exposure to lead worldwide. From a public health point of view, inorganic lead exposure has become a serious problem in both occupational and environmental settings (Calderón-Salinas *et al.*, 1996a; Todd *et al.*, 1996; Wakefield, 2002; ATSDR, 2007). Lead can be absorbed as a result of inhalation, oral ingestion or dermal exposure, but the last of these is much less efficient than the other two. After absorption into the bloodstream most of the lead taken up and bound to erythrocytes. The freely diffusible plasma fraction is distributed extensively throughout tissues and the highest concentrations are found in bones, teeth, the liver, lungs, kidneys, brain and spleen. Lead has estimated half-lives of 35 days in blood, 40 days in soft tissue and 20-30 years in bone (Brito *et al.*, 2002). It is mainly excreted via renal and gastrointestinal pathways (NTP, 2003; ATSDR, 2007). The toxicity associated with lead is widely known. However, the biochemical and molecular mechanisms of lead toxicity are poorly understood. This metal is linked to a wide range of physiological, biochemical and behavioral dysfunctions (Calderón-Salinas *et al.*, 1996b; Courtois *et al.*, 2003). The molecular basis of lead toxicity involves: covalent binding to proteins (Goering, 1993; Navarro-Moreno *et al.*, 2009), oxidative damage (Gurer-Ohrnan *et al.*, 2004; Rendón-Ramírez *et al.*, 2007) and interaction with stereospecific sites for divalent cations such as calcium ions (Ca^{2+}). The last of these mechanisms has effects that impair a range of different, biologically significant, calcium-dependent processes. These include intracellular signaling, divalent metal transport, energy metabolism, enzymatic processes, apoptosis, ionic conduction, cell adhesion, protein maturation and genetic regulation (Garza *et al.*, 2006). Lead ions (Pb^{2+}) are transported to their targets by erythrocytes via Ca^{2+} transport systems, competing with Ca^{2+} (Calderón-Salinas *et al.*, 1999a, b). As a result of this competition, Ca^{2+} influx decreases, a phenomenon that may alter Ca homeostasis (Quintanar-Escorza *et al.*, 2007).

Recent epidemiological studies have reported that even at low levels lead has a graded association with several ill-health outcomes such as hypertension, developmental defects, neurological problems, cognitive impairment, kidney damage and anemia (Muntener *et al.*, 2003; Ekong *et al.*, 2006; Menke *et al.*, 2006; Bemmel *et al.*, 2011). Olewińska *et al.* (2010) have shown that occupational exposure to lead is associated with damage to DNA. Both DNA damage and repair appear to be modulated by interactions between environmental and genetic factors. The response to environmental factors often depends on specific genetic polymorphisms (Dušinská and Collins., 2008). One of the most studied genes that can affect the toxicity of lead codes for δ-aminolevulinic acid dehydratase (δ-ALAD). This enzyme is the second one in the heme biosynthetic pathway and plays a role in the pathogenesis of lead poisoning (Onalaja *et al.*, 2000). When δ-ALAD activity is deficient, due to lead poisoning, erythrocyte synthesis is inhibited and blood hemoglobin concentration falls. In the second step of heme synthesis, δ-ALAD catalyzes the formation of porphobilinogen from two molecules of δ-aminolevulinic acid (δ-ALA). δ-ALAD is the most sensitive enzyme to lead in the heme pathway and has a high affinity for the metal. Lead binds to the enzyme's SH group, which normally binds zinc, preventing it from binding to δ-ALA (Warren *et al.*, 1998). Because lead effectively inhibits δ-ALAD activity, resulting in δ-ALA accumulation in blood and urine, urinary δ-ALA has also been used as a biomarker for lead exposure and as a marker of early biological effects of lead (Sithisarankul *et al.*, 1998; Warren *et al.*, 1998; Sakai, 2000). Studies indicate that the strong affinity to amino acid thiol groups (SH) is a characteristic shared by zinc, cadmium, mercury, and lead (Valle and Ulmer., 1972; Huang *et al.*, 2004; Nunes-Tavares *et al.*, 2005). Of all the enzymes that are inhibited by Pb^{2+} binding to active center -SH groups, it is δ-ALAD about which most is known (Bernard and Lauwerys, 1987).

It is also well known that the activity of some thiol-containing enzymes may be altered by thiol/disulfide exchange between the protein sulfhydryl groups and disulfides (Gilbert, 1984). Creatine kinase (CK; EC 2.7.3.2) is a thiol-containing enzyme that catalyzes the reversible transfer of the phosphoryl group from phosphocreatine to ADP, regenerating ATP. This enzyme exerts a key role in cellular energy metabolism of tissues with high energy requirements (Wallimann *et al.*, 1992). There are distinct CK isoenzymes, which are compartmentalized specifically in places where energy is released (mitochondria) or used (cytosol) (Friedman and Perryman., 1991). Adenylate kinase (AK; EC 2.7.4.3) is a thiol-containing enzyme like δ-ALAD and CK, catalyzing the reversible transfer of phosphoryl between ATP and AMP (Willemoes and Kilstrup, 2005). This enzyme, along with CK, is responsible for the enzymatic phosphotransfer network; in other words, it is responsible for the transfer of the phosphate of ATP where it is produced (mitochondria) to the place where it is consumed (cytosol) (Dzeja and Terzic, 2003). Both enzymes, CK and AK, are intimately associated in such a way that when one enzyme activity is reduced, the activity of the other enzyme is enhanced (Dzeja *et al.*, 1999, 2002). Lead affects various hematological systems, such as heme biosynthesis and catabolism of pyrimidine nucleotides. These metabolic disorders can be used as indicators for biological monitoring of lead exposure, on the basis of dose-effect relationships between blood lead concentrations (BPb) and the biochemical effects of lead. In this study, we investigated the effect of Pb^{2+} on the activity of the thiol-enzymes δ-ALAD, CK, AK and on levels of the non-enzymatic antioxidant defense mechanism glutathione (GSH) in human erythrocytes from lead-exposed workers and demonstrate that CK activity can be used for biological monitoring of lead exposure.

Materials and Methods

Subjects

We recruited 22 male lead-exposed workers 20-50 years old (mean 35 years) and 21 normal volunteers. All volunteers provided informed consent before venous blood samples were collected and treated with heparin, during their routine physical examinations. These workers were employed in manufacturing and recycling of automotive batteries in Rio Grande do Sul, Brazil. Control subjects were fifteen males 20-48 years old (mean 34 years) and six females 18-40 years old (mean 29 years) with no history of lead exposure. Controls underwent clinical blood analysis and abnormal findings were not found. Exclusion criteria were as follows: history, or current physical findings of serious cardiovascular, renal, hepatic, endocrine, metabolic or gastrointestinal diseases or previous pharmacological treatment. All subjects provided informed consent in writing and participation was voluntary. The study was approved by Feevale University Ethics Commission.

Blood analysis

Venous blood was used for hematological testing. Blood was drawn by venous puncture and collected in EDTA tubes. The following parameters were measured using a Siemens automatic hematology system: red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (CHCM) and white blood cell count (WBC).

Determination of lead levels in blood

Blood lead (BPb) was quantified as follows. Samples were diluted to 1:10 with a dilution solution containing 0.1% Triton X-100 (v/v) and 1% HNO_3 (v/v) in Milli-Q water. Lead was analyzed using a Perkin Elmer Analyst 600 graphite furnace atomic absorption spectrometer with a pyrolytic graphite-coated furnace and a permanent matrix modifier composed of tungsten, rhodium, and ammonium hydrogen phosphate. The injection volume was 20µl of the diluted sample. The temperatures used for the drying phase were 120°C.5·s^{-1} (rise time 10s) followed by 200°C.5·s^{-1} (rise time 5s); 600°C.20·s^{-1} (rise time 10s) for ashing; 1700°C.3·s^{-1} (step mode) for atomization; and 2200°C.4·s^{-1} (rise time 1s) for cleaning. Argon 99.999% (v/v) (White Martins, Brazil) was used as carrier gas at a flow rate of 250ml·min^{-1} for all phases except atomization. The detection and quantification limits of the method employed are 0.06 and 0.15µg·dl^{-1}, respectively.

Preparation of erythrocytes

Blood was collected by venous puncture into heparinized tubes. Each sample was centrifuged at 800g for 10min and the plasma and white cells were carefully removed by aspiration to avoid loss of erythrocytes. The packed cells were washed three further times at 700g for 5min with isotonic buffer. After this process, the erythrocytes were hemolyzed by adding distilled water at a dilution of 1:5 in a tube, then each sample was centrifuged at 800g for 10min and the supernatant was separated for biochemical analysis.

Erythrocyte CK activity

Aliquots from heparinized blood were used for determination of creatine kinase (CK) activity. CK activity was assessed in lysed erythrocytes in accordance with the preparation of erythrocytes (above). The reaction mixture contained the following final concentrations: 60mM Tris-HCl buffer, pH 7.5, 7mM phosphocreatine, 9mM $MgSO_4$, and ~1µg protein in a final volume of 0.13ml. . After 30min of pre-incubation at 37ºC, the reaction was started by the addition of 0.42µmol ADP. The reaction was stopped after 10min incubation by the addition of 1µmol p-hydroxymercuribenzoic acid. The reagent concentrations and the incubation time were chosen to assure linearity of the enzymatic reaction. Appropriate controls were carried out to measure chemical hydrolysis of phosphocreatine. The creatine formed was estimated according to the colorimetric method of Hughes (1962). The color was developed by the addition of 0.1ml of 2% α-naphthol and 0.1ml 0.05% diacetyl in a final volume of 1ml and read at 540nm after 20min. Results were expressed as mmol of creatine formed per min per mg protein.

Erythrocyte δ-ALAD activity

δ-aminolevulinic acid dehydratase activity was assayed as described by Berlin and Schaller (1974). Aliquots from heparinized blood were used for determination of δ-ALAD activity. δ-ALAD activity was assessed in lysed erythrocytes in accordance with the preparation of erythrocytes (above). Modified Ehrlich's reagent was used to react with the porphobilinogen (PBG) final product to yield a pink-colored compound, which was measured at 555nm. Activity was expressed as µmol PBG/min/lit of erythrocyte.

Erythrocyte AK activity

Aliquots from heparinized blood were used for determination of adenylate kinase **(**AK) activity. AK activity was assessed in lysed erythrocytes. AK activity was measured with a coupled enzyme assay with hexokinase (HK) and glucose 6-phosphate dehydrogenase (G6PD), according to Oliver (1955) with the modifications introduced by Dezja *et al.* (1999). The reaction mixture contained 100mM KCl, 20mM HEPES, 20mM glucose, 4mM $MgCl_2$, 2mM $NADP^+$, 1mM EDTA, 4.5U/ml of HK, 2U/ml of G6PD and 1µg of protein homogenate. The reaction was initiated by the addition of 2mM ADP and the reduction of $NADP^+$ was followed at 340nm for 3min in a spectrophotometer. ADP, $NADP^+$, G6PD and HK were dissolved in water. Reagents concentration and assay time (3min) were chosen to ensure linearity of the reaction. Results were expressed in µmol of ATP formed per min per mg of protein.

Reduced GSH concentrations

Reduced glutathione (GSH) concentrations were measured according to Browne and Armstrong (2002). Erythrocytes were hemolyzed with 1µl Triton X-100 and 10min later precipitated with 20% trichloroacetic acid (w/v). After centrifugation, the supernatant aliquots were diluted in 20 volumes of (1:20, v/v) 100mM sodium phosphate buffer pH 8.0, containing 5mM EDTA. Of this preparation, 100µl were incubated with an equal volume of o-phthaldialdehyde (1mg/ml methanol) at room temperature during 15min. Fluorescence was measured using excitation and emission wavelengths of 350 and 420nm, respectively. A calibration curve was prepared with standard GSH (0.01-1mM) and concentrations are expressed as nmol of GSH/mg of protein.

Protein determination

Erythrocyte protein content was determined using the method described by Lowry and co-workers (1951), using bovine albumin as the standard.

Statistical analysis

Data were analyzed using Student's t test for independent samples. Dose-dependent effects were analyzed by linear regression. All data were analyzed using the Statistical Package for the Social Sciences (SPSS 17.0 for Windows; Leech *et al.*, 2005).

Results

Table I shows the results of those clinical analyses for which there was a significant difference between the

TABLE I
EFFECT OF LEAD ON BLOOD WHITE CELL COUNT, RED CELL COUNT, HEMOGLOBIN, HEMATOCRIT, MEAN CORPUSCULAR VOLUME, MEAN CORPUSCULAR HEMOGLOBIN AND MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION IN 21 NORMAL VOLUNTEERS AND 22 LEAD-EXPOSED WORKERS

	Control	Lead-exposed
WBC (/µl)	8010 ±1333	7836 ±1510
RBC (106/µl)	4.59 ±0.44	3.78 ±0.31 ***
HGB (g/dl)	14.38 ±0.33	12.88 ±0.41 ***
HCT (%)	42.28 ±3.5	37.0 ±2.51 ***
MCV (fl)	84.61 ±2.63	83.86 ±2.83
MCH (pg)	30.9 ±1.75	25.36 ±1.22 ***
MCHC (g/dl)	34.2 ±1.64	32.86 ±1.78 *

Data are expressed as mean ±SD. Different from controls to: ***p<0.0001, *p<0.05 (Student's t test).

individuals in the exposed and control groups. The following results were all significantly lower in lead-exposed individuals than in controls: RBC ($t_{(41)}$=6.88, ***p<0.0001); HGB ($t_{(41)}$=13.08, **p< 0.0001); HCT ($t_{(41)}$=5.67, **p<0.0001); MCH ($t_{(41)}$=12.06, **p<0.0001); and MCHC ($t_{(41)}$=2.63, *p<0.05). There was no significant difference between the two groups in terms of WBC ($t_{(41)}$=0.940, p=0.692) or MCV ($t_{(41)}$=0.90, p=0.371).

As shown in Table II, blood lead concentrations were significantly higher in lead-exposed individuals than in controls ($t_{(41)}$=-26.64, **p<0.0001) while CK ($t_{(31)}$=2.87, **p<0.01) and δ-ALAD ($t_{(28)}$=9.26, **p<0.0001) activities were both significantly lower in lead-exposed individuals than in controls. We also found that lead had no effect on AK activity ($t_{(30)}$=1.52, p=0.24) and erythrocyte glutathione content was reduced ($t_{(26)}$=3.04, **p<0.01) in lead-exposed individuals compared with normal volunteers. Furthermore, lead significantly inhibited CK (t=6.89, β=-0.43, *p<0.05) and δ-ALAD (t=16.71, β=-0.85, **p<0.0001) activities in a dose-dependent manner. Figures 1 and 2 illustrate the strength of the relationship between blood lead levels and biomarkers of lead toxicity, including CK and δ-ALAD activity. Figure 3 illustrates the lead dose-dependent effect analyzed by linear regression for lead-exposed individuals *vs* normal volunteers; the figure reveals a significant regression for CK against δ-ALAD activity (t=14.71, β=0.58, **p<0.001). These results indicate apparent dose-effect relationships between CK and δ-ALAD activity. Figure 4 illustrates the lead dose-dependent effect analyzed by linear regression for lead-exposed individuals *vs* normal volunteers. This figure illustrates a significant regression for blood lead levels against GSH content (t=23.51, β=-0.85, p<0.0001). Figures 5 and 6 illustrate a significant regression for CK activity against GSH content (t= 10.68, β=0.54, p<0.01) and for δ-ALAD activity against GSH content (t=6.96, β=0.84, p<0.0001), respectively. These results indicate apparent dose-effect relationships between GSH content and CK activity and between GSH content and δ-ALAD activity.

TABLE II
BLOOD LEAD CONCENTRATION, Δ-ALAD, CK AND AK ACTIVITY IN ERYTHROCYTES FROM NORMAL VOLUNTEERS AND LEAD-EXPOSED WORKERS

	Control (n=12-21)	Lead-exposed (n=16-22)
Blood lead concentration	1.88 ±0.39	61.9 ±10.3***
δ-ALAD activity	29.1 ±7.1	9.2 ±4.7***
CK activity	6.28 ±1.2	5.12 ±1.12***
AK activity	3.68 ±0.79	3.24 ±0.62
GSH content	1.33 ±0.85	0.678 ±0.15***

Data are expressed as mean ±SD for experiments performed in duplicate. Different from controls to: ***p<0.0001 (Student's t test). Results were expressed as $\mu g \cdot dl^{-1}$ (blood lead concentration), μmol of porphobilinogen (PBG) transformed per min/liter of erythrocyte (δ-ALAD activity), mmol of creatine transformed per min/mg of protein (CK activity) and μmol of ATP formed per min/mg of protein (AK activity).

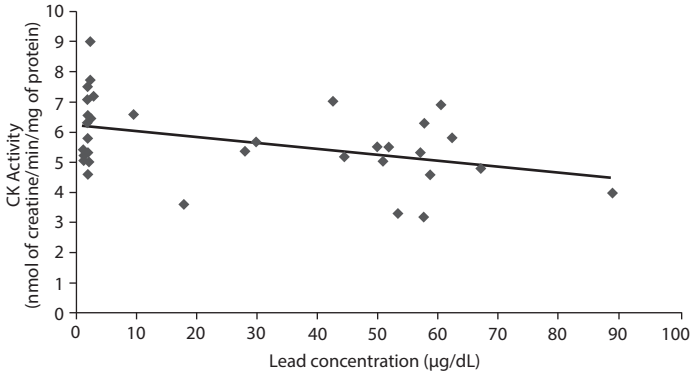

Figure 1. Lead dose-dependent effects were analyzed by linear regression for 17 lead-exposed individuals and 16 normal volunteers. This figure illustrates a significant regression for blood lead levels against CK activity; a new biomarker of lead toxicity (t=6.89, β=-0.43, *p<0.05).

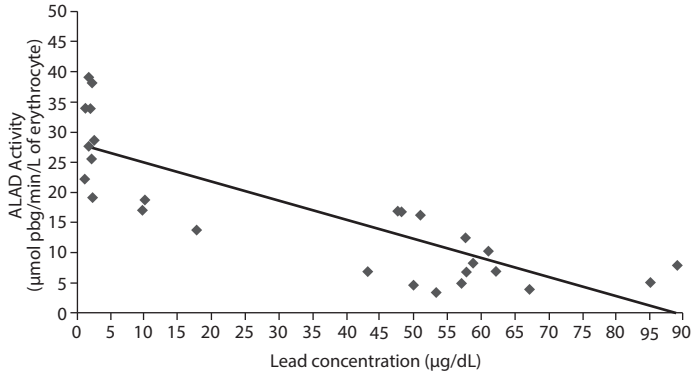

Figure 2. Lead dose-dependent effects were analyzed by linear regression for 18 lead-exposed individuals and 12 normal volunteers. This figure illustrates a significant regression for blood lead levels against δ-ALAD activity; a classic biomarker of lead toxicity (t=16.71, β=-0.85, p<0.0001).

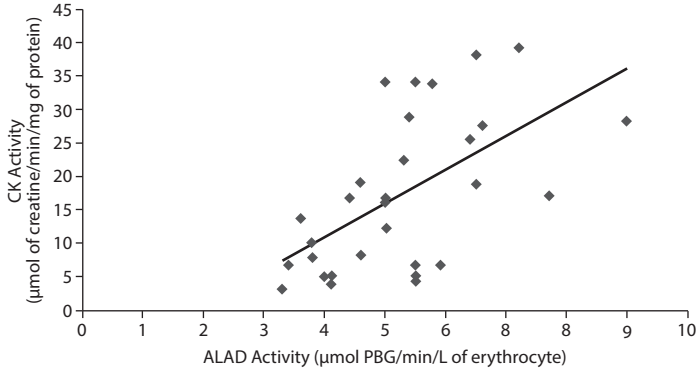

Figure 3. Lead dose-dependent effects were analyzed by linear regression for 17 lead-exposed individuals and 13 normal volunteers. This figure illustrates a significant regression for CK against δ-ALAD activity (t=14.71, β=0.58, **p<0.001).

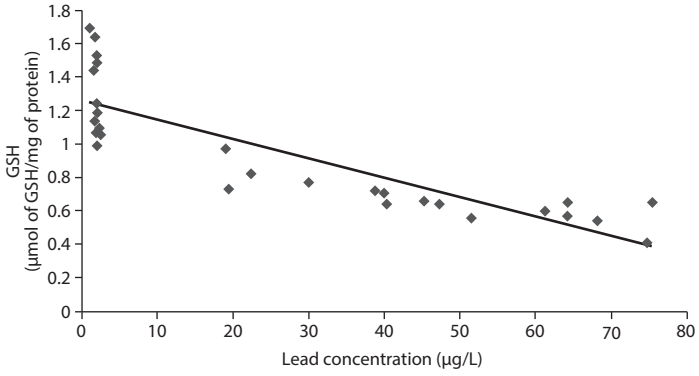

Figure 4. Lead dose-dependent effects were analyzed by linear regression for 16 lead-exposed individuals and 12 normal volunteers. This figure illustrates a significant regression for BPb levels against GSH content (t=23.51, β=-0.85, p<0.0001).

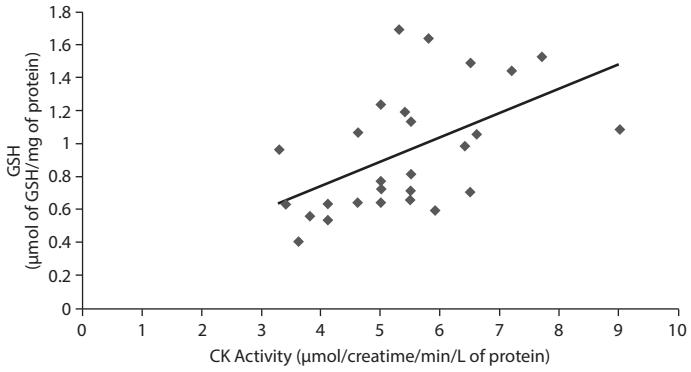

Figure 5. Lead dose-dependent effects were analyzed by linear regression for 16 lead-exposed individuals and 12 normal volunteers. This figure illustrates a significant regression for CK activity against GSH content (t= 10.68, β= 0.54, p<0.01).

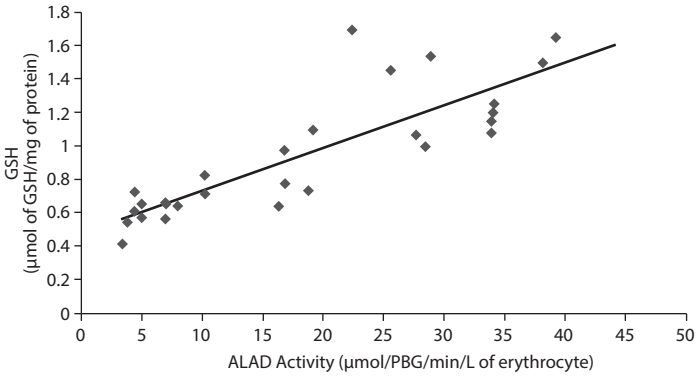

Figure 6. Lead dose-dependent effects were analyzed by linear regression for 16 lead-exposed individuals and 12 normal volunteers. This figure illustrates a significant regression for δ-ALAD activity against GSH content (t=6.96, β=0.84, p<0.0001).

DISCUSSION

In this study we investigated the effect of lead on the activity of the thiol-enzymes CK and δ-ALAD in human erythrocytes from lead-exposed workers. The values for several red-cell parameters (hemoglobin, hematocrit, red blood cell count and mean corpuscular volume) were lower in lead-exposed individuals than in controls. These results suggest that lead inhibits CK and δ-ALAD activity by interacting with their thiol groups. Inhibition of these enzymes and GSH depletion can induce erythrocyte cell death through hemolysis. The results indicate an apparent dose-effect relationship between GSH content and CK activity and between GSH content and δ-ALAD activity. Therefore, simultaneous suppression of CK and δ-ALAD activity could severely impair both erythrocyte metabolism and antioxidant defenses (GSH), with severe consequences for cell function and survival. Inhibition of these enzymes can induce erythrocyte cell death through hemolysis. The results indicate an apparent dose-effect relationship between BPb and CK activity and between BPb and δ-ALAD activity.

Lead toxicity leads to free radical damage via two separate, although related, pathways: 1) generation of reactive oxygen species (ROS), including hydroperoxides, singlet oxygen and hydrogen peroxide; and 2) direct depletion of antioxidant reserves (Ercal *et al.*, 2011). In any biological system where ROS production increases, antioxidant reserves are depleted. One of the effects of lead exposure impacts glutathione metabolism (Lepper *et al.*, 2010). Glutathione plays an important role in antioxidant defense and redox regulation (Wu *et al.*, 2004). It not only reacts with free radicals, but can also form conjugates with numerous substances, including heavy metals (Meister, 1994). It is possible that the reduction in concentrations of GSH and non-protein thiols in the liver and kidneys after exposure to Pb^{2+} could be the result of lead's high degree of affinity for SH groups (Gurer *et al.*, 1999). On the other hand, it is known that GSH depletion induces cell death by apoptosis (Armstrong and Jones, 2002). Erythrocytes have a high affinity for lead, binding 99% of that present in the bloodstream. Lead has a destabilizing effect on cell membranes, and in RBC the effect is to decrease cell membrane fluidity and increase the erythrocyte hemolysis rate. Hemolysis appears to be the end result of ROS-generated lipid peroxidation in the RBC membrane (Lawton and Donaldson, 1991). Hypochromic or normochromic anemia is a hallmark of lead exposure resulting from ROS generation and subsequent erythrocyte hemolysis (Gurer and Ercal, 2000). Lead, in common with silver, mercury, and copper, is considered to be a strong hemolytic agent capable of causing erythrocyte destruction through formation of lipid peroxides in cell membranes (Farant and Wigfield, 1982).

In addition to membrane peroxidation, lead exposure causes hemoglobin oxidation, which can also cause RBC hemolysis. The mechanism responsible for this reaction is lead-induced inhibition of δ-ALAD. Lead's toxic effects are manifest in depressed heme formation and δ-ALAD is the enzyme that is most sensitive to this depression (Farant and Wigfield, 1982). Feksa *et al.* (2012) found that pyruvate kinase (PK) behaves similarly to δ-ALAD, contributing further harm to erythrocyte survival systems. Pyruvate kinase is an enzyme that catalyzes the conversion of phosphoenolpyruvate (PEP) to pyruvate, producing adenosine triphosphate (ATP) from adenosine diphosphate (ADP) (Feksa *et al.*, 2012). Our results suggest that there is a strong correlation between inhibition of CK, δ-ALAD and induction of cell death by hemolysis. This is manifest in the alterations to several hematological parameters, such as HGB, HCT, RBC and MCV, which were lower in lead-exposed individuals than in controls. However, further studies to evaluate the activity of thiol-containing enzymes such as CK and δ-ALAD in occupationally lead-exposed workers are still needed. In this study it is shown that CK behaves similarly to δ-ALAD, contributing further harm to erythrocyte survival systems. The AK activity did not change in the presence of lead. We can explain this effect because both enzymes, CK and AK, which are responsible for transferring phosphate from ATP that is produced to the location where it is consumed, are intimately associated; when one enzyme activity is reduced, the activity of the other enzyme is enhanced (Dzeja *et al*., 1999, 2002). The adenylate kinase is responsible for the reversible conversion between phosphates of ATP, ADP and AMP (Bae and Phillips 2006). AK doubles the energy potential of ATP, having the ability of regenerate ATP from two ADP and by the regulation of processes involving adenine nucleotides (Noma, 2005; Willemoes and Kilstrup, 2005). CK has the main function in energy metabolism, where it works like a buffer system of levels of cellular ATP; it catalyzes the reversible transfer of the phosphoryl group of phosphocreatine to ADP, regenerating ATP (Warchala *et al*., 2006). Inhibition of CK and PK activity (Feksa *et al*., 2012) and no change in activity of AK in the erythrocyte can help avoid severe consequences for cell function and survival. The study of the mechanisms by which lead acts may contribute to a better understanding of the symptoms caused by this metal.

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INHIBICIÓN DE ENZIMAS CON GRUPOS TIOL EN ERITROCITOS DE TRABAJADORES EXPUESTOS AL PLOMO

Thereza Luciano Trombini, Evandro Oliveira, Daiane Bolzan Berlese, Renato Minozzo, Tássia de Deus, Cristina Deuner Muller, Rafael Linden, Vandré Casagrande Figueiredo, Clóvis Milton Duval Wannmacher, Greicy Michelle Marafia Conterato y Luciane Rosa Feksa

RESUMEN

Se considera que el plomo (Pb^{2+}) entra a los eritrocitos por intercambio aniónico y permanece en la célula unido a grupos tiol. La creatina quinasa (CK) y la adenilato quinasa (AK) son enzimas con grupos tiol que tienen un rol central en la homeóstasis energética de eritrocitos. La dehidratasa del ácido δ-aminolevulínico (δ-ALAD) es la segunda enzima en la vía biosintética del hemo y juega un papel en la patogénesis del envenenamiento plúmbico. Se estudió el efecto del Pb^{2+} en la actividad de CK, AK y δ-ALAD en eritrocitos de trabajadores expuestos al plomo. La muestra incluyó 22 trabajadores varones expuestos y 21 voluntarios normales. Los primeros trabajaban en manufactura y reciclaje de baterías automotrices. Se estudiaron parámetros básicos de eritrocitos y se hicieron contajes de leucocitos totales. Se determinaron las actividades de CK, AK y δ-ALAD, y las concentraciones sanguíneas de Pb^{2+}. Los individuos expuestos tuvieron niveles significativamente más altos de Pb^{2+} sanguíneo que los controles. Los niveles de actividad de CK y δ-ALAD fueron significativamente más bajos en sujetos expuestos, mientras que el plomo no afectó la actividad de AK. Los individuos expuestos tuvieron valores menores en varios parámetros eritrocíticos. Los resultados indican una aparente relación dosis-dependiente entre Pb^{2+} sanguíneo y actividad de CK y δ-ALAD, y que el plomo inhibe la actividad de δ-ALAD y CK al interactuar con sus grupos tiol. Es entonces posible que el plomo perturbe la homeóstasis energética, asociándose a una reducción de metabolismo energético que contribuye a la disfunción celular observada en individuos expuestos al plomo.

INHIBIÇÃO DAS ENZIMAS COM GRUPOS TIOL EM ERITRÓCITOS DE TRABALHADORES EXPOSTOS AO CHUMBO

Thereza Luciano Trombini, Evandro Oliveira, Daiane Bolzan Berlese, Renato Minozzo, Tássia de Deus, Cristina Deuner Muller, Rafael Linden, Vandré Casagrande Figueiredo, Clóvis Milton Duval Wannmacher, Greicy Michelle Marafia Conterato e Luciane Rosa Feksa

RESUMO

O chumbo (Pb^{2+}) entra no eritrócitos através de permuta aniônica e permanece na célula através da ligação a grupos tiol. A creatina quinase (CK) e adenilatoquinase (AK) são duas enzimas contendo grupos tiol que exercem papel fundamental para a homeostase energética celular em eritrócitos. Ácido δ-aminolevulínico desidratase (δALAD) é a segunda enzima da via biossintética do heme e desempenha um papel na patogênese de envenenamento por chumbo. Investigamos o efeito do Pb^{2+} sobre a atividade das δALAD, CK e AK em eritrócitos de trabalhadores expostos ao chumbo. A amostra foi composta por 22 trabalhadores expostos do sexo masculino e 21 voluntários normais. Os trabalhadores expostos trabalham na fabricação e reciclagem de baterias automotivas. Parâmetros de hemácias foram avaliados, contagem de células brancas, a atividade da CK, AK e δALAD e as concentrações sanguíneas de chumbo foram determinados. Indivíduos expostos ao chumbo tinham níveis significativamente mais elevados do que os controles. Os níveis de atividade da CK e de δALAD foram significativamente menores nos indivíduos expostos. O chumbo não teve efeito sobre a atividade de AK. Indivíduos expostos apresentaram valores menores do que os controles para os parâmetros dos glóbulos vermelhos. Estes resultados indicam uma relação dose-efeito entre a atividade da CK, δ-ALAD e chumbo, o cual inibe a atividade da δ-ALAD e CK, interagindo com os seus grupos tiol. É, portanto, possível que o chumbo altere a homeostase energética e pode estar ligada com a redução no metabolismo energético contribuindo para a disfunção celular observada nestes indivíduos expostos em chumbo.