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C. R. Biologies 330 (2007) 581–588
COMPTES RENDUS BIOLOGIES
http://france.elsevier.com/direct/CRASS3/

Pharmacology, toxicology / Pharmacologie, toxicologie

Antioxidant enzymes activities and bilirubin level in adult rat treated with lead

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Received 10 April 2007; accepted after revision 25 May 2007

Available online 28 June 2007

Presented by Pierre Buser

Abstract

Lead toxicity is closely related to its accumulation in several tissues and its interference with bioelements, whose role is critical for several biological processes. Recently, oxidative stress has been proposed as a possible mechanism involved in lead toxicity. This study was carried out to investigate the effect of dose-dependent lead exposure on haematological and oxidative stress parameters. Adult male 'Wistar' rats (150–200 g) were divided into three groups: group [Pb 5] and group [Pb 15] received respectively 5 mg Pb^{2+} ($n = 16$) and 15 mg Pb^{2+}/kg b.w. ($n = 16$) as lead acetate solution i.p. for a period of seven days. Group [T] ($n = 16$) served as control and received 15 mg Na^{+}/kg b.w. as sodium acetate solution i.p. for the same period. All animals were sacrificed 24 h after the last injection. Blood superoxide dismutase (SOD) and blood glutathione peroxidase (GPx) activities and plasma bilirubin level were measured. Liver was quickly excised for the estimation of alteration in lipid peroxidation indices (MDA). Lead exposure induces, in both treated groups, a marked decline in haematocrit and haemoglobin levels ($p < 0.01$) when compared to control. The results show also a significant decrease ($p < 0.01$) in SOD activity, but only in group [Pb 15]. SOD activity did not decrease in group [Pb 5] in comparison with control ($p > 0.05$). However, lead exposure caused a light increase in GPx activity in group [Pb 15], which remains non-significant ($p > 0.05$) compared to control. Group [Pb 5] did not record significant changes in the activity of GPx. Lead exposure for a period of seven days resulted in a significant ($p < 0.05$) increase in bilirubin level in group [Pb 15] compared to control. The bilirubin level from rats of group [Pb 5] did not reach a statistical significance. Changes in liver MDA content in lead-exposed rats from [Pb 5] and [Pb 15] groups did not reach a statistical ($p < 0.05$) significance. It is concluded that lead induces oxidative stress in a dose-dependent manner. No dose-dependent response was observed in blood GPx activity and liver MDA content. These results could be due to the short duration of the treatment. ***To cite this article: A. Annabi Berrahal et al., C. R. Biologies 330 (2007).***

Résumé

Incidence de la toxicité du plomb sur l'activité des enzymes antioxydantes et du taux plasmatique de la bilirubine chez le rat adulte. Le plomb étant un élément non essentiel à la vie des cellules eucaryotes, son introduction dans l'organisme (par inhalation de poussières de plomb ou par ingestion d'aliments contenant du plomb) modifie la biologie des cellules, et plus particulièrement celle des globules rouges, en perturbant une myriade de voies métaboliques et de processus physiologiques. Parmi les mécanismes impliqués dans l'induction de la toxicité du plomb, nous nous intéressons au stress oxydant au niveau érythrocytaire.

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1631-0691/$ – see front matter
doi:10.1016/j.crvi.2007.05.007

Ainsi, nous avons procédé à la détermination des paramètres hématologiques et au dosage des deux principales enzymes antioxydantes, la superoxyde dismutase (SOD) et la glutathion peroxydase (GPx), ainsi que d'un antioxydant naturel non enzymatique, la bilirubine totale. Afin d'évaluer l'ampleur du stress oxydant au niveau tissulaire, nous avons procédé à la détermination de l'un des produits finaux de la peroxydation lipidique au niveau hépatique : le malondialdéhyde (MDA). Le protocole expérimental repose sur l'administration, pendant 7 j, par IP, d'une solution d'acétate de plomb à raison de 5 mg Pb/kg de PC [Pb 5] et 15 mg Pb/kg de PC [Pb 15] pour les rats mâles Wistar traités (150 à 200 g), et d'une solution d'acétate de sodium, à raison de 15 mg Na^+/kg de PC, pour les rats témoins. Les résultats montrent une diminution importante des taux de l'hématocrite et de l'hémoglobine chez les deux groupes de rats traités [Pb 5] et [Pb 15] par rapport au groupe des rats témoins [T] ($p < 0{,}01$). Par ailleurs, on note une diminution significative de l'activité de la SOD, mais seulement chez le groupe [Pb 15] ($p < 0{,}01$). En revanche, la GPx montre une légère augmentation de son activité chez les deux groupes de rats traités, activité qui reste tout de même non significative. Par ailleurs, l'exposition au plomb entraîne une augmentation du taux de la bilirubine totale chez le groupe traité à la plus forte dose ($p < 0.05$), alors que le groupe [Pb 5] ne montre aucune variation significative par rapport au groupe [T]. Au niveau hépatique, le dosage du MDA ne montre aucune différence significative chez les deux groupes [Pb 5] et [Pb 15] par rapport au groupe [T]. Le plomb affecte considérablement les érythrocytes d'une manière dose-dépendante. Les variations observées dans les résultats peuvent être expliquées par la courte durée de l'exposition. ***Pour citer cet article : A. Annabi Berrahal et al., C. R. Biologies 330 (2007).***

Keywords: Lead; SOD; GPx; Bilirubin; Lipid peroxidation; Rats

Mots-clés : Plomb ; SOD ; GPx ; Bilirubine ; Peroxydation lipidique ; Rats

1. Introduction

Lead is naturally present in the lithosphere in negligible quantity, due to rock wind erosion or volcanic rejections [1].

Nowadays, lead is being a ubiquitous environmental contaminant due to its significant role in modern industry [2].

However, both occupational and environmental exposures remain a serious problem in many developing and industrializing countries [3].

Effectively, lead constitutes the most abundant non-essential element in the human organism, due to its dispersion in ambient air, in many foods, in drinking water, and in dust. Its toxicity is closely related to its accumulation in certain tissues and its interference with the bioelements, whose role is critical for several physiological processes. It has many undesired effects, including neurological [4,5], behavioural [6,7], immunological [8–11], renal [12–14], hepatic [13], and especially haematological [15,16] dysfunctions.

However, biochemical and molecular mechanisms of lead toxicity are poorly understood [2]. Various mechanisms were suggested to explain them: inhibition of the calcium-pump, a transport protein [3], disturbances in mineral metabolism, inactivation of several enzymes, demyelinisation of nervous tissues, etc. Recently, oxidative stress has been proposed as another possible mechanism involved in lead toxicity. According to many workers, lead-induced damage may result from disturbance of the prooxidant and antioxidant balance that is found in cells [15].

Generation of highly reactive oxygen species (ROS) such as the hydroxyl radical [OH°], hydrogen peroxide [H_2O_2], superoxide anion [$O_2{}^-$] and lipid peroxides [LPO°] after lead exposure has been reported [5].

Based on the above considerations, this study was carried out to investigate the effects of dose-dependent exposure to lead on oxidative stress parameters in rat blood.

About 99% of the lead present in the blood is bound to erythrocytes. They have a high affinity for lead and contain the majority of the lead found in the blood stream, which makes them more vulnerable to oxidative damage than many other cells. Moreover, erythrocytes can spread lead to different organs of the body [16]. Therefore, we tried to investigate the effects of dose-dependent lead exposure on an end-product of lipid peroxidation known as MDA in hepatic cells.

2. Materials and methods

2.1. Chemicals

Lead (II) acetate was purchased from Prolabo (France). Butylated hydroxytoluene (BHT), 2-thiobarbituric acid (TBA), Coomassie G250 and bovine serum albumin (BSA) were obtained from Sigma Chemicals Co. (Germany). All other chemicals were of the highest grade available.

2.2. Animals

All experiments were performed with 'Wistar' male rats weighing 150–200 g, which were obtained from the Tunisian Society of Pharmaceutical Industries.

The animals were housed in clean plastic cages and allowed to acclimatize in the laboratory environment for a week ($T = 22 \pm 2\,^{\circ}C$ with 12-h dark/light cycle and ventilation system).

In the experimental period, animals had access to food and water ad libitum.

2.3. Experimental design

The animals were randomly assigned into three groups of 16 individuals and treated for seven days. The first group [T] served as control and received daily 15 mg Na^{+}/kg b.w. i.p. as sodium acetate solution.

The second [Pb 5] and third [Pb 15] groups received daily, respectively, 5 mg and 15 mg Pb^{2+}/kg b.w. i.p. as lead acetate solution for the same period.

Throughout the experience, the rats were daily weighed.

At the end of the treatment, 24 h after the last dose of lead, animals were killed by decapitation without preliminary anaesthesia, and arteriovenous blood was taken quickly. The liver was removed by transverse abdominal incision and kept frozen at $-80\,^{\circ}C$.

2.4. Preparation of plasma and tissue extract

Blood collected on heparin was taken and let rest for 30 min at room temperature. Plasma was obtained by centrifugation at 1000 *g* for 10 min at 4 °C, and stored at $-80\,^{\circ}C$ in aliquots until use.

Livers were excised immediately, washed with ice-cold physiologic saline solution (0.9%), blotted dry, and weighed. Tissues were homogenized for 30 s in 10 volumes of ice-cold 10 mM phosphate buffered saline (PBS, pH 7.4). Homogenates were centrifuged at 10 000 *g* for 10 min at 4 °C to remove cell debris, nuclei, and mitochondria. Resulting supernatants served for malondialdehyde (MDA) measurements.

2.5. Haematological parameters

2.5.1. Haemoglobin concentration

Haemoglobin was measured colorimetrically according to Drabkin and Austin's method [17] through its transformation to cyanmethemoglobin under the action of potassium ferricyanide and potassium cyanide. Haemoglobin concentration was expressed in g/l of blood.

2.5.2. Haematocrit content

Haematocrit measurements were carried out in capillary tubes centrifuged with HEMATOCRIT 20 Hettich for 15 min at 1000 rpm.

2.6. Assay of SOD activity

The superoxide dismutase (SOD) (E.C.1.15.1.1) level in erythrocytes was determined using the Ransod Kit supplied by the Randox Laboratories (Ardmore, Northern Ireland, UK).

Xanthine and xanthine oxidase were used to generate superoxide anion, which reacts with 2-(4-indophenyl)-3-(4-nitro-phenyl)-5-phenyl tetrazolium chloride (INT) to form a red formazan dye. Changes in the absorbance were determined at 505 nm during the first three minutes of the reaction. Enzyme activity in the sample was calculated from a standard curve. One unit of SOD is defined as the amount that inhibits the INT reaction by 50%. Specific activity was defined as Units/g haemoglobin.

2.7. Assay of GPx activity

Glutathione peroxidase (GPx) (E.C.1.11.1.9) level in erythrocytes was based on that of Paglia and Valentine [18]. After the addition of cumene hydroperoxide, the conversion of NADPH to $NADP^{+}$ by glutathione reductase and generated oxidized glutathione was continuously monitored spectrophotometrically at 340 nm for 2 min. GPx activity was expressed in units/g of haemoglobin.

2.8. Plasma total bilirubin assay

Total bilirubin concentration was measured colorimetrically according to Jendrassik and Grof [19]. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotised sulphanilic acid.

Changes in absorbance were read at 578 nm against their samples blank. Total bilirubin was expressed in mg/dl of plasma.

2.9. Lipid peroxidation in liver

The lipid peroxidation product in livers was determined by TBARS (thiobarbituric acid reactive substances) expressed as the extent of malondialdehyde

(MDA) production [20,21]. The samples were suspended in PBS pH 7.4, mixed with butylated hydroxytoluene (BHT)-TCA solution (1% w/v BHT dissolved in 20% TCA), and centrifuged at 1000 *g* for 10 min. The supernatant was then mixed with 0.5 N HCl and 120 mM TBA (2-thiobarbituric acid) in 26 mM Tris, and heated in a water bath at 80 °C for 15 min. After cooling, the absorbance of the resulting chromophore was determined at 532 nm using a BIORAD UV-visible spectrophotometer (Smart Spec 3000), and MDA production was determined by using an extinction coefficient of $1.56 \times 10^5\ M^{-1}\,cm^{-1}$, and expressed in nmol MDA/g of proteins.

2.10. Protein assay

The protein content of supernatants was spectrophotometrically estimated according to the method of Bradford [21] using bovine serum albumin as standard.

2.11. Statistical analysis

All results were expressed as mean ± standard deviation. Comparisons between the groups were performed by one-way ANOVA followed by Student *t*-test. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Body weight of rats

There was a steady decrease in body weight of rats of the two treated groups compared to the respective values of the control group ($p < 0.01$) (Table 1).

Statistical analysis of growth rates is shown in Fig. 1. For the two groups treated with different doses of lead acetate, we noted a significant decrease ($p < 0.01$) in growth rates with increasing the dose of lead. However, growth suppression in group [Pb 15] was considerably greater than that observed in group [Pb 5] ($p < 0.01$).

3.2. Haematological parameters

As is shown in Figs. 2 and 3, lead administration induces, in both treated groups [Pb 5] and [Pb 15], a marked decline in haematocrit and haemoglobin levels ($p < 0.01$) when compared to control.

3.3. Antioxidant enzymes

– A significant decrease in SOD activity was recorded in group [Pb 15] compared with control ($p < 0.01$), but the activity of this enzyme did not decrease significantly in group [Pb 5] (Fig. 4).
– Lead exposure caused a light increase in GPx activity in RBCs of rats treated with 15 mg Pb/kg b.w., which remains non significant compared to control. The group treated with 5 mg Pb/kg b.w. did not record changes in the activity of GPx compared to control (Fig. 5).

Table 1
Body weight of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight)

Control ($n = 16$)	Pb 5 ($n = 18$)	Pb 15 ($n = 18$)
Body weight (g):		
Initial weight (g): 174.94 ± 10.77	167.35 ± 4.65	181.25 ± 7.09
Final weight (g): 191.69 ± 10.44	166.53 ± 4.36[a]	155.38 ± 5.46[a/b]

Results are expressed as mean ± SD. $N = 16$ rats for each group.
[a] $p < 0.01$, compared to the corresponding value of control group.
[b] $p < 0.01$, compared to the corresponding value of group [Pb 5].

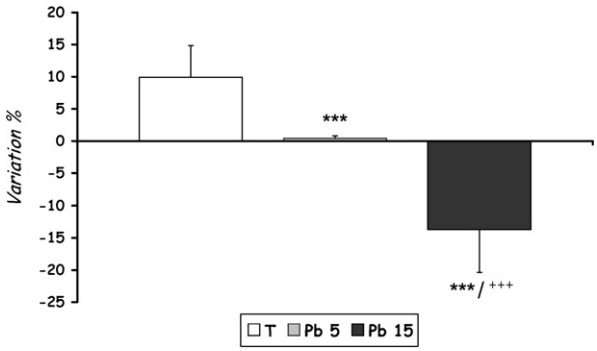

Fig. 1. Growth-rate evolution of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group. $^{+++}p < 0.01$ compared to the corresponding value of group [Pb 5].

3.4. Bilirubin levels in plasma

We noted a significant increase in the bilirubin level ($p < 0.05$) in the group of rats treated with 15 mg Pb/kg b.w. compared to control.

The bilirubin level from rats of the group treated with 5 mg Pb/kg b.w. increased with lead treatment, but did not reach a statistical significance (Fig. 6).

3.5. MDA levels

The lipid peroxide level from rats of group [Pb 15] increased with lead treatment, but the effect did not reach statistical significance. No change was noted in

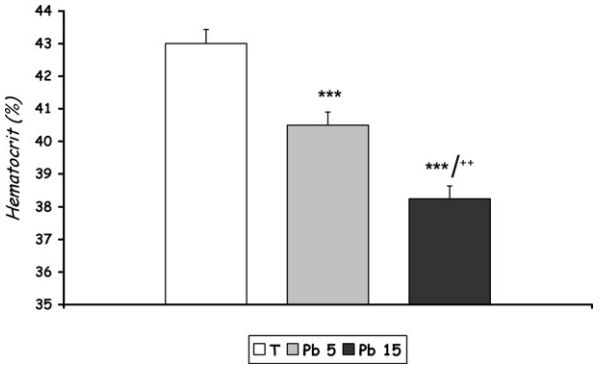

Fig. 2. Haematocrit level of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group. $^{++}p < 0.05$ compared to the corresponding value of group [Pb 5].

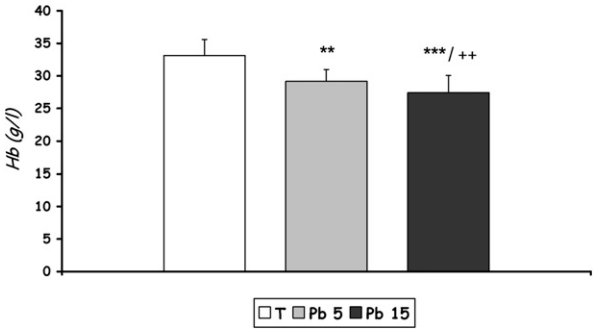

Fig. 3. Haemoglobin level of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group. $^{**}p < 0.05$ compared to the corresponding value of [T]. $^{***}p < 0.01$ compared to the corresponding value of [T]. $^{++}p < 0.05$ compared to the corresponding value of [Pb 5].

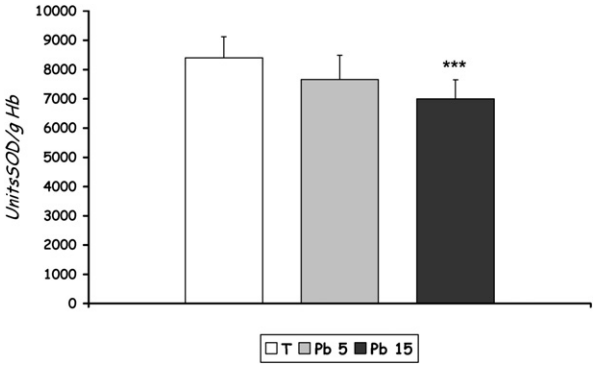

Fig. 4. SOD activity in erythrocytes of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group. $^{***}p < 0.01$ compared to the corresponding value of [T].

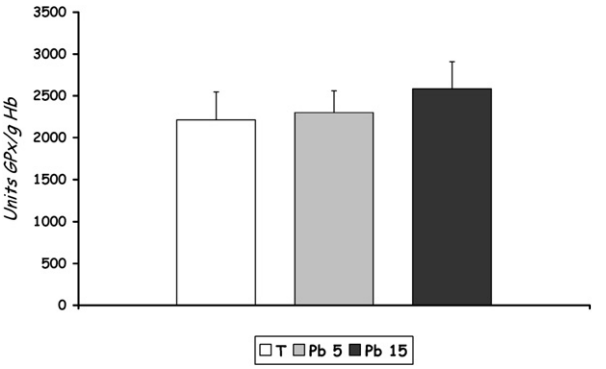

Fig. 5. GPx activity in erythrocytes of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group.

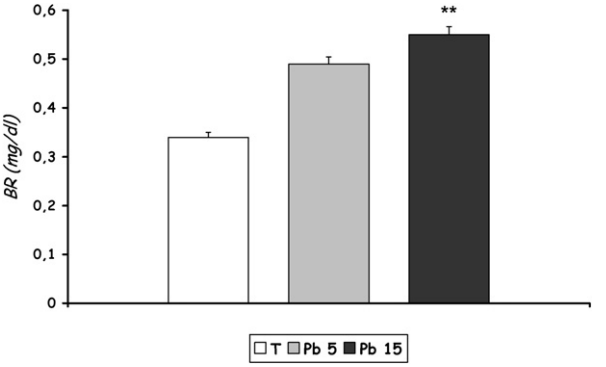

Fig. 6. Total bilirubin level in plasma of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group. $^{**}p < 0.05$, compared to the corresponding value of [T].

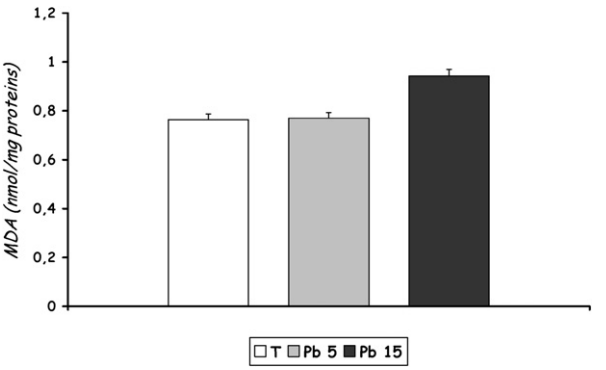

Fig. 7. Lipid peroxidation expressed as MDA in liver homogenate of controls and Pb-exposed rats treated for seven days by i.p. administration of lead acetate (5 mg and 15 mg Pb/kg body weight). Results are expressed as mean ± SD. $N = 16$ rats for each group.

the level of MDA in the liver of rats treated with 5 mg Pb/kg b.w. (Fig. 7).

4. Discussion

This study showed that adult male Wistar rats treated with different doses of lead acetate for seven days cause a significant decrease in body weight. This result is in agreement with several studies [22,23], which suggested that the reduced growth was due to reduced food consumption via lead effects on the satiety set-point.

The haematological system has been proposed as being an important target for lead-induced toxicity. Lead is known to interfere with haeme and haemoglobin synthesis and to affect erythrocyte morphology and survival [24].

Anaemia observed during lead poisoning in this study is thought to result from inhibition of haeme synthesis and a decreased life span of the erythrocytes [25]. This shortening of life span was observed by Teramaya [26] in experiments with rats and it is thought to be probably due to the direct toxic effect of lead upon the cell membrane [25]. Lead, furthermore, interferes with iron utilization for haeme formation in the mitochondria, and radio-iron studies showed that lead competes with iron for the incorporation into red blood cells. The precise mechanism of action of lead at stake in cellular metabolism remains largely unknown [24].

On the other hand, it has been shown that accumulation of δ-ALA induces oxidative stress in Chinese hamster ovary cells, where the induction was found to be reversed by incubating the cells with a well-known antioxidant, *N*-acetylcysteine [27].

Moreover, free radicals produced in the presence of heavy metals contribute to haemoglobin denaturing and precipitation, leading to anaemia [16].

These data suggest that anaemia observed in lead poisoning is caused by lead both directly and indirectly by the accumulation of δ-ALA [27].

However, much remains to be clarified about the exact nature of the mechanism by which lead induced haemolysis and shortening of the life span of circulating erythrocytes [28].

In the present study, lead is shown to alter the oxidative stress-related parameters both in erythrocytes and in liver tissue. SOD, which requires copper and zinc for its activity, was found to decrease in lead-administered rats. SOD is believed to play a major role in the first line of antioxidant defence by catalyzing the dismutation of superoxide anion radicals to form hydrogen peroxide H_2O_2 and molecular oxygen O_2 [29]. Sivaprasad et al. [16] showed that oxidative stress created by the metal favours increased production of superoxide anions. Under oxidative stress, SOD can behave in two different ways: initially and when the stress is moderated, the cells act by suppressing the SOD, but if the stress lasts a long time and favours increased production of ROS, the enzyme is exhausted and its concentration falls [30]. Effectively, in our case, decreased SOD activity observed at only one group [15 mg Pb/kg b.w.] could be explained by the massive production of superoxide anions, which override enzymatic activity and lead to the fall of its concentration in erythrocytes. Mylorie et al. [31] suggested that this could be due to a lead-induced copper deficiency. According to Pfafferoot et al. [32], the low activity of SOD could also be due to the inactivation of the enzyme by crosslinking or damage to DNA.

Selenium-dependent GPx catalyze the reduction of a variety of hydroperoxides (ROOH and H_2O_2) using GSH, thereby protecting mammalian cells against oxidative damage. Hunaiti and Soud [28] showed that the activities of glutathione S-transferase, glutathione peroxidase, and reductase, as well as the content of blood GSH, had decreased with an increasing lead concentration in the blood of some occupational workers exposed to this metal. In our study, we rather note an increase in GPx activity, which remains non-significant in the group [15 mg Pb/kg b.w.] compared to controls ($p > 0.05$). Just like SOD, GPx behaves in two different ways under oxidative stress, and this result can be explained by the short duration of the treatment.

Bilirubin is also regarded as a member of the antioxidant family, even though it is known to have toxic effects at high concentrations [33]. Bilirubin has been regarded for many years as cytotoxic, mainly because of its association with neonate jaundice and its possibility of provoking irreversible brain damage at high concentrations [34]. It is only since the early 1990s that a physiological role for bilirubin as a potent antioxidant has emerged. Stocker et al. [35] noted that bilirubin possesses strong antioxidant potential against peroxyl radicals. At physiologic oxygen tension, bilirubin surpassed α-tocopherol as the most potent protector against lipid peroxidation. In plasma, it can act synergically with vitamin E to protect lipid membranes from the peroxidation initiated within the lipid phase [35,36]. The study carried out by Miréles et al. [37] showed that erythrocytes subjected to oxidative stress and incubated with high levels of bilirubin (45–60 mg/dl) with physiological levels of BSA (3 g/dl) induced: (1) a significant concentration-dependent decrease in the fluorescence decay of Cis-PnA, a polyunsaturated fatty acid that can intercalate in cell membrane, with lipid peroxidation; (2) a 50% decrease in G6PD activity and in

Na^+/K^+ ATPase activity; (3) an increase in protein oxidation. These data suggest that bilirubin, at high levels (>30 mg/dl), may exacerbate oxidative stress, although the mechanism by which this occurs is not yet clear.

Bilirubin could be also an important cytoprotector for tissues that are less equipped for antioxidant defence, like myocardium and nervous tissues [33]. Doré et al. [38] showed that increase bilirubin formation due to activation of HO-2 (constitutive isoform of HO) protects against hydrogen peroxide-induced neurotoxicity. It has been also demonstrated that intracellular bilirubin concentrations can be locally and temporarily increased by induction of HO-1 (inducible isoform of HO) or rapid activation of HO-2, so as to resist short- and long-lasting oxidative stress [34]. It has been proposed that the specific induction of HO-1 by various forms of oxidative stress was part of the defensive mechanism mounted by cells against stress injury to decrease the levels of potential prooxidants and to increase the concentrations of active bile pigments that can act as antioxidants [39–41]. HO-1 up-regulation is followed by increased bilirubin production, altogether determining the adaptive response of cells to oxidative stress [39].

The study undertaken by Noricga et al. [42] showed that administration of bilirubin to rats reduces δ-ALA-induced lipid peroxidation, restores GSH content and the activities of the antioxidant enzymatic system to normal levels, providing evidence that bilirubin can act as an efficient antioxidant and free-radical scavenger.

Based on the above information, we adhere to the idea that bilirubin can act in vivo as efficient scavenger of ROS and that bilirubin plays a key physiological role in cytoprotection against oxidant-mediated damage. However, it is important to mention that there is a threshold of cytoprotective effects of bilirubin, as shown in the study undertaken by Miréles et al. [37].

The oxidative stress has also been implicated to contribute to lead-associated tissue injury in the liver, kidney, brain and other organs [13]. Our study showed an increase in MDA content in liver of rats treated with the highest dose of lead [Pb 15], suggesting an increase in lipid peroxidation in hepatic cells. This result is in agreement with the study undertaken by Patra et al. [13], who recorded an increase in MDA content in the liver and the brain of rats subjected to subchronic exposure to lead for a period of four weeks. Pagliara et al. [43] showed that lead-induced liver hyperplasia was followed by apoptosis mediated by oxidative stress in Kupffer cells. The group treated with 5 mg Pb/kg b.w [Pb 5] recorded no changes in MDA content in liver compared to controls, suggesting that this may be due to the short duration of the treatment.

References

[1] M. Falcy, A. Hesbert, D. Jargot, J.-C. Protois, M. Reynier, M. Schneider, P. Serre, Plomb et ses composes minéraux : tiré à part des cahiers de notes documentaires : Hygiène et sécurité du travail n° 170 ; FT n° 59, 1998.

[2] M.G. Shalan, M.S. Mostafa, M.M. Hassouna, S.E. Hassab El-Nabi, A. El-Rafaie, Amelioration of lead toxicity on rat liver with vitamin C and silymarin supplements, Toxicology 206 (2005) 1–15.

[3] G. Yücebilgiç, R. Bilgin, L. Tamer, S. Tükel, Effects of lead on Na^+–K^+ ATPase and Ca^{2+} ATPase activities and lipid peroxidation in blood of workers, Int. J. Toxicol. 22 (2003) 95–97.

[4] E.G. Moreira, G.J.M. Rosa, S.B.M. Barros, V.S. Vassilieff, I. Vassilieff, Antioxidant defense in rat brain regions after developmental lead exposure, Toxicology 169 (2001) 145–151.

[5] K. Soltaninejad, A. Kebriaeezadeh, B. Minaiee, S.N. Ostad, R. Hosseini, E. Azizi, M. Abdollahi, Biochemical and ultrastructural evidences for toxicity of lead through free radicals in rat brain, Hum. Exp. Toxicol. 22 (2003) 417–423.

[6] E.G. Moreira, I. Vassilieff, V.S. Vassilieff, Developmental lead exposure: behavioral alterations in the short and long term, Neurotox. Teratol. 23 (2001) 489–495.

[7] M. De Marco, R. Halpern, H.M.T. Barros, Early behavioral effects of lead perinatal exposure in rat pups, Toxicology 211 (2005) 49–58.

[8] S. Razani-Boroujerdi, B. Edwards, M.L. Sopori, Lead stimulates lymphocyte proliferation through enhanced T cell–B cell interaction, Pharmacol. Exp. Ther. 288 (1999) 714–719.

[9] N. Ercal, R. Neal, P. Treeratphan, P.M. Lutz, T.C. Hammond, P.A. Dennery, D.R. Spitz, A role for oxidative stress in suppressing serum immunoglobulin levels in lead-exposed Fisher 344 rats, Arch. Environ. Contam. Toxicol. 39 (2000) 251–256.

[10] T.L. Bunn, P.J. Parsons, E. Kao, R.R. Dietert, Gender-based profiles of developmental immunotoxicity to lead in the rat: assessment in juveniles and adults, J. Toxicol. Environ. Health, Part A 64 (2001) 223–240.

[11] T.L. Bunn, P.J. Parsons, E. Kao, R.R. Dietert, Exposure to lead during critical windows of embryonic development: differential immunotoxic outcome based on stage of exposure and gender, Toxicol. Sci. 64 (2001) 57–66.

[12] M. Loghman-Adham, Renal effects of environmental and occupational lead exposure, Environ. Health Perspect.105 (9) (1997) 928–938.

[13] R.C. Patra, D. Swarup, S. Dwivedi, Antioxidant effects of α-tocopherol, ascorbic acid and L-methionine on lead-induced oxidative stress to the liver, kidney and brain in rats, Toxicology 162 (2001) 81–88.

[14] I. Vargas, C. Castillo, F. Posadas, B. Escalante, Acute lead exposure induces renal haeme oxygenase-1 and decreases urinary Na^+ excretion, Hum. Exp. Toxicol. 22 (2003) 237–244.

[15] H.M. Mousa, A.A. Al-Qarawi, B.H. Ali, H.A. Abdel Rahman, S.A. ElMougy, Effect of lead exposure on the erythrocytic antioxidant levels in goats, J. Vet. Med. A 49 (2002) 531–534.

[16] R. Sivaprasad, M. Nagaraj, P. Varalakshmi, Combined efficacies of lipoic acid and meso-2,3-dimercaptosuccinic acid on lead-induced erythrocyte membrane lipid peroxidation and antioxidant status in rats, Hum. Exp. Toxicol. 22 (2003) 183–192.

[17] D.L. Drabkin, J.H. Austin, Spectrophotometric studies. I. Spectrophotometric constants for common hemoglobin derivatives in human, dog and rabbit blood, J. Biol. Chem. 98 (2) (1932) 719–733.

[18] D.E. Paglia, W.N. Valentine, Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase, J. Lab. Clin. Med. 70 (1967) 158–169.
[19] L. Jendrassik, P. Grof, Biochem. Z. 297 (1938) 81.
[20] J.A. Buege, S.D. Aust, Microsomal lipid peroxidation, Meth. Enzymol. Biomembranes, Part C 52 (1978) 302–310.
[21] M.M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye-binding, Anal. Biochem. 72 (1976) 248–254.
[22] P.B. Hammond, D.J. Minnema, R. Shukla, Lead lowers the set point for food consumption and growth in weanling rats, Toxicol. Appl. Pharmacol. 106 (1990) 80–87.
[23] D.J. Minnema, P.B. Hammond, Effect of lead exposure on patterns of food intake in weanling rats, Neurotoxicol. Teratol. 16 (1994) 623–629.
[24] B. Jacob, B. Ritz, J. Heinrich, B. Hoelscher, H.E. Wichmann, The effect of low-level blood lead on hematologic parameters in children, Environ. Res. 82 (2000) 150–159.
[25] C.D. Klassen, Heavy Metals and Heavy Metal Antagonists, Goodman and Gilman's Pharmacological basis of therapeutics, USA, 1996.
[26] K. Terayama, Effects of lead on electrophoretic mobility membrane sialic acid, deformability and survival of rat erythrocytes, Ind. Health 31 (1993) 113–126.
[27] R. Neal, P. Yang, J. Fiechtl, D. Yildiz, H. Gurer, N. Ercal, Prooxidant effects of δ-aminolevulinic acid (δ-ALA) on Chinese hamster ovary (CHO) cells, Toxicol. Lett. 91 (1997) 169–178.
[28] A.A. Hunaiti, M. Soud, Effect of lead concentration on the level of glutathione, glutathione S-transferase, reductase and peroxidase in human blood, Sci. Total Environ. 248 (2000) 45–50.
[29] J.-M. Matés, Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology, Toxicology 153 (2000) 83–104.
[30] S.A. Levine, P.M. Kidd, Antioxidant Adaptation: Its Role in Free Radical Pathology, Biocurrents division, Allergy Research Group, San Leonardo, CA, USA, 1996.
[31] A.A. Mylorie, H. Collins, C. Umbles, J. Kyle, Erythrocyte superoxide dismutase activity and other parameters of copper status in rats ingesting lead acetate, Toxicol. Appl. Pharmacol. 82 (1986) 512–520.
[32] C. Pfafferoot, H.J. Meiselman, P. Hochstein, The effect of MDA on erythrocyte deformability, Blood 159 (1982) 12–15.
[33] K. Hagymàsi, I. Kocsis, G. Lengyel, P. Sipos, J. Fehér, A. Blàzovics, Further evidence of altered redox status of hyperbilirubinemic patients: role of bilirubin in Gilbert syndrome, Acta Biol. Szeged. 47 (1–4) (2003) 131–134.
[34] M.L. Tomaro, A.M.C. Batlle, Bilirubin: its role in cytoprotection against oxidative stress, Int. J. Biochem. Cell Biol. 34 (2002) 216–220.
[35] R. Stocker, A.F. McDonagh, A.N. Glazer, B.N. Ames, Antioxidant activities of bile pigments: biliverdin and bilirubin, Methods Enzymol. 186 (1990) 301–309.
[36] G. Marilena, New physiological importance of two classic residual products: carbon monoxide and bilirubin, Biochem. Mol. Med. 61 (1997) 136–142.
[37] L.C. Miréles, M.A. Lum, P.A. Dennery, Antioxidant and cytotoxic effects of bilirubin on neonatal erythrocytes, Pediatr. Res. 45 (3) (1999) 355–362.
[38] S. Doré, M. Takahashi, C.D. Ferris, L.D. Hester, D. Guastella, S.H. Snyder, Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury, Proc. Natl Acad. Sci. USA 96 (1999) 2445–2450.
[39] J.E. Clark, R. Foresti, C.J. Green, R. Motterlini, Dynamics of heme oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress, Biochem. J. 348 (2000) 615–619.
[40] J.O. Ossola, M.L. Tomaro, Heme oxygenase induction by UVA radiation: a response to oxidative stress in rat liver, Int. J. Biochem. Cell Biol. 30 (1998) 285–292.
[41] J.O. Ossola, G. Kristoff, M.L. Tomaro, Heme oxygenase induction by menadione bisulfite adduct-generated oxidative stress in rat liver, Comp. Biochem. Phys. 127 (2000) 91–99.
[42] G.O. Noricga, M.L. Tomaro, A.M.C. Del Batlle, Bilirubin is highly effective in preventing in vivo δ-aminolevulinic acid-induced oxidative cell damage, Biochem. Biophys. Acta 1638 (2003) 173–178.
[43] P. Pagliara, A. Chionna, E.C. Carla, S. Caforiao, L. Dini, Lead nitrate and gadolinium chloride administration modify hepatocyte cell surfaces, Cell. Tissue Res. 312 (1) (2003) 41–48.