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Acetylcholinesterase activity and antioxidant capacity of zebrafish brain is altered by heavy metal exposure

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ABSTRACT

Pollution is a world problem with immeasurable consequences. Heavy metal compounds are frequently found as components of anthropogenic pollution. Here we evaluated the effects of the treatment with cadmium acetate, lead acetate, mercury chloride, and zinc chloride in acetylcholinesterase activity and gene expression pattern, as well as the effects of these treatments in antioxidant competence in the brain of an aquatic and well-established organism for toxicological analysis, zebrafish (*Danio rerio*, Cyprinidae). Mercury chloride and lead acetate promoted a significant decrease in acetylcholinesterase activity whereas they did not alter the gene expression pattern. In addition, the antioxidant competence was decreased after exposure to mercury chloride. The data presented here allowed us to hypothesize a signal transmission impairment, through alterations in cholinergic transmission, and also in the antioxidant competence of zebrafish brain tissue as some of the several effects elicited by these pollutants.

1. Introduction

The pollutant emissions are increasing worldwide and bringing huge health and environmental problems, particularly in aquatic milieu. The pollution is frequently composed of a mixture of heavy metals, including the three most expressive: cadmium (Cd^{2+}), lead (Pb^{2+}), and mercury (Hg^{2+}); and the second most important trace metal in the body, zinc (Zn^{2+}) (Coleman, 1992; Monnet-Tschudi et al., 2006; Vallee and Falchuk, 1981).

Cadmium is incorrectly disposed in the environment as a result of anthropogenic activities, as mining exploration, as a constituent of color pigments and re-chargeable nickel–cadmium batteries (Jarup, 2003). The cadmium effects on health are countless, and vary from kidney damage, bone effects and also many types of cancer, being classified by the IARC (International Agency for Research on Cancer) as carcinogenic to humans (Group 1) (International Agency for Research on Cancer, 1993).

Another metal which the general population is exposed is the lead, being the mines, industries of glass, food bowls, and wine considered important sources of its emission. In relation to its health effects, they are well established, including its action in various neurotransmitter systems (Cory-Slechta, 1995). There is evidence of the carcinogenic potential of lead compounds, which ranks it as a compound probably carcinogenic to humans (Group 2A) (International Agency for Research on Cancer, 2006).

Mercury is used since the pre-history as a pigment, passing through a cure for syphilis in the 1800s and arriving in the present as diuretics, dental amalgam fillings, thermometers and several uses that despise its toxicity. A high intake of mercury by humans comes together with an elevated consumption of fish. When in contact with animals, mercury can cause lung damage, neurological and psychological disturbances and also, but in a lesser extent, development of cancer, being classified by the IARC as possibly carcinogenic to humans (Group 2B) (International Agency for Research on Cancer, 1993).

Although the transition metal zinc plays a neuromodulatory role in the CNS, studies reported that the increase of cytosolic Zn^{2+} concentrations, in large part via Ca^{2+} channels, triggers several downstream mechanisms which culminate in neuronal cell death (Sheline et al., 2002; Cai et al., 2006). In contrast to the very low levels of free intracellular Zn^{2+}, toxic exposures to this metal

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strongly rises $[Zn^{2+}]_i$ to 400–600 nM (Sensi et al., 1999). At this concentration, Zn^{2+} can decrease the activity of key glycolytic enzymes, such as GAPDH (Krotkiewska and Banas, 1992) and phosphofructokinase in purified forms (Ikeda et al., 1980). Studies also reported that Zn^{2+} induces mitochondrial dysfunction by inhibiting the activities of enzymes involved in electron transport, leading to an increase in neuronal reactive oxygen species and, consequently, oxidative stress (Kim et al., 1999; Manev et al., 1997; Noh et al., 1999). Furthermore, a recent data showed that Zn^{2+}-mediated neurotoxicity is dependent of intracellular NAD^+ levels and the sirtuin activity, indicating that alterations in energy metabolic pathways could be regulated at transcriptional level (Cai et al., 2006).

The cholinergic system, with acetylcholine (ACh) as the neurotransmitter, is involved in cognitive processes, through the activation of metabotropic muscarinic and ionotropic nicotinic cholinergic receptors. The reaction responsible for the maintenance of levels of ACh is catalyzed by two cholinesterases (ChE): acetylcholinesterase (AChE) (E.C. 3.1.1.7) and butirylcholinesterase (BuChE) (E.C. 3.1.1.8). zebrafish (*Danio rerio*) is an emergent vertebrate model for studying several biological events, such as neurochemical alterations promoted by heavy metal toxicity (Senger et al., 2006). This teleost possesses only the gene for AChE, which is responsible for the whole ACh degradation, being the BuChE absent. The AChE gene has already been identified, cloned and functionally detected in the zebrafish brain (Bertrand et al., 2001). Acetylcholinesterase is an important biomarker for several environmental contaminants in zebrafish (Rico et al., 2006; Senger et al., 2006). In addition, it is also known the important role of this enzyme in diseases with an increasing incidence in the elderly population, such as Alzheimer disease (Han et al., 2007; Kim et al., 2008).

There is evidence of the interaction between heavy metals as mercury and lead and the etiology of neurodegenerative diseases, since many of these metals can cross the blood brain barrier and accumulate in the brain, promoting the generation of oxidative stress and alterations in the metabolism of some proteins associated with the development of neurodegenerative diseases, such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis (reviewed in Monnet-Tschudi et al., 2006).

Therefore, considering the increase of the pollution, incorrect disposal of heavy metals as industrial effluents, the immersion of organisms in this impaired environment and the possible consequences of this exposure, the aim of the present study was to investigate the effects of four heavy metal compounds in AChE activity and its gene expression pattern. Furthermore, we have analyzed the effects of some of these heavy metal treatments in parameters related to antioxidant defenses and lipid peroxidation in the brain of an aquatic and well-established organism for toxicological analysis, zebrafish.

2. Materials and methods

2.1. Chemicals

Zinc chloride ($ZnCl_2$, CAS number 7648-85-7) was purchased from Nuclear (Brazil) and cadmium acetate [$Cd(CH_3COO)_2$, CAS number 543-90-8], mercury chloride ($HgCl_2$, CAS number 7487-94-7) and lead acetate [$Pb(CH_3COO)_2$, CAS number 301-04-2] were purchased from QM (Brazil). Trizma Base, EDTA, EGTA, sodium citrate, Coomassie Blue G, bovine serum albumin, acetylthiocholine, 5,5′-dithiobis-2-nitrobenzoic acid (DNTB), HEPES, BHT (99%), 2,2′-azobis(2-methylpropionamidine) dihydrochloride (ABAP) and 1,1,3,3-tetramethoxypropane were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA). KCl and SDS (90%) were purchased from Labsynth (Brazil). Tetramethoxypropane (TMP) and 2′,7′-dichlorodihydrofluorescein diacetate were purchased from Acros Organics (Morris Plains, NJ, USA) and Molecular Probes Inc. (Eugene, OR, USA) respectively. $MgCl_2$ and Acetic acid 99.7% were purchased from Isofar and Vetec (Brazil) respectively. TRIzol, GelRed and Taq DNA polymerase were purchased from Invitrogen Corp. (Carlsbad, CA, USA). All other reagents used were of analytical grade.

2.2. Animals

Adult and healthy zebrafish of both sexes were obtained from specialized supplier (Redfish Agroloja, RS, Brazil) and kept in standard conditions (tap water treated with Tetra's AquaSafe® to neutralize chlorine, chloramines, and heavy metals present in tap water that can be harmful to fish continuously aerated water, 25 ± 2 °C, under a 14–10 h light/dark cycle photoperiod) in 50 L housing tank for at least 2 weeks to acclimate before the experiments. The animals were maintained healthy and free of any signs of disease and fed three times a day with TetraMin Tropical Flake Fish. The Ethics Committee of Pontifical Catholic University of the Rio Grande do Sul (PUCRS) approved the protocol under the number 0703854-CEUA.

2.3. In vivo treatments

The animals were separated in groups of 12 animals and housed in 3 L tanks with the respective treatment. The animals were transferred to the test aquarium filled with reverse osmosis water to avoid the presence of any metal in the tap water and also to avoid the use of the metal chelant Tetra's AquaSafe®. The animals were kept in continuously aerated water, 25 ± 2 °C, under a 14–10 h light/dark cycle photoperiod, fed three times a day with TetraMin Tropical Flake fish. The treatments were as follows: mercury chloride or lead acetate at a final concentration of 20 μg/L, which has been chosen in previous studies from our laboratory (Senger et al., 2006) based on reportings about the aquatic environment (Berzas Nevado et al., 2003, Jha et al., 2003; Ram et al., 2003). The concentrations of zinc chloride (5 mg/L) or cadmium acetate (0.1 mg/L) were chosen according the National Council for the Environment (Brazil) (Resolution 357/2005), that allow the disposal of these heavy metal concentrations as industrial effluents in the environment. Control group was kept in the same conditions as the other groups, but without the addition of any metals in the reverse osmosis water. The animals were maintained in the test aquarium for 24, 96 h and 30 days for acute, subchronic and chronic exposures, respectively. The water of the tanks was changed every two days to guarantee the concentration desired of the heavy metal treatment.

2.4. In vitro assays

Mercury chloride and lead acetate (1–250 μM) were added to the reaction medium before preincubation with the enzyme and maintained throughout the enzyme assays described in the Section 2.3. Zinc chloride and lead acetate were not analyzed *in vitro* because they were already analyzed by Senger et al. (2006). Each metal was added to the reaction medium at 75 nM, 150 nM, 500 nM, 1000 nM, 25 μM and 250 μM; these concentrations were chosen in order to analyze the effect of a wide spectrum of concentrations, ranging from the concentration of heavy metal found in the test tank water, which was already shown in the environment (Berzas Nevado et al., 2003; Jha et al., 2003) to higher concentrations chosen based on previous studies showing the effect of these metals on other enzymes involved in nucleoside/nucleotide metabolism (Aikawa et al., 1980; Senger et al., 2006). Control group was performed with no addition of metal in the enzyme assay.

2.5. Determination of acetylcholinesterase activity

Zebrafish were cryoanesthetized and immediately euthanized by decapitation, and whole brains were homogenized on ice in 60 vol. (v/w) of 0.05 M Tris–HCl, pH 8.0, using a Teflon-glass homogenizer. For the AChE activity analysis, a pool of 3 brains was considered as a sample. To ensure the consistence of our results, the tests were performed at least in quadruplicates, using a total of 12 animals per group. AChE activity was determined according to the method of Ellman et al. (1961) with minor modifications. Briefly, the activity on the homogenate was measured by determining the rate of hydrolysis of acetylthiocholine iodide (ACSCh, 0.88 mM) in a final volume of 300 μL, with 33 μL of 100 mM phosphate buffer, pH 7.5, and 2 mM DTNB. In this solution, 5 μg of protein of each sample were added and pre-incubated at 25 °C for 10 min. The reaction was started with the addition of the substrate acetylthiocholine, and as soon as the substrate was added the hydrolysis and the formation of the dianion of DTNB were analyzed in 412 nm for 2.5 min (in intervals of 30 s) using a microplate reader. AChE activity was expressed as micromole of thiocholine (SCh) released per hour per milligram of protein.

2.6. Semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated from a pool of 5 zebrafish brains constituting each group using TRIzol reagent according manufacturer instructions. The RNA was quantified spectrophotometrically and all samples were adjusted to 160 ng/μL. cDNA species were synthesized with SuperScript™ First-Strand (Synthesis System for RT-PCR, Invitrogen), in accordance with the suppliers. PCR reactions were performed as previously described in Rico et al. (2006). The AChE and β-actin gene amplifications were conducted in a final volume of 20 μL, with 0.1 μM of each primer, 0.2 μM of dNTP, 2 mM of $MgCl_2$ and 0.5 U of Taq DNA polymerase. The PCR reaction were performed following the conditions below: 2 min at 94 °C, 1 min at 60 °C or 58.5 °C for AChE and β-actin gene respectively, 1 min at 72 °C for 35 cycles. A post-extension period of 10 min at 72 °C was performed. A negative control for the PCR product was conducted. The PCR products were analyzed in a 1% agarose gel in an UV transilluminator using GelRed 10×. The band intensities were analyzed in a semi-quantitative way using Image J software. The primers used for the gene amplification were CCAAAAGAATAGAGATGCCATGGACG (forward) and TGTGATGTTAAGCAGA CGAGGCAGG (reverse) for AChE (Rico et al., 2006) and GTCCCTGTACGCCTCTGGTCG (forward) and GCCGGACTCATCGTACTCCTG (reverse) for β-actin (Chen et al., 2004). To ensure the consistence of our results, the tests were performed at least in quadruplicates, using a total of 20 animals per group.

2.7. Antioxidant capacity against peroxyl radicals

Total antioxidant competence against peroxyl radicals was evaluated through reactive oxygen species (ROS) determination in tissues samples treated or not with a peroxyl radical generator (Amado et al., 2009). Briefly, on a white 96-well microplate, 10 μL of brain homogenates were disposed into the wells, six wells per sample. The reaction buffer (127.5 μL) containing 30 mM HEPES (pH 7.2), 200 mM KCl and 1 mM $MgCl_2$ were added to the wells containing the samples. In three of the six wells of each sample, 7.5 μL of 2,2′-azobis 2 methylpropionamidine dihydrochloride (ABAP; 4 mM) were added. In the other three wells the same volume of ultrapure water was pipetted. After this, the microplate was put into a fluorescence microplate reader (Victor 2, Perkin Elmer), programmed to keep temperature at 35 °C. At this temperature, peroxyl radicals are produced by thermal decomposition of ABAP (Winston et al., 1998). Immediately before microplate reading, it was added in each well 10 μL of the fluorescent probe 2′,7′-dichlorofluorescein diacetate (H_2DCF-DA) in a final concentration of 40 μM, according to the methodology employed by Ferreira-Cravo et al. (2007). H_2DCF-DA is deacetylated and the product H_2DCF is oxidized by ROS to the fluorescent compound DCF, which is detected at wavelengths of 488 and 525 nm, for excitation and emission, respectively. Fluorescence readings (fluorescence units or FU) were performed every 5 min during 30 min. Total fluorescence production was calculated by integrating the fluorescence units (FU) along the time of the measurement, after adjusting FU data to a second order polynomial function. The results were expressed as area difference of FU × min in the same sample with and without ABAP addition and standardized to the ROS area without ABAP (background area). The relative difference between ROS area with and without ABAP was considered a measure of antioxidant capacity, with high area difference meaning low antioxidant capacity, since high fluorescence levels were obtaining after adding ABAP, meaning low competence to neutralize peroxyl radicals (Amado et al., 2009).

2.8. Measurement of lipid peroxidation

Lipid peroxidation was measured through determination of thiobarbituric acid reactive substances (TBARS), following the methodology of Oakes and Van der Kraak (2003). Brain homogenates (10 μL) were added to a reaction mixture made with 150 μL of 20% acetic acid, 150 μL of thiobarbituric acid (0.8%), 50 μL of Milli Q water and 20 μL of sodium dodecyl sulfate (SDS, 8.1%). Samples were heated at 95 °C during 30 min and after cooling by 10 min, 100 μL of Milli Q water and 500 μL of n-butanol was added. After centrifugation (3000 × *g* during 10 min at 15 °C), the organic phase (150 μL) was placed in a microplate reader and the fluorescence registered after excitation at 515 nm and emission of 553 nm. The concentration of TBARS (nmol/mg of wet tissue) was calculated employing tetramethoxypropane (TMP) as standard.

2.9. Protein determination

Protein was measured with two different methods in accordance with the sensitivity required for the analysis. For the AChE activity analysis, protein was measured by the Coomassie Blue method considering bovine serum albumin as standard (Bradford, 1976). For the antioxidant procedures, total protein content was measured by the Biuret method using a commercial Total Protein Kit (Doles Inc. Brazil) in accordance with the supplier instructions.

2.10. Statistical analysis

Data for the enzymatic and antioxidant analyses were expressed as means ± S.E.M. and analyzed by one-way analysis of variance (ANOVA), following the post hoc test of Tukey, considering $P < 0.05$ as significant. Before ANOVA, its assumptions (normality and variances homogeneity) were checked.

3. Results

3.1. Acetylcholinesterase enzymatic activity and gene expression

As shown in Fig. 1, the effect of zinc chloride, cadmium acetate, mercury chloride and lead acetate were investigated on AChE activity in zebrafish brain. Despite a slight augment of the activity after 24 h exposure, zinc chloride did not affect the enzyme activity ($P > 0.05$) (Fig. 1A). Similarly to zinc chloride, cadmium acetate did

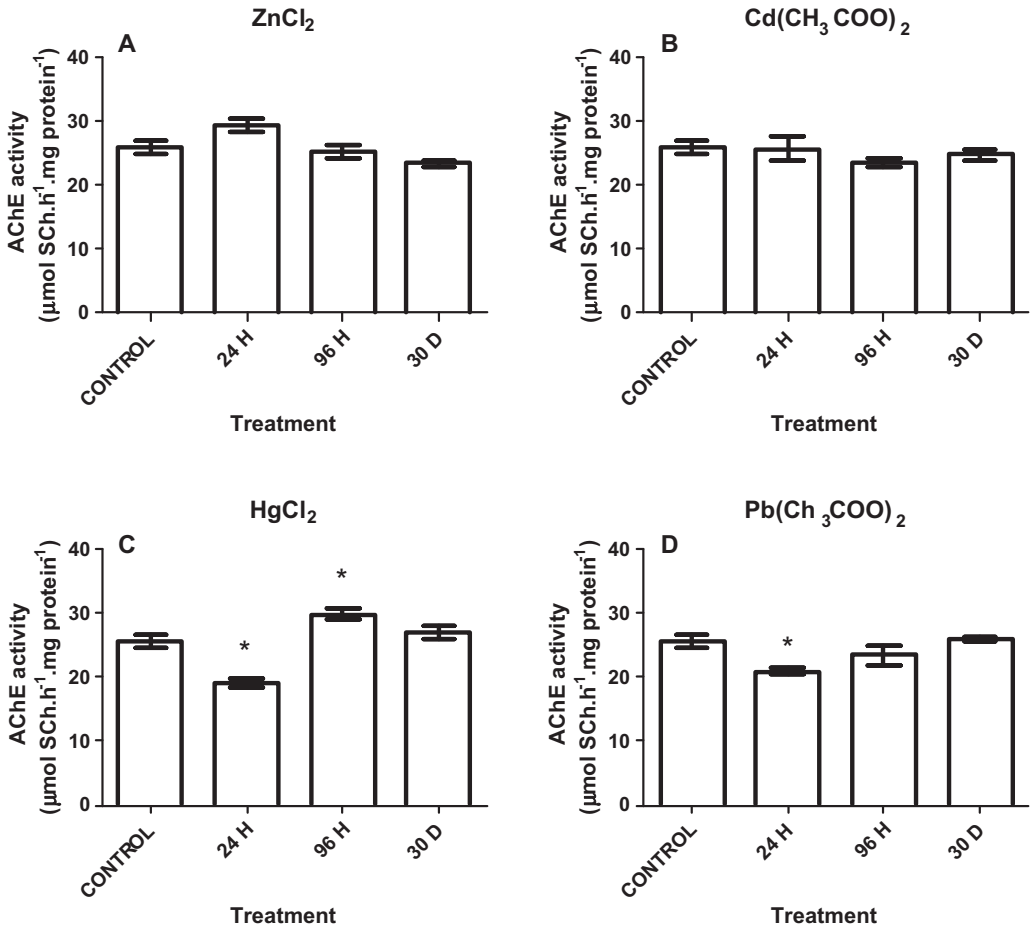

Fig. 1. *In vivo* effect of different treatments (24 h, 96 h, and 30 days) with $ZnCl_2$ (A), $Cd(CH_3COO)_2$ (B), $HgCl_2$ (C), and $Pb(CH_3COO)_2$ (D) in AChE activity in zebrafish brain. Data represent means ± S.E.M of at least three independent experiments. *Significantly different from control group (ANOVA followed by Tukey's test as post hoc, $P < 0.05$).

not alter the enzyme activity in the concentration tested (Fig. 1B; $P > 0.05$). The two compounds that altered AChE activity were lead acetate and mercury chloride. In relation of the effects of the treatment with mercury chloride, we observed a reduction (25%, $P < 0.05$) of the AChE activity in the 24 h-treated animals, following an elevation (16%, $P < 0.05$) of the AChE activity in the 96 h-treated animals in relation to control. This alteration is stabilized after 30 days of treatment (Fig. 1C). Lead acetate induced a significant reduction (18%, $P < 0.05$) of the AChE activity in the 24 h-treated animals, following a progressive restoration of the normal activity after 96 h and 30 d of treatment (Fig. 1D). We also evaluated the gene expression of this enzyme after 24 h treatment with lead acetate and mercury chloride and after 96 h of mercury chloride treatment. As shown by Fig. 2, there were no significant changes ($P > 0.05$) in the AChE mRNA transcript levels after lead acetate and mercury chloride exposure.

3.2. *In vitro* effects of heavy metal treatments on Acetylcholinesterase activity

According to Fig. 3, neither mercury chloride nor lead acetate interfered directly in the enzyme at the concentrations reached with the *in vivo* treatment. Only mercury chloride at higher concentrations tested *in vitro* (25 and 250 μM) promoted significant decrease on AChE activity (77% and 55%, respectively) (Fig. 3A). Although we have observed a significant effect on AChE activity, it is important to emphasize that these concentrations are much higher than the mercury levels detected in the aquatic environment.

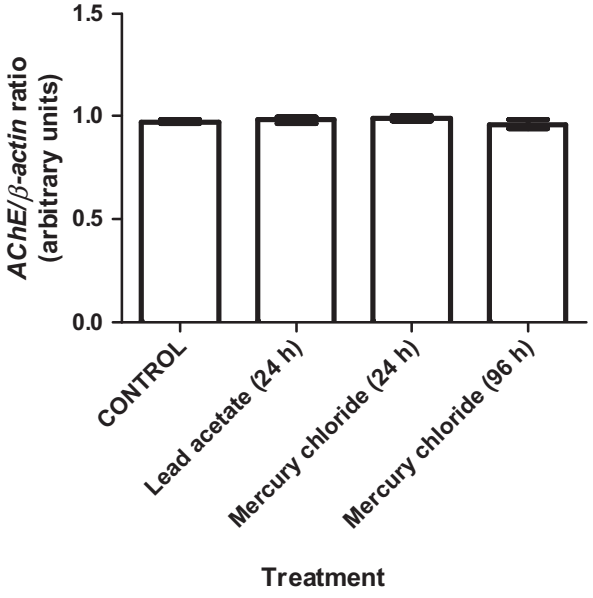

Fig. 2. Relative gene expression pattern of AChE after $Pb(CH_3COO)_2$ (24 h) and $HgCl_2$ (24 and 96 h) treatments in zebrafish brain. Data represent means ± S.E.M of the AChE vs. β-actin mRNA ratio of at least four independent experiments. The results were analyzed by densitometry using Image J 1.37 for Windows.

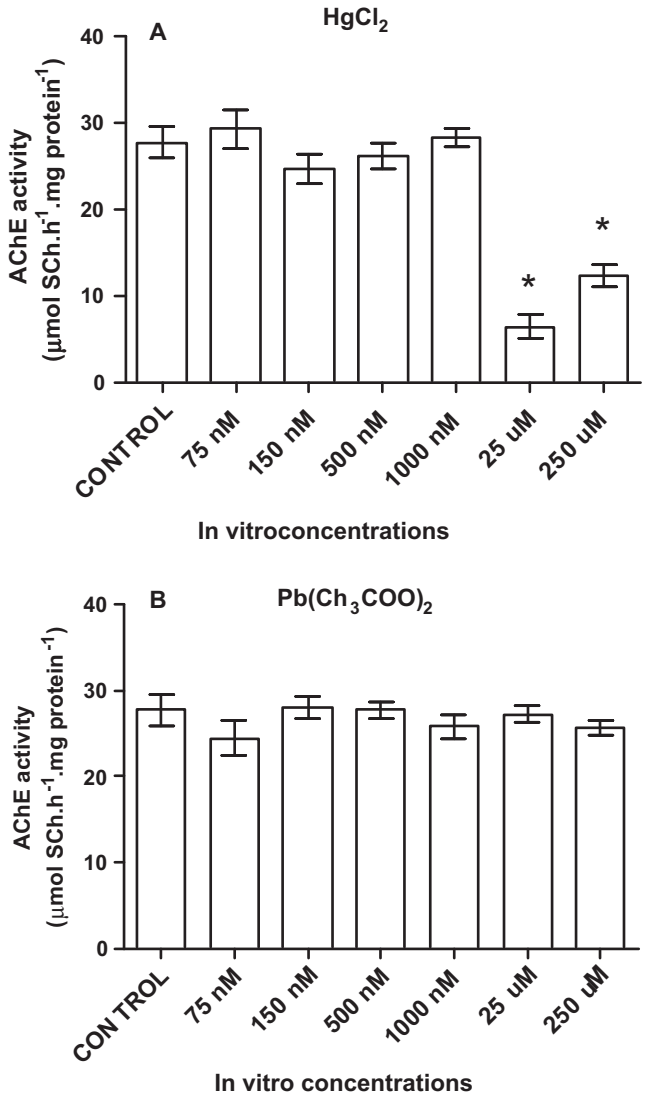

Fig. 3. *In vitro* effect of varying concentrations of $HgCl_2$ (A) and $Pb(CH_3COO)_2$ (B) in AChE activity in zebrafish brain. Data represent means ± S.E.M of four independent experiments. *Significantly different from control group (ANOVA followed by Tukey's test as post hoc, $P < 0.05$).

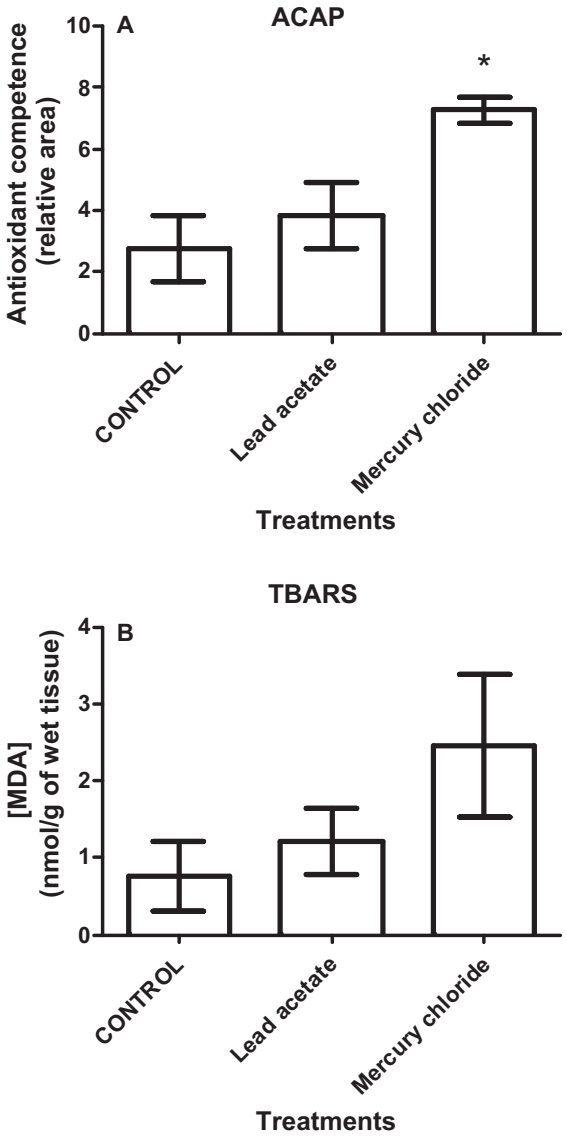

Fig. 4. Total antioxidant capacity against peroxyl radicals (A) and lipid peroxides content expressed as thiobarbituric acid reactive substances (TBARS) (B) in zebrafish brain after 24 h treatments with $Pb(CH_3COO)_2$ and $HgCl_2$. Data represent means ± S.E.M of at least three independent experiments. *Significantly different from control group (ANOVA followed by Tukey's test as post hoc, $P < 0.05$).

3.3. Antioxidant analysis

Samples of animals treated for 24 h with mercury chloride showed a decrease in total antioxidant competence ($P < 0.05$) against peroxyl radicals (Fig. 4A) as a result of an augmented relative area (162%) when compared to the control group ($P < 0.05$). The treatment with lead acetate for 24 h was not able to interfere in the total antioxidant competence, as shown by a relative area statistically similar to the control group ($P > 0.05$). No differences in the TBARS content (Fig. 4B) were observed under the experimental conditions.

4. Discussion

In present study, we have evaluated the effect of different treatments with four heavy metal compounds (zinc chloride, cadmium acetate, lead acetate, and mercury chloride) on the AChE activity and gene expression in zebrafish brain. In the concentrations tested, only the animals treated with lead acetate and mercury chloride have shown alterations in the AChE activity. In addition, we have shown the effects of the treatments above in the antioxidant competence and lipid peroxidation in zebrafish brain and the treatment with mercury chloride had the ability to reduce the antioxidant competence against peroxyl radicals.

For the *in vivo* treatments, we have tested an environmental low concentration of $HgCl_2$ and $Pb(CH_3COO)_2$, which corresponds to the dose found in aquatic environment (Berzas Nevado et al., 2003; Jha et al., 2003). The results demonstrated an inhibition on acetylcholinesterase after 24 h of exposure to Hg^{2+} and Pb^{2+}. This effect was progressively restored after 96 h of exposure to these metals, being possible to observe an increase of acetylcholine hydrolysis after 96 h-exposure to Hg^{2+}. However, after chronic treatment (30 days), acetylcholine hydrolysis returned to the control level in both Hg^{2+} and Pb^{2+} treatments. The normalization of the enzyme activity after chronic treatment can be due to compensatory response of the cell to the toxic effects induced by the metals after short-period exposures. Thus, it is possible to observe that the exposure to low concentrations of these metals in the aquatic environment interferes with the acetylcholine hydrolysis differing with the time and level of exposure. In addition, for the *in vitro* experiments, a direct inhibitory effect on

AChE activity was only observed at high concentrations of Hg^{2+} whereas lower doses of this metal did not alter the enzyme activity. Therefore, the effects observed after *in vivo* treatment have leaded us to investigate a possible indirect mechanism able to affect AChE after exposure to these contaminants.

There is evidence that heavy metals can affect diverse post-translational modifications of proteins, which are a decisive step for some proteins to achieve its correct folding and enzyme activity. One action attributed to heavy metals is the disruption of zinc-fingers motif containing proteins. Zinc finger proteins are the largest class of transcription factors, which, in the presence of this ion, shows the correct folding and stability allowing the binding to nucleic acids to regulate transcription (Zawia et al., 2000; Zeng and Kagi, 1995). This motif is present in a number of critical brain specific proteins and the substitution of zinc by environmental heavy metal ions, mainly as a consequence of lead exposure can induce structural and functional changes in these proteins, contributing to the cellular degeneration, disturbed gene expression, signal transduction, and DNA repair (Zawia et al., 2000). Considering this hypothesis, we have evaluated the possible influence of the heavy metals tested in AChE gene expression. AChE transcript levels were not affected by the treatment with heavy metals, suggesting other metabolic target for the treatments. General consensus holds that the harmful effects of Cd^{2+}, Hg^{2+} and Pb^{2+} mainly result from their interaction with proteins; some metals such as chromium, nickel and platinum are also known to interact with DNA (Sharma et al., 2008). Sharma et al. (2008) have shown that heavy metal ions are potent inhibitors of protein folding, suggesting that the interference of metal ions with non native forms of proteins might result in quantitative deficiencies of the affected proteins and in the formation of proteotoxic aggregates, which can contribute to explain the pleiotropic symptomatology of heavy metal poisoning (Hu, 2005; Kosnett, 2007; Waisberg et al., 2003). Since we did not detect alterations on AChE transcript levels, potential harmful effect of metals on DNA structure are unlikely. However, it is not possible to exclude that Hg^{2+} and Pb^{2+} might induce toxic effects through their influence in the protein folding.

Oxygen, a vital fuel for all eukaryotic organisms is also a reason of concern. This occurs by its ability to continuously generate reactive oxygen species (ROS) that can be extremely harmful to cell constituents when in high cellular levels. However, organisms have a protective machinery composed of enzymatic and non-enzymatic defenses (Halliwell and Gutteridge, 2007), that are responsible to counteract the actions of ROS and prevent oxidative stress. These actions range from lipid peroxidation, protein oxidation, enzyme inactivation and DNA breakage up to carcinogenesis, ageing and neurodegenerative diseases, which occur when ROS formation exceeds antioxidant defense capability or disrupt redox signalling, affecting cell functionality (reviewed in Monnet-Tschudi et al., 2006). There is evidence that environmental contaminants can alter antioxidant status of the cell, being the antioxidant and oxidative stress parameters used as biomarkers of pollution (Halliwell and Gutteridge, 2007; Regoli et al., 2002). Because of their multitargeted actions, heavy metals can increase ROS levels by the perturbation of diverse pathways, including enzymatic processes, mitochondrial functions, and endogenous antioxidant defense mechanisms.

The stress oxidative analysis was performed only in groups where a kinetic alteration has occurred. Here we have analyzed the effects of 24 h-treatment with mercury chloride and lead acetate in the total ROS production to look for general alterations rather than be restricted to one unique component of this pathway (Amado et al., 2009). Mercury chloride has reduced the antioxidant competence, as shown by a higher relative area than the control group in the analysis of competence against peroxyl radicals. Therefore, it is possible to suggest that the oxidative damage induced by Hg^{2+} treatment in zebrafish brain may be involved in the inhibitory effect observed on AChE activity. Alterations in neurotransmission systems can explain some neurotoxicological characteristics of the heavy metals. Hence, the identification of biological alterations related to the cholinergic systems during metal exposure may render some important insights about the neurochemical and molecular targets involved in neutoxicity promoted by heavy metals.

In summary, we have shown that the heavy metal targets occur at different levels, such as inducing alterations in the enzyme involved in the control of cholinergic transmission and also in the antioxidant competence of the tissue, which are a small portion of the wide spectrum of actions promoted by these pollutants.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

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