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Toxicology

Effectiveness of $(PhSe)_2$ in protect against the $HgCl_2$ toxicity

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ABSTRACT

This work investigated the preventive effect of diphenyl diselenide [$(PhSe)_2$] on renal and hepatic toxicity biomarkers and oxidative parameters in adult mice exposed to mercury chloride ($HgCl_2$). Selenium (Se) and mercury (Hg) determination was also carried out. Mice received a daily oral dose of $(PhSe)_2$ (5.0 mg/kg/day) or canola oil for five consecutive days. During the following five days, the animals were treated with a daily subcutaneous dose of $HgCl_2$ (5.0 mg/kg/day) or saline (0.9%). Twenty-four hours after the last $HgCl_2$ administration, the animals were sacrificed and biological material was obtained. Concerning toxicity biomarkers, Hg exposure inhibited blood δ-aminolevulinic acid dehydratase (δ-ALA-D), serum alanine aminotransferase (ALT) activity and also increased serum creatinine levels. $(PhSe)_2$ partially prevented blood δ-ALA-D inhibition and totally prevented the serum creatinine increase. Regarding the oxidative parameters, Hg decreased kidney TBARS levels and increased kidney non-protein thiol levels, while $(PhSe)_2$ pre-treatment partially protected the kidney thiol levels increase. Animals exposed to $HgCl_2$ presented Hg content accumulation in blood, kidney and liver. The $(PhSe)_2$ pre-treatment increased Hg accumulation in kidney and decreased in blood. These results show that $(PhSe)_2$ can be efficient in protecting against these toxic effects presented by this Hg exposure model.

Introduction

Mercury (Hg) is a non-essential metal which can be found in elemental, organic and inorganic forms [1,2]. Studies have shown that mercury chloride ($HgCl_2$), an inorganic form of this element [3,4], can cause damage to the organs of several known animals, such as mice and rats [5–7]. Its toxicity is mainly expressed in renal tissue, since the kidneys are the primary route to toxic metal elimination [8,9], not to mention Hg has high affinity by sulfhydryl groups binding to biomolecules containing this radical [10]. Likewise, Hg interaction with sulfhydryl groups decreases the ability of antioxidant agents, such as glutathione (GSH), in neutralizing reactive oxygen species (ROS), consequently leading to lipid peroxidation [11].

Several drugs and compounds have been tested with the objective of reducing Hg damage [6,11]. Previous studies performed by our research group have shown the ability of zinc and copper, essential trace elements, in protecting against the Hg toxicity [12–18].

Another important trace element in the metabolism is selenium (Se). Like Hg, Se can be found in elemental, organic and inorganic forms [19]. Se participates in various metabolic routes, especially those that involve the antioxidant system; for example, Se is present in active sites of antioxidant enzyme glutathione peroxidase (GPx) [20,21]. It is also known that Se alters the Hg distribution in the organism, and due to this effect Hg toxicity can be reduced [22]. Moreover, Se can reduce the Hg toxicity through the prevention of oxidative damage [23].

Diphenil disselenide $(PhSe)_2$ is an organoselenium compound with various pharmacologic activities such as antidepressant [24] and anti-inflammatory [25]. This compound has shown antioxidant activity against lipid peroxidation induced by different agents in different tissues [26–31]. Moreover, $(PhSe)_2$ was shown to be efficient in protecting hematological alterations induced by Hg [32].

Thus, considering that Se is an essential element with beneficial antioxidant property, the present study evaluated the protective effect of $(PhSe)_2$ against renal and hepatic damage induced by $HgCl_2$ in mice, as well as on oxidative parameters. Still, Hg and Se levels in kidney, liver and blood were determined to evaluate the influence of the element redistribution.

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Materials and methods

Chemicals

Reagents used in this study were obtained from Sigma Chemical Co. (St. Louis, MO) and standard commercial suppliers. Commercial kits were used for the biochemical dosages, those of which were obtained from Kovalent do Brasil Ltda (Rio de Janeiro, Brazil) and Labtest Diagnóstica S.A. (Minas Gerais, Brazil). $(PhSe)_2$ was prepared according to the method described by Paulmier [33].

Animals

Adult male Swiss mice were obtained from the animal house of the Federal University of Santa Maria and transferred to our breeding colony. They were kept on a 12 h light/dark cycle, a controlled temperature of $22 \pm 2^{\circ}$ C and with free access to food (Guabi, RS, Brazil) and water. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil (Process number 039/2014). All efforts were made to minimize the number of animals used and their suffering.

Experimental protocol

The animals were weighed and treated for five consecutive days with $(PhSe)_2$ (5.0 mg/kg) or canola oil (oil, vehicle) by oral gavage (v.o.). During the following five days, the animals were weighed and treated with $HgCl_2$ (5.0 mg/kg) or saline (sal, vehicle) by subcutaneous injection (s.c.). Animals were distributed in four experimental groups: oil–sal (control group), Se–sal (Se group), oil–Hg (Hg group) and Se–Hg (Se–Hg group). Twenty-four hours after the last $HgCl_2$ administration, mice were weighed and killed by cardiac puncture. Blood, renal and hepatic tissues samples were collected and processed according to the technique to be performed.

Tissue preparation

To obtain the serum for urea and creatinine levels, aspartate (AST) and alanine aminotransferase (ALT) activities determination, blood samples were collected in tubes without anticoagulant and centrifuged at $2000 \times g$ for 10 min at room temperature. For the δ-ALA-D activity assay, blood samples were collected in tubes with heparin and then transferred to a recipient with distilled water, in 1:4 (v/v) proportions, under constant agitation on ice bath for 10 min to full hemolysis. Kidneys and liver were quickly removed and placed on ice and homogenized in 5 and 7 volumes, respectively, of Tris–HCl buffer (10 mM, pH: 7.4) to obtain the homogenate. The homogenates were centrifuged at $8000 \times g$ for 30 min at 4 °C and the low-speed supernatants (S1) were separated and used for δ-ALA-D activity and TBARS level determination. Furthermore, a portion of blood, kidney and liver was removed and frozen at −20 °C until Hg and Se level determination.

Biochemical determinations

All biochemical determinations were carried out by spectrophotometry using a Biospectro – SP-22 spectophotometer.

δ-ALA-D activity

This technique was performed according to Sassa [34] by measuring the rate of product formation (porphobilinogen, PBG), as previously described by Peixoto et al. [14]. The incubation system was previously pipetted and after addition of 100 μL of hepatic S1 or 200 μL of blood hemolyzed or renal S1, the incubation was initiated and carried out for 30, 60 and 90 min for liver, blood and kidney, respectively, at 39 °C. The reaction was then stopped by the addition of trichloroacetic acid (TCA) 10% containing $HgCl_2$ 0.05 M and the PBG formation was measured with Ehrlich's reagent, using the molar absorption coefficient of 6.1×10^4 for Ehrlich-PBG salt. The absorbance was determined spectrophotometrically at 555 nm, and the specific enzymatic activity was expressed as nmol PBG/h/mg protein.

ALT and AST activity

ALT activity was determined in a medium containing buffer 55.8 mM, α-ketoglutaric acid 1.67 mM, L-alanine 83.3 mM, sodium azide 12.8 mM and 25 mL of serum incubated at 37 °C for 30 min. The reaction was stopped by adding HCl 0.45 mM. The color reactive (2,4-dinitrophenylhydrazine 0.45 mM) was then added and the medium was incubated for 20 min at room temperature. The color was intensified by NaOH 0.33 mM and the absorbance was determined spectrophotometrically at 505 nm. The activity (U/L) was calculated by comparing with a calibration curve utilizing sodium pyruvate as standard. The AST activity was determined similarly to ALT enzyme, except that the L-aspartic acid 83.3 mM was used as a substrate and that the medium was incubated at 37 °C for 60 min.

Urea levels

Urea levels were determined using a Labtest commercial kit. 10 μL of serum sample was added to a medium containing phosphate buffer (19.34 mM pH 6.9), sodium salicylate (58.84 mM), sodium nitroprusside (3.17 mM), and urease (≥12.63 UK/L) for 5 min at 39 °C. The reaction was then stopped by adding oxidant solution (final concentrations: NaOH 0.07 M and sodium hypochlorite 3.01 mM) and incubated for another 5 min for color development. The absorbance was determined at 600 nm.

Creatinine levels

Creatinine levels were determined using a Labtest commercial kit. The estimation of creatinine serum levels was carried by measuring the quantity of the product formed (creatinine picrate), and using creatinine as standard. 50 μL of serum sample was added to a medium containing picric acid (20.2 mM) and NaOH (145.4 mM) and incubated at 37 °C and the absorbance was measured spectrophotometrically at 510 nm.

TBARS assays

The lipid peroxidation was determined according to the method described by Ohkawa et al. [35] through the measurement of the thiobarbituric acid-reactive species (TBARS). 200 μL of renal or hepatic S1 was incubated with 300 μL of thiobarbituric acid (TBA) (0.8%), 200 μL of SDS (8.1%) and 500 μL of acetic acid buffer (2.5 M, pH 3.4) for 2 h at 95 °C. For blood TBARS determination, an aliquot of heparinized blood was homogenized in trichloroacetic acid (TCA) 40%, in 2:1 (v/v) proportions, followed by centrifugation at $2000 \times g$ for 10 min to obtain the supernatant (S1). 250 μL of S1 was incubated with 600 μL of TBA (0.6%), 600 μL of phosphoric acid (1%) and 50 μL of distillated water for 2 h at 95 °C and the absorbance was measured spectrophotometrically at 532 nm. A curve using malondialdehyde (MDA) as standard was constructed in order to express the results in nmol MDA/mg protein.

Total thiol and non-protein thiol (NPSH) level determination

Liver and kidney thiol (SH) levels were determined in S1 as previously described by Ellman [36]. For non-protein thiol determination, the protein fraction contained in S1 was precipitated using TCA 4% in 1/1 proportion, followed by centrifugation at $2000 \times g$ for 10 min, in order to obtain the S2 that was used for analysis. The absorbance was determined at 412 nm. A curve using glutathione as