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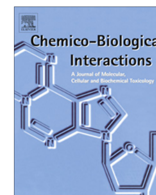

Protective effects of diphenyl diselenide in a mouse model of brain toxicity

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ABSTRACT

Interest in organoselenide chemistry and biochemistry has increased in the past three decades, mainly due to their chemical and biological activities. Here, we investigated the protective effect of the organic selenium compound diphenyl diselenide $(PhSe)_2$ (5 μmol/kg), in a mouse model of methylmercury (MeHg)-induced brain toxicity. Our group has previously demonstrated that the oral and repeated administration (21 days) of MeHg (40 mg/L) induced MeHg brain accumulation at toxic concentrations, and a pattern of severe cortical and cerebellar biochemical and behavioral. In order to assess neurotoxicity, the neurochemical parameters, namely, mitochondrial complexes I, II, II–III and IV, glutathione peroxidase (GPx) and glutathione reductase (GR) activities, the content of thiobarbituric acid-reactive substances (TBA-RS), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and brain-derived neurotrophic factor (BDNF), as well as, metal deposition were investigated in mouse cerebral cortex. Cortical neurotoxicity induced by brain MeHg deposition was characterized by the reduction of complexes I, II, and IV activities, reduction of GPx and increased GR activities, increased TBA-RS and 8-OHdG content, and reduced BDNF levels. The daily treatment with $(PhSe)_2$ was able to counteract the inhibitory effect of MeHg on mitochondrial activities, the increased oxidative stress parameters, TBA-RS and 8-OHdG levels, and the reduction of BDNF content. The observed protective $(PhSe)_2$ effect could be linked to its antioxidant properties and/or its ability to reduce MeHg deposition in brain, which was here histochemically corroborated. Altogether, these data indicate that $(PhSe)_2$ could be consider as a neuroprotectant compound to be tested under neurotoxicity.

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1. Introduction

Selenium is a fundamental component of the living cells of a variety of organisms with antioxidant properties [1–5], and it is necessary for the expression of at least 25 selenium-dependent enzymes, including the powerful antioxidant glutathione peroxidase (GPx), which protects macromolecules from peroxidative damage [6], the thioredoxin reductase [7] and several other selenoproteins that modulate the cellular redox and antioxidant status [8,9].

Interest in compounds containing selenium has increased in the past three decades, mainly due to their biological activities (for a revision see Ref. [4]). The pharmacological properties of organoselenium compounds have been extensively reported after the identification of ebselen [2-phenyl-1,2-benzisoselenazole-3 (2H)-one] as a mimetic of GPx [10]. In this scenario, diphenyl diselenide $(PhSe)_2$, is the simplest of the synthetic diaryl diselenides, and it has been reported to possess antioxidant, antinociceptive, antiinflammatory, antihyperglycemic, antiatherogenic, hepatoprotective, antiulcer, antidepressant-like and anxiolytic-like actions [11–19].

The antioxidant activity of $(PhSe)_2$ may be related to its thiol peroxidase-like activity, where the reaction catalyzed by the organoselenium compound is similar to that catalyzed by the antioxidant enzyme, GPx [4]. This antioxidant property is of particular significance, in living cells, because it decomposes hydrogen peroxide, phospholipid hydroperoxide and other organic hydroxyperoxides, preventing the formation of reactive and toxic hydroxyl, and lipoperoxyl radicals [20]. Based on this, it appears that $(PhSe)_2$ could behave as a potent protective compound in models of

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toxicity, mainly in tissues with high rates of oxygen consumption and high content of easily peroxidizable long-chain polyunsaturated fatty acids, which are particularly vulnerable to oxidative damage [21]. Thus, $(PhSe)_2$ appears to be a promising neuroprotective agent, based on the potential antioxidant activity, and also, on its low toxicity, which has been demonstrated in rabbits [22,23] and rodents [24]. Furthermore, $(PhSe)_2$ is less toxic than the related compound ebselen [25]. In line with this, ebselen has been already used in clinical trials and consistently demonstrated to reduce brain damage in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage, and improved the outcome of acute ischemic stroke [26–28].

Taking into account the potential pharmacological properties of organoselenium compounds and the lower toxicity of $(PhSe)_2$, when compared to ebselen or the inorganic forms of selenium [2,29], as well as the scarcity of studies on molecular mechanisms related to $(PhSe)_2$-induced neuroprotection, the present study aimed to analyze the potential neuroprotective effect of $(PhSe)_2$ on neurochemical parameters in brain from MeHg-poisoned mice.

2. Experimental procedures

2.1. Animals and reagents

Male Swiss albino mice of 60 days of life obtained from the Central Animal House of the Centre for Biological Sciences, Universidade Federal de Santa Catarina, Florianópolis – SC, Brazil, were used. The animals were maintained on a 12-h light/dark cycle (lights on 07:00–19:00 h) in a constant temperature (22 ± 1 °C) colony room, with free access to water and protein commercial chow (Nuvital-PR, Brazil). The experimental protocol was approved by the Ethics Committee for Animal Research (PP00084/CEUA) of the Universidade Federal de Santa Catarina, Florianópolis – SC, Brazil. The experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize the number of animals used and their suffering.

The chemicals were of analytical grade and purchased from Sigma (St. Louis, MO, USA), except methylmercury (II) chloride which was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA), the monoclonal antibody against 8-hydroxy-2′-deoxyguanosine and polyclonal antibody against BDNF that were obtained from JaICA® (Fukuroi, Shizuoka, Japan) and Chemicon® (Temecula, CA, USA), respectively, and 3,3′-diaminobenzidine from Dako Cytomation (Glostrup, Denmark). $(PhSe)_2$ was prepared and characterized by our group as previously described [30]. The chemical purity of $(PhSe)_2$ was determined by GC/HPLC (99.9% of purity).

The biochemical measurements were performed in a Varian Cary 50 spectrophotometer (Varian Inc., Palo Alto, CA, USA) with temperature control. For brain tissue preparations an Eppendorf 5415 R (Eppendorf, Hamburg, Germany) centrifuge was used. The microscopic analyses were performed with a Nikon microscope (Nikon, USA).

2.2. Treatments

Twenty-four mice were randonly divided into four experimental groups and received vehicle (controls), MeHg (40 mg/L), $(PhSe)_2$ (5 μmol/kg) and MeHg plus $(PhSe)_2$ during 21 days. Our group has previously demonstrated that the oral and repeated (21 days) administration of MeHg 40 mg/L induced MeHg brain accumulation at toxic concentrations of 3–5 μg/g tissue (3–5 ppm) [29,31], which could be translated into a brain concentration of 15–30 μM [32]. The toxicant was diluted in tap water, and was freely available. $(PhSe)_2$ was dissolved on dimethylsulfoxide (DMSO) and subcutaneously administered [33,34]. Proper control animals received vehicle injections (1 mL/kg body weight).

The liquid and solid consumptions did not significantly differ between the groups (data not shown).

In some experiments MeHg (10 and 50 μM) was dissolved in DMEM, $(PhSe)_2$ (1 μM) in DMSO and exposed to astroglioma C6 cells.

2.3. Tissue preparation

Animals were sacrificed by decapitation without anesthesia 24 h after the last subcutaneous administration. The brain was rapidly excised on a Petri dish placed on ice and the cerebral cortex was dissected, weighed and kept chilled until homogenization, which was performed using a ground glass type Potter-Elvejhem homogenizer. The maximum period between the tissue preparation and enzyme analysis was always less than a week.

2.4. Brain preparations for measuring the respiratory chain complex activities

Mouse cerebral cortex was homogenized in 10 volumes of phosphate buffer (pH 7.4), containing 0.3 M sucrose, 5 mM MOPS, 1 mM EGTA and 0.1% bovine serum albumin. The homogenates were centrifuged at 1500*g* for 10 min at 4 °C and the pellet was discarded. The supernatant was centrifuged at 17,000*g* for 10 min at 4 °C in order to isolate the mitochondrial fraction present in the pellet, which was finally dissolved in the same buffer [35].

2.5. Brain preparations for measuring the oxidative stress parameters

Brain tissue was homogenized in 5 volumes (1:5, w/v) of 20 mM sodium phosphate buffer (pH 7.4) containing 140 mM KCl. Homogenates were centrifuged at 750*g* for 10 min at 4 °C to discard nuclei and cell debris [36,37]. The pellet was discarded and the supernatant, a suspension of mixed and preserved organelles, including mitochondria, was separated and immediately used for the analyses.

2.6. Maintenance and treatment of cell line

The astroglioma C6 cell line was obtained from the American Type Culture Collection (Rockville, Maryland, USA). The cells were seeded in flasks and cultured in Dulbecco's modified Eagle's medium (DMEM) (pH 7.4) containing 5% fetal bovine serum, sterile antimycotic solution 100×: penicillin 100 IU/mL, streptomycin 0.1 mg/mL and amphotericin 0.25 μg/mL, in a 95% O_2 and 5% CO_2 humidified atmosphere, at 37 °C. Exponentially growing cells were detached from the culture flasks using 0.05% trypsin/ethylenediaminetetracetic acid and seeded in 96-well plates (5×10^4 - cells/well) [38]. After cells reached confluence, the culture medium was removed by suction and the cells were exposed to 10 and 50 μM MeHg for 1 h, and the reduction of MTT (for assessing cellular viability) was used as a neurotoxicity endpoint. In addition, cells were also exposed to $(PhSe)_2$, before, during or after MeHg exposure, as follows:

(a) 24 h 1 μM $(PhSe)_2$ (controls).
(b) 24 h 1 μM $(PhSe)_2$ + 1 h 10 or 50 μM MeHg (pre-treatment).
(c) 1 h 1 μM $(PhSe)_2$ + 1 h 10 or 50 μM MeHg (concomitant exposure).
(d) 1 h 10 or 50 μM MeHg + 24 h 1 μM $(PhSe)_2$ (post-treatment).

2.7. Preparation of mouse cortical slices

Mice were euthanized by decapitation and had their cerebral cortex dissected on ice in Krebs–Ringer bicarbonate buffer (KRB;

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