



Diphenyl diselenide regulates Nrf2/Keap-1 signaling pathway and counteracts hepatic oxidative stress induced by bisphenol A in male mice[☆]

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ABSTRACT

Bisphenol A (BPA) is a chemical toxicant that has deleterious effects on human. BPA causes oxidative stress in tissues, including the liver. Diphenyl diselenide (PhSe)₂ improves the antioxidant response via activation of the nuclear factor (erythroid-derived 2)-like 2 (Nrf2)/Kelch-like ECH-associated protein (Keap 1) pathway in macrophage cells. In the present study, we investigated whether (PhSe)₂ counteracts hepatic oxidative stress induced by BPA in male and female Swiss mice. Three-week-old mice received by the intragastric (i.g.) route BPA (5 mg/kg) from 21st to 60th postnatal day (PND). At PND 61, the mice were treated with (PhSe)₂ (1 mg/kg, i.g.) for seven days. Parameters of hepatic damage and oxidative stress were determined in male and female mice. The results show that BPA increased the activity of aspartate aminotransferase in female mice, and in male mice the activity of alanine aminotransferase was increased. Male and female mice had an increase in fat mass accumulation. Male mice showed an increase in hepatic oxidative damage of proteins and a decrease in non-enzymatic (ascorbic acid and non-protein thiol) and enzymatic (superoxide dismutase) defenses, which are consistent with oxidative stress status. Male mice were more susceptible than female mice to hepatic oxidative stress induced by BPA. BPA decreased Nrf2/Keap1 protein content in male mice. (PhSe)₂ reduced hepatic oxidative stress induced by BPA in male mice. Our results demonstrate that male mice were more susceptible to hepatic oxidative stress induced by BPA than female mice. (PhSe)₂ regulated Nrf2/Keap-1 signaling pathway and counteracted hepatic oxidative stress induced by BPA in male mice.

1. Introduction

Endocrine disrupting chemicals (EDCs) are substances able to alter synthesis, transport and binding of endogenous hormones (Harris et al., 2017). Bisphenol A (BPA) is an EDC used in polycarbonate plastics and epoxy resins manufacturing (Beltifa et al., 2017). Consequently, this compound is present in domestic products, such as baby bottles, toys, food and drink containers (Geens et al., 2012). It is important to mention that BPA can undergo the leaching process, migrate from various polycarbonate plastics, evaporate in the air or blend to drink and food (Beltifa et al., 2017). This creates possible routes of human exposure to BPA through oral, inhalation or dermal. Because of

indiscriminate use and the consequent exposure to BPA from different sources (Liu et al., 2017), it becomes useful to investigate its deleterious effects.

BPA exposure has been associated with abnormal production of reactive oxygen species (ROS) in tissues, including the mouse liver (Bindhumol et al., 2003). In addition, it has been demonstrated that BPA alters liver morphology and transaminase activities (Kazemi et al., 2017), but usually these reports have limited mechanistic details. The nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and Kelch-like ECH-associated protein 1 (Keap 1) pathway is a key regulatory mechanism in oxidative stress (Deshmukh et al., 2017). These signals regulate expression of about 100 genes with functions directly linked with cell

Abbreviations: BPA, bisphenol A; PhSe₂, diphenyl diselenide; Nrf2, nuclear factor (erythroid-derived 2)-like 2; Keap1, Kelch-like ECH-associated protein 1; i.g., intragastric; EDCs, endocrine disrupting chemicals; NPSH, non-protein thiol; PND, postnatal day; δALA-D, δ aminolevulinic acid dehydratase; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; S1, supernatant; i.p., intraperitoneal; DNPH, dinitrophenylhydrazine; DTNB, 5,5-dithiobis(2-nitrobenzoic acid); NADPH, nicotinamide adenine dinucleotide phosphate; GSH, reduced glutathione; GR, glutathione reductase; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS, sodium dodecyl sulfate; EGTA, ethylene glycol tetraacetic acid; JNK, jun N-terminal kinases; PKC, protein kinase C; GSK3, β-glycogen synthase kinase 3; PI3K/AktPKB, phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase b

[☆] EDC- Endocrine disrupting chemicals; BPA – Bisphenol A; ROS -Reactive oxygen species; Nrf2- nuclear factor (erythroid-derived 2)-like 2; Keap1- Kelch-like ECH-associated protein 1.

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survival and redox state. The major mediator of this response is Nrf2 which can induce transcription of detoxifying enzymes, such as superoxide dismutase and glutathione peroxidase (Osburn et al., 2006).

Selenium is an essential trace element nutritionally important to mammals (Ewan, 1976). In the last decades, the pharmacological studies of synthetic

organoselenium compounds revealed these molecules as promising antioxidants, enzyme inhibitors and hepatoprotective agents (Nogueira and Rocha, 2011). In this context, diphenyl diselenide (PhSe)₂ is one of the most studied organoselenium compounds due to its multi-target properties. (PhSe)₂ has been reported to have hepatoprotective (Carvalho et al., 2013), antidepressant (Quines et al., 2016) and memory improving (Jardim et al., 2017) actions.

Considering what was mentioned before and that (PhSe)₂ improves the antioxidant response via activation of the Nrf-2 pathway in macrophage cells (Mancini et al., 2014), we hypothesized whether this organoselenium compound counteracts the hepatic oxidative stress induced by BPA in male and female mice.

2. Materials and methods

2.1. Animals

The experiments were carried out using male and female Swiss mice (initial weight 10–12 g). The animals were from 5 different litters (5 mothers). The animals were kept under controlled condition with constant temperature (22 ± 2 °C) and in a light/dark cycle with lights turned on at 7:00 A.M. Mice were maintained in white cages with free access to water and food (Guabi, RS, Brasil). In order to avoid contamination of underused environmental the animals received water in glass bottles. The experiments were performed according with the EC Directive 86/609/EEC for animal experiments and the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil and registered under the number 1660280915/2016. All efforts were made to reduce the number of animals to a minimum.

2.2. Drugs

BPA (Fig. 1) was purchased from Sigma (St. Louis, MO, USA) and (PhSe)₂ (Fig. 1) was prepared in our laboratory according to the method described in the literature (Paulmier, 1986). The ¹H NMR and ¹³C NMR spectra analyses showed analytical and spectroscopic data in full agreement with (PhSe)₂ assigned structure. The chemical purity (99.9%) was determined by GC/MS. All other chemicals were obtained of analytical grade or from standard commercial suppliers.

BPA was dissolved in canola oil and administered by the intragastric (i.g.) route at a dose of 5 mg/kg of body weight to mice (Gassman, 2017; Jang et al., 2012; Jardim et al., 2017; Luo et al., 2013). (PhSe)₂ was also dissolved in canola oil and administered i.g. at a dose of 1 mg/kg of body weight to mice. The dose of (PhSe)₂ was chosen based on our previous study (Jardim et al., 2017), this dose did not cause weight loss or side effects in mice. All drugs were administered to mice in a constant volume of 10 mL/kg.

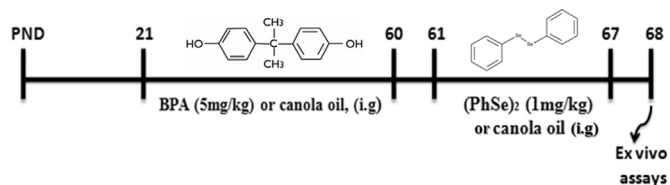


Fig. 1. Experimental design. BPA or canola oil was daily administered i.g. to mice from postnatal day 21 (PND 21) to PND 60. Treatment with (PhSe)₂ started at PND 61 and finished at PND 67.

2.3. Experimental design

The schematic experimental design representation is illustrated in Fig. 1.

At the postnatal day 21 (PND 21), the animals were randomly divided in four different groups of male or female as following: control (vehicle, 10 mL/kg), (PhSe)₂ (1 mg/kg), BPA (5 mg/kg), BPA (5 mg/kg) + (PhSe)₂ (1 mg/kg). The exposure to BPA was carried out in mice from PND 21 to PND 60. Mice of control and (PhSe)₂ groups received canola oil by the i.g. route, once a day, whereas those from the BPA and BPA + (PhSe)₂ groups received 5 mg/kg BPA. After the BPA last administration, mice of (PhSe)₂ and BPA + (PhSe)₂ groups were treated with (PhSe)₂ at the dose of 1 mg/kg (i.g.) and those of control and BPA groups received canola oil (i.g.). The body weight was daily monitored to adjust the dosage of BPA or (PhSe)₂ and to calculate the body weight gain (n = 5 per group). The individual body weight gain was calculated by the difference between the baseline body weight, obtained before the beginning of treatment, and the body weight at the end of treatment.

After the end of treatment with (PhSe)₂ or canola oil, the animals were anaesthetized with a single intraperitoneal (i.p.) injection of pentobarbital (200 mg/kg) for blood sample collection. The animals were killed by cervical dislocation and the samples of livers were collected. The liver and plasma samples were quickly frozen at -80 °C for further analyses. Besides, the abdominal and epididymal fat were removed and weighed to calculate the ratio of fat mass to total body weight (n = 6–7 per group).

2.4. Sample preparation

The blood was centrifuged x 2500g for 10 min in order to obtain the plasma fraction and used to determine the activities of aspartate aminotransferase (AST) (n = 6–7 per group) and alanine aminotransferase (ALT) (n = 6–7 per group).

Samples of livers were homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, w/v) for carbonyl protein content determination (n = 5–6 per group). For other techniques, the homogenate was centrifuged for 10 min at 2.500 × g to yield a low-speed supernatant (S1). Samples of S1 were used to determine the δ aminolevulinic acid dehydratase (δALA-D) activity (n = 6–7 per group), ascorbic acid (n = 6–7 per group) and non-protein thiol (NPSH) levels (n = 7–8 per group) and the activities of superoxide dismutase (SOD) (n = 5–6 per group), catalase (CAT) (n = 6 per group) and glutathione peroxidase (GPx) (n = 5–6 per group). For the western blot assay, samples of livers were collected only from male mice (n = 6 per group).

2.5. Parameters of hepatic toxicity

2.5.1. AST and ALT activities

It was determined the plasma AST and ALT activities, which are biochemical markers of acute hepatic toxicity. AST and ALT activities were determined using the colorimetric method described previously (Reitman and Frankel, 1957). All assays were carried out using commercial kits (LABTEST, Diagnostic S.A. Minas Gerais, Brazil). AST and ALT activities were expressed as UI/L.

2.5.2. δ-δALA-D activity

δ-δALA-D has a high sensitivity to oxidative stress and can be used as an indirect biomarker of organoselenium compounds toxicity (Rocha et al., 2012). The enzyme assay was determined in samples of livers according the literature some modifications (Sassa, 1982). S1 (100 μL) was pre-incubated for 10 min at 37 °C in a medium containing 1 M phosphate buffer (pH 6.8). The enzymatic reaction was started with the addition of the substrate (δ-ALA) and incubated for 1 h at 37 °C. The reaction stopped with the addition of a trichloroacetic solution (10%) with 10 mM of HgCl₂. The product was measured using Erlich's reagent

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