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p-Methoxyl-diphenyl diselenide protects against cisplatin-induced renal toxicity in mice

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ABSTRACT

The present study was designed to investigate the effects of *p*-methoxyl-diphenyl diselenide $(OMePhSe)_2$ on oxidative stress and renal damage parameters of mice exposed to cisplatin. $(OMePhSe)_2$ (50 and 100 mg/kg/day) was orally administered to mice for six consecutive days. On the third day after the beginning of $(OMePhSe)_2$ treatment, the renal toxicity was induced by injecting cisplatin (10 mg/kg intraperitoneal) in mice. $(OMePhSe)_2$ treatment (50 mg/kg) partially reduced plasma urea and creatinine levels increased by cisplatin. Histopathological examination of kidneys showed that $(OMePhSe)_2$ ameliorated renal injury caused by cisplatin. $(OMePhSe)_2$ attenuated the decrease in reduced glutathione (GSH) and ascorbic acid (AA) levels, the inhibition of glutathione peroxidase (GPx), glutathione S-transferase (GST), glutathione reductase (GR) and catalase (CAT) activities caused by cisplatin in kidney. $(OMePhSe)_2$ treatment partially protected against the inhibition of renal δ-aminolevulinic dehydratase (δ-ALA-D) activity caused by cisplatin. No alteration in renal lipid peroxidation levels was found in cisplatin and/or $(OMePhSe)_2$ groups. $(OMePhSe)_2$ was effective against the increase in reactive species (RS) levels caused by the cisplatin exposure. Based on the renoprotective and antioxidant actions of $(OMePhSe)_2$ we suggest that this organoselenium compound could be considered a feasible candidate to protect against toxicity commonly encountered in cisplatin exposure.

1. Introduction

Cancer globally ranks as the second-most leading cause of mortality (Howland et al., 2006). Cisplatin (cis-dichlorodiammine-platinum II) is a synthetic anticancer drug extensively clinically used in the treatment of several human malignancies. Anticancer activity of cisplatin is attributed to the conversion to a complex, which forms an interstrand cross-link with double-strand DNA to prevent DNA synthesis (Baek et al., 2003). The most common adverse effect limiting the efficacy of this drug is renotoxicity which develops primarily in the S3 segment of the proximal tubule. About 25–35% of patients experience a significant decline in renal function after a single dose of cisplatin (Luke et al., 1992).

Although the exact mechanism of cisplatin-induced renotoxicity is not well understood, according to the previous reports, some mechanisms as depletion of sulfhydryl (SH) groups, impaired antioxidant defense system and mitochondrial dysfunction by inhibition of complexes I–IV of the respiratory chain in proximal tubules could contribute to renotoxicity resulting from cisplatin (Baek et al., 2003). Recently, El-Beshbishy and collaborators (2011) reported that alpha lipoic acid, an antioxidant, protected against cisplatin-induced nephrotoxicity through antioxidant and antiapoptotic mechanisms combined with initiation of mRNA expression of antioxidant genes. The potential protective effect of several natural and synthetic antioxidants has been investigated in different models of nephrotoxicity induced by cisplatin (Ali and Al Moundhri, 2006; Chakraborty et al., 2011). Additionally, the fact that the depletion of sulfhydryl groups is reported as one of the mechanisms of renotoxicity induced by cisplatin (Baek et al., 2003) and that the inhibition of δ-ALA-D activity has been demonstrated in experimental models of renotoxicity (Corte et al., 2009; Brandão et al., 2010) and in patients with chronic renal failure (Da Silva et al., 2007) motivated us to investigate the role of δ-ALA-D activity in cisplatin model of renal toxicity. δ-ALA-D, a

Abbreviations: $(OMePhSe)_2$, *p*-methoxyl-diphenyl diselenide; GSH, reduced glutathione; AA, ascorbic acid; GPx, glutathione peroxidase; GST, glutathione S-transferase; GR, glutathione reductase; CAT, catalase; δ-ALA-D, δ-aminolevulinic dehydratase; SH, sulfhydryl; SDS, sodium dodecyl sulfate; TBARS, thiobarbituric acid reactive species; MDA, malondialdehyde; TCA, trichloroacetic acid; OPA, o-phthalaldehyde; GSSG, oxidized glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; ROS, reactive oxygen species; NMR, nuclear magnetic resonance; GC/MS, gas chromatography/mass spectrometry; DCHF-DA, 2′,7′-dichlorofluorescein diacetate; DCF, dichlorofluorescein.

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sulfhydryl- and zinc-containing enzyme, catalyzes the asymmetrical condensation of two molecules of δ-ALA to produce PBG an intermediary in heme biosynthesis (Jaffe, 1995). The inhibition of δ-ALA-D may unfavorably affect the heme biosynthesis pathway that, in turn, may have pathological consequences (Bechara et al., 1993) such as anemia. *p*-Methoxyl-diphenyl diselenide $(OMePhSe)_2$ is an antioxidant compound with antinociceptive and neuroprotective actions (Jesse et al., 2009; Nogueira and Rocha, 2010; Pinton et al., 2011). In addition, our research group demonstrated the protective effect of $(OMePhSe)_2$ in lethal acute liver failure induced by lipopolysaccharide and D-galactosamine in mice (Wilhelm et al., 2009). In this way, we designed this study to investigate the effects of $(OMePhSe)_2$, an organoselenium compound, on oxidative stress and renal parameters of mice exposed to cisplatin.

2. Materials and methods

2.1. Animals

Adult Swiss male mice, weighing 25–35 g, were obtained from a local breeding colony. Mice were kept in a separate animal room, on a 12 h light/dark cycle with lights on at 7:00 a.m., at room temperature (22 ± 1 °C), with free access to food and water. 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.

2.2. Drugs

Cisplatin was purchased from Sigma (St. Louis, USA) and dissolved in saline. $(OMePhSe)_2$ (Fig. 1) was prepared in our laboratory according to the literature method (Paulmier, 1986). Analysis of the ^1H NMR and ^{13}C NMR spectra showed that $(OMePhSe)_2$ obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of compound (99.9%) was determined by GC/MS. This drug was dissolved in canola oil. $(OMePhSe)_2$ and cisplatin were administered at a constant volume of 10 ml/kg of body weight. All other chemicals were of analytical grade.

2.3. Ex vivo experiments

2.3.1. Exposure

Animals were randomly divided into six groups of six animals each. Animals of **group 1** received daily canola oil (10 ml/kg; per oral route, p.o.). **Groups 2** and **5** received daily $(OMePhSe)_2$ at a dose of 50 mg/kg (p.o.). **Groups 3** and **6** received every day $(OMePhSe)_2$ at a dose of 100 mg/kg (p.o.). These treatments were performed for six consecutive days. At the third day of treatment with $(OMePhSe)_2$, renotoxicity was induced in animals of **groups 4**, **5** and **6** by injecting a single dose of cisplatin (10 mg/kg, i.p.) (Ajith et al., 2007; Fouad et al., 2008). The doses of $(OMePhSe)_2$ were chosen based on a previous study (Wilhelm et al., 2009). The protocol of mouse treatment is given below:

Group (1) canola oil (10 ml/kg, p.o.) and saline solution (10 ml/kg, i.p.);
Group (2) $(OMePhSe)_2$ (50 mg/kg, p.o.) and saline solution (10 ml/kg, i.p.);
Group (3) $(OMePhSe)_2$ (100 mg/kg, p.o.) and saline solution (10 ml/kg, i.p.);
Group (4) canola oil (10 ml/kg, p.o.) and cisplatin (10 mg/kg; i.p.);
Group (5) $(OMePhSe)_2$ (50 mg/kg, p.o.) and cisplatin (10 mg/kg; i.p.);
Group (6) $(OMePhSe)_2$ (100 mg/kg, p.o.) and cisplatin (10 mg/kg; i.p.).

As shown in Scheme 1, 72 h following cisplatin administration, all mice were anesthetized for blood collection by heart puncture. Heparin was used as anticoagulant in order to obtain the plasma (hemolyzed plasma was discharged). The plasma was used for determination of urea and creatinine levels. After this procedure, animals were killed by cervical dislocation and kidneys were removed and dissected.

The right kidney was fixed in 10% neutral buffered formalin for histopathological examination. The left kidney was homogenized in 50 mM Tris–HCl, pH 7.5 (1/10, w/v), centrifuged at 2400*g* for 15 min to obtain the low-speed supernatant

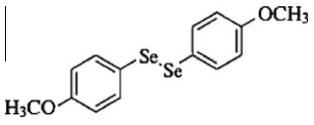
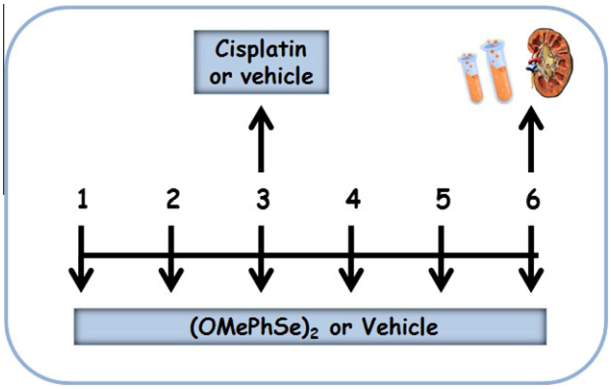


Fig. 1. Chemical structure of $(OMePhSe)_2$.



Scheme 1. Protocol treatment.

(S_1). S_1 was used for all biochemical assays, except to determinate the reduced glutathione (GSH) levels, for which the samples of kidneys were homogenized in 0.1 M $HClO_4$ and centrifuged at 5000*g* for 10 min.

2.3.2. Histopathological analysis

For light microscopy examination, right kidneys (from four animals per group) were embedded in paraffin, sectioned at 4 μm and stained with hematoxylin and eosin (Nath et al., 2000).

2.4. Biochemical assays

2.4.1. Renal Markers of Damage

Plasma urea (Mackay and Mackay, 1927) and creatinine (Jaffe, 1886) levels were analyzed using commercial Kits (Labtest, Diagnostica S.A., Minas Gerais, Brazil). The urea and creatinine levels were expressed as mg/dl.

2.4.2. Lipid peroxidation levels

An aliquot of S_1 (200 μl) was added to the reaction mixture containing 500 μl thiobarbituric acid (0.8%), 200 μl sodium dodecyl sulfate (SDS, 8.1%) and 500 μl acetic acid (pH 3.4) and was incubated for 2 h at 95 °C. Thiobarbituric acid reactive species (TBARS) were determined as described by Ohkawa et al. (1979). Malondialdehyde (MDA) reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm. The lipid peroxidation levels were expressed as nmol MDA equivalents/mg protein.

2.4.3. Reactive species (RS) levels

RS levels were determined by a spectrofluorimetric method, using 2′,7′-dichlorofluorescein diacetate (DCHF-DA) assay (Loetchutinat et al., 2005). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCF) is measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 15 min after the addition of DCHF-DA to the medium. RS levels were expressed as arbitrary units (AU).

2.4.4. Ascorbic acid (AA) levels

The determination of AA levels was performed as described by Jacques-Silva et al. (2001), with some modifications. Briefly, S_1 was precipitated in 10% trichloroacetic acid (TCA) solution. An aliquot of S_1 following TCA precipitation (300 μl), at a final volume (575 μl) of the solution, was incubated for 3 h at 38 °C, then 500 μl of H_2SO_4 (65% v/v) were added to the medium. The reaction product was determined using a color reagent containing dinitrophenyl hydrazine (4.5 mg/ml) and $CuSO_4$ (0.075 mg/ml) at 520 nm. AA content is related to tissue amount (μmol AA/g tissue).

2.4.5. Reduced glutathione (GSH) levels

GSH levels were determined by spectrofluorimetric method in accordance with Hissin and Hilf (1976) using o-phthalaldehyde (OPA) as fluorophore. S_1 (100 μl) was incubated with 100 μl of OPA (0.1% in methanol) and 1.8 ml of 0.1 M phosphate buffer (pH 8.0) for 15 min at room temperature in dark. Fluorescence was measured with a fluorescence spectrophotometer at excitation wavelength of 350 nm and at emission wavelength of 420 nm. GSH levels were expressed as nmol/g tissue.

2.4.6. Glutathione peroxidase (GPx) activity

GPx activity in S_1 was assayed spectrophotometrically by the method described by Wendel (1981), through the GSH/NADPH/glutathione reductase system, by the dismutation of H_2O_2 at 340 nm. In this assay, the enzyme activity is measured indirectly by means of NADPH decay. H_2O_2 is decomposed, generating oxidized glutathione (GSSG) from reduced GSH. GSSG is regenerated back to GSH by the GR present in the assay media, at the expense of NADPH. The enzymatic activity was expressed in nmol NADPH/min/mg protein.