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Introduction of trifluoromethyl group into diphenyl diselenide molecule alters its toxicity and protective effect against damage induced by 2-nitropropane in rats

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Abstract

This study was undertaken to investigate if the introduction of trifluoromethyl (F_3C) group into diphenyl diselenide $(PhSe)_2$ molecule causes acute toxicity in rats. It was further examined if the presence of F_3C group in $(PhSe)_2$ molecule alters the protective effect caused by $(PhSe)_2$ against damage induced by 2-nitropropane (2-NP) in rats. To investigate the potential oral toxicity of $(PhSe)_2$ and $(F_3CPhSe)_2$, rats received a single oral application of $(PhSe)_2$ (7.8–312 mg/kg) or $(F_3CPhSe)_2$ (11.2–448 mg/kg). Calculated lethal dose (LD_{50}) for $(PhSe)_2$ and $(F_3CPhSe)_2$ was estimated to be 312 mg/kg (= 1 mmol/kg) and 234 mg/kg (= 0.52 mmol/kg), respectively. Oral administration of $(PhSe)_2$ in rats did not change plasma alanine and aspartate aminotransferase activities (AST and ALT) as well as urea and creatinine levels. The introduction of F_3C group into $(PhSe)_2$ molecule increased AST activity in plasma of rats. $(PhSe)_2$ (3.2 mg/kg = 10 μmol/kg) and $(F_3CPhSe)_2$ (4.48 mg/kg = 10 μmol/kg) protected ALT, AST and γ-glutamyl transferase (γ-GT) activities against the increase caused by oral administration of 2-NP (120 mg/kg) in rats. $(PhSe)_2$ and $(F_3CPhSe)_2$ ameliorated hepatic catalase activity altered by 2-NP in rats. These results indicate that the chemical alteration into $(PhSe)_2$ molecule introduced toxicity and altered its protective effect against damage induced by 2-NP in rats.

Keywords: Selenium; Diphenyl diselenide; *m*-Trifluoromethyl-diphenyl diselenide; 2-Nitropropane; Hepatic damage; Liver; Antioxidant

1. Introduction

Selenium is largely known to develop its biological activity as an integral part of functional selenoproteins, including glutathione peroxidase and thioredoxin reductase, which catalyze reactions essential to the protection of cellular components against oxidative and free radical damage (Rotruck et al., 1973; Burk and Lane, 1983; Bock et al., 1991).

The interest in organoselenides chemistry and biochemistry has increased in the last three decades mainly due to the fact that organoselenium compounds having a potential selenol moiety are good antioxidant candidates (Arteel and Sies, 2001). In addition to their antioxidant property (Muller et al., 1984; Meotti et al.,

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2004), selenium compounds were found to have neuroprotective (Porciúncula et al., 2001; Rossato et al., 2002) antihypertensive, anticancer, antiviral, immunosuppressive, antimicrobial and anti-inflammatory properties (Sies, 1993; Schewe, 1995; May, 1999; Nogueira et al., 2003a; Zasso et al., 2005).

Recent data from our research group reported that diphenyl diselenide $(PhSe)_2$, the simplest of diaryl diselenides, is active as a glutathione peroxidase mimic and is a secure drug when administered acutely to mice at the doses that have anti-inflammatory, antinociceptive, antidepressant-like and anti-ulcer activities (Meotti et al., 2004; Nogueira et al., 2003a; Zasso et al., 2005; Savegnago et al., 2007a, b). Furthermore, the hepatoprotective effect of organoselenium compounds, among them $(PhSe)_2$, on liver damage induced by 2-nitropropane (2-NP) in rats has been reported (Borges et al., 2005, 2006).

More recently, we have closely investigated whether the introduction of functional groups (e.g. chloro, fluoro or methoxyl) into the aromatic ring of $(PhSe)_2$ alters its effect. In fact, $(PhSe)_2$ causes tonic–clonic seizures in mice and the introduction of functional groups into the aromatic ring of this compound (*p*-chloro, *m*-trifluoromethyl, *p*-methoxyl) reduces or abolishes the appearance of seizure episodes (Nogueira et al., 2003b).

In view of this, it would be of considerable significance if the introduction of functional groups into the aromatic ring of $(PhSe)_2$ could provide alternatives to current therapeutic agents. Therefore, the purpose of the present study was to investigate if the introduction of F_3C group into $(PhSe)_2$ ring: (i) causes acute toxicity in rats; (b) alters the protective effect of $(PhSe)_2$ against the damage induced by 2-NP in rats.

2. Materials and methods

2.1. Chemicals

$(PhSe)_2$ and *m*-trifluoromethyl diphenyl diselenide $(F_3CPhSe)_2$ (Fig. 1) were prepared according to the literature methods (Paulmier, 1986). Analysis of the ^1H NMR and ^{13}C NMR spectra showed that $(PhSe)_2$ and $(F_3CPhSe)_2$ presented analytical and spectroscopic data in full agreement with their assigned structures. The chemical purity of $(PhSe)_2$ and $(F_3CPhSe)_2$ (99.9%) was determined by GC/HPLC. Organoselenium compounds were dissolved in canola oil. 2-NP was obtained from Sigma. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

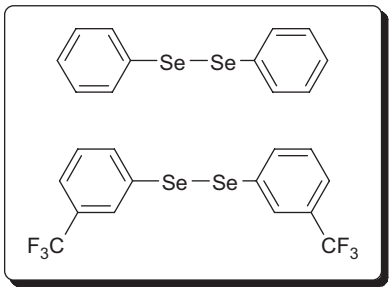

Fig. 1. Chemical structures of diphenyl diselenide (a) and *m*-trifluoromethyl-diphenyl diselenide (b).

2.2. Animals

Male adult albino Wistar rats (200–250 g) from our own breeding colony were used. The animals were kept in a separate animal room, on a 12 h light/dark cycle (with lights on at 6:00 h), at a room temperature of $22 \pm 2\,^{\circ}C$ and with free access to food 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.

2.3. General toxicity and lethal dose

To investigate if the introduction of F_3C group into $(PhSe)_2$ ring alters $(PhSe)_2$ toxicity, five to eight animals per group were usually tested in the experiments. Rats received a single oral application by gavage of $(PhSe)_2$ (7.8–312 mg/kg), $(F_3CPhSe)_2$ (11.2–448 mg/kg) or vehicle (1 ml/kg, canola oil). After administration, animals were observed up to 72 h to determine the lethal dose (LD_{50}) of $(PhSe)_2$ or $(F_3CPhSe)_2$. After 72 h of exposure, rats were anesthetized for blood collection by heart puncture. Plasma was obtained by centrifugation at 2000*g* for 10 min (hemolyzed plasma was discarded) and used for biochemical assays of aspartate aminotransferase (AST), alanine amino transferase (ALT) and urea and creatinine levels.

2.4. Damage induced by 2-NP

Rats received a single oral dose of 2-NP (120 mg/kg; dissolved in canola oil) (groups 2, 5 and 6) on Tuesday, Thursday and Saturday for 2 weeks, according to Fiala et al. (1987). On Monday, Wednesday and Friday animals received orally $(PhSe)_2$ (3.12 mg/kg = 10 μmol/kg) or $(F_3CPhSe)_2$ (4.8 mg/kg = 10 μmol/kg) groups (3, 4, 5 and 6) for 2 weeks. They received selenide and 2-NP treatments every other day. The doses of $(PhSe)_2$ and $(F_3CPhSe)_2$ were based on LD_{50} values of $(PhSe)_2$. The control group received only vehicle (canola oil, 1 ml/kg) (group 1). Five to eight animals per group were usually tested in the experiments.

The protocol of rat treatments is given below:

Group 1 – Canola oil (p.o.) plus canola oil (1 ml/kg, p.o.)