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Convulsant effect of diphenyl diselenide in rats and mice and its relationship to plasma levels

Marina Prigol, Ricardo F. Schumacher, Cristina WayneNogueira, Gilson Zeni*

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

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ABSTRACT

Diphenyl diselenide [$(PhSe)_2$], an organoselenium compound, presents pharmacological and toxicological properties in rodents. The aim of this study was to carry out the determination and quantification of $(PhSe)_2$ in plasma after oral administration (p.o.) of this compound (500 mg/kg), dissolved in canola oil, in rats and mice. The second objective was to verify the involvement of different routes of administration ((p.o.), intraperitoneal (i.p.) and subcutaneous (s.c.)) and vehicle solutions (canola oil and dimethyl sulfoxide (DMSO)) in the appearance of seizure episodes and in the plasmatic levels of $(PhSe)_2$ in rats and mice. Analysis of $(PhSe)_2$ in blood samples was performed by gas chromatography/flame ionized detector system (GC/FID). Rat and mouse peak plasma $(PhSe)_2$ levels were 13.13 and 10.11 μg/ml (C_{max}), respectively, and occurred at 0.5 h (T_{max}) post-dosing. The use of different administration routes (p.o., i.p. and s.c.) and vehicle solutions (canola oil or DMSO) in rats and mice indicated that the appearance of seizures and $(PhSe)_2$ plasmatic levels are dependent of administration routes (i.p. > p.o. > s.c.), vehicle solutions (DMSO > canola oil) and animal species (mice > rat).

1. Introduction

Selenium is an essential trace element nutritionally important to mammals, with physiological roles as a structural component of several antioxidant enzymes involved in the peroxide decomposition (Rayman, 2000; Ursini and Bindoli, 1987). Recent studies about selenium species distribution and metabolic transformation in biological tissues have been reported. These studies show the biological transformation of Se from inorganic Se compounds and selenoamino acids in foods, into excreted selenocompounds via key metabolic intermediates (Schomburg et al., 2004; Suzuki, 2005). The biotransformation involves diffusion of Se compounds into the intestine (Mc Connell and Cho, 1965), transport to organs and utilization to biosynthesize selenoproteins via selenophosphate and selenocysteine, and transformation into selenosugars to be excreted into the breath and urine (Kobayashi et al., 2002; Suzuki et al., 2005).

A number of novel pharmaceutical agents derived from selenium or designed to influence specific aspects of selenium metabolism are under development (Mugesh et al., 2001; Nogueira et al., 2004; Rosa et al., 2007). In spite of extensive literature describing pharmacological properties of organoselenium compounds, little is known about their mode of action (Machado et al., 2006; Prigol et al., 2008a; Rocha et al., 2005; Rosa et al., 2003). Organoselenium compounds are products with a double face due to their contrasting behaviour, which depends on the dose used (Nogueira et al., 2004). Recently the biological activities of diphenyl diselenide $(PhSe)_2$ have been reported and this compound has emerged as a candidate for therapeutic purposes. On one hand, $(PhSe)_2$ has been proven effective against neurotoxicity (Ghisleine et al., 2003), hyperglycemia (Barbosa et al., 2006), lipid peroxidation (Meotti et al., 2004; Luchese et al., 2007), inflammation (Savegnago et al., 2007a), nociception (Savegnago et al., 2007b), anxiety-like (Savegnago et al., 2007c) and depressant-like (Ghisleni et al., 2008; Savegnago et al., 2008) models using rats and mice. On the other hand, $(PhSe)_2$ has been reported to cause neurotoxicity in rodents (Nogueira et al., 2003a; Prigol et al., 2007, 2008b) and inhibition of sulfhydryl enzymes (Barbosa et al., 1998; Maciel et al., 2000; Meotti et al., 2003; Borges et al., 2005; Nogueira et al., 2003b; Prigol et al., 2007). In addition, a different toxicity across species induced by $(PhSe)_2$ has been documented, since the chemical failed to produce any adverse effects in rats, despite a much higher dose. In contrast, a similar dose of the compound induces seizures and death in mice (Nogueira et al., 2003a). The reasons for differential toxicity between mice and rats are presently unknown. Although Maciel et al. (2003) have reported that acute treatment with $(PhSe)_2$ caused a significant increase in Se concentration in liver, kidney and brain, information related to the analysis of $(PhSe)_2$ in biological matrices are limited. Therefore, the literature dealing with detection and quantification of $(PhSe)_2$ concentration in plasma of rats and mice

* Corresponding author. Tel.: +55 55 3220 8140; fax: +55 55 3220 8978.
E-mail address: gzeni@quimica.ufsm.br (G. Zeni).

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is scarce.

The aim of this study was to carry out the determination and quantification of $(PhSe)_2$ after oral administration of this compound, dissolved in canola oil, in plasma of rats and mice. Since seizures induced by $(PhSe)_2$ are dependent of species and route of administration (Nogueira et al., 2003a) the second objective of this study was to verify the involvement of different routes of administration (oral (p.o.), intraperitoneal (i.p.) and subcutaneous (s.c.)) and vehicle solutions (canola oil and dimethyl sulfoxide (DMSO)) in the plasmatic levels of $(PhSe)_2$ and in the appearance of seizure episodes in rats and mice.

2. Materials and methods

2.1. Chemicals

$(PhSe)_2$ was prepared in our laboratory according to the literature method (Paulmier, 1986). Analysis of the ^1H NMR and ^{13}C NMR spectra showed that $(PhSe)_2$ obtained presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of compound (99.9%) was determined by GC/HPLC and was stable under storage conditions at room temperature, humidity and light.

2.2. Animals

Male adult Wistar rats (200–250 g) and male adult Swiss mice (25–30 g) obtained from a local breeding colony were used. The animals were kept in a separate animal room, on a 12 h light/12 dark cycle, in an air conditioned room (22 ± 2 °C). Commercial diet (GUABI, RS, Brazil) and tap water were supplied *ad libitum*. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Brazil.

2.3. Preparation of $(PhSe)_2$ solution

The solution was weekly prepared and the compound was dissolved in canola oil or DMSO. The concentration of $(PhSe)_2$ was analyzed prior to use. The solutions were stored at 2–8 °C and allowed to warm up to room temperature before use. The adult rats and mice were given a single p.o., i.p. or s.c. dosage of 500 mg/kg $(PhSe)_2$. The dosage of $(PhSe)_2$ used in this study is far above the therapeutic range (Savegnago et al., 2007a, 2008), because $(PhSe)_2$ causes neurotoxicity only at very high doses. Therefore, the choice of the $(PhSe)_2$ dosage was based on previous studies dealing with dose–response for appearance of seizures episodes (Nogueira et al., 2003; Prigol et al., 2007).

$(PhSe)_2$ solutions were administered to animals in the morning between 8:00 a.m. and 9:30 a.m. at a dosing volume of 10 ml/kg. The feed was available *ad libitum* and animals were not fasted prior to dosing.

2.4. Experimental design

2.4.1. Determination and quantification of $(PhSe)_2$ in plasma of rats and mice

$(PhSe)_2$ dissolved in canola oil was administered to animals once a day per oral route by gavage. Four animals per group (for each time-point) were anesthetized and blood samples (1 ml) were collected from each animal by heart puncture, in heparin tubes, at 0 (for blank blood sample), 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after the gavage dose.

Blood samples were centrifuged at 3000 rpm for 5 min. The plasma was separated and stored chilled until assay.

2.4.2. Involvement of administration route and vehicle solution in the appearance of seizure episodes and in plasma concentration of $(PhSe)_2$ in rats and mice

The endpoint seizure was chosen to correlate the involvement of different routes of administration and vehicle solutions with plasmatic levels of $(PhSe)_2$ in mice and rats. To this end, $(PhSe)_2$ at the dose of 500 mg/kg dissolved in canola oil or DMSO was administered by i.p., s.c. or p.o. route in mice and rats.

Appearance of seizures was quantified as previously described by Prigol et al. (2007). In short, animals were observed for 1 h in Plexiglas chambers for the appearance of tonic-clonic seizures lasting more than 5 s. The latency for the onset of the first tonic-clonic seizures episode was also recorded. Animals which did not display seizures (1 h) were considered protected.

Three animals per group were anesthetized and blood samples were collected from each animal subsequently to the seizure episodes. Blood samples were obtained as above described in Section 2.4.1.

2.5. Determination and quantification of $(PhSe)_2$ in plasma samples

Determination of $(PhSe)_2$ concentrations in plasma were made in all samples, collected at the time of euthanasia. The plasma (200 μl) was mixed to ethyl acetate in the ratio of 1:1 (v/v) to each tube. The extraction was performed by vortex-mixing the tubes for 3 min. After, the samples were centrifuged at 3000 rpm for 5 min, the supernatants were separated and filtrated through a membrane (0.45 μm pore size) Millipore®. The filtrated was transferred to a clean test tube and 1 μl aliquot was injected into chromatographic system for analysis. Qualitative and quantitative analysis of $(PhSe)_2$ in plasma samples were conducted by gas chromatography (CG 2010 Shimadzu®) associated to flame ionized detector system (FID) and using a 5% diphenyl/95% dimethyl column, 30 m × 0.25 mm × 0.25 μm, from Restek®. The limit of detection (LOD) for $(PhSe)_2$ was 0.5 μg/ml, and the limit of quantification (LOQ) was 1 μg/ml. The standard curve for $(PhSe)_2$ was linear (R_2 = 0.99887) over the concentration range 1–100 μg/ml. The intra- and interday coefficients of variation for drug was <10%. Assay recovery for drug ranged between 90% and 110% of the target at all the concentrations tested with a coefficient of variation <10% to analysis.

The plasma concentration (C), the maximum plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly obtained from plasma data.

2.6. Statistical comparisons

Analysis of variance was performed using one-way (ANOVA) (latency to seizure and plasma $(PhSe)_2$ levels), followed by the Duncan's multiple range. Data are expressed as means ± S.E.M. Values of $p < 0.05$ were considered statistically significant.

Table 1
Plasmatic parameters after oral administration $(PhSe)_2$ dissolved in canola oil in rats and mice.

	Rats	Mice
C_{max} (μg/ml)	13.13 ± 1.29	10.11 ± 1.26
T_{max} (h)	0.5 ± 0.00	0.5 ± 0.00

Data are reported as means ± S.E.D; n = 4.

3. Results

As shown in Table 1, the rat and mouse peak plasma $(PhSe)_2$ levels were 13.13 and 10.11 μg/ml (C_{max}), respectively and occurred at 0.5 h (T_{max}) post-dosing 500 mg/kg $(PhSe)_2$ p.o., dissolved in canola oil. Plasma levels of $(PhSe)_2$ for mice and rats decreased 1 h after

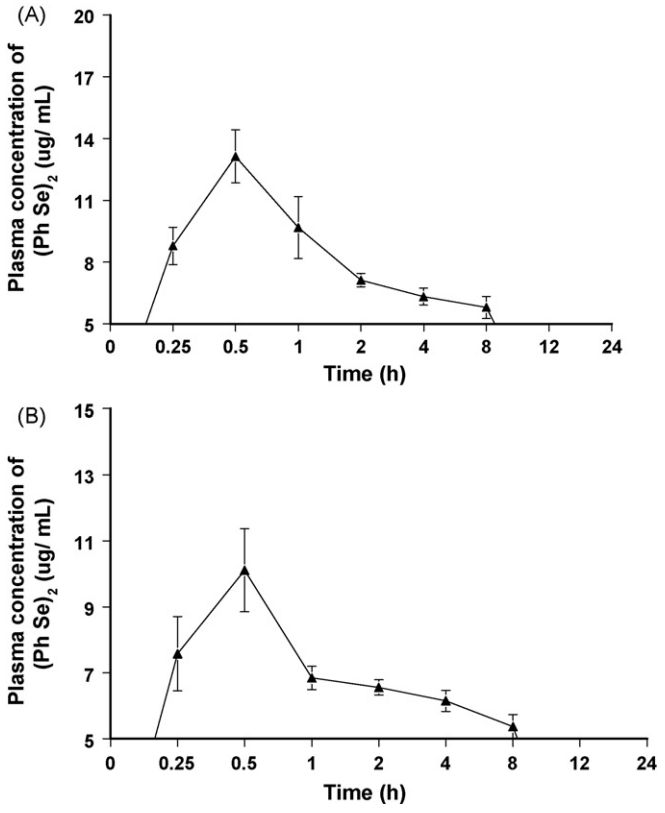

Fig. 1. Plasma concentration–time profile of $(PhSe)_2$ (μg/ml) in rats (a) and mice (b) after oral administration of $(PhSe)_2$ at the dose of 500 mg/kg (▲).