Available online at www.sciencedirect.com
ScienceDirect
Life Sciences 80 (2007) 1865–1872
Life Sciences
www.elsevier.com/locate/lifescie

A biochemical and toxicological study with diethyl 2-phenyl-2-tellurophenyl vinylphosphonate in a sub-chronic intraperitoneal treatment in mice

Daiana Silva Ávila, Priscila Gubert, Cristiane Lenz Dalla Corte, Diego Alves, Cristina Wayne Nogueira, João Batista Teixeira Rocha, Félix Alexandre Antunes Soares *

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

Received 24 November 2006; accepted 15 February 2007

Abstract

Diethyl-2-phenyl-2-tellurophenyl vinylphosphonate (DPTVP) is an organotellurium compound with low toxicity after subcutaneous administration in mice. This study evaluated possible in vivo and ex vivo toxicological effects of daily injections of DPTVP for 12 days in mice, using the intraperitoneal administration. This route potentially increases the pharmacokinetics of absorption, distribution, metabolism and toxicity of DPTVP. Treatment with DPTVP (0, 30, 50, 75, 100, 250, 350 or 500 μmol/kg) were not associated with mortality or body weight loss. Nevertheless, the liver and liver-to-body weight ratio increased in groups treated with 350 and 500 μmol/kg of DPTVP. However, plasmatic aspartate and alanine aminotransferase activities (classical markers of hepatotoxicity) were not increased after diethyl-2-phenyl-2-tellurophenyl vinylphosphonate administration. Hepatic, renal and cerebral thiobarbituric acid reactive substances (TBARS), δ-ALA-D activity and Vitamin C levels were not modified after DPTVP treatment. Renal and hepatic superoxide dismutase (SOD) and catalase (CAT) were unchanged after DPTVP treatment. Conversely, SOD activity significantly increased in brain in groups treated with 50, 75, 100 and 500 μmol/kg of DPTVP treated groups. Our findings corroborates that brain is a potential target for organochalcogen action. The absence of severe overt signs of toxicity after sub-chronic exposure to DPTVP reinforces the necessity for more detailed pharmacological studies concerning this new organotellurium compound.

Keywords: Tellurium; Toxicity; Mice; Intraperitoneal injections; Antioxidant enzymes; Toxicity biomarkers

Introduction

Despite the growing use of organotellurium compounds in the chemical and biochemical fields, there has been little concern about their toxicity. In fact, these compounds have been shown as promising and useful alternatives for numerous synthetic operations in organic synthesis, especially vinylic tellurides (Petragnani, 1994; Zeni et al., 2003, 2006; Comasseto et al., 1997). Consequently, it is important to advance our understanding about their toxicological properties.

Previous studies have indicated that organotellurium compounds are potentially toxic and lethal to rodents at low doses (Meotti et al., 2003; Savegnago et al., 2006). Indeed, tellurides can cause citotoxicity (Sailer et al., 2003, 2004; Iwase et al., 2004; Rooseboom et al., 2002), hepatotoxicity (Meotti et al., 2003), glutamatergic system alterations (Nogueira et al., 2001, 2002), and teratogenic effects (Stangherlin et al., 2005). In addition, organotellurium compounds can inhibit cysteinyl-containing enzymes, such as Na^+/K^+ ATPase (Borges et al., 2005), δ-ALA-D (Maciel et al., 2000; Nogueira et al., 2003a,b; Barbosa et al., 1998) and squaleno monooxigenase (Laden and Porter, 2001), Thus, the toxicity of organotellurides can be related to the interaction with thiol groups of important biomolecules (Nogueira et al., 2004), the replacement of selenium in selenoproteins, such as thioredoxin (Engman et al., 2000), and the capacity of the tellurium compounds to induce the formation of reactive oxygen species (Chen et al., 2001).

In spite of this, there has been a considerable interest in organotellurium compounds as potential antioxidants in living systems against several pro-oxidant agents, such as hydrogen

* Corresponding author. Tel.: +55 55 3220 8140; fax: +55 55 3220 8031.
E-mail address: felix_antunes_soares@yahoo.com.br (F.A.A. Soares).

0024-3205/$ - see front matter © 2007 Published by Elsevier Inc.
doi:10.1016/j.lfs.2007.02.033

peroxide, peroxynitrite, hydroxyl radicals and superoxide radical anion (Ren et al., 2001; Wieslander et al., 1998; Engman et al., 1995; Briviba et al., 1998; Kanski et al., 2001; Jacob et al., 2000), since these compounds may mimic glutathione peroxidase activity (GPX) (Andersson et al., 1993; Engman et al., 1992, 1994). This property is thought to be due to oxidation of Te from the divalent to the tetravalent state. Besides, tellurides are promising antitumoral drugs and their chemoprotective effects can be related to their cytotoxic properties and to their ability to inhibit important enzymes necessary for the tumor growth. (Rao et al., 1996; Cunha et al., 2005; Engman et al., 2000). These pharmacological properties are much more evident in organotellurium compounds than in selenium or sulfur analogues, making these compounds extremely attractive in medical therapies.

Diethyl 2-phenyl-2-tellurophenyl vinylphosphonate (DPTVP) is an asymmetric telluride used as an intermediate of great synthetic potential because it combines the chemical reactivity of vinylic tellurides and vinylic phosphonates (Zeni et al., 2003; Braga et al., 2001). In a previous study, this compound, showed a potent antioxidant activity in vitro and an unexpected very low toxicity in vivo, when injected subcutaneously (Ávila et al., 2006). The in vitro antioxidant activity was similar to that of diphenyl ditelluride, which is much more toxic than DPTVP.

Considering the low in vivo toxicity exhibited by DPTVP in s.c. protocol (Ávila et al., 2006), we were encouraged to investigate its possible toxicological/pharmacological effects using a different via of administration (intraperitoneal). Considering our intend we exposed rodents to DPTVP in a sub-chronic treatment and we evaluated biochemical and toxicological parameters, such as the level of lipid peroxidation, δ-ALA-D activity, antioxidant system (catalase and superoxide dismutase activity and Vitamin C), the levels of glutathione (GSH) and hepatic damage (AST and ALT).

Materials and methods

Chemicals

DPTVP (Fig. 1) synthesis was performed by addition of alkynylphosphonates to a solution of sodium organyl tellurolate, prepared by the reduction of diorganyl ditellurides with sodium borohydride in ethanol at room temperature (Braga et al., 2000).

Animals

Adult mice from our own breeding colony were maintained in an air conditioned room (20–25 °C) under natural lighting conditions with water and food (Guabi-RS, Brasil) ad libitum. All experiments were conducted in accordance with the Guiding Principles for the Use of Animals in Toxicology, adopted by the Society of Toxicology in July 1989.

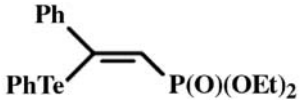

Fig. 1. Chemical structure of diethyl-2-phenyl-2-tellurophenyl vinylphosphonate.

Table 1
Organ weight/body weight ratio (g/g) of animals treated for twelve days with diethyl-2-phenyl-2-tellurophenyl vinylphosphonate

Dose (μmol/kg)	Liver	Kidney	Brain
0	0.052±0.003	0.013±0.001	0.013±0.001
30	0.054±0.003	0.013±0.001	0.015±0.001
50	0.060±0.003	0.014±0.001	0.014±0.001
75	0.058±0.003	0.014±0.001	0.015±0.001
100	0.052±0.003	0.014±0.001	0.015±0.001
250	0.058±0.003	0.014±0.001	0.015±0.001
350	0.064±0.004 *	0.014±0.001	0.014±0.001
500	0.066±0.003 *	0.015±0.001	0.014±0.001

Data are expressed as mean±S.E.M for eight animals in each group.
* Indicates statistical difference from control group ($p<0.05$, Duncan post-hoc test).

Animal's treatment

Mice were divided into eight groups and daily weighted and treated for 12 days with diethyl-2-phenyl-2-tellurophenyl vinylphosphonate 0 (canola oil), 30, 50, 75, 100, 250, 350 and 500 μmol/kg) intraperitoneally (i.p.—10 mol/kg). After the 12th day, animals were anesthetized to the collection of blood via heart puncture and killed by decapitation.

Tissue preparation

Brain, liver and kidney were removed, weighed, homogenized (1:7, 1:10, 1:7, respectively) in Tris–HCl 10 mM buffer, pH 7.4 and centrifuged at 4000 ×*g* for 10 min at 4 °C. The low speed supernatant fraction obtained (S1) was maintained in ice-cold for the assays. Separately, small quantities of brain, liver and kidney were separated to perform GSH/GSSG levels determination.

Ex vivo assays

Lipid peroxidation

Lipoperoxidation in liver, kidney and brain S1 was assessed by the measurement of thiobarbituric reactive substances (TBARS, Ohkawa et al., 1979). TBARS were determined spectrophotometrically at 532 nm after one hour of incubation with SDS 8.1%, Acetic acid/HCl buffer and thiobarbituric acid 0.6% at 95 °C, using Malondialdehide as standard.

δ-ALA-D activity

Mice δ-ALA-D activity was assayed according to the method of Sassa (1982), by measuring the rate of product (porphobilinogen, PBG) formation, except that 84 mM potassium phosphate buffer, pH 6.4, and 2.5 mM ALA were used. All experiments were carried out after a 15 min preincubation of S1 with the medium, starting the reaction by adding the substrate, aminolevulinic acid. Incubation was carried out for 1, 2 and 3 h for liver, kidney and brain, respectively, at 37 °C. The reaction