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Organoselenium compounds have been pointed out asl therapeutic agents. In contrats, the potential therapeutic of tellurides has not yet been demonstrated. The present study evaluated in a comparative way the potential toxicological effects of diphenyl diselenide $(PhSe)_2$ and diphenyl ditelluride $(PhTe)_2$ in mice after in vivo administration. Genotoxicity (as determined by comet Assay) and mutagenicicity were used as end-points of toxicity. Subcutaneous administration of high doses of $(PhSe)_2$ or $(PhTe)_2$ (500 μmol/Kg) caused distinct genotoxicity in mice. $(PhSe)_2$ significantly decreased the DNA damage index after 48 and 96 hours of its injection ($p<0.05$). In contrast, (PhTe) caused a significant increase in DNA damage ($p<0.05$) after 48 and 96 hours of intoxication. $(PhSe)_2$ did not show mutagenicity but $(PhTe)_2$ exhibited an increase in mutagenicicity as detected by an increase in the micronuclei frequency. Thus, this study demonstrated that after acute in vivo exposure ditelluride caused genotoxicity in mice; which may be associated with pro-oxidant effects of diphenyl ditelluride. These results indicated that exposure to ditelluride can be genotoxic to mice and that the use of this compound and possibly other related tellurides must be carefully controlled.

Diphelyl Ditelluride Causes Acute Genotoxicity in Adult Mice, Whereas Diphenyl Diselenide Has a Protective Effect

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Abstract

Organoselenium compounds have been pointed out asl therapeutic agents. In contrats, the potential therapeutic of tellurides has not yet been demonstrated. The present study evaluated in a comparative way the potential toxicological effects of diphenyl diselenide $(PhSe)_2$ and diphenyl ditelluride $(PhTe)_2$ in mice after in vivo administration. Genotoxicity (as determined by comet Assay) and mutagenicicity were used as end-points of toxicity. Subcutaneous administration of high doses of $(PhSe)_2$ or $(PhTe)_2$ (500 μmol/Kg) caused distinct genotoxicity in mice. $(PhSe)_2$ significantly decreased the DNA damage index after 48 and 96 hours of its injection ($p<0.05$). In contrast, (PhTe) caused a significant increase in DNA damage ($p<0.05$) after 48 and 96 hours of intoxication. $(PhSe)_2$ did not show mutagenicity but $(PhTe)_2$ exhibited an increase in mutagenicicity as detected by an increase in the micronuclei frequency. Thus, this study demonstrated that after acute in vivo exposure ditelluride caused genotoxicity in mice; which may be associated with pro-oxidant effects of diphenyl ditelluride. These results indicated that exposure to ditelluride can be genotoxic to mice and that the use of this compound and possibly other related tellurides must be carefully controlled.

Keywords: Organotellurium, Organoselenium, Genotoxicity, Mutagenicity and Cytotoxicity.

Introduction

Selenium (Se) and Tellurium (Te) belongs to the chalcogen family, sharing similar electronic configuration and some chemical properties with sulfur (S) (Comasseto et al., 1997; Comasseto, 2010). Se has a fundamental role in several living organisms as component of several antioxidant enzymes, including glutathione peroxidase and thioredoxin reductase (Arner et al., 2000; Nogueira & Rocha., 2011). Despite its biological role, the excess of selenium can be toxic due its ability to generate free radicals and catalyze thiol oxidation (Barbosa et al., 1998; Nogueira, Zeni & Rocha, 2004; Rocha et al., 2012; Hassan & Rocha, 2012; Kade et al. 2013). The excess of free radical formation can damage mammalian tissues including thiol containing enzymes that are sensitive to pro-oxidant situations (Rocha et al., 2012 ; Rosa et al., 2007; Maciel et al., 2000). Diphenyl diselenide $(PhSe)_2$, (Fig. 1) is a simple and stable organoselenium compound widely used in organic synthesis and it has been proposed as good candidate for pharmacological and therapeutic purposes (Nogueira, Zeni & Rocha, 2004; Rosa et al. 2007; Nogueira & Rocha, 2011) . $(PhSe)_2$ exhibits thiol peroxidase-like activity superior to that of ebselen, an organoselenium compound that was used in clinical trial as antioxidant and mimetic of native glutathione peroxidase enzyme (Nogueira & Rocha., 2011; Kade & Rocha, 2013; Kade et al. 2013). However, exposure to high doses of $(PhSe)_2$ can deplete thiols in different tissues and can be neurotoxic to rodents (Maciel et al., 2000).

There are reports that trace amounts of Te are present in body fluids such as blood and urine (Chasteen et al., 2009). Te has also been found in the form of tellurocysteine and telluromethionine in several proteins in bacteria, yeast and fungi but no telluroproteins have been identified in animal cells (Bienert et al., 2008). Thus, in contrast to selenium, tellurium does not have any biological function (Taylor, 1996). Literature has demonstrated immunomodulatory, antioxidant and anticancer properties of various organotellurides (Nogueira, Zeni & Rocha, 2004; Avila et al., 2012), semisynthetic tellurosubtilisin (Mao et al., 2005) and dendrimeric organotellurides (Francavilla et al., 2001). More sophisticated telluride molecules were synthesized from polystyrene nanoparticle via microemulsion polymerization. The nanoenzyme showed higher efficiency and provided a platform for the synthesis and designing of polymeric nanoparticles as excellent model of enzyme mimics (Huang et al., 2008). Organotellurium compounds can also mimic glutathione peroxidase activity (Engman et al., 1995) and, consequently, these compounds can be potential antioxidants, effective against some cell damaging agents, such as hydrogen peroxide, peroxynitrite, hydroxyl radicals and superoxide anions (Anderssonet al., 1994; Kanski et al., 2001; Jacob et al., 2000).

Recently, our research group demonstrated that organoselenium and organotellurium present hemolytic and genotoxic effects in human blood cells (Santos et al., 2009), which is in accordance with results published by other laboratories in experimental models of bacteria and rodents (Degrandi et al., 2010). Similarly both organoselenides and tellurides can be toxic in different in vivo and in vitro models of

64 animal pathologies (Maciel et al., 2000; Taylor, 1996; Stangherlin et al., 2009; Moretto et al., 2007;
65 Heimfarth et al., 2011; Heimfarth et al., 2012 a; Heimfarth et al., 2012 b; Comparsi et al., 2012) and is
66 found to be extremely toxic to mice upon acute or prolonged exposure (Maciel et al., 2000; Heimfarth et
67 al., 2012 b ; Comparsi et al., 2012). The toxicity of tellurides can be associated with their pro-oxidant
68 activity, particularly, the oxidation of thiol- and selenol-groups of important body proteins (Nogueira, Zeni
69 & Rocha, 2004; Comparsi et al. 2012; Hassan & Rocha 2012).
70 Following our interest to determine the boundary between the potential protective and toxic properties of
71 organochalcogens, the present study was designed to evaluate the toxic potential of $(PhSe)_2$ and $(PhTe)_2$ in
72 in mice. We have determined the genotoxicity and mutagenicity of these compounds after acute
73 administration to swiss male mice using DNA damage and micronuclei frequency as end-points of
74 toxicity.

MATERIAL AND METHODS

Chemicals

The chemical structure of organochalcogens tested in this study is shown in (Fig.1) diphenyl diselenide and (II) diphenyl ditelluride. The compounds were dissolved in canola oil immediately before use. $(PhSe)_2$ and $(PhTe)_2$ were obtained from Sigma-Aldrich. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Animals

Male Swiss adult mice weighing 30-40 g were obtained from our own breeding colony (Animal house-holding, UFSM- Brazil). Animals were kept in separate animal cages, on a 12-h light/dark cycle, at a room temperature of (23ºC ± 3) 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 of the Federal University Of Santa Maria, Brazil (23081.002435/2007-16).

Mice were divided in six groups (n=4) and received a single subcutaneous injections of (1) canola oil (Control group 48h, mice were euthanized 48 hours after the oil injection); (2) diphenyl ditelluride (500 µmol/kg in canola oil, euthanized 48 hours after injection) ; (3) diphenyl diselenide (500 µmol/kg in canola oil, euthanized 48 hours after injection); (4) canola oil (Control group 96h, mice were euthanized 96 hours after injection); (5) diphenyl ditelluride (500 µmol/kg in canola oil, euthanized 96 hours after injection) and (6) diphenyl diselenide (500 µmol/kg in canola oil, euthanized 96 hours after injection). The doses were based in a previous acute toxicological study as reported by Maciel et al. 2000.

Sample preparation for Comet Assay

After the treatment, animals were anesthesized with ketamine and 2.5 ml blood samples were collected by heart puncture and immediately euthanized by decaptation. Mice blood leukocytes were isolateds and used in the comet test but no pre-incubation was carried out (Santos et al. 2009(a); (b); Meinerz et al. 2011).

Micronucleus test

In micronucleus test (MN), two samples of total blood from each animal were placed in a microscope slides and air dried at room temperature. Slides were stained with 5% May-Grunwald-Giemsa for 5 min. The criteria used for the identification of MN were a size smaller than one-third of the main nucleus, no attachment to the main nucleus, and identical color and intensity as in the main nucleus. MN were counted in 2000 cells with well-preserved cytoplasm and calculated as: % MN = number of cells containing micronucleus X 100 / total number of cells counted. Micronuclei presence was determined by three investigators that were blind to the animal treatments.

Comet assay

Comet assay is a rapid, simple and sensitive technique for measuring DNA breaks and repair in single cells. This test has been used to investigate the effect of many toxic agents on DNA (Collins et al., 2002; Blasiak et al., 2004). The comet assay was performed under alkaline conditions according procedure of Santos et al. 2009 (a) and Santos et al., 2009 (b). The slides obtained from white cells of treated mice were analyzed under blind conditions by at least two individuals. DNA damage is presented as DNA damage index (DI). The DNA damage was calculated from cells in different damage classes (completely undamaged: 100 cells × 0 to maximum damaged − 100 cells × 4). Damage index is illustrated in Figure 2 and classes were determined considering the DNA tail and DNA migration length.

Statistical analysis

Data are expressed as mean ± SD from 5 independent experiments performed in duplicate or triplicate. Statistical analysis was performed using Kruskawallis test followed by Dun´s test. Results were considered statistically significant when $p<0.05$.

RESULTS

No animal died during the experimental period. After 48 hours of diselenide or ditelluride treatment, mice did not show typical symptoms associated with toxicity such as stereotypical behavior, ataxia, diarrhea, increased dieresis or abdominal writings. However, after 96 hours, the group treated with $(PhTe)_2$ presented diarrhea, low level of motor activity and decrease of body weight (data not shown); which is in accordance with previous finding from our laboratory (Maciel et al. 2001).

Comet assay

After *in vivo* administration, diphenyl diselenide caused a significant decrease in DNA damage index (DI) both after 48 and 96 hours. In contrast, diphenyl ditelluride caused a significant increase in DNA damage index (DI). After 48 hours, the damage caused by ditelluride was about 25 and 100% higher than control and diphenyl diselenide groups, respectively (Table 4). After 96 hours, the DI caused by diphenyl ditelluride was about 30 and 90% higher than control and diselenide treated mice (Table 4).

Micronucleus test

After 48 or 96 hours of a single dose of diphenyl ditelluride, a significant increase in the number of micronuclei was observed in male adult mice when compared with control and diphenyl diselenide group (Figure 3). Diphenyl diselenide did not modify the number of micronuclei when compared to control group (Figure 3).

DISCUSSION

The results presented here indicates a clear toxic effect of $(PhTe)_2$ when compared with $(PhSe)_2$. Tellurium (Te) has the potential of redox cycling which leads to formation of reactive oxygen species (ROS) thus triggering oxidative damage to bio-significant molecules from proteins to lipids and nucleic acids (Maciel et al.,2000 ;Nogueira, Zeni & Rocha, 2004; Santos et al., 2009; Degrandi et al., 2010; Sailer et al., 2004). Organotellurium-induced intracellular ROS accumulation has been reported to be the cause of cell death in HL-60 and different type of cancer cells (McNaughton et al., 2004; Juan et al., 2010; Ding et al.,2002; Rigobello et al., 2009). In contrast, exposure of mice to $(PhSe)_2$ caused a significant decrease in the DNA damage index (DI) both after 48 and 96 hours of drug administration as shown in Table 1. The protective effect can be attributed to its anti-oxidant or GPx like activity (Nogueira & Rocha, 2011).

As observed in DNA damage test, the toxic behavior of $(PhTe)_2$ was completely different than $(PhSe)_2$ in micronucleus assay. The frequency of mutations, showed by an increase of micronuclei frequencies, reinforce the toxicity of $(PhTe)_2$. It is important to note that $(PhSe)_2$ did not modify the number of micronuclei, when compared to control group (Figure 3). Previous studies have also demonstrated mutagenicicity of $(PhTe)_2$ at higher concentrations in V79 cells (Rosa et al., 2007). While more recently, we have reported the mutagenicity of another Te-containing organic compound, (*S*)-dimethyl 2-(3-(phenyltellanyl) propanamido) succinate in mice leucocytes (Meinerz et al., 2011). These effect of tellurides can be associated with their pro-oxidant properties (Nogueira, Zeni & Rocha, 2004).

In conclusion, the results presented here indicate that diphenyl ditelluride is toxic to mice, whereas at the same dose diphenyl diselenide had protective effects. This data supports studies that have been published about the toxicological and pharmacological effects of organochalcogens in different pathological models. These effects may be linked to the pro-oxidant activity exhibited by organotellurium compounds. In effect, our data indicated that diphenyl diselenide can have protective effects after in vivo administration to mice, which can be related to its antioxidant properties, whereas diphenyl ditelluride is much more toxic than diphenyl diselenide. Furthermore, in view of the genotoxic effect of $(PhTe)_2$, the indication in the literature that organotellurides could be therapeutically active compounds must be revisited taking into consideration the potential toxicity of this element. Accordingly, additional studies will be needed to elucidate the mechanism(s) by which $(PhTe)_2$ mediates its toxicity and whether or not distinct chemical forms of organotellurides can have similar toxic effect in animal models.

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Figure 1

Structure of Diphenyl Diselenide and Diphenyl Ditelluride

Fig. 1 Structure of diphenyl diselenide and diphenyl ditelluride.

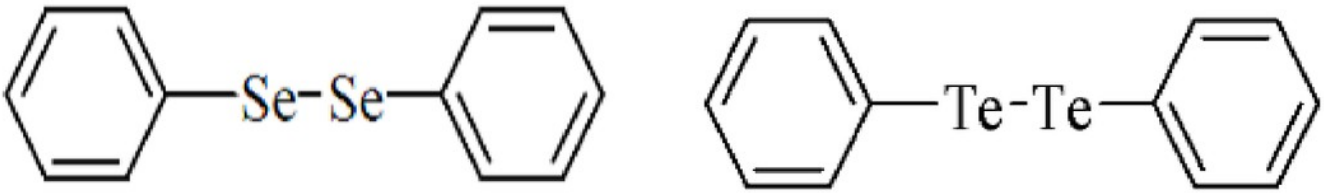

Figure 2

DNA damage quantification

Classifications of DNA damage in human leukocytes. DNA damage index was calculated from cells in different damage levels, which were classified in the visual score by the measurement of DNA migration length and in the amount of DNA in the tail. The level 5 was excluded of our evaluation. pared.

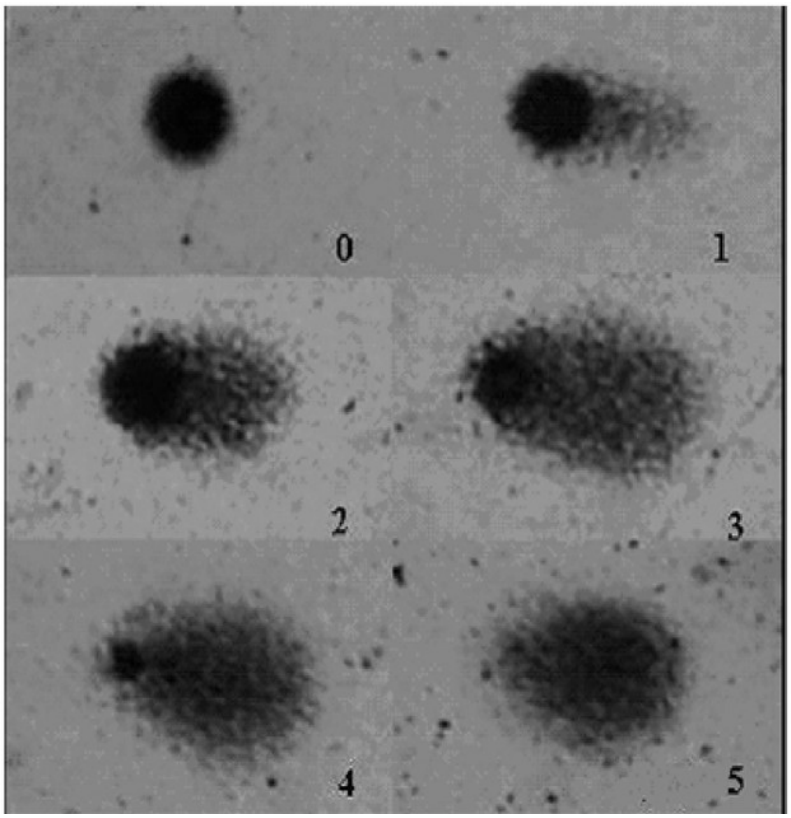

Figure 3

Micronuclei Frequency after Treatment with Diselenide and Ditelluride

Figure 3. Frequency of Micronuclei (MN) cells in mice exposed to $(PhTe)_2$ or $(PhSe)_2$. Mice were exposed to a sigle dose of diselenide or ditelluride (500 µmol/kg, s.c.). Forty eight and 96 hours after the injection, blood cells were examined for the presence of micronuclei. Data are expressed as mean±SD for 5 mice per group. * Denoted $p > 0.01$ as compared to control group; # Denoted $p > 0.01$ as compared to diphenyl diselenide.

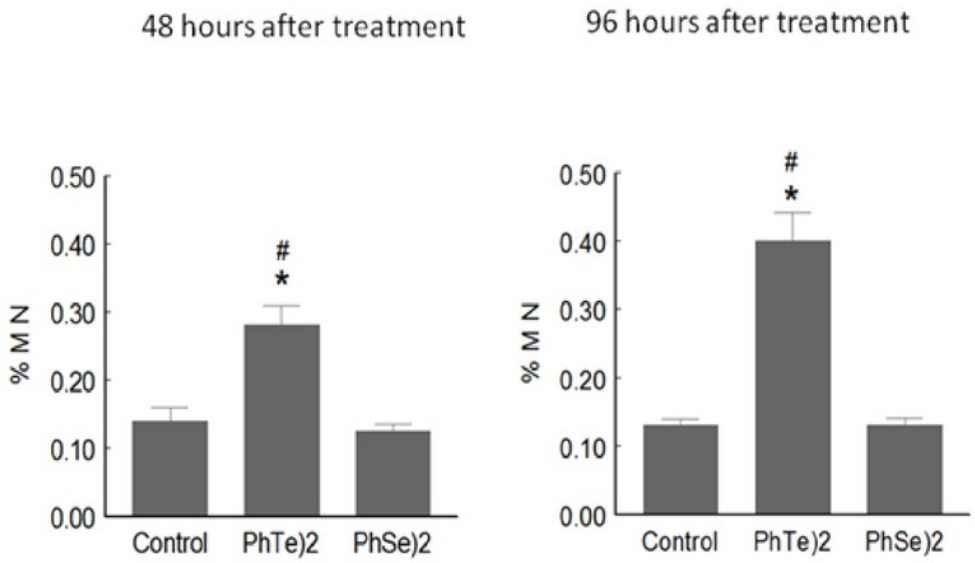

Table 1(on next page)

DNA Damage Levels in Leucocytes from Mice Treated with Diselenide or Ditelluride

Table 1. Distribution of damage levels in mice leukocytes exposed to diphenyl diselenide and diphenyl ditelluride (500 µmol/kg, s.c.) DNA damage is presented as DNA damage index (DI). Data are expressed as means for five independent experiments. Statistical analysis by Kruskawalis test followed by Dun´s test.

Compound	Hours of Exposition	Damage levels of DNA					DI
		0	1	2	3	4	
Control	**48h**	61.0±0.5	19.6±2.0	13.4±1.4	4.5±0.8	1.0±0.5	63.0±2.5[a]
$(PhSe)_2$	**48h**	77.2±3.6	11.8±1.6	6.6±1.3	3.8±1.1	0.6±0.2	40.8±7.8[b]
$(PhTe)_2$	**48h**	48.0±9.7	32.3±9.6	13.0±3.2	5.0±1.0	1.6±0.6	80.0±9.3[c]
Control	**96h**	63.5±0.5	20.7±6.5	12.5±5.5	3.7±0.5	0.0±0.0	58.0±4.6[a]
$(PhSe)_2$	**96h**	80.0±2.0	10.0±2.0	5.0±3.0	3.0±0.6	2.0±2.0	40.0±1.1[b]
$(PhTe)_2$	**96h**	59.5±3.5	19.0±7.0	12.0±3.0	9.2±0.8	1.6±0.5	76.0±1.2[c]

1 **Table 1.** Distribution of damage levels in mice leukocytes exposed to diphenyl diselenide and diphenyl
2 ditelluride (500 µmol/kg, s.c.)
3 .

4 DNA damage is presented as DNA damage index (DI). Data are expressed as means for five independent
5 experiments. Statistical analysis by Kruskawalis test followed by Dun´s test.