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# Comparative Deposition of Diphenyl Diselenide in Liver, Kidney, and Brain of Mice

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The essential trace element selenium (Se) is a micronutrient that plays a major role in preventing lipid peroxidation *in vivo*. Se is an essential component of several enzymes with physiological antioxidant properties, including different classes of glutathione peroxidase (Fohé et al. 1973; Rotruck et al. 1973; Ursini et al. 1982). Furthermore, in animal experiments Se supplementation reduces the incidence of both chemically induced and spontaneously occurring cancers (Ganther 1999), and causes a dramatic decrease in the incidence of coronary artheriosclerosis (Salonen et al. 1988) and of ischemic metabolic changes (Kondoh et al. 1999). However, ingestion of high quantities of Se can be toxic for mammals (Kim and Mahan 2001).

In 1941, Painter first proposed that inorganic Se toxicity was due to its interaction with thiols. Organic Se compounds can also oxidize –SH groups by a variety of mechanisms (Barbosa et al. 1998; Chen and Ziegler 1994; Ganther 1968; Farina et al. 2001, 2002). Diaryldiselenides compounds are simple synthetic intermediaries in organic synthesis (Braga et al. 1997) which increase the risk of exposure in the workplace. Furthermore, based on their thiol-peroxidase like activity (Wilson et al. 1989), diaryldiselenides have been considered as possible potential therapeutic agents (Jaques-Silva et al. 2001, Rossato et al. 2002).

In recent work, we showed that *in vivo* exposure to diphenyl diselenide inhibits δ-Aminolevulinate dehydratase (δ-ALA-D) (Maciel et al. 2000), a thiol-containing enzyme, which is essential for all aerobic organisms because participates in the biosynthesis pathway of prosthetic groups of physiologically significant proteins such as hemoglobin and cytochromes (Folmer et al. 2002, Jaffe et al. 1995, Sassa 1998). However, data concerning Se-distribution in tissues after exposure to diphenyl diselenide are lacking in the literature. Taking into account the potential pharmacological use of diorganyl diselenides, the aim of this work was investigate the Se deposition in different tissues after *in vivo* exposure to diphenyl diselenide.

## MATERIALS AND METHODS

Adult male mice (3 months old) weighing 30 to 45 g 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,.Brazil) ad libitum.

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Exposure to diphenyl diselenide was performed essentially as described by Maciel et al. (2000). In acute exposure (a single dose), mice were weighed and injected subcutaneously with dimethyl sulfoxide (DMSO) (control, 5 mL/kg) or 1 mmol/kg diphenyl diselenide (in an equivalent volume of DMSO) and killed 24 hours after drug injection. For chronic exposure (14 doses), mice were weighed and injected subcutaneously once a day with DMSO (2.5 mL/kg) or 125 or 250 μmol/kg diphenyl diselenide and killed 24 hours after the last injection.

Total Se assays were made using a Perkin-Elmer (Norwalk, CT, USA) model 3030 atomic absorption spectrometer equipped with an MHS-10 hydride-generation system. A Se hollow cathode lamp was used as a light source, operated at a current of 16 mA (Perkin-Elmer lamp). The wave-length was set to 196.0 nm, and a spectral band-width of 2.0 nm was selected. The decomposition step was performed using a commercial microwave system specially designed for sample decomposition under pressure (Provecto Systems, model DGT 100, maximum pressure of 130 atm and 1,000 W of power), provided with six vessels of polytetrafluotethylene. Nitric and hydrochloric acids were distilled twice in a sub-boiling system (Berghof). All other reagents were of analytical grade (p.a., Merck, Darmstadt, Germany). Distilled and deionized water (maximum conductivity of 1.3 μS/cm) was used. Sodium tetrahydroborate solution (1%, w/v) was prepared by dissolving $NaBH_4$ powder in 1% (w/v) sodium hydroxide. The solution was filtered and prepared fresh daily. A commercial standard of 1000 mg/l Se stock solution (Merck) was used to prepare solutions of lower concentration. After sacrifice, liver, kidney and brain samples were removed from the animals and homogenized and subsequently were analyzed. About 0.3 g of each sample was placed inside the polytretraflourethylene vessel and 4 ml of $HNO_3$ (65%, v/v) were added. After 30 min, the vessel was capped and heated twice for 8 min at 500 W in a microwave oven. After cooling to room temperature in a cold water-bath, the polytetrafluorethylene vessels were opened to release the pressure. A 0.5 ml aliquot of $H_2O_2$ (30%, w/w) recapped tightly, was placed in a microwave oven again and heated for 5 min at 600 W. This last step was repeated one more time. The digests thus obtained were treated by the method of Lan et al. (1994); digests were transferred to glass tubes, 5 ml of 6 mol/l HCl were added and the mixture was placed in a boiling water-bath for 30 min to reduce any selenate to selenide. After cooling to room temperature, the solution was quantitatively transferred to a 25 ml volumetric flask and made up to volume with 1 mol/l HCl. Se was then determined by hydride generation atomic absorption spectrometry (HGAAS). Aliquots of different sample volumes (according to Se concentration) were dispensed into a reaction vessel connected to the MHS-10 system. The $NaBH_4$ solution in the reservoir was pumped into the vessel for 15 sec., during which time the hydride selenide generated was swept with argon into the quartz cell for absorbance measurements. The reference solutions of Se added to the digests were recovered to ascertain the results from the analytical curve. The additions were performed to obtain two or three times the original value without addition (results were close to a total recovery from 97% up to 101% and no difference in linearity from the analytical curve was observed). The concentrations of reference Se solutions were 0.75, 1.00, 1.25, 1.50, 2.00 and 2.50 μg/l. Dilution with 1 mol/l HCl was made when necessary. The detection limit was 0.5 μg/l and the

characteristic mass was 80 pg. The analytical curve was linear from 0.50 up to 2.50 μg/l. Blank solutions were prepared and analyzed together with the samples.

Data were analyzed by one- and two-way ANOVA, followed by Duncan's Multiple Range Test when appropriate. Correlation coefficients were determined by linear regression analysis. Differences were considered to be significant when $P \leq 0.05$.

## RESULTS AND DISCUSSION

Acute treatment with 1 mmol/kg diphenyl diselenide did not affect the mice body weight. In contrast, chronic exposure to diphenyl diselenide caused a significant decrease in animals body weight gain (data not show). The decrease in body weight was similar to that published previously (Maciel et al. 2000). Acute treatment with 1 mmol/kg diphenyl diselenide did not affect the liver-, kidney- or brain-to-body weight ratio (data not shown). Brain- and kidney-to-body weight ratios were not changed by chronic exposure to diphenyl diselenide (data not shown). In contrast, chronic exposure to diphenyl diselenide caused a two-fold increase by in liver-to-body weight ratio (data not shown). The increase in this ratio was similar to that published previously (Maciel et al. 2000).

Acute treatment with 1 mmol/kg diphenyl diselenide caused a significant increase in Se concentration in liver (3.1-fold; $p<0.01$), kidney (2.7-fold; $p<0.01$), and brain (3.1-fold; $p<0.01$) (Figure 1). In addition, total Se deposition in kidney and liver was significantly higher than in brain ($p<0.01$). In fact, after a single dose of diphenyl diselenide, the concentration of Se deposition (μg/g) in liver and kidney was 3 to 4 times higher than that found in brain.

Chronic exposure (14 doses) to diphenyl diselenide caused a dose dependent increase in Se-deposition in liver (4.9 –fold for 125 μmol/kg, $p<0.01$ and 8.7-fold for 250 μmol/kg), in brain (1.8–fold for 125 μmol/kg, $p<0.01$ and 3.1-fold for 250 μmol/kg) (Figure 2) and in kidney (3.0–fold for 125, $p<0.01$ μmol/kg and 3.5-fold for 250 μmol/kg) (Figure 2). A statistically significant positive correlation was observed between the dose administrated and Se-deposition in liver ($r = 0.93$ $p<0.01$), kidney ($r = 0.83$, $p<0.05$), and brain ($r = 0.96$, $p<0.001$). Deposition of Se after chronic exposure to diphenyl diselenide was higher in liver, followed by kidney and brain. In fact, total Se levels in liver were 2.3- and 3.6-fold higher than in the kidney of mice treated with 125 and 250 μmol/kg of diphenyl diselenide, respectively. Se levels in liver were about 14-fold higher than in brain for animals injected with 125 and 250 μmol/kg.

The results of the present investigation demonstrated that deposition of Se in three organs varied depending on the period of treatment. In fact, after a single dose of diphenyl diselenide, liver and kidney accumulated similar quantities of Se (Figure 1). However, after 14 days of exposure, deposition was higher in liver than in kidney (Figure 2). Several factors affect excretion of a compound, including molecular weight and charge. Aromatic compounds, such as chlorinated

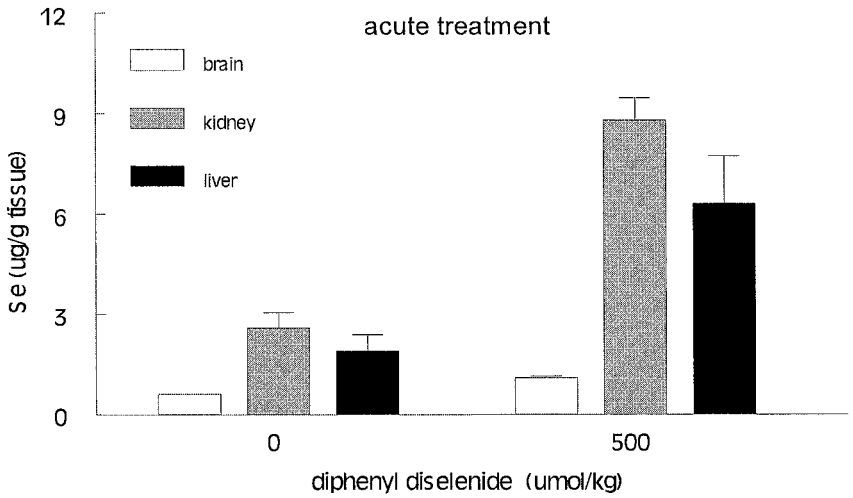


**Figure 1**. Total Se content of liver, kidney and brain after acute treatment with diphenyl diselenide (500 μmol/kg). Four to five samples were used for Se determination in each group. All samples were run in triplicate Tissue was prepared as described in Materials and Methods. ANOVA showed a significant effect of Se treatment ($p<0.01$) for all tissues. Data are presented as mean ± SD.

biphenyls, a molecular weight higher than 200 determines a preferential excretion via bile instead of in urine (Trimbell, 1991). In analogy, diphenyl diselenide, which has a molecular weight superior to 300, is expect to be predominantly excreted in the bile. Consequently, the deposition of Se predominantly in the liver can be related to the fact that body attempting to excrete diphenyl diselenide in the bile. Brain also accumulated Se after exposure to diphenyl diselenide, but the level attained in brain was considerably lower than that found in liver and kidney.

For years it has been known that inorganic and organic forms of Se disturb heme biosynthesis and normal erythropoiesis in rodents (Hogan and Jackson 1986; Hogan and Pendleton 1995). The disturbance of hemoglobin synthesis caused by Se can be casually linked to ALA-D inhibition (Hogan and Pendleton 1995). In line with this, our group demonstrated that acute and chronic exposure to diphenyl diselenide causes inhibition of ALA-D and a decrease in hemoglobin levels in mice (Maciel et al. 2000; Jacques-Silva et al. 2001). The pattern of enzyme inhibition paralleled the deposition of total Se observed in the present investigation. Consequently, we may speculate that the inhibition of ALA-D is related to total Se deposition in mouse tissues.

Diphenyl diselenides are simple synthetic compounds with thiol peroxidase-like activity (Wilson et al. 1989) and may have catalytic antioxidant activity by decomposing reactive peroxides. However, the therapeutic value of this substance must be considered with caution because the accumulation of this compound in liver can result in hepatotoxicity.

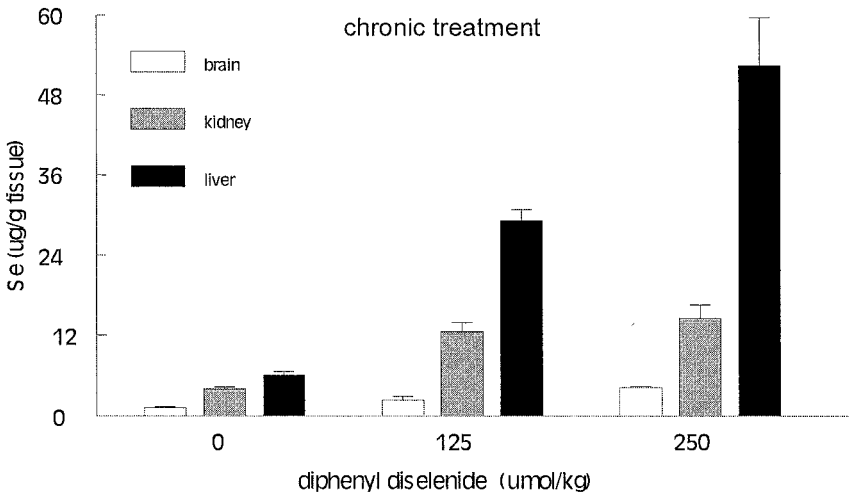


**Figure 2**. Total Se content in liver, kidney and brain from mice submitted to chronic treatment with diphenyl diselenide. Three samples were used for the tissue in each group. All samples were run in triplicate Tissue was prepared as described in Materials and Methods. Two-way ANOVA showed a significant main effect of Se treatment ($p<0.01$) in all tissues. Data are presented as mean ± SD.

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