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DMPS and *N*-acetylcysteine induced renal toxicity in mice exposed to mercury

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Abstract

Acute effects of mercuric chloride ($HgCl_2$) were evaluated on mice. Mice received a single dose of $HgCl_2$ (4.6 mg/kg, subcutaneously) for three consecutive days. Thirty minutes after the last injection with $HgCl_2$, mice received one single injection of 2,3-dimercapto-1-propanesulfonic acid (DMPS) or *N*-acetylcysteine (NAC) or diphenyl diselenide $(PhSe)_2$. DMPS, NAC and $(PhSe)_2$ were utilized as therapy against mercury exposure. At 24 h after the last $HgCl_2$ injection, blood, liver and kidney samples were collected. δ-Aminolevulinate dehydratase (δ-ALA-D) and Na^+, K^+ ATPase activities, thiobarbituric acid-reactive substances (TBARS), non-protein thiols (NPSH) and ascorbic acid concentrations were evaluated. Plasma aspartate (AST) and alanine (ALT) aminotransferase activities, as well as urea and creatinine levels were determined. The group of mice exposed to Hg + $(PhSe)_2$ presented 100% of lethality. Exposure with $HgCl_2$ caused a decrease on the body weight gain and treatments did not modify this parameter. δ-ALA-D, AST and ALT activities, TBARS, ascorbic acid levels and NPSH (hepatic and erythrocytic) levels were not changed after $HgCl_2$ exposure. $HgCl_2$ caused an increase in renal NPSH content and therapies did not modify these levels. Mice treated with $(PhSe)_2$, Hg + NAC and Hg + DMPS presented a reduction in plasma NPSH levels. Creatinine and urea levels were increased in mice exposed to Hg + NAC, while Hg + DMPS group presented an increase only in urea level. Na^+, K^+ ATPase activity was inhibited in mice exposed to Hg + DMPS and Hg + NAC. In conclusion, therapies with $(PhSe)_2$, DMPS and NAC following mercury exposure must be better studied because the formation of more toxic complexes with mercury, which can mainly damage renal tissue.

Introduction

Inorganic mercury is widely used in certain types of batteries and continues to be an essential component of fluorescent light bulbs (Clarkson 1997). The toxic effects of mercury on human and animal systems are well documented (WHO 1976, 1990). It is known that mercuric chloride (Hg^+) can stimulate lipid peroxidation by enhancing H_2O_2 formation in mitochondria (Lund *et al.* 1991).

A method for the detoxification of mercury that is widely recommended is its transformation into a chelate complex (Jones 1994). 2,3-Dimercapto-1-propanesulfonic acid (DMPS), a chelating agent, has been shown to be effective for the treatment of mercury intoxication in animals and humans (Aposhian *et al.* 1995). However, Nogueira and colaborators (2003c) have reported that DMPS inhibited hepatic δ-aminolevulinate dehydratase (δ-ALA-D) activity from mice and the inhibition

potency of DMPS is increased in the presence of mercury and cadmium. Since mercury induces oxidative stress (Huang *et al*. 1996), it is believed that antioxidants should be one of the most important components of an effective treatment against mercury intoxication.

N-acetylcysteine (NAC), a sulfhydryl containing antioxidant (Moldeus *et al*. 1986), is possibly one of the most widely investigated compounds that has beneficial effects on clinical conditions in which free radicals are involved (Berend 1985). Selenium is an essential element with physiological antioxidant properties, appearing as a selenocysteine (Bock *et al*. 1991), a structural component of several enzymes involved in peroxide decomposition, including glutathione peroxidase (Flohé *et al*. 1973; Rotruck *et al*. 1973) and phospholipid hydroperoxide glutathione peroxidase (Ursini *et al*. 1982). Organic forms of selenium have been suggested as possible antioxidant agents because they exhibit glutathione peroxidase-like activity (Nogueira *et al*. 2004). Conversely, Nogueira *et al*. (2003a) demonstrated that diphenyl diselenide $(PhSe)_2$ inhibits δ-ALA-D activity from human blood by interacting with SH groups of the enzyme.

Because Hg^+ has great affinity for SH groups of endogenous biomolecules, which may contribute to its toxicity (Clarkson 1997), δ-ALA-D and Na^+, K^+-ATPase, sulfhydryl containing enzymes, could be sensitive to mercury exposure. Accordingly, Rocha *et al*. (1993, 1995) reported that δ-ALA-D activity was inhibited after mercuric chloride administration. This enzyme plays a fundamental role in most aerobic organisms by participating in heme biosynthesis pathway (Sassa 1998) and its inhibition can lead to δ-ALA accumulation, which in turn can enhance the generation of free radicals, aggravating oxidative damage to cellular components (Pereira *et al*. 1992; Bechara 1996).

In addition, Na^+, K^+-ATPase could also be sensitive to oxidizing agents (Thévenod & Friedmann, 1999; Folmer *et al*. 2004; Borges *et al*. 2005). Na^+, K^+-ATPase is an enzyme embedded in the cell membrane and responsible for the active transport of sodium and potassium ions. This process regulates the cellular Na^+/K^+ concentrations and hence their gradients across the plasma membrane, which are required for vital functions such as membrane co-transports, cell volume regulation and membrane excitability (Doucet 1988; Jorgensen 1986). Several reports have shown the effects of metals such as mercury (Klonne *et al*. 1988; Anner *et al*. 1992) cadmium and lead (Pedrenho *et al*. 1996; Garcia & Corredor 2004) on Na^+, K^+-ATPase activity.

In the present study, we examined the effects of DMPS, $(PhSe)_2$ and NAC on acute mercury-poisoning in mice. Thereby, we evaluated the effect of mercury and therapies on δ-ALA-D and Na^+, K^+-ATPase activities, thiobarbituric acid-reactive substances (TBARS), non-protein thiol and ascorbic acid levels on mice tissues. The parameters that indicate hepatic (aspartate (AST) and alanine (ALT) aminotransferase activities in plasma) or renal (urea and creatinine levels) damage were also examined.

Materials and methods

Chemicals

Mercuric chloride ($HgCl_2$) was obtained from Merck (Darmstadt, Germany). 2,3-Dimercapto-1-propanesulfonic acid (DMPS), *N*-acetylcysteine (NAC), δ-aminolevulinic acid (δ-ALA) and *p*-dimethylaminobenzaldehyde were purchased from Sigma (St. Louis, MO, USA). Diphenyl diselenide was synthesized according to Paulmier (1986). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of diphenyl diselenide (99.9%) was determined by GC/HPLC. All other chemicals were of analytical grade and obtained from standard commercial suppliers. $(PhSe)_2$ was dissolved in DMSO (dimethylsulfoxide).

Animals

Male adult Swiss albino mice (30–35 g) from our own breeding colony were used. The animals were kept on separate animal rooms, on a 12 h light/dark cycle, at a room temperature of 22 °C, 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, Federal University of Santa Maria, Brazil.

Exposure

A group of six to eight mice was usually tested in each experiment. Mice received one daily injection of mercuric chloride ($HgCl_2$), subcutaneously, at the dose of 4.6 mg/kg (dissolved in saline at 0.46 mg/ml) for three consecutive days (Emanuelli *et al*. 1996; Perottoni *et al*. 2004b). Thirty minutes after the last injection with mercury, mice received one injection of DMPS at the dose of 400 μmol/kg intraperitonealy (i.p) (Santos *et al*., 2004) or NAC (300 mg/Kg, i.p) or diphenyl diselenide (100 μmol/kg, s.c) (Nogueira *et al*. 2003b).

The protocol of mice treatment is given below:

Group 1 (control): saline (s.c.) + DMSO (s.c.) + saline (i.p.)
Group 2 (DMPS): saline (s.c.) + DMPS (i.p.) + saline (i.p.)
Group 3 $(PhSe)_2$: saline (s.c.) + $(PhSe)_2$ (s.c.) + saline (i.p.)
Group 4 (NAC): saline (s.c.) + NAC (i.p.) + DMSO (s.c.)
Group 5 (Hg): $HgCl_2$ (4.6 mg/kg, s.c.) + DMSO (s.c.) + saline (i.p.)
Group 6 (Hg + DMPS): $HgCl_2$ (4.6 mg/kg, s.c.) + DMPS (i.p.) + saline (i.p.)
Group 7 (Hg + $(PhSe)_2$): $HgCl_2$ (4.6 mg/kg, s.c.) + $(PhSe)_2$ (s.c.) + saline (i.p.)
Group 8 (Hg + NAC): $HgCl_2$ (4.6 mg/kg, s.c.) + NAC (i.p.) + DMSO (s.c.)

At 24 h after the last $HgCl_2$ injection, the blood samples were collected directly from the ventricle of the heart in animals anesthetized. Subsequently, mice were sacrificed and liver and kidney were removed.

Mortality index

The effects of DMPS, $(PhSe)_2$ and NAC on mortality index of mice exposed to mercury were evaluated 24 h after therapies.

Body weight

Effects of DMPS and NAC on body weight gain of mice exposed to mercury were evaluated. The body weight gain of mice was monitored for the whole course of the experiment.

δ-ALA-D activity

δ-ALA-D activity was assayed by the method of Sassa (1982) by measuring the rate of product (porphobilinogen) formation except that 45 mM potassium phosphate buffer, pH 6.4 and 2.5 mM of aminolevulinic acid (ALA) were used (Barbosa *et al*. 1998). Incubations were carried out for 30 (liver) and 60 min (kidney) at 39 °C. The reaction product was determined using modified Ehrlich's reagent at 555 nm, with a molar absorption coefficient of 6.1×10^4 per M for the Ehrlich-porphobilinogen salt.

Determination of non-protein thiols (NPSH)

NPSH in kidney, liver, erythrocytes and plasma were determined by the method of Ellman (1959). To determine NPSH in liver and kidney, the homogenate was centrifuged at 4000 × *g* at 4 °C for 10 min and the supernatant (500 μl) was mixed (1:1) with 10% trichloroacetic acid (500 μl). After centrifugation, the protein pellet was discarded and free – SH groups were determined in the clear supernatant.

The erythrocyte samples (300 μl) were hemolyzed with 10% Triton (100 μl) and precipitated with 200 μl of 10% trichloroacetic acid. After centrifugation, free – SH groups were determined in the supernatant. NPSH determination in plasma was carried out without sample treatment.

Determination of thiobarbituric acid-reactive substances (TBARS)

Lipid peroxidation was performed by the formation of TBARS during an acid-heating reaction as previously described by Draper and Hadley (1990). Briefly, the samples were mixed with 1 ml of 10% TCA and 1 ml of 0.67% thiobarbituric acid subsequently they were heated in a boiling water bath for 15 min. The absorbance was read at 532 nm and the data expressed as nmol malondialdehyde (MDA)/g tissue.

Ascorbic acid determination

Ascorbic acid determination was performed as described by Jacques-Silva *et al*. (2001). Protein (tissues) was precipitated in 10 volumes of a cold 4% trichloroacetic acid solution. An aliquot of

sample (300 μl), in a final volume of 1 ml of the solution, was incubated at 38 °C for 3 h, then 1 ml H_2SO_4 65% (v/v) was added to the medium. The reaction product was determined using a color reagent containing 4.5 mg/ml dinitrophenyl hydrazine and $CuSO_4$ (0.075 mg/ml).

Aspartate (AST) and alanine aminotransferase (ALT) activities

Plasma AST and ALT enzymes were used as the biochemical markers for the early acute hepatic damage. AST and ALT activities were determined using a commercial Kit (LABTEST, Diagnostica S.A, Minas Gerais, Brazil).

Urea and creatinine levels

Renal function was analyzed using a commercial Kit (LABTEST, Diagnostica S.A, Minas Gerais, Brazil) by determining plasma urea and creatinine.

Na^+, K^+-ATPase activity

Immediately after the mouse sacrifice, the kidney was removed and the homogenate was prepared in 0.05 M Tris–HCl buffer (pH 7.4). The homogenate was centrifuged and supernatant was used for assay of protein Na^+, K^+-ATPase. The reaction mixture for Na^+, K^+-ATPase activity assay contained 6 mM $MgCl_2$, 100 mM NaCl, 20 mM KCl, 1 mM EDTA and 40 mM Tris–HCl, pH 7.4, in a final volume of 500 μl. The reaction was initiated by the addition of ATP to a final concentration of 3.0 mM. Controls were carried out under the same conditions with the addition of 1 mM ouabain. Na^+, K^+-ATPase activity was calculated by the difference between the two assays. Released inorganic phosphate (Pi) was measured by the method of Fiske & Subbarow (1925).

Statistical analysis

Data are expressed as mean ± S.E.M. Statistical analysis was performed to compare treatment groups to respective control groups using a one-way analysis of variance followed by the Duncan's multiple range test when appropriate. Values of $P < 0.05$ were considered statistically significant. For comparing the mortality index, a non-parametric chi-square test was applied.

Table 1. Effects of DMPS, $(PhSe)_2$ and NAC on mortality index of mice exposed to mercury.

	Mortality index
control	0/16
DMPS	0/16
$(PhSe)_2$	0/16
NAC	0/16
Hg	1/19
Hg + DMPS	2/19
Hg + $(PhSe)_2$	23/23[a, b]
Hg + NAC	2/20

[a]Denoted $P < 0.01$ as compared to the control group (chi-square test).
[b]Denoted $P < 0.01$ as compared to the Hg group (chi-square test).

Results

Mortality index

Results indicated that mice exposed to mercury plus $(PhSe)_2$ presented 100% of lethality (Table 1). For this reason, other parameters were not evaluated in this group (Hg + $(PhSe)_2$). In the other groups, there was no significant difference in the mortality index.

Body weight

The body weight gain of the mice was monitored for the whole course of the experiment. Mice exposed to mercury presented a reduction in the body weight gain when compared to the control group. Therapies with NAC and DMPS were not effective in restoring the body weight (Table 2).

δ-ALA-D activity

Results demonstrated that acute mercury exposure did not affect renal and hepatic δ-ALA-D activities. Therapies with DMPS and NAC did not modify enzyme activity when compared to the control and Hg groups (data not shown).

Lipid peroxidation

Renal and hepatic malondialdehyde levels, a parameter of TBARS formation, remained unchanged after mercury exposure. Therapies did not modify MDA levels when compared to the control and Hg groups (data not shown).

Table 2. Effects of DMPS and NAC on body weight gain of mice exposed to mercury.

	Body weight gain (g)
control	0.55 ± 0.58 (1.6)
DMPS	−0.48 ± 0.64 (−1.4)
$(PhSe)_2$	−0.81 ± 0.64 (−2.3)
NAC	−0.31 ± 0.49 (−0.9)
Hg	−1.85 ± 0.56 (−5.2)[a]
Hg + DMPS	−1.91 ± 0.97 (−5.6)[a]
Hg + NAC	−2.46 ± 0.98 (−6.9)[a]

Data are mean ± S.E.M. from six to eight animals in each group. ()% of the body weight change.
[a]Denoted $P < 0.05$ as compared to the control group (one-way ANOVA/Duncan).

Ascorbic acid concentration

Mercury exposure did not modify renal and hepatic ascorbic acid levels. DMPS and NAC therapies did not change ascorbic acid levels when compared to the control and Hg groups (data not shown).

Non-protein thiols (NPSH)

Results indicated that mercury exposure increases renal NPSH levels (186.61%) when compared to the control group ($P < 0.01$). Therapies did not change renal NPSH levels in comparison to the Hg group (Figure 1). Hepatic NPSH levels remained unchanged after mercury exposure and therapies did not modify these levels (Figure 1). Mercury expo- sure and therapies did not change erythrocytes NPSH content (Figure 2). Mice exposed to $(PhSe)_2$ presented a decrease in plasma NPSH levels (30.19%) when compared to the control group ($P < 0.05$). Hg–DMPS and Hg–NAC groups also presented a decrease in plasma NPSH levels (about 30%) when compared to the control group ($P < 0.05$) (Figure 2).

Plasma AST and ALT activities

Neither mercury nor therapies with DMPS and NAC altered AST and ALT activities when compared to the control group (Table 3).

Urea and creatinine levels

It is surprising that the urea and creatinine concentrations are not significantly elevated in Hg treated mice. The absolute values (76.16 mg/dL for urea and 0.51 mg/dl for creatinine) are elevated though not significantly due to the large scatter (Table 3). Conversely, Hg + DMPS and Hg + NAC groups presented an increase in urea level (101.88 and 127.90%, respectively) when compared to the control group ($P < 0.01$). One-way analysis yielded an increase in urea level for Hg + DMPS and Hg + NAC groups (45.42 and 64.17%, respectively) when compared to the Hg

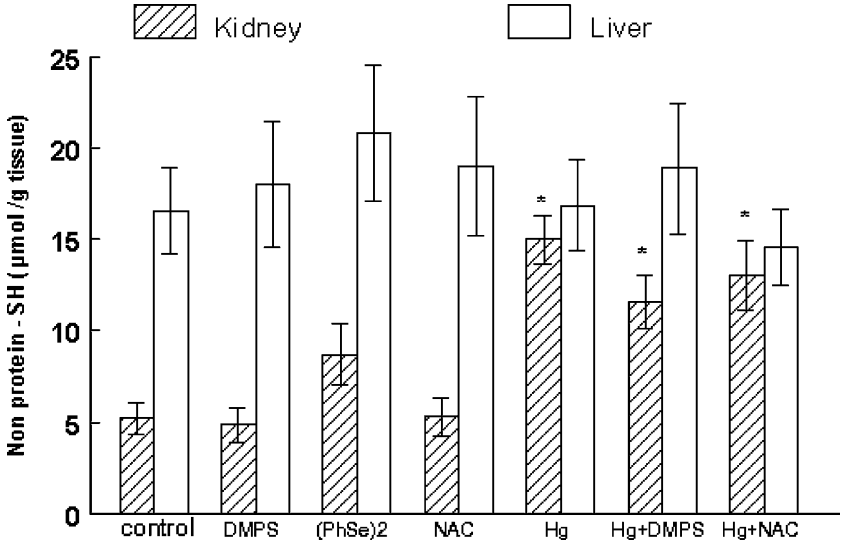

Figure 1. Effects of NAC and DMPS on non-protein thiol group content in liver and kidneys of mice exposed to mercury. Data are reported as mean ± S.E.M. of six to eight animals per group. (*) Denoted $P < 0.01$ as compared to the control group (one-way ANOVA/Duncan).

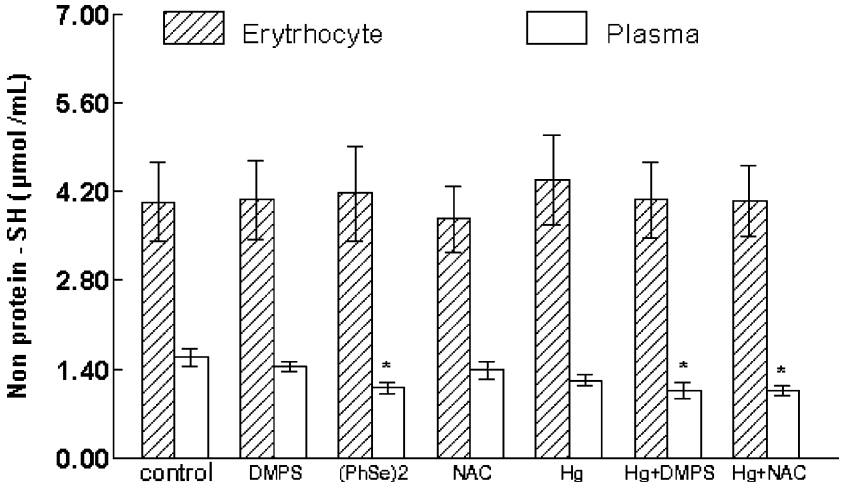

Figure 2. Effects of NAC and DMPS on non-protein thiol group content in erythrocytes and plasma of mice exposed to mercury. Data are reported as mean ± S.E.M. of six to eight animals per group. (*) Denoted $P < 0.05$ as compared to the control group (one-way ANOVA/Duncan).

Table 3. Effects of DMPS and NAC on urea and creatinine levels and on AST and ALT activities of mice exposed to mercury.

	Urea (mg/dl)	Creatinine (mg/dl)	AST (IU/l)	ALT (IU/l)
Control	54.86 ± 3.30	0.24 ± 0.023	114.33 ± 10.99	33.11 ± 3.08
DMPS	49.85 ± 5.38	0.33 ± 0.08	118.80 ± 16.99	33.20 ± 2.22
(PhSe)2	35.21 ± 2.97	0.32 ± 0.075	151.49 ± 40.04	28.40 ± 2.90
NAC	56.05 ± 5.21	0.40 ± 0.063	117.75 ± 28.75	36.25 ± 7.40
Hg	76.16 ± 10.89	0.51 ± 0.158	111.80 ± 19.39	23.55 ± 4.07
Hg + DMPS	110.75 ± 13.43[a, b]	0.62 ± 0.11	103.14 ± 19.24	28.85 ± 2.25
Hg + NAC	125.03 ± 14.68[a, b]	1.13 ± 0.23[a,b]	174.75 ± 18.30	37.77 ± 12.95

Data are mean ± S.E.M. from six to eight animals in each group.
[a]Denoted $P < 0.01$ as compared to the control group (one-way ANOVA/Duncan).
[b]Denoted $P < 0.05$ as compared to the mercury group (one-way ANOVA/Duncan).

group ($P < 0.05$) (Table 3). The results also demonstrated that mice exposed to Hg + NAC presented an increase in creatinine levels (370.83 and 121.57%, respectively) when compared to the control ($P < 0.01$) or Hg ($P < 0.05$) groups (Table 3).

Na^+, K^+-ATPase activity

Mercury exposure did not change renal Na^+, K^+-ATPase activity (Figure 3). Conversely, Na^+, K^+-ATPase activity was inhibited in mice exposed to Hg + DMPS and Hg + NAC (44.74 and 47.54%, respectively) in comparison to the control group ($P < 0.05$). One-way analysis revealed that Hg + DMPS and Hg + NAC groups presented Na^+, K^+-ATPase activity reduced (45.29 and 48.07%, respectively) when compared to the Hg group ($P < 0.05$).

Discussion

Inorganic mercury has a non-uniform distribution after absorption, being accumulated mainly in kidneys (Emanuelli *et al.* 1996; Klaassen 1996). In the current study, mercury toxicity was evidenced by the reduction in the body weight gain after mercury exposure, whereas therapies with DMPS or NAC were not effective in restoring the body weight in mice exposed to mercury. In addition, we observed for the first time that mice exposed to mercury and treated with $(PhSe)_2$ presented 100% of lethality, suggesting a toxic synergistic effect

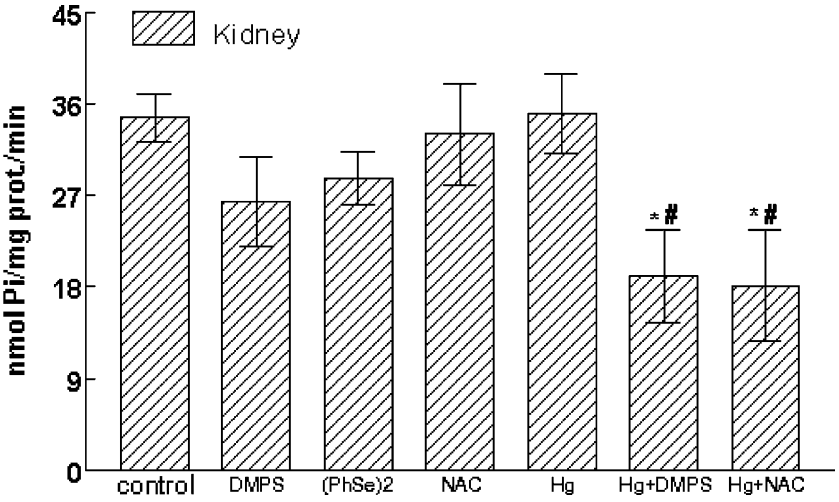

Figure 3. Effects of NAC and DMPS on renal Na^+, K^+-ATPase activity of mice exposed to mercury. Data are reported as mean ± S.E.M. of six to eight animals per group. (*) Denoted $P < 0.05$ as compared to the control group (one-way ANOVA/Duncan). (#) Denoted $P < 0.05$ as compared to the mercury group (one-way ANOVA/Duncan).

between these two compounds. Since mercury and $(PhSe)_2$ did not present renal and hepatic toxicity when given alone, we can infer that toxicity observed in mice simultaneously exposed to Hg and $(PhSe)_2$ is related to a complex formed between Hg and $(PhSe)_2$, which could have pro-oxidant activity. In addition, we have demonstrated that $(PhSe)_2$ presents chelating activity in mice exposed to cadmium (Santos *et al.* 2005b). Thus, the complex formed between cadmium and $(PhSe)_2$ appears to be different from Hg–$(PhSe)_2$ because diphenyl diselenide protected against toxicity induced by cadmium (Santos *et al.* 2005a), whereas Hg–$(PhSe)_2$ complex induced lethality in mice.

Our results also demonstrated that acute mercury exposure did not change δ-ALA-D activity and TBARS levels. These results are, at least in part, in accordance with previous studies that did not verify alterations in hepatic δ-ALA-D activity and TBARS levels in rats after mercury exposure (Perottoni *et al.* 2004a, b). Accordingly, Farina *et al.* (2003) have reported that acute mercuric chloride exposure did not alter renal and hepatic δ-ALA-D activity, as well as TBARS levels 24 after mercury poisoning.

In this study, renal NPSH levels were increased in mice after mercury exposure and were not modified by treatment with DMPS and NAC. Mercury is able to increase glutathione reductase (GR) activity under *in vivo* conditions (Lash & Zalups 1996). This increment can be related, at least in part, to the direct oxidative effects of mercury on endogenous glutathione (GSH), which leads to the enhancement in the GR activity. Although renal GR activity was not measured in our protocols, the observed increase in renal NPSH levels can be interpreted as a pathophysiological response to preserve the homeostasis of intracellular thiol status. Another important data found in this study was that $(PhSe)_2$, Hg + DMPS and Hg + NAC groups presented decreased NPSH levels in plasma, but not in erythrocytes. Previous studies have demonstrated that GSH content varies in different tissues and even within different intracellular compartments, it can be inferred that characteristic redox states exist in different tissues. Thus, the variation between plasma and erythrocytes NPSH levels can be explained by differences in some of the factors that modulate the GSH content. For instance, GSH levels in cells are primarily dependent upon the rates of biosynthesis and utilization in oxidation/reduction reactions. Amounts of the precursor amino acids as cysteine and the activity of the enzyme glutamate–cysteine ligase are the key factors affecting GSH synthesis (Rebrin *et al.*, 2005).

Mercury exposition associated with therapies (DMPS and NAC) induced renal toxicity that was clearly evidenced by an increase in urea and creatinine levels. Regarding the renal parameters evaluated, NAC seems to be the most hazardous therapy utilized in this study. Moreover, the association of Hg with DMPS or NAC inhibited the renal Na^+, K^+-ATPase activity, supporting

the renal toxicity induced by both therapies. Na^+, K^+-ATPase is a sulfhydryl-containing enzyme, it is probable that the complex formed between Hg and DMPS or between Hg and NAC causes a Na^+, K^+-ATPase inhibition by oxidizing sulfhydryl groups critical for the enzyme activity. Accordingly, chemical interactions between metals (cadmium and mercury) and thiols have been described earlier (Rivera *et al.* 1989; Aposhian and Aposhian 1990). Nogueira and co-workers (2003c) have reported the formation of a complex (metal–chelator) between cadmium or mercury and DMPS. In addition, previous studies have reported that mercury–thiol complexes present prooxidant activity higher than isolated components (Miller & Woods 1993, Putzer *et al.* 1995).

N-acetylcysteine (NAC) is a thiol-containing antioxidant (Moldeus *et al.* 1986) and ROS scavenging (Aruoma *et al.* 1989). Some studies have indicated that NAC has also chelating activity with regard to diverse heavy metals (Banner *et al.* 1986). Thus, DMPS, NAC and $(PhSe)_2$ could exert their toxic effects due to the formation of complexes with mercury, which would be more toxic than the isolated components.

The hypothesis that Hg–DMPS or Hg–NAC complexes are transported by renal tubules more easily than Hg and therefore develop a stronger toxicity must be considered. In fact, several authors have reported that renal uptake of inorganic mercury involves a mechanism localized along the proximal tubule and appears to be dependent on the organic anion transport system (Zalups & Lash 1994; Zalups & Minor 1995; Zalups & Barfuss 1998). This transport system does not transport large proteins, while that small molecular conjugates of mercury are likely transported (Zalups 1998). Thus, the organic anion transport system may transport inorganic mercury conjugated with thiols (GSH, cysteine, NAC or DMPS) and increase mercury toxicity.

Therefore, the results obtained indicated that mercury exposition associated with therapies (DMPS and NAC) caused renal toxicity, which was evidenced by an increase in urea and creatinine levels and an inhibition in renal Na^+, K^+-ATPase activity. In addition, $(PhSe)_2$ therapy caused 100 % of lethality in mice exposed to mercury. In conclusion, we believe that therapies with $(PhSe)_2$, DMPS and NAC following mercury exposure must be better studied because the formation of more toxic complexes with mercury, which can mainly damage renal tissue.

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