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Research report

Diphenyl diselenide, a simple organoselenium compound, decreases methylmercury-induced cerebral, hepatic and renal oxidative stress and mercury deposition in adult mice

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ABSTRACT

Oxidative stress has been pointed out as an important molecular mechanism in methylmercury (MeHg) intoxication. At low doses, diphenyl diselenide ($(PhSe)_2$), a structurally simple organoselenium compound, has been shown to possess antioxidant and neuroprotective properties. Here we have examined the possible in vivo protective effect of diphenyl diselenide against the potential pro-oxidative effects of MeHg in mouse liver, kidney, cerebrum and cerebellum. The effects of MeHg exposure (2 mg/(kg day) of methylmercury chloride 10 ml/kg, *p.o.*), as well as the possible antagonist effect of diphenyl diselenide (1 and 0.4 mg/(kg day); *s.c.*) on body weight gain and on hepatic, cerebellar, cerebral and renal levels of thiobarbituric acid reactive substances (TBARS), non-protein thiols (NPSH), ascorbic acid content, mercury concentrations and activities of antioxidant enzymes (glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD)) were evaluated after 35 days of treatment. MeHg caused an increase in TBARS and decreased NPSH levels in all tissues. MeHg also induced a decrease in hepatic ascorbic acid content and in renal GPx and CAT activities. Diphenyl diselenide (1 mg/kg) conferred protection against MeHg-induced hepatic and renal lipid peroxidation and at both doses prevented the reduction in hepatic NPSH levels. Diphenyl diselenide also conferred a partial protection against MeHg-induced oxidative stress (TBARS and NPSH) in liver and cerebellum. Of particular importance, diphenyl diselenide decreased the deposition of Hg in cerebrum, cerebellum, kidney and liver. The present results indicate that diphenyl diselenide can protect against some toxic effects of MeHg in mice. This protection may be related to its antioxidant properties and its ability to reduce Hg body burden. We posit that formation of a selenol intermediate, which possesses high nucleophilicity and high affinity for MeHg, accounts for the ability of diphenyl diselenide to ameliorate MeHg-induced toxicity.

1. Introduction

Mercury (Hg) is a persistent pollutant that is widespread in the environment [10]. All sources of environmental mercury represent a potential risk for human poisoning, because the methylation of inorganic mercury to methylmercury (MeHg) in waterways can result in MeHg accumulation in the aquatic food chain. Consequently, dietary fish consumption can represent a common source for human exposures [3,7,13]. MeHg has been shown by many investigators to have toxic effects in animals, as well as in humans, and its toxicity is thought to be mediated by multiple, yet not fully understood mechanisms. One general mechanism that has been demonstrated to play an important role in MeHg toxicity is an exacerbation of oxidative stress, which can be related, at least in part, to MeHg's high affinity for endogenous thiols [2,15,25,31,35]. In line with this, multiple studies have indicated that both in vitro and in vivo exposure to methylmercury can deplete GSH [36,38].

Selenium is an essential element for mammalian cells because it is a component of selenium-proteins, namely, antioxidant selenoenzymes, such as glutathione peroxidase and thioredoxin reductase [26,27]. For the case of inorganic Hg, inorganic Se(IV) has an important influence in Hg fate and can profoundly affect Hg distribution and deposition in mammals [19]. In fact, Se(IV) can be metabolized to selenide by erythrocytes, which, in turn, has high affinity for Hg

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and thus can form a stable and biologically inert complex with Hg [39]. However, the possible protective role of dietary selenium on MeHg neurotoxicity has been recently questioned [9]. In contrast to inorganic selenium, data about a possible protective role of organic forms of selenium on MeHg toxicity are scarce. Recently, one study demonstrated that selenomethionine could reduce MeHg toxicity by a mechanism that does not involve changes in Hg deposition in critical organs [34]. Thus, Se can likely counteract or minimize Hg toxicity via two distinct processes, namely, one involving changes in the distribution and deposition of Hg and another involving the modulation of antioxidant seleno-enzymes.

In the last 3 decades, the concept that selenium- and tellurium-containing molecules may be better nucleophiles (and therefore antioxidants) than classical antioxidants has led to the design of synthetic organoselenium compounds and to the demonstration that ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one) may serve as a therapeutic antioxidant agent for the treatment of pathologies associated with oxidative stress [26]. These promising results have stimulated the interest in the area of synthesis and reactivity of organochalcogens compounds with particular emphasis on their biological properties [4,14,26,27]. Notably, diaryl diselenides has been shown to possess important antioxidant properties [26,27] and diphenyl diselenide (the simplest of the diaryl diselenides) has been shown to possess thiol peroxidase activity (antioxidant) and protective properties both in vitro and in vivo models of experimental toxicity [6,22–24,23,27]. However, its potential beneficial properties against MeHg-induced toxicity have yet to be investigated.

Taking into account the fact that MeHg, an important environmental contaminant, can cause cellular damage due to its pro-oxidative properties and that diphenyl diselenide is a potent antioxidant with potential therapeutic use [11], the aim of the present study was to evaluate the effects of exposure to MeHg and/or diphenyl diselenide on TBARS, non-protein thiols (NPSH) and ascorbic acid levels, as well as on CAT, SOD and GPx activities in different tissues of adult mice. Furthermore, since data about the potential protective effect of organoseleno compounds, particularly for diselenides, is scarce, we also determined the effect of diphenyl diselenide on mercury deposition in liver, kidney and brain (cerebrum and cerebellum) in order to gain a better understanding on the possible interaction of diselenides and MeHg after in vivo exposure to these compounds.

2. Materials and methods

2.1. Chemicals

Methylmercury(II) chloride was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Diphenyl diselenide $(PhSe)_2$ was synthesized according to the literature methods [30] and was dissolved in soy bean oil. All other chemicals were of the highest available commercial grade.

2.2. Animals

Adult Swiss Albino mice (male), 60 days old and weighting approximately 33–38 g, were maintained at 22 ± 2 °C, on a 12:12-h light/dark cycle, with free access to food and water.

2.3. Treatment

Fifty-five mice were divided in six experimental groups: control (n = 10), MeHg (in this group about 20% of the treated animals died; n = 10), diphenyl diselenide 1 mg/kg (n = 8), diphenyl diselenide 0.4 mg/kg (n = 8), MeHg + diphenyl diselenide 1 mg/kg (n = 10), and MeHg + diphenyl diselenide 0.4 mg/kg (n = 9). The mice were treated daily for 35 days by oral gavage with MeHg and/or subcutaneous injections of diphenyl diselenide. Diphenyl diselenide was dissolved in soy bean oil for subcutaneous administrations, 10 ml/kg and the administered doses (0.4 and 1 mg/kg) were based on a previous study by our group [13]. MeHg (10 ml/kg) was dissolved in a $NaHCO_3$ solution (25 mM) for gavage administrations and its dose (2 mg/kg) was based on Miyamoto et al. [21]. Control mice (group A) received a daily dose of a $NaHCO_3$ solution 25 mM (10 ml/kg) plus a daily injection of soy oil (10 ml/kg).

2.4. Tissue preparation

After the treatment period (35 days), mice were killed. Livers, cerebra, kidneys and cerebella were quickly removed, placed on ice and homogenized in 10 volumes of Tris–HCl 10 mM, pH 7.4.

2.5. Lipid peroxidation

TBARS (thiobarbituric acid reactive species) were determined in tissue homogenates by the method of Ohkawa [28] with minor modifications [32], in which malondialdehide (MDA), an end product of fatty acid peroxidation, reacts with thiobarbituric acid (TBA) to form a colored complex.

2.6. Determination of non-enzymatic antioxidant defenses

GSH was measured as nonprotein thiol (NPSH). To determine NPSH, 500 μl of 10% trichloroacetic acid was added to 500 μl of homogenates. After centrifugation (4000 × g at 4 °C for 10 min), the protein pellet was discarded and free sulfhydryl groups (–SH) were determined in the clear supernatant and, ascorbic acid (Vitamin C) determination was performed as described by Jacques-Silva [16].

2.7. Enzymatic antioxidant defenses

CAT, SOD and GPx activities were determined according to Aebi [1], Misra and Fridovich [20] and Pagalia and Valentine [29], respectively.

2.8. Hg analysis

Tissue levels of total Hg were measured by cold vapor atomic absorption spectrometry according to Moretto et al. [22].

2.9. Statistical analysis

Differences between the groups were analyzed by one-way ANOVA. followed by Duncan's multiple range test when appropriated. Statistical differences in the various groups were determined with one-way randomized ANOVA design. When the overall test of significance (P < 0.05) led to a rejection of the null hypothesis, post hoc multiple range test was performed. Statistical analyses were performed with CSS:Statistica software (StatSoft Inc., Tulsa, OK).

3. Results

At the end of the treatment, body weight gain, liver and kidney weights were significantly lower in MeHg-exposed mice when compared to the control group (one-way ANOVA, followed by the Duncan multiple range test, P < 0.05). Simultaneous treatment with MeHg and diphenyl diselenide reduced the loss in body and organs weights caused by MeHg alone (Table 1).

Table 1
Effect of MeHg on body weight gain of mice.

Group	Initial body weight(g)	Liver weight (g)	Kidney weight (g)	Cerebrum weight (g)	Cerebellum weight (g)	Body weight gain (g)
Control	33.6 ± 1.27	1.90 ± 0.10	0.70 ± 0.03	0.33 ± 0.012	0.13 ± 0.007	12.9 ± 1.77
MeHg	32.5 ± 1.71	0.99 ± 0.17*	0.52 ± 0.04*	0.31 ± 0.010	0.11 ± 0.005	−4.83 ± 2.32*
Se 1 mg/kg	32.2 ± 2.78	2.37 ± 0.14#	0.67 ± 0.04	0.33 ± 0.002	0.12 ± 0.005	10.0 ± 1.84
MeHg + Se 1 mg/kg	32.5 ± 0.91	2.34 ± 0.05	0.63 ± 0.02	0.30 ± 0.005	0.12 ± 0.005	4.38 ± 1.12#
Se 0.4 mg/kg	34.5 ± 2.70	2.10 ± 0.10	0.65 ± 0.04	0.33 ± 0.011	0.12 ± 0.007	13.7 ± 2.03
MeHg + Se 0.4 mg/kg	36.5 ± 2.15	2.05 ± 0.19	0.69 ± 0.04	0.32 ± 0.006	0.12 ± 0.004	3.33 ± 3.66#

Values represent mean ± S.D. for body weight gain between 0 and 35 days of treatment (n = 8–10 mice/group). (*, #) Values statistically different compared to control (P < 0.05) by one-way ANOVA (means with different symbols differ from each other), followed by the Duncan multiple range test.