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Dietary selenium protect against redox-mediated immune suppression induced by methylmercury exposure

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ABSTRACT

The antagonism between selenium (Se) and mercury (Hg) has been widely recognized, however, the protective role of Se against methylmercury (MeHg) induced immunotoxicity and the underlying mechanism is still unclear. In the current study, MeHg exposure (0.01 mM via drinking water) significantly inhibited the lymphoproliferation and NK cells functions of the female Balb/c mice, while dietary Se supplementation (as Se-rich yeast) partly or fully recovered the observed immunotoxicity, indicating the protective role of Se against MeHg-induced immune suppression in mice. Besides, MeHg exposure promoted the generation of the reactive oxygen species (ROS), reduced the levels of nonenzymic and enzymic antioxidants in target organs, while dietary Se administration significantly diminished the MeHg-induced oxidative stress and subsequent cellular dysfunctions (lipid peroxidation and protein oxidation). Two possible mechanisms of Se's protective effects were further revealed. Firstly, the reduction of mercury concentrations (less than 25%, modulated by Se supplementation) in the target organs might contribute, but not fully explain the alleviated immune suppression. Secondly and more importantly, Se could help to maintain/or elevate the activities of several key antioxidants, therefore protect the immune cells against MeHg-induced oxidative damage.

1. Introduction

Mercury (Hg) has been recognized as a global metal contaminant over decades. It is well known that methylmercury (MeHg) is more bioavailable than inorganic Hg (Wang et al., 2010), and could be biomagnified in top-level predators (including human) through trophic transfer (Clarkson, 2002; Syversen and Kaur, 2012). As a consequence, organic methylmercury (MeHg) is widely reported as the major Hg species in biota (including human beings, Zhang et al., 2010), accumulating mainly via food consumption. In mammals, MeHg could transfer cross the blood–brain barrier and placenta, thus induce adverse effects on the central nervous system of adults and children (Clarkson, 1993). Apart from the well-recorded neurotoxicity, immune system has recently been noticed as another sensitive target for MeHg (i.e., induce immunosuppression and autoimmunity, Haggqvist et al., 2005).

To prevent the potential risk of MeHg exposure, the U.S. Food and Drug Administration recommends the pregnant women and young children to restrict their fish consumption (especially predatory fish). However, fish is an excellent source for a series of nutrients: omega-3 fatty acid, vitamins, high-quality proteins, and minerals (such as selenium), which are essential for our health. Selenium has received the most attention among those nutrients, because it is essential for the normal function of selenoproteins (selenocysteine and selenomethionine, Reeves and Hoffmann, 2009) involved in numerous biological functions, e.g., preventing oxidative damage, maintaining homeostasis of thyroid hormone, enhancing immune functions (Hoffmann and Berry, 2008; Ralston and Raymond, 2010). Fortunately, the enriched Se status has been widely reported to alleviate, reduce, and/or reverse MeHg-induced symptoms of toxicity (Cuvin-Aralar and Furness, 1991), in fish (Bjerregaard et al., 2011), birds (Stoewsand et al., 1974) and rats (Ralston et al., 2008; Sakamoto et al., 2013). The current mercury advisories focus only on the mercury level in fish, but do not consider the Se–Hg interactions. Understanding the mechanisms responsible for the protective effects of Se would help the reassessment of the potential risks versus nutritional benefits from consuming fish.

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Among several possible pathways proposed to explain the antagonism between MeHg and Se (as reviewed by Khan and Wang, 2009), the molecular mechanism likely involve the formation of MeHg–Se compounds. The intracellular speciation of MeHg is commonly dominated by MeHg–SR (–SR = amino acid containing S, Harris et al., 2003; Lemes and Wang, 2009) complexes. However, when the labile MeHg–SR reaches the active sites of selenoproteins, the –SR would be replaced by –SeR via ligand exchange process because selenium has even greater affinity for Hg than sulfur, forming unavailable MeHg-selenocysteine (MeHg–Sec) complexes (Rabenstein and Reid, 1984; Arnold et al., 1986). Therefore, MeHg is recognized as a highly specific, irreversible inhibitor of selenium-dependent enzymes. Since selenoenzymes are essential for preventing oxidative stress, sequestration of Se by MeHg diminishes the ability of exposed organisms to counteract reactive oxygen species (ROS), which might induce oxidative damage. Selenium supplementation could directly maintain the selenoenzyme activities and their synthesis, thus prevent the cellular oxidative damage (Ralston et al., 2008). Such mechanism has been widely confirmed in the brain and neuroendocrine tissues (Whanger, 2001), however seldom explored in immune system. Besides, selenium has also been reported to reduce the Hg body burden via modulating the Hg biokinetics, e.g., reducing the retention of MeHg in fish (Bjerregaard et al., 2011) and shrimp (Bjerregaard and Christensen, 2012), augmenting the elimination of Hg through urine in human (Li et al., 2012). Such process might also contribute to the protective effect of Se.

In the present study, the protective role of dietary Se supplementation on MeHg-induced immunotoxicity to female Balb/c mice was investigated, by evaluating the lymphocyte performance (i.e., proliferation indexes of T cells and B cells) and the lymphocyte function (i.e., the activity of natural killer (NK) cells). Two possible mechanisms underlying the protective role of Se were further explored. Firstly, dietary Se might modulate MeHg biodynamics to reduce Hg burden in target immune organs (spleen and thymus). Secondly, selenium supplementation might impair the inhibited selenoenzymes activities (due to MeHg-induced Se deficiency), therefore eliminate or reverse oxidative stress to immune cells in target organs (spleen and thymus). Specifically, the oxidative stress were evaluated by measuring ROS formation, as well as the activities of antioxidants, including primary enzymatic antioxidant (i.e., superoxide dismutase (SOD) and glutathione peroxidase (GPx, Se-dependent enzyme)) and the levels of non-enzymatic antioxidant (i.e., reduced glutathione (GSH)). The oxidative damage to target organs were assessed by monitoring the cellular dysfunctions, e.g., lipid peroxidation (indicated by malondialdehyde (MDA)) and protein oxidation (indicated by protein thiol).

2. Methods and materials

2.1. Drinking water and diets preparation

The MeHg-contaminated drinking water was prepared by dissolving methylmercury chloride (MeHgCl) (obtained from Sigma–Aldrich, St Louis, MO, USA) in Milli-Q water at a concentration of 0.01 mmol/L. The mice diets with different levels of supplemental selenium, 2 mg/kg (hereafter referred to as Se-adequate diets), and 6 mg/kg (hereafter referred to as Se-rich diets), were prepared by homogeneously mixing Se-enriched yeast (containing approximate 2000 mg selenium/kg) with the basal diet. The actual Se and background Hg concentrations in the mice diets were 0.16 mg Se/kg and 0.067 mg Hg/kg (basal diets), 2.28 mg Se/kg and 0.037 mg Hg/kg (Se-adequate diets), and 6.37 mg Se/kg and 0.065 mg Hg/kg (Se-rich diets), measured by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7700).

In the present study, the Se-enriched yeast was chosen as supplemental Se for the mice, considering three factors: (1) The molecular forms of Se that predominate in natural food (including Se-enriched yeast) are amino acid, i.e., selenomethione (SeMet) and selenocysteine (Sec). (2) Organic Se is more bioavailable but less toxic than inorganic Se (e.g. selenate and selenite, Alfthan et al., 1991; Rayman, 2004). (3) It was reported that organic Se (e.g., SeMet, Sec and Se-enriched yeast) rather than inorganic Se could significantly promote the elimination of MeHg in fish (Bjerregaard et al., 2011) and human (Li et al., 2012), therefore reduce MeHg-induced toxicity.

2.2. Animals and experimental setup

Female Balb/C mice (5 weeks old, 16–18 g) (Shanghai Super – B&K laboratory animal Corp. Ltd) were housed in Laboratory Animal Center of Tongji University under a 12:12-h light-to-dark cycle at 20 ± 3 °C and 50 ± 15% relative humidity. After acclimatization for one week, the mice were assigned into different groups according to the following experimental setup.

In experiment 1, the potential protective effects of dietary Se supplementation on MeHg-induced immunotoxicity were evaluated via examining the alteration of immune cell functions after 30 days' exposure. In this experiment, female Balb/c mice ($n = 30$) were randomly assigned into 6 groups: control group (the mice were fed with sterilized water and basal diets, $n = 5$); MeHg group (the mice were fed with MeHg-contaminated sterilized water and basal diets, $n = 5$); Se-adequate group (the mice were fed with Se-adequate diets and sterilized water, $n = 5$); Se-rich group (the mice were fed with Se-rich diets and sterilized water, $n = 5$); MeHg + Se-adequate group (the mice were simultaneously fed with MeHg-contaminated sterilized water and Se-adequate diets, $n = 5$); MeHg + Se-rich group (the mice were simultaneously fed with MeHg-contaminated sterilized water and Se-rich diets, $n = 5$). During the exposure period, the body weight of the mice was recorded every other day. At the end of the experiment, the proliferation performance of T and B lymphocytes and the activity of NK cells were monitored.

In experiment 2, the possible mechanisms behind the observed protective effects of dietary Se were further explored. In this experiment, female Balb/c mice ($n = 40$) were randomly assigned into 4 groups: control group (the mice were fed with sterilized water and basal diets, $n = 10$), MeHg group (the mice were fed with MeHg-contaminated sterilized water and basal diets, $n = 10$); Se-rich group (the mice were fed with Se-rich diets and sterilized water, $n = 10$); MeHg + Se-rich group (the mice were simultaneously fed with MeHg-contaminated sterilized water and Se-rich diets, $n = 10$). At the end of 30 days' exposure, half individuals in each group were used to investigate the inter-organ distribution of Hg by measuring the total Hg (tHg) concentrations in different tissues (liver, kidney, brain, thymus, spleen, muscle and blood). Samples from the remaining individuals were used to assess the effects of dietary Se supplementation on the balance between the oxidative stress (indicated by ROS formation) and the antioxidative responses (via measuring the activities of enzymatic and non-enzymatic antioxidants) accompanied with MeHg exposure. The cellular dysfunctions due to oxidative stress to the lipids and proteins of immune cells in target organs (thymus and spleen) were evaluated. Specifically, the protein thiol was measured as the index of protein oxidation, while malondialdehyde (MDA) was detected as indicator of lipid peroxidation.

During the whole exposure period, to minimize the potential MeHg loss via adsorption and volatilization, the drinking water was replaced every other day with freshly prepared solutions. All animal care procedures and experimental protocols were performed in accordance with current China legislation and approved by the Animal Committee of Tongji University School of Medicine, Shanghai, China (TJmed-012-03).

2.3. Sample preparation

After sampling blood from the eyes of each individual into plastic heparinized tubes, the mice were killed by dislocation of their necks. All the tissues (liver, kidney, brain, thymus, spleen and muscle) were removed aseptically and weighed. The relative weights of organs were further calculated as organ weight (mg)/body weight (g). The body weight gain (g) = the final bodyweight (g) – the initial bodyweight (g).

Single-cell suspensions of the sample tissues were prepared as described earlier (Burchiel et al., 2004) with modification for further measurements. Briefly, the thymus or spleen samples were homogenized by forcing through 400-μm stainless steel mesh. Cells were harvested in RPMI1640 medium and erythrocytes were lysed with a lysing buffer (BD, Franklin Lakes, NJ). After washing for three times, the cells were re-suspended in RPMI1640 medium supplemented with 10% fetal calf serum and adjusted to the required cell densities using a Countess Automated Cell Counter (Invitrogen).

2.4. Lymphocyte proliferation assay

The mitogens concanavalin A (ConA) and lipopolysaccharide (LPS) were chosen to stimulate T and B cell proliferation, respectively. Methyl thiazolyl tetrazolium (MTT, Sigma–Aldrich, St Louis, MO, USA) assay as described in previous studies (Mosmann, 1983; Visconti et al., 1991) were used to determine T and B cell proliferation. In short, 100 μl of splenocytes or thymocytes suspensions (2×10^6 cells/ml) together with ConA or LPS solution were added into a 96-well microtiter plate at a final concentration of 5 μg/ml ConA or 10 μg/ml LPS. Each sample was tested in triplicated wells. The complete RPMI1640 medium was used as the control. The microtiter plates were incubated at 37 °C in a 5% CO_2 humidified incubator (Sanyo, Japan). After 48 h, 20 μl of MTT (5 mg/ml PBS) was added to each well and