Regulatory Toxicology and Pharmacology 70 (2014) 720–727

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Safety evaluation of Se-methylselenocysteine as nutritional selenium supplement: Acute toxicity, genotoxicity and subchronic toxicity

Hui Yang, Xudong Jia *

Key Laboratory of Food Safety Risk Assessment of Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing 100021, China

ARTICLE INFO

Article history:
Received 7 August 2014
Available online 31 October 2014

Keywords:
Selenium
Se-methylselenocysteine
Safety evaluation
Food supplements
Benchmark dose

ABSTRACT

The significant toxicity of selenium emphasizes the need to assess the health risk of various selenocompounds as nutritional supplements. Se-methylselenocysteine (SeMC) was recently reported to be more bioactive but the toxicological effects have not been sufficiently characterized. This study aimed to evaluate the safety of SeMC and provide the Acceptable Daily Intake (ADI) for its use in human diet. Our results demonstrated that SeMC, with the Median Lethal Dose (LD_{50}) of 12.6 and 9.26 mg/kg BW in female and male mice, was of high potent of health hazard under acute oral exposure, but a battery of tests including Ames test, micronucleus assay and mouse sperm malformation assay suggested that SeMC was not genotoxic. The repeated dose study indicated little systemic toxicity of SeMC at supernutritional levels (0.5, 0.7, 0.9 mg/kg BW/day) after 90-day oral exposure. Importantly, the 95% lower confidence value of Benchmark Dose (BMDL) was estimated as 0.34 mg/kg BW/day according to the elevated relative liver weight. The ADI for human was established at 3.4 μg/kg BW/day. The results suggested greater safety of SeMC as a nutritional selenium supplement, but health risk needs to be further evaluated when SeMC is applied beyond this level to achieve cancer chemoprevention.

1. Introduction

As an essential micronutrient for human, selenium has been studied for nearly sixty years since its biological function was discovered in 1957 (Schwarz, 1976). It has been proven that selenium is a critical component of proteins required for various biological functions, such as antioxidant defense, reduction of inflammation, thyroid hormone production, fertility (Brown and Arthur, 2001; Whanger, 2002). Due to its essentiality for mammalian life, selenium has been utilized as a feed supplement for livestock especially in geographical areas that are naturally low in selenium. In addition, selenium is also suggested to be used for nutritional fortification in human food to prevent or rectify selenium deficiency (e.g. Keshan disease in China) (Alfthan et al., 2014; Chen, 2012; Kieliszek and Blazejak, 2013). However, selenium may induce toxic reactions at levels several times that normally ingested in the human diet (Thomson, 2004; Yang and Xia, 1995). Epidemiological studies and case reports have shown that chronic dietary exposure to selenium compounds (500 μg selenium per day) is associated with several adverse health effects in humans such as disruption of endocrine function, impartment of immunity, hepatoxicity and amyotrophic lateral sclerosis (Bratter and Negretti de Bratter, 1996; Vinceti et al., 2001). Currently, the tolerable upper intake level (UL) of selenium has been set at 400 μg/day, which is about 7 times the recommended dietary allowance (RDA) in the United States (55 μg/day) (FNB, 2000). However, it was believed that intake of selenium above normal nutritional range can confer more health benefits. For example, selenium has been previously suggested possessing anticancer activity at supernutritional levels (e.g. 200 μg/day) (Thomson, 2004). Thus, the biological study on selenium in the last century has been marked by the controversial balance between efficacy and safety.

It has been proven that the toxicity of selenium is dependent on the chemical speciation (WHO/IPCS, 1987). Elemental selenium and most metallic selenides have relatively less toxicity because of the low bioavailability, which on the other hand limited their utility in feed and food nutritional supplementation. By contrast, selenates, selenites and organoselenium compounds, such as selenomethionine (SeMet), selenocysteine and methylselenocysteine are widely used as nutritional selenium source but they are all toxic in large doses. In general, SeMet and SeMet enriched yeast are more effective in increasing body selenium levels and less toxic than inorganic selenium. The reason may lie on the non-specific incorporation of selenomethionine into proteins as the

* Corresponding author at: 7 Panjiayuan Nanli, Beijing 100021, China.
E-mail address: jiaxudong@cfsa.net.cn (X. Jia).

http://dx.doi.org/10.1016/j.yrtph.2014.10.014
0273-2300/

amino acid methionine and further providing reversible selenium storage in organs and tissues (Schrauzer, 2003). However, the excessive incorporation can also lead to structural malformation or loss of enzymatic activity in some sulfur-containing proteins due to the replacement of sulfur in sulfhydryl groups or thiols (critical for disulfide bond formation) with selenium (Stadtman, 1990).

Se-methylselenocysteine (SeMC), another naturally occurring organoselenium compound firstly identified in *Astragalus bisulcatus* (Trelease et al., 1960) and later found from many other plants (Freeman et al., 2012; Lyi et al., 2005), was reported to be less toxic and more bioactive than inorganic or other organic selenium compounds (Hoefig et al., 2011). Because SeMC is not readily incorporated into proteins and accumulates in a free pool after ingestion, it has been considered a better form of nutritional selenium supplements (Neuhierl and Bock, 1996). In addition, recent studies indicated that SeMC conferred remarkable protection against breast cancer (El-Bayoumy and Sinha, 2004; Ip et al., 2000; Unni et al., 2005; Zhang and Zarbl, 2008), prostate cancer (Sinha et al., 2014; Zhang et al., 2010) and colorectal carcinoma (Cao et al., 2014). SeMC can be a similar or better selenium source than SeMet and supplies methylselenol, an active metabolite recognized essential for the anticancer effect (Ip et al., 2002; Zhan et al., 2013), much more efficiently in organs than SeMet (Suzuki et al., 2006). It is promising that SeMC can be developed as a pharmaceutical drug that can be used in chemoprevention and clinical intervention of human cancers. However, a common concern in the uses of SeMC either for nutritional supplementation or for cancer chemoprevention is the safety risk rising from significant toxicity of selenium, and this has not been well elucidated yet.

In this study, we aimed to evaluate the toxicity of SeMC through multiple *in vitro* and *in vivo* experiments. Median Lethal Dose (LD_{50}) was established for the classification of acute oral toxicity; bacterial reverse mutation assay, mouse bone marrow micronucleus assay and mouse sperm malformation assay were performed to determine the genotoxicity of SeMC; a 90-day feeding experiment was carried out to investigate the subchronic oral toxicity, and the benchmark dose (BMD) approach was applied to estimate a point of departure for the hazard risk assessment of SeMC.

2. Materials and methods

All aspects in this project involving animal care, use, and welfare were performed in compliance with the Food and Drug Administration (FDA) principles of GLP and in accordance with the FDA Guidance for Industry and Other Stakeholders, "Toxicological Principles for the Safety Assessment of Food Ingredients Redbook 2000" (FDA, 2000). All animal study protocols have been approved by the Office of Laboratory Animal Welfare, National Institute for Nutrition and Food Safety (Beijing, China).

2.1. Test substance

L-Se-methylselenocysteine (SeMC), molecular weight 182.08, water-soluble white powder with purity >96%, was provided by Chuanqi Pharm Incoporation (Jiangxi, China).

2.2. Acute toxicity study

The conventional method was used for the acute oral toxicity test of SeMC as described in Organization for Economic Co-operation and Development (OECD) guideline (OECD, 1987). Fifty BABL/c mice (six weeks old) were randomly divided into five groups, 10 mice per group and 5 mice for each sex. Base on the result of a range finding test, animals were treated with SeMC, solubilized in purified water, at 2.15, 4.64, 10.0, 21.5, 46.4 mg/kg BW by a single gavage. General health observations and mortality were monitored on a daily base throughout 14 days after treatment. Median Lethal Dose (LD_{50}) was calculated on the basis of animal death at different dose levels (Horn, 1956).

2.3. Genotoxicity study

2.3.1. Bacterial reverse mutation assay (Ames test)

Four characterized histidine-dependent stains *of Salmonella typhimurium* (TA97, TA98, TA100, and TA102) were utilized for bacterial reverse mutation assay. S9 microsomal fraction of rat liver homogenate as the metabolic activation system was prepared according to previous method (Mortelmans and Zeiger, 2000). Bacteria in agar plate were treated with SeMC, solubilized in purified water, at concentrations of 0, 1.6, 8.0, 40, 200, 1000 μg/plate with or without S9 metabolic activation. The standard mutagens were used as positive controls in experiments, i.e. sodium azide (NaN3, 1.5 μg/plate) for TA100 without S9, 2-aminofluorene (2-AF, 10 μg/plate) for TA97, TA98, and TA100 with S9, 4-nitro-o-phenylenediamine (4NOPD, 20 μg/plate) for TA97 and TA98 without S9, mitomycin (MMC, 2.5 μg/plate) for TA102 with S9, and 8-dihydroxyanthraquinone (DHAQ, 50 μg/plate) for TA102 without S9. After incubation for 48 h at 37 °C, the revertant colonies were counted manually. The experiment was repeated twice in the same condition.

2.3.2. Mouse bone marrow micronucleus assay

Fifty BABL/c mice (six weeks old) were randomly divided into five groups, 10 mice per group and 5 mice for each sex. Animals were treated with SeMC at 1.15, 2.31 and 4.63 mg/kg BW by gavage, twice with 24 h interval. Cyclophosmide (40 mg/kg BW) was used as positive control while purified water as negative control. At 6 h after the second gavage, animals were euthanized and sternum aseptically removed. The contents of the spinal canal were squeezed out and diluted with calf serum, then smeared on the slides. After fixation with methanol and Giemsa staining, Red blood cells (RBC) and polychromatic erythrocytes (PCE) were observed under microscopy. The number of PCE was counted from 200 RBC in each animal and the ration of PCE/RBC was calculated. For each animal, 1000 PCE were examined for the incidence of micronucleated PCE.

2.3.3. Mouse sperm malformation assay

Twenty-five male BABL/c mice (six weeks old) were randomly divided into five groups, 5 mice per group. Animals were treated with SeMC at 0, 1.15, 2.31 and 4.63 mg/kg BW, or cyclophosmide at 40 mg/kg BW by gavage, once a day for 5 days. Thirty days after the last gavage, animals were euthanized and both epididymides surgically removed. The epididymides were cut into pieces in saline then centrifuged at 1000 r/min for 7 min. The sperm suspension was applied on slide and dried in air. The slides were fixed with methanol and stained with 1.5% Eosin, and subsequently examined under microscopy. The spermatozoa with morphological abnormalities were counted from 1000 spermatozoa per animal for the calculation of malformation rates.

2.4. 90-day repeated dose study

2.4.1. Study design

Groups of 10 male and 10 female weaning Sprague–Dawley rats (six weeks old) were given SeMC by daily gavage at doses of 0.5, 0.7 and 0.9 mg/kg BW in a vehicle of purified water (5 ml/kg BW) for 90 days. The doses were designed according to the LD_{50} from acute oral toxicity study, and the highest dose was set as about 10% of LD_{50}. Animals in the control group were given purified water for the same period. General clinical observations were recorded daily.