Ecotoxicology and Environmental Safety 156 (2018) 34–40

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Maternal dietary exposure to selenium nanoparticle led to malformation in offspring

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ARTICLE INFO

Keywords:
Nanotoxicology
Selenomethionine
Selenite
Dietary
Medaka
Teratogenesis

ABSTRACT

Selenium (Se) is an essential element and its biological activity is related to its speciation. It is also well-known that in excess it can cause teratogenesis in fish and birds. In this study we compared dietary toxicity of elemental selenium nanoparticles (SeNPs) with selenite and selenomethionine (Se-Met). Japanese medaka (*Oryzias latipes*) was used as a laboratory model to determine Se effects on adults and their offspring. Adult females were individually exposed using a dry diet fortified with 0, 10 or 20 μg/g of the three Se species for 7 days and then allowed to breed for 3 days. Fertilization rate and the proportion of malformed offspring were examined. The three Se diets led to significant increase in maternal tissue Se concentration in the order of Se-Met > > selenite > SeNP. However, in terms of proportion of malformed offspring, the effect of Se-Met = selenite > SeNP. The malformations included pericardial edema and craniofacial changes, which were typical for Se toxicity. The mismatch of maternal ovary Se concentration and proportion of malformed offspring suggested total Se concentration is a poor predictor of toxicity and teratogenesis. Comparing expression of four genes related to oxidative stress in maternal tissue also showed that there were significant differences in expression patterns between three Se diets in the order of selenite = SeNP > Se-Met. Our results showed that SeNPs cause similar toxicity as other Se species but require further study to elucidate the underlying mechanism.

1. Introduction

Selenium (Se) is an essential micronutrient that maintains physiological homeostasis (Janz, 2012). At least 25 selenoproteins have been identified in humans and animals, including important enzymes iodothyronine deiodinases 1–3 and glutathione peroxidase (Weekley et al., 2013). In supra-nutritional levels, however, selenium is a toxicant causing deformities in yolk-producing species, such as birds and fishes. Lemly (2002a) have suggested that dietary Se above 3 μg/g causes elevated Se concentrations in developing eggs. When Se concentration in eggs exceed 10 μg/g prevalence of teratogenic deformities increased rapidly (Woock et al., 1987). Under elevated Se concentrations, hatchability of eggs was not affected even though high incidence of deformities was reported to cause mortality of larvae/fry (Gillespie and Baumann, 1986). Hazardous Se exposures to aquatic life, particularly to fish, have been widely reported. For example, irrigation of seleniferous soils resulted in high selenium concentrations in evaporative ponds in California (Hamilton, 2004) which led to increase in Se concentrations in plants, invertebrates and fish from the ponds to 22–175 μg Se/g dry weight, which was 12–130 times higher than a nearby control area. Another example is at the Belews Lake in North Carolina where liquid waste from a local coal-fired power plant released return flow from slurry containing high concentrations of selenium (150–200 μg Se/L) into the waterbody (Hamilton, 2004; Lemly, 2002b). This led to bioaccumulation of Se in the food chains and led to reproductive impairment and tissue pathology in resident fish. One the Se toxicity most commonly referred to is the teratogenic deformities, including deformities in the eye, jaw, spine and edema (Lemly, 2002b). Developmental abnormalities in young fish persisted in 1996, 10 years after the stop of Se-laden wastewater entering the lake (Lemly, 2002b).

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https://doi.org/10.1016/j.ecoenv.2018.02.073

Se mainly exists as inorganic Se species (selenate and selenite) and organic Se species (e.g. selenomethionine (Se-Met)) in the aquatic environment (Janz et al., 2010). Organic Se species are considered more toxic to fish than inorganic Se (Janz et al., 2010). Toxicity of Se mainly manifests through maternal deposition of Se into eggs leading to subsequent assimilation and toxicity in the developing embryo (Janz et al., 2010). Teratogenic deformities occur as the embryo develops. Typical deformities occur in the heart, craniofacial, and spinal regions (Lemly, 2014).

Two potential mechanisms have been proposed to explain Se toxicity. The first is the simple substitution of selenium for sulfur during protein assembly (Lemly, 2002b). Oxidative stress has also been proposed to be a potential toxic mechanism of Se as various Se species can cause oxidation of cellular thiols (Lavado et al., 2012). This was demonstrated by the generation of superoxide radical when Se-Met was incubated with cells or the enzyme methioninase through the generation of methylselenol yet Se-Met alone did not generate superoxide radical (Spallholz et al., 2004). Therefore antioxidant enzymes, such as glutathione S-transferase (GST), catalase, superoxide dismutase (SOD) and glutathione reductase (GR) were often used as a biomarker of Se toxicity.

Interest in selenium nanoparticles (SeNP) has been increasing in the recent years due to their excellent bioactivities. SeNPs showed significant anti-cancer properties and can significantly inhibit cancer cell growth at μM range (e.g. Wu et al., 2013). They have also shown immunomodulation properties in a number of livestock species at μg/g level, including broilers (Cai et al., 2012) and sheep (Sadeghian et al., 2012). More and more studies have proposed to use SeNP as an animal feed supplement to boost livestock immune capacity (see review by Sarkar et al., 2015). This application has high potential of releasing SeNP into the environment through spillage during the mixing of SeNP into animal feed, disposal of leftover feed and/or egestion of unabsorbed NP from livestock. However, toxicity of SeNP was different from other Se species and is not well understood. For example, acute mortality test using zebrafish embryos showed that SeNP can be more toxic or less toxic than selenite depending on the type of SeNP and their surface stabilizer (Mal et al., 2017). Previous studies of SeNP aquatic toxicity have mostly focused on water-exposure. However, it is also known that maternal exposure to Se can result in deformities in offspring in yolk-forming animals like fish (e.g. Chernick et al., 2016). Therefore this study aimed at investigating how maternal dietary SeNP exposure affects medaka embryo viability and development.

2. Materials and methods

2.1. Medaka culture

A breeding colony of orange-red (OR) medaka (*Oryzias latipes*) was maintained at PolyU Shenzhen Base under approved animal care and maintenance protocols by the institute. Adult fish were maintained in closed recirculating water conditions at 25 °C under a 14:10 light: dark cycle, fed three times per day with Otohime B1 dry diet (Pentair Aquatic Eco-Systems, USA) and supplemented twice daily with *Artemia* nauplii.

2.2. Selenium nanoparticle synthesis and characterization

Chitosan-selenium nanoparticles (CTS-SeNPs) were created using methods modified from Wu et al., (2012, 2013). Chitosan (CTS) solution (0.25%) was mixed with aqueous sodium selenite solution (2.5 mM). Ascorbic acid (100 mM) was added dropwise into the mixture under stirring in the dark. After reconstituting with ultrapure water to 25 mL, the mixture was stirred and allowed to react for 12 h under room temperature before extensive dialysis (*M*w cutoff: 8000). The resultant CTS-SeNPs existed as monodispersed spherical particles in aqueous solution. These CTS–SeNPs were characterized by transmission electron microscopy (TEM; JEOL 2010 + Horiba EX-250, USA) and NanoSight NS300 (Malvern Instruments Limited, USA) for particle size distribution. Elemental composition of the SeNP was characterized by EDX under TEM. In NanoSight, NP size distribution were measured by taking average of 3 measurements. Total Se concentration of this CTS-SeNP stock was determined by ICP-MS.

2.3. Diet preparation

Base dry diet (Otohime B1) was fortified with 10 and 20 μg/g of seleno-L-methionine (Sigma-Aldrich), selenite (Sigma-Aldrich) and CTS-SeNP. The desired concentration of Se of each compound was dissolved into MilliQ water (Millipore, USA) to make a 20 mL solution respectively. This solution was poured over 20 g of dry food in a clean Petri dish and thoroughly mixed by kneading it to ensure the liquid was evenly distributed and well incorporated. The mixture was then freeze-dried overnight or as long as needed. Freeze-dried food was broken apart gently passing through a 100 μm sieve to ensure the particle size was suitable for the medaka fish. A control diet was made using the same protocol without the addition of any Se. All the diets were stored in 50 mL centrifuge tubes at 4 °C.

Total Se concentration of the base diet and all experimental diets were determined by ICP-MS (Agilent 7500). The base diet contained 3.78 μg Se /g dw and was comparable to the value reported (4.07 μg Se /g dw) by Chernick et al. (2016) on the same base diet. Fortification resulted in additional 10 and 20 μg/g of Se to the experimental diet accurately (Table 1).

2.4. Parental exposure regime

The following exposure regime was repeated for each of the Se treatment and embryo collections. Adult male and female medaka of reproductive age (6–9 months) were randomly selected from the breeding colony. Reproductive status of individuals was determined by isolating together a male and a female and observing their reproductive output and fertilization rate for 3 days under 14:10 light: dark at 25 ± 1 °C. Only breeding individuals were selected for the experiment. The pairs was then separated and each female were individually maintained in 1 L of US EPA moderately hard water (US EPA, 2002). Female fish were then divided up randomly into groups of 20 and exposed to one of 7 treatment diets respectively (control; 10, 20 μg/g selenite; 10, 20 μg/g selenomethionine; 10, 20 μg/g CTS-SeNP) for 7 days. Females were allowed to feed ad libitum for 5 min twice daily and unconsumed feed were removed using a net. A daily 50% water change was carried out for exposure groups. Preliminary studies showed that under this feeding regime to fish consume a ration of about 1.5–2% bw per day (data not shown). Males were fed on control diet during this period. After 7 days, 10 females from each treatment were euthanized by MS-222 overdose and organs were extracted by necropsy. Tissue from 5 individuals were used for measuring Se concentration and the remaining 5 were used for studying gene expression. The remaining 10 females of each treatment were again individually paired up with males and allowed to breed for 3 days to study the effect of maternal Se

Table 1
Total selenium concentration (mean ± SD) in all experimental diets expressed as μg/g dry weight.

Diet	Total selenium concentration (μg/g dw)
Base diet (control)	3.78 ± 0.26
Selenite 10 μg/g	13.62 ± 0.37
Selenite 20 μg/g	22.54 ± 0.33
Selenomethionine 10 μg/g	12.63 ± 0.48
Selenomethionine 20 μg/g	21.79 ± 0.44
CTS-SeNP 10 μg/g	12.48 ± 0.38
CTS-SeNP 20 μg/g	23.81 ± 0.31