Reproductive Toxicology 65 (2016) 272–282

Contents lists available at ScienceDirect

Reproductive Toxicology

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

Maternal methylmercury from a wild-caught walleye diet induces developmental abnormalities in zebrafish

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ARTICLE INFO

Article history:
Received 5 February 2016
Received in revised form 3 August 2016
Accepted 16 August 2016
Available online 17 August 2016

Keywords:
MeHg
Maternal exposure
Zebrafish
Offspring
Toxicity

ABSTRACT

Maternal methylmercury (MeHg) exposure from a contaminated diet causes adverse effects in offspring, but the underlying mechanism(s) remains unclear. In the present study, we investigated the effects of maternal dietary MeHg-exposure on the offspring, using the zebrafish (*Danio rerio*) as a model system. Female zebrafish were exposed to MeHg (0.88–3.10 ppm) by consuming a diet made from wild-caught walleye originally intended for human consumption. While dietary MeHg exposure did not significantly influence fecundity, offspring showed increases in morphologic alterations and mortality, neurobehavioral dysfunction, and dysregulation of global gene expression. Gene expression analysis suggested that MeHg might affect neuronal and muscular development *via* dysregulation of genes related to transcriptional regulation (such as *supt5h*) and cell cycle (such as *ccnb1*). Results from this study provide evidence that food intended for human consumption, with relatively modest levels of MeHg, may induce adverse effects in offspring.

1. Introduction

Methylmercury (MeHg) is ubiquitous in the environment and undergoes bioaccumulation and biomagnification resulting in high concentrations in large predatory fish and carnivorous sea mammals [1]. Humans are primarily exposed to MeHg through the consumption of contaminated fish; therefore, individuals that consume fish as their major protein source may be exposed to much higher concentrations of MeHg than the average person. Thus, this subpopulation endures the highest risk associated with mercury (Hg) exposure. Fish consumption by women prior to or during pregnancy is of particular concern as the benefits of fish consumption (*e.g.* long-chain polyunsaturated fatty acids) must be weighed against the risk of exposing the developing fetus to significant amounts of MeHg [2–4]. It is well established in both human and animal models, that prenatal exposure to high doses of MeHg can lead to widespread brain damage and impaired neurological development resulting in defects ranging from severe cerebral palsy and cognitive disabilities to subtle deficiencies in motor function, sensory responses, learning and memory [5–7]. The effects of low-dose MeHg exposure are more subtle, ranging from impaired motor function and sensory defects to slight deficiencies in learning and memory [5,6,8–11]. Several mechanisms have been proposed for MeHg-induced toxicity: 1) oxidative stress [12,13]; 2) inhibition of neural differentiation and dysregulation of neural migration [14–16]; 3) interactions with neurotransmitter receptors and alterations in the release of neurotransmitters [17,18]; 4) inhibition of ion channels and the resulting changes in intracellular ions [19–21] and; 5) irreversible inhibition of selenoenzymes which compromises selenium's biological availability [22,23]. Even with this extensive compilation of cellular and molecular data, the

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http://dx.doi.org/10.1016/j.reprotox.2016.08.010
0890-6238/

mechanisms responsible for MeHg-induced neurotoxicity remain elusive [24,25].

In the present study we used the zebrafish (*Danio rerio*) as a model system to determine the health risks, and associated molecular mechanisms, posed to offspring following maternal dietary MeHg exposure. The zebrafish is a powerful and well-established vertebrate model system for developmental biology and toxicology with a rapidly expanding repertoire of genetic and genomic tools. In this study, adult female zebrafish were exposed to dietary MeHg *via* wild-caught walleye intended for human consumption. Since walleye are one of the top predatory fish species naturally found in the Great Lakes region, they accumulate relatively high levels of MeHg. Walleye is a popular sport fish and can be a major source of MeHg for humans in the Midwestern United States of America (USA). Here we evaluate the effects of dietary exposure to MeHg on reproduction and offspring health. Female zebrafish were fed fish-based diets with known concentrations of MeHg. The offspring of these females were tested for developmental abnormalities including morphologic alterations, neurobehavioral dysfunction, and dysregulation of global gene expression. Results from this study will allow us to better understand the potential risks of MeHg exposure to developing embryos, and to refine fish consumption advisories for human populations.

2. Methods

2.1. Animals and exposures

All zebrafish were handled according to protocols approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Wisconsin-Milwaukee (UWM). Zebrafish were housed at a density of ≤4 fish/L in 28 °C dechlorinated and filtered municipal water in the Aquatic Animal Facility of the Children's Environmental Health Sciences Core Center at UWM. Adult female TL zebrafish (Tupfel long fin, ZIRC, Eugene, OR, USA) and male GL zebrafish (Golden long fin, Ekkwill Farms, FL, USA) were used in this study. Zebrafish were exposed to MeHg by consuming a diet made from lyophilized wild-caught walleye (*Sander vitreus)* fillets from northern Wisconsin, which were originally intended for human consumption. Chemical analyses by atomic absorption spectrometry were performed on each lyophilized walleye diet to confirm that Hg was the only significant contaminant in the diet as well as to determine the Hg dose (Supplementary Table 1). Since previous studies have shown that more than 95% of the total Hg in fish tissue is in the form of MeHg [26,27], we nominated the concentration of total Hg as equivalent to the concentration of MeHg herein. Hammerschmidt and Sandheinrich have shown a clear correlation between maternal MeHg consumption and egg MeHg levels [28]. Therefore, in order assess the effects of maternal dietary MeHg exposure female TL zebrafish were fed lyophilized walleye (4% body weight per day) containing 0.88, 1.90, or 3.10 ppm (or μg/g) of MeHg (referred to as low, medium, and high, respectively) while male GL zebrafish were fed normal Biodiet starter food (Bio-Oregon, Westbrook, ME; 4% body weight per day).

Females were fed lyophilized walleye diets for a total of four weeks (week 1–4); before and after this time period they were fed normal Biodiet starter food (4% body weight per day). Condition factor (CF) and ovosomatic index (OSI) were assessed in the exposed individuals at weeks 0 (before), 2 and 4 (during) and 6 (after) of the experiment. CF is a commonly used indicator of overall fish health and nutrient availability, and is calculated by [mass (mg)/length^3 (mm)] × 100 [29]. OSI is a commonly used indicator of reproductive health in female fish, which denotes the contribution of the ovary to the total mass of the fish, and is calculated by [ovary mass (mg)/total body mass (mg)] × 100. N = 10–26 depending on sampling date and exposure dose [30]. Female zebrafish that were fed walleye diets were spawned weekly throughout the study (weeks 0–7). The coloration difference between the adult TL females and GL males allowed the sexes to be separated easily after spawning and returned to their appropriate tanks for continued dietary exposure. Eggs were counted and reported as the average number of eggs produced per female. Early-life stage (ELS) toxicity in embryos was quantified as previously described [31]. Briefly, embryos were observed daily, and their health was assessed and given a score of 0–4 based upon the presence of characterized endpoints of toxicity (0 = normal, 1 = slight, generally one morphologic defect, 2 = moderate, generally two morphologic defects, 3 = severe, more than two morphologic defects, and 4 = dead) to establish a cumulative ELS toxicity score. Commonly observed morphological defects included cardiac edema, lack of air bladder inflation, and malformations of the face, trunk, tail, or fins. Some offspring from each treatment group were raised to adulthood with no further mercury exposure for behavioral testing.

2.2. Total Hg measurement

Total Hg in the ovary, viscera, and carcass of adult female zebrafish (n = 2-10, depending on the availability of animals) was quantified as previously described by Gerstenberger and Dellinger [32]. Briefly, samples were placed in a solution containing 4 ml of concentrated sulfuric acid and 1 ml of concentrated nitric acid heated to 80–90 °C. Samples were then allowed to digest for 15 min or until all the fish tissue was dissolved. Once cooled, 15 ml of 5% potassium permanganate and 8 ml of potassium persulfate were added to each sample. The samples were then allowed to oxidize overnight. Prior to analysis, 10 ml of hydroxylamine hydrochloride/10% sodium chloride solution and 5 ml of stannous chloride were added to each sample. Thereafter, samples were analyzed using an Instrumentation Laboratory Video 12 spectrophotometer (Instrumentation Laboratory, Bedford, MA, USA), and the maximal absorbance at a wavelength of 253.7 nm was recorded. The measurement of total Hg in the eggs was carried out as described by Weber et al. [33]. Briefly, chorion-intact embryos (n = 100; 3 replicates from each exposure group) were collected at 24 h post-fertilization (hpf) and rinsed twice in Hg^{2+}-free E2 embryo medium (15 mM NaCl, 0.5 mM KCl, 1 mM $MgSO_4$, 150 μM KH_2PO_4, 50 μM Na_2HPO_4, 1 mM $CaCl_2$, 0.7 mM $NaHCO_3$), placed in 7.0 ml Teflon microvials, and then acid digested in a microwave oven (MARS 5, CEM Corp., Matthew, NC, USA). Calibration standards (1–100 ppb Hg^{2+}) were prepared from a 10-μg/ml (in 5% ICP-MS grade HNO_3) Hg^{2+} standard solution (CertiPrep, Metuchen, NJ, USA) and a calibration curve was constructed. Mercury was analyzed with a MicroMass Platform inductively coupled plasma-mass spectrophotometer (ICP-MS) (Manchester, UK) equipped with a CETAC ASX 500 autosampler (Waters Corp, Medford, MA, USA) under MassLynx NT software (Waters Corporation, MA, USA) control for elemental measurements. All analyses were measured in the SIR Mode (Single Ion Recording) for 60 s.

2.3. Evaluation of the visual startle response

The visual startle response assay was performed according to the methods described by Weber et al. [33]. Briefly, adult fish (4–6 months old; n = 8 per exposure) were placed into a dimly lit stationary glass container (10-cm diameter and 5-cm depth). Surrounding the glass container was a white rotating PVC plastic drum with a black bar. The speed of the rotating drum was adjusted so that the fish experienced 50 encounters with the black bar in 5 min (10 rpm). Observation of the black bar by the fish caused a startle response-characterized as a clearly identified C-start escape maneuver. Each