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Selenium induces the demethylation of mercury in marine fish☆

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ABSTRACT

The antagonistic effect of selenium (Se) on mercury (Hg) toxicity has been known for decades. Earlier studies mainly focused on Hg-Se interaction based on biokinetics and bioaccumulation, but the influences of Se on *in vivo* biotransformation of methylmercury (MeHg) have not been well understood. We conducted a 42-day exposure study to investigate the dynamic changes of MeHg and its primary degradation product - inorganic mercury (IHg) - in different organs of black seabream (*Acanthopagrus schlegeli*) exposed to different dietary Se levels. A physiologically based pharmacokinetic (PBPK) model was then developed to describe the biotransformation and disposition of MeHg under the influence of Se. Our results demonstrated that Se significantly increased the transformation from MeHg into IHg, thereby decreasing the accumulation of MeHg. The simulation further showed that the intestine was the major site for demethylation, with an estimated rate 1.5-fold higher in high Se treatment than in low Se treatment. However, the hepatic demethylation rate was extremely low and comparable between the two treatments (0.012–0.015 d^{-1}). These results strongly suggested that the intestine instead of the commonly assumed liver was the major site for Hg-Se interaction. Furthermore, Se did not show significant influences on the distribution and elimination of MeHg, but promoted the uptake and elimination of the generated IHg from demethylation. Therefore, Se-induced demethylation especially in the intestine played an important role in mitigating the MeHg accumulation. This study provided new sight to elucidate the Hg-Se interaction in fish.

1. Introduction

Mercury (Hg) has attracted public concern for decades due to its widespread distribution and persistence in the environment (Driscoll et al., 2013). Fish consumption is the primary pathway for Hg exposure to humans. Methylmercury (MeHg), the most toxic form of Hg, can be easily accumulated by fish owing to its efficient assimilation and difficulty of elimination (Wang, 2012). The elevated level of MeHg in fish poses a potential risk rather than a benefit (Kim et al., 2016). Selenium (Se), a necessary micronutrient, has been shown to interact with Hg in a variety of organisms (Belzile et al., 2006; Bjerregaard and Christensen, 2012; Truong et al., 2014). The Hg-Se interaction has commonly been described as antagonistic due to the protective effects of Se on Hg toxicity (Yang et al., 2008; Bjorklund et al., 2017). However, synergistic effects have also been reported (Lemire et al., 2010; Penglase et al., 2014). This inconsistent phenomenon may be caused by the differences in the chemical species, dosing concentrations, administration methods and organism species (Wyatt et al., 2016). However, the underlying mechanisms in Hg-Se interaction have not been thoroughly understood.

The antagonistic effects of Se on Hg in fish, particularly the decreased MeHg bioaccumulation, have been widely observed in both laboratory and field studies (Deng et al., 2008; Peterson et al., 2009; Huang et al., 2013). It has been reported that Se can reduce MeHg bioavailability (thus decrease assimilation) or increase the elimination of MeHg, leading to an overall decreased MeHg bioaccumulation (Huang et al., 2013; Amlund et al., 2015). The proposed mechanism is due to the formation of biologically inert MeHg-selenol complexes (Khan and Wang, 2009). Conversely, Dang and Wang (2011) observed that both assimilation and elimination kinetics of MeHg was not affected when fish were either pre- or co-exposed to dietary Se. Thus, the counteraction of Se on MeHg accumulation is complicated and explanations for different observations are still open to questions. More importantly, most earlier studies focused on Hg-Se antagonism in fish based on uptake, distribution and elimination, but generally ignored the

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biotransformation of MeHg. In fact, significant *in vivo* MeHg demethylation was recently demonstrated in fish and can directly influence the relative abundance of IHg versus MeHg (Wang et al., 2017). Under *in vitro* conditions, Se can mediate the demethylation process via the formation of bis(methylmercuric)selenide, which finally decomposed into HgSe(s) (Khan and Wang, 2010). It has also been suggested that the demethylation reaction initiated by selenoamino acids occurred in mammals and birds (Eagles-Smith et al., 2009; Lyytikainen et al., 2015). If *in vivo* MeHg demethylation could be induced or enhanced by Se, the bioaccumulation of MeHg by fish would decrease. However, little is known about the effects of Se on MeHg demethylation within the fish body. The only related study was that selenoneine (a Se-containing antioxidant compound) could accelerate the excretion and demethylation of MeHg in zebrafish embryos (Yamashita et al., 2013). It is still unclear that which organ is the major site for Hg-Se antagonism in fish. In mammals and birds, the liver is considered to play an important role in detoxifying MeHg via demethylation with the aid of Se (Yasutake and Hirayama, 2001; Lailson-Brito et al., 2012; Gajdosechova et al., 2016). For fish, the low percentage of MeHg over total mercury in the liver was usually observed to be coupled with high Se burden (Yang et al., 2010; Arroyo-Abad et al., 2016), suggesting that MeHg may be demethylated and bound as Hg-selenide in hepatic cells. However, the decreased MeHg percentage in the liver could be due to MeHg inter-organ transportation from liver to muscle (Wang et al., 2013). The digestive tract is another important site for Hg-Se interaction. The formation of MeHg-selenol complexes in the gut lumen has been proposed to decrease bioavailability of MeHg at the intestinal epithelium (Huang et al., 2013). Moreover, the intestine has been suggested to play a predominant role in MeHg biotransformation by marine fish (Wang et al., 2017). Se in the food may also take part in intestinal demethylation of MeHg, and this could be another pathway for Se counteracting with MeHg toxicity. However, the Se-mediated MeHg biotransformation in the intestine has never been considered. Therefore, there is a need to investigate whether Se could mediate MeHg demethylation in fish and find out where this process occurs.

Physiologically based pharmacokinetic (PBPK) model can be developed to estimate the dose to target tissue by taking into account the rate of absorption, distribution and storage in tissues, metabolism and excretion on the basis of interplay among critical physiological, physicochemical and biochemical determinants (U. S. EPA, 2006). PBPK modeling has been successfully utilized to describe the disposition of Hg as well as other metals in fish, and helps to elucidate the roles of different compartments in handling metals (Franco-Uria et al., 2010; Wang and Wang, 2015, 2016). Based on the PBPK modeling, Wang et al. (2017) found that MeHg demethylation occurred in marine fish and intestine played the most important role in this process. However, this tool has not been used for studying the behaviors of Hg and the responses of organs under different Se conditions. Our study investigated the dynamic changes of MeHg and its primary degradation product (IHg) in different organs of black seabream (*Acanthopagrus schlegeli*) exposed to different levels of Se. Based on the determinations on the related Hg kinetics by PBPK modeling, we also aimed to illustrate the effects of Se on MeHg biotransformation and disposition in fish and evaluate the responses of specific organ. The study can help to develop a more complete understanding of Hg-Se interaction in fish.

2. Material and methods

2.1. Fish and diet

The black seabream (*Acanthopagrus schlegeli*) were chosen since they are widely distributed in the coastal environment and can be easily acclimated in the laboratory. Juvenile fish (10 cm in length, 15 g in fresh weight) were collected from a fish farm located in Sai Kung, Hong Kong, and were acclimated for 2 weeks in the sand-filtered seawater at 25 °C. The physicochemical characters of the seawater were 32 psμ (practical salinity units), pH 8.0, 4.0 mg L^{-1} DOC (dissolved organic carbon) and 0.3 ng L^{-1} dissolved Hg. The fish were fed with clean commercial food pellets (containing 38% crude protein, 7% fat, 5% fiber and 8% ash, New Life International, Inc.) at a rate of 0.016 g dry weight g^{-1} wet weight d^{-1}. The measured total mercury (THg), methylmercury (MeHg) and total selenium (TSe) in clean fish diet were 0.035 ± 0.002 μg g^{-1} dry weight (dw), 0.022 ± 0.005 μg g^{-1} dw and 1.16 ± 0.15 μg g^{-1} dw, respectively. The MeHg concentration in the spiked fish diet was set as 1.0 μg g^{-1} dw, whereas TSe concentrations were set as 5.0 (Low Se treatment) and 10.0 (High Se treatment) μg g^{-1} dw, respectively. The two levels of TSe were chosen since Se concentrations in realistic invertebrates were in the range of 1–20 μg g^{-1} dw (Stewart et al., 2004; Chapman et al., 2010). We also used selenomethionine (SeMet) to spike the fish diet because it is the most predominant chemical form in natural prey (Janz, 2012). Two types of spiked food pellets were prepared in this study: SeMet only and MeHg-SeMet mixed. The spiked fish diet was obtained by incubating ~100 g of clean food pellets into 125 mL newly prepared solution (SeMet or MeHg-SeMet added into distilled water). After 4 h incubation, the pellets were dried at room temperature under dark conditions for 2 days. The measured concentrations of THg and MeHg in the spiked fish diet were 1.05 ± 0.10 μg g^{-1} dw and 1.01 ± 0.06 μg g^{-1} dw, respectively, and TSe concentrations in Low Se and High Se treatments were 4.3 ± 0.54 μg g^{-1} dw and 10.7 ± 0.84 μg g^{-1} dw, respectively.

2.2. Treatments and sampling

There were three treatments in the present study: control (no MeHg and Se added), Low Se (1 μg g^{-1} dw MeHg + 5 μg g^{-1} dw Se) and High Se (1 μg g^{-1} dw MeHg + 10 μg g^{-1} dw Se). Fish were randomly captured and transferred into four aquariums (size of 60 × 30 × 45 cm^3) for each treatment. There were 20 fish in each aquarium for Low Se and High Se treatments and 4 fish in each aquarium for the control treatment. In the previous two treatments, the fish were fed with MeHg + SeMet spiked food pellets for 12 days (exposure for MeHg), and then were fed with SeMet spiked fish diet for 30 days (depuration for MeHg). Fish in the control treatment were fed with clean commercial fish diet (as described above) during the entire experimental period (42 days). Feeding was carried out twice a day with the ingestion rate set to be 0.016 g dry weight g^{-1} wet weight d^{-1} and feeding time lasted for around 1 h. The fish diet was consumed by fish very fast and almost no food pellets was left (<5%) at the end of feeding. The feces were timely siphoned off to ensure that the water was not contaminated by the residual MeHg. For all aquariums, the seawater was cycled at a flow rate of 3 L/min to ensure that the water was clean. During the entire experiment, there was no fish mortality in the high Se and low Se treatments and the performance (feeding behavior, swimming) of fish was normal. This indicated that fish health was not affected by these levels of Se or Hg.

Fish in the Low Se and High Se groups were sampled at 0, 3, 6, 9, 12, 13, 15, 17, 20, 24, 28, 32, 37 and 42 d, whereas those in the control group were sampled at the initial and end of experiment. Four fish (one from each aquarium) were randomly collected for each treatment at each time point. Fish were narcotized in cold ice water and then dissected into intestine, gills, liver and carcass (the rest part of fish body majorly composed of muscle tissue). The blood was sampled by cutting off the caudal fin and absorption by

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