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Importance of Molar Ratios in Selenium-Dependent Protection Against Methylmercury Toxicity

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Abstract The influence of dietary selenium (Se) on mercury (Hg) toxicity was studied in weanling male Long Evans rats. Rats were fed AIN-93G-based low-Se torula yeast diets or diets augmented with sodium selenite to attain adequate- or rich-Se levels (0.1, 1.0 or 15 μmol/kg, respectively) These diets were prepared with no added methylmercury (MeHg) or with moderate- or high-MeHg (0.2, 10 or 60 μmol/kg, respectively). Health and weights were monitored weekly. By the end of the 9-week study, MeHg toxicity had impaired growth of rats fed high-MeHg, low-Se diets by approximately 24% ($p<0.05$) compared to the controls. Growth of rats fed high-MeHg, adequate-Se diets was impaired by approximately 8% ($p<0.05$) relative to their control group, but rats fed high-MeHg, rich-Se diets did not show any growth impairment. Low-MeHg exposure did not affect rat growth at any dietary Se level. Concentrations of Hg in hair and blood reflected dietary MeHg exposure, but Hg toxicity was more directly related to the Hg to Se ratios. Results support the hypothesis that Hg-dependent sequestration of Se is a primary mechanism of Hg toxicity. Therefore, Hg to Se molar ratios provide a more reliable and comprehensive criteria for evaluating risks associated with MeHg exposure.

Keywords Selenium · Mercury · Methylmercury · Toxicity · Brain · Blood

Introduction

Although low-level methylmercury (MeHg) exposures have not been associated with adverse effects, the consequences of exposures to toxic quantities include lethality, growth inhibition, motor defects, oxidative damage to specific brain regions, and related

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pathological consequences [1–3]. However, the molecular mechanisms responsible for these toxic effects remain incompletely defined, making it difficult to evaluate risks associated with low-level MeHg exposures from seafood consumption [1–4]. Selenium (Se)-dependent protection against Hg toxicity has been recognized for 50 years [5] and has since been demonstrated in all species evaluated [6–22], but the molecular mechanism responsible for Se-dependent protective effects remains as incompletely defined as those of Hg toxicity. However, because of the high binding affinity (10^{45} M) between Hg and Se [23], direct Hg sequestration by Se has often been assumed responsible for the mechanism of Se's protective effect [1, 24, 25].

If Se-dependent protection occurs through Hg sequestration, the molar concentrations of Se and Hg in protected tissues need to approach or exceed a 1:1 stoichiometry to be effective. Therefore, many studies have employed diets with toxic Se concentrations to produce Se-dosing regimes that approached a 1:1 stoichiometry with toxic MeHg doses. Because the physiological importance of selenium was not identified until the late 1980s [26], many of the researchers of these earlier studies viewed Se as a cocontaminant rather than as an essential nutrient and failed to recognize the healthy range of dietary Se concentrations (approximately 0.1–25 μmol Se/kg; 0.01–2 ppm Se). Consequently, the toxic Se concentrations used in some studies could have been more rapidly deleterious to the health of the experimental animals than the MeHg itself, which may explain occasional contradictions in results. Likewise, poor understanding of the importance of selenium's roles in physiology and the distinctions in biochemical pathways of the different molecular forms of selenium may have also confused the issue of selenium's involvement in protection against Hg toxicity.

Selenium is a nutritionally essential element required for synthesis of 25–35 proteins with various functions [27–29], including preventing or reversing oxidative damage. Se-dependent enzymes (selenoenzymes) occur with tissue-specific distributions in all cells of all animals that possess a nervous system. However, selenoenzyme functions appear to be especially important in the brain [30, 31] and endocrine organs [32] as Se concentrations and selenoenzyme activities in these tissues exhibit remarkable homeostatic preservation. The Se contents and certain selenoenzymes in these tissues are preferentially maintained at or near optimal concentrations, but the amounts in somatic tissues such as blood, kidney or liver diminish or increase in response to dietary Se intakes [33, 34].

Adverse impacts of Hg toxicity on Se-dependent physiological processes in the brain and during development have been demonstrated in multiple models. Selenoenzyme activities are compromised by high levels of Hg or MeHg exposure [10, 35–39], but supplemental dietary Se maintains or increases these selenoenzyme activities and prevents or diminishes the oxidative damage that otherwise accompanies exposure to toxic amounts of Hg. Therefore, it is also hypothesized that rather than sequestering Hg, Se's protection against Hg toxicity is achieved by offsetting the loss of Se-dependent enzyme activities occurring as a consequence of Hg-dependent Se sequestration [10, 22, 36, 40, 41]. As selenoenzymes normally protect the nervous system from oxidative damage, this hypothesis can more accurately explain the many aspects of both Hg toxicity and the Se-dependent protective effect against Hg toxicity.

Many previous studies of Hg to Se interactions used acute exposures of physiologically inappropriate dosages, often administered parenterally. As the MeHg issue primarily concerns fish consumption, it is more appropriate to examine interactions between MeHg at concentrations that reflect the range of low, moderate, and toxic exposures in relation to dietary Se at nutritionally meaningful levels. The low-Se torula yeast basal diets used in this study were supplemented with sodium selenite to attain either adequate-Se concentrations that support healthy selenoenzyme activities in somatic tissues or rich but nontoxic Se

levels known to augment total Se and selenoenzyme activities in the blood, liver, and kidney. These concentrations reflect the range of dietary Se intakes that occur in nature. It is important to recognize that organic and inorganic forms are all reduced to inorganic selenide during selenocysteine synthesis [33], making sodium selenite an appropriate dietary form for these studies. Although the low dietary Se level of the torula yeast basal diet is known to greatly diminish Se and selenoenzyme activities of somatic tissues, maintenance on this diet is not associated with overt health consequences. Likewise, Se concentrations and selenoenzyme activities are maintained at near-normal levels in brain and endocrine tissues [33]. It is noteworthy that the rich-Se diet used in this study contained 15 μmol Se/kg, a concentration similar to that present in ocean fish [42].

The present experiment was designed to examine the influence of molar Hg to Se ratios and the dose- and time-dependent protective effects of the nutritional range of dietary Se against the development of MeHg toxicity. To accomplish this goal, the effects of dietary Hg to Se molar ratios on Hg toxicity and the distribution of Hg and Se were studied in the hair, blood, and brain tissues of rats. As current risk assessment criteria are exclusively based on Hg concentrations, an additional goal of this study was to examine the potential value of using the Hg to Se molar ratio as a more accurate criterion for assessing risk associated with MeHg exposure.

Materials and Methods

Diets and Animal Treatment

Diets used in this study were AIN-93G torula yeast-based diets formulated with balanced nutrient compositions [43] that were prepared by Teklad (Madison, Wisconsin) and supplied containing low, adequate- or rich-Se concentrations. These diet products were prepared to be 1% low in fatty acids to allow for MeHg additions. The low-Se (0.1 μmol Se/kg) basal diet was augmented with graduated amounts of sodium selenite to create the diets containing adequate- or rich-Se levels, 1.0 or 15 μmol Se/kg, respectively.

Upon receipt, the control diets without any added MeHg were supplemented with 1% safflower oil. Test diets were supplemented with MeHgCl dissolved in safflower oil to attain final MeHg concentrations at moderate or high levels: approximately 10.0 or 60 μmol MeHg/kg, respectively. Safflower oil prepared with or without added MeHg was added to diets at 10 g/kg final weight in 1.5-kg batches that were mixed for 5 min with a commercial mixer and stored frozen until use. The analyzed Se concentration in the low-Se diet (nominally 0.1 μmol Se/kg; 0.006 ppm Se) was 0.11±0.04 μmol Se/kg. The Se concentrations in the basal diets supplemented to adequate-Se levels (nominally 1.0 μmol Se/kg; 0.06 ppm) was 0.81±0.18 and rich-Se diets (nominally 15 μmol Se/kg; 1.34 ppm Se) were 17.26±2.76 μmol Se/kg). Although no MeHg was added to the basal diet, analysis revealed that Hg was present at 0.2±0.02 μmol Hg/kg (approximately 0.044 ppm Hg), similar to previously observed background levels present in other purified diets [44]. Diets supplemented with safflower oil containing MeHg at moderate or high levels had analyzed values of 7.77±0.97 and 60.85±1.97 μmol Hg/kg, respectively, approximately 10 and 60 μmol MeHg/kg, equivalent to approximately 1.5 and approximately 12 ppm Hg, respectively.

Three-week-old weanling male Long Evans rats were purchased from Charles River Laboratories (Boston, Massachusetts) and individually housed in polyethylene plastic cages with aspen bedding and open wire tops. Rats were fed rat chow for 1 week of acclimation

before distribution into nine weight-matched groups (six to seven rats per group; mean weights at start of feeding study = 121 ± 12 g). Rat groups were randomly assigned to dietary treatments in a 3 (dietary Se) × 3 (dietary MeHg) factorial design. Rats were provided with deionized water and their designated diets supplied ab libitum. Treatment groups were housed in an animal facility with room temperature maintained at 28°C, humidity 53% with a 12-h light/12-h dark cycle. Rat health and body weights were assessed at weekly intervals throughout the study.

On day 63 of the study, rats were anesthetized with ketamine rompun (1:1.37) delivered by intraperitoneal injection at a dosage of 1 μl/g. Hair samples were collected from adjacent patches of black and white hair of anesthetized animals using stainless steel electronic clippers. After collection of hair samples, rats were euthanized by cardiac exsanguination using syringes prepared with EDTA anticoagulant. Brains were wrapped in labeled aluminum foils and flash frozen in liquid nitrogen. Brain and blood samples were stored at −80°C until analysis.

Elemental Analysis in the Hair, Blood, and Brain

Hair samples were washed according to the method developed by the International Atomic Energy Agency [45]. Hair samples (0.5 g) were washed with 100 ml of 1:1 *v*/*v* acetone/methanol four times for 20 min each. After washing, the samples were transferred to a polyethylene filter crucible attached to a vacuum pump and rinsed with 1.5 l of doubly distilled water. The samples were then dried overnight at 105°C and stored desiccated.

Brain samples were freeze-dried, homogenized into powder, and stored desiccated. Molar concentrations of Hg and Se in dry brain tissues were later corrected to reflect their wet weight concentrations. Hair, blood, and brain samples were distributed in aliquots of 0.5–1.0 g weighed to 0.0001 g into acid-washed 20-ml borosilicate digestion tubes. Sample batches included quality control digestion blanks and certified reference materials. Standard reference materials of human hair, BCR-397 (Geel, Belgium), IAEA-086, (Vienna, Austria); blood, UTAK-2 (UTAK Laboratories, Valencia, California); and dog fish muscle, DORM-2 (dogfish muscle certified reference material DORM-2, National Research Council of Canada, Ottawa, Ontario, Canada) were digested and analyzed along with sample batches. Analytical precision was assessed by performing duplicate digestion samples, analysis duplicates, and standard spike recoveries every 10 samples.

Blood sample batches were digested with 0.5 ml of perchloric acid ($HClO_4$) and 2 ml of nitric acid (HNO_3). Loosely capped digestion sample tubes were predigested at room temperature overnight before heating the samples in a dry, hot block heater at 90–100°C for 8–9 h to complete the digestion. Sample solutions were then diluted into the range of calibration and analyzed for Hg by cold-vapor atomic absorption (AA) using a CETAC 6000A (CETAC Technologies, Omaha, Nebraska) and for Se using a heated graphite furnace AA using a Perkin Elmer 5100 with Zeeman correction (Perkin Elmer, Norwalk, Connecticut). The digestion procedure for hair and brain samples was similar to the blood sample digestion except 0.25 ml of concentrated H_2SO_4 was used instead of $HClO_4$.

Statistical Analysis

Diet-dependent differences in growth and elemental concentrations of Hg and Se in the hair, blood, and brain tissues of different dietary treatment groups were compared using a *t*-test. All data were tested with Stata Release 9 (*Stata Statistical Software*: Release 9, StataCorp LP., College Station, Texas).

Results

Dietary Se in the Prevention of MeHg-Dependent Growth Inhibition

Results were compared using *t*-tests of differences of independent means or Mann–Whitney nonparametric tests, depending on the normality of samples. The growth of rat treatment groups provided diets of differing Se and MeHg compositions, shown in Fig. 1. There were

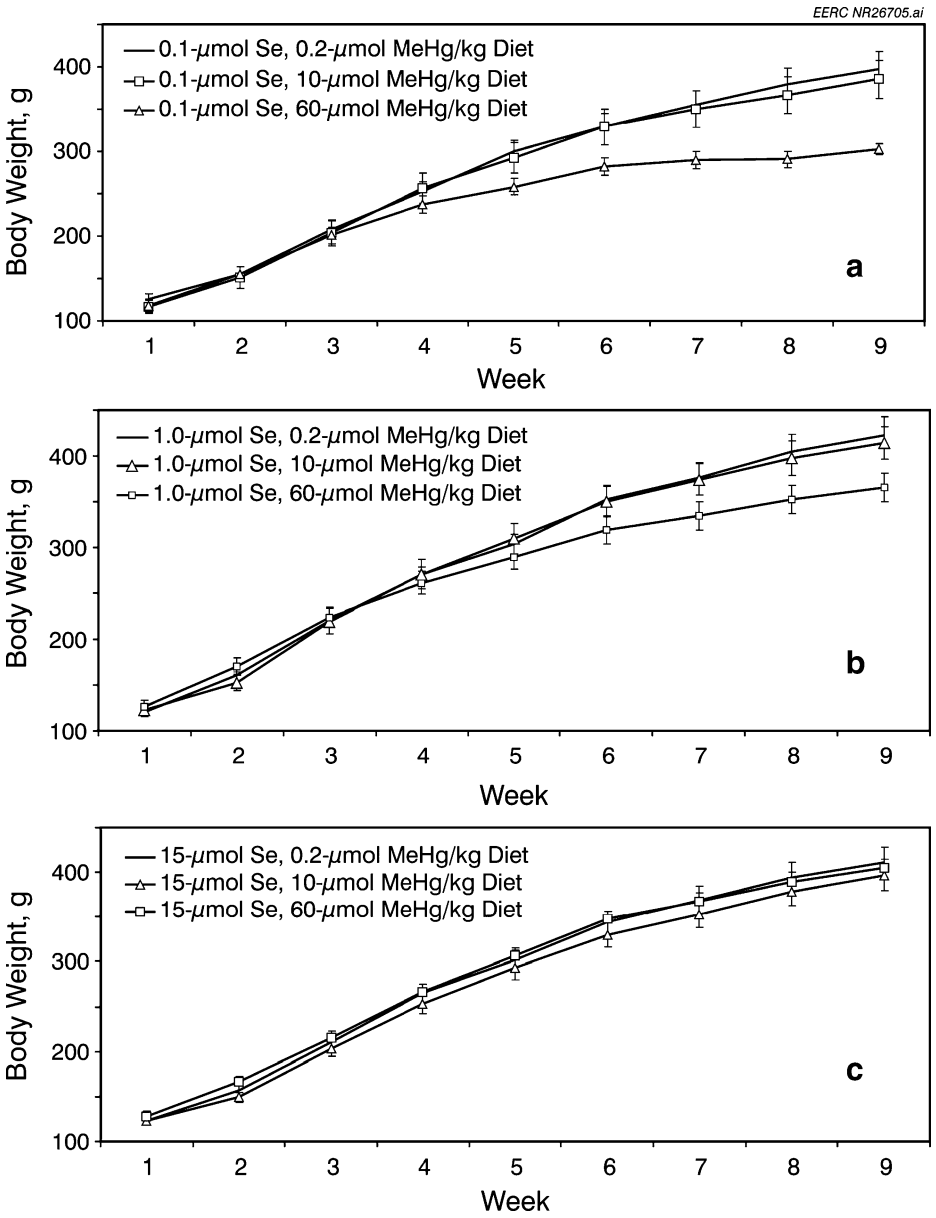

Fig. 1 Growth of rats fed graduated quantities of MeHg and Se (**a**, **b**, **c**)

no Se-dependent differences in growth of rats fed diets with low- or moderate-level MeHg ($p>0.05$). However, among groups treated with high-MeHg diets, there were Se-dependent influences that became increasingly apparent as the study progressed. Among rats fed low-Se diets, growth of the group fed high (60 μmol/kg) MeHg was impaired significantly ($p<0.05$) by week 5 (Fig. 1a) and by the end of the study the growth of the low-Se, high-MeHg exposed rats was approximately 25% less than that of their control group fed low-Se diets without added MeHg ($p<0.01$). Rats fed adequate-Se diets (Fig. 1b) that were exposed to high-MeHg grew perceptibly less than their respective control group, but the difference did not become significant until the final week when their mean weights were 14% less than the controls ($p<0.05$). Rats fed high-MeHg along with Se-rich diets (Fig. 1c) did not show MeHg-dependent growth inhibition relative to their control group at any time during the study.

Dietary MeHg and Se Effects on Elemental Accumulation in Tissues

The Hg contents in hair samples collected at the end of week 9 are detailed in Table 1. Hair color did not influence Hg deposition in hair from Long Evans rats ($p>0.05$) so only black hair results are shown. Hair Hg was significantly related to dietary MeHg intakes ($p<0.01$), but increasing dietary Se tended to be accompanied by increases in Hg distribution into hair in rats fed high-MeHg diets. Dietary MeHg was proportionally reflected by Hg measured in blood and brain contents ($p<0.01$) in all cases, and the influence of dietary Se on Se contents of blood was similarly sensitive.

The influences of dietary Hg and Se on each other's distributions in the blood are listed in Table 1 and depicted in Fig. 2. Effects of dietary Se on deposition of Se in hair were not measured as hair Se is not accepted as a valid criterion of Se status. Blood Se of rats fed adequate-Se, low-MeHg diets was 8.56±0.80 μM. Blood Se in rats fed low-Se diets diminished to approximately 93% ($p<0.05$) of those observed in rats fed adequate-Se. These concentrations are proportionally similar to the relative Se concentration in the diets. The blood Se in rats fed Se-rich diets without added MeHg almost doubled. In contrast to low-Se rats, the twofold increase in blood Se does not proportionally reflect the 15-fold greater concentration of Se in diets. Dietary Se influenced Hg distribution in the blood, as shown in Table 1 and Fig. 2. Compared to blood Hg levels in the low-Se group, Hg concentrations in the blood of rats fed moderate-MeHg/kg diets increased approximately 50% when diets contained adequate ($p<0.001$) or rich amounts of Se ($p<0.001$). Among rats fed 60 μmol MeHg/kg, blood Hg was approximately 40% higher ($p<0.01$) in rats fed rich-Se diets.

Dietary MeHg influenced Se concentrations in the blood (Table 1 and Fig. 2). Compared to their controls, blood Se in the adequate-Se, high-MeHg group diminished by 10%. In contrast, Se concentrations in the blood of rats fed rich-Se diets increased by approximately 40% when MeHg/kg was present in the diet at either 10 μmol ($p=0.01$) or 60 μmol ($p<0.01$), possibly as the result of retention of insoluble HgSe complexes.

Dry matter comprised 21.8±0.7% of total brain weights, but no significant differences in dry to wet weight ratios were noted between treatment groups. Treatment-dependent effects on Hg and Se concentrations in brains are shown in Table 1 and Fig. 3. Brain Se of rats fed adequate-Se diets was 2.39±0.18 μmol Se/kg. After 9 weeks on a low-Se diet, brain Se concentrations had diminished only slightly, although the Se in the blood supplying the brain had diminished by 93%. The Se in brains of rats fed rich-Se diets increased by approximately 30% relative to rats fed adequate-Se diets, although their diets contained 15 times as much Se.

Table 1 Effects of Dietary Methylmercury and Selenium on Growth and Mercury and Selenium Distribution in Hair, and Brains of Rats

Diet composition			*n*	Body weights week 9	Hair (μmol Hg/kg)	Blood (μM Hg)	Blood (μM Se)	Blood Hg to Se ratio	Brain (μM Hg)	Brain (μM Se)	Brain Hg to Se ratio
Se MeHg (μmol/kg)		Hg to Se ratio in diet									
0.1	0.2	2	7	397±56	0.5±0.03	0.60±0.02	0.60±0.24	0.01±0.09	0.07±0.10	2.06±0.17	0.03±0.06
0.1	10	100	7	385±58	52.4±13.3	25.18±3.62	0.53±0.16	52.02±19.10	1.12±0.39	1.89±0.18	0.60±0.23
0.1	60	600	7	302±17	1,970.8±216.8	273.64±72.07	0.41±0.07	682.12±197.54	20.55±4.82	1.49±0.15	14.00±3.91
1	0.2	0.2	6	423±49	0.8±0.3	n.d.	8.56±0.80	–	0.07±0.14	2.39±0.18	0.03±0.06
1	10	10	6	414±43	52.9±17.1	37.12±4.31	8.14±0.92	4.58±0.51	2.11±0.91	2.52±0.26	0.87±0.42
1	60	60	6	365±42	2,030.9±99.1	351.07±62.51	5.30±0.79	66.75±12.23	17.82±1.27	2.38±0.33	7.56±0.67
15	0.2	0.01	6	411±41	1.5±1.5	n.d.	16.06±4.89		−0.12±0.06	3.09±0.80	0.04±0.02
15	10	0.67	6	397±43	77.0±40.9	38.00±6.14	22.29±2.23	1.72±0.33	4.55±1.89	4.38±0.74	1.04±0.32
15	60	4	7	406±24	2,269.7±408.1	379.43±61.21	23.14±2.62	16.70±3.85	36.62±4.74	10.40±1.23	3.56±0.58

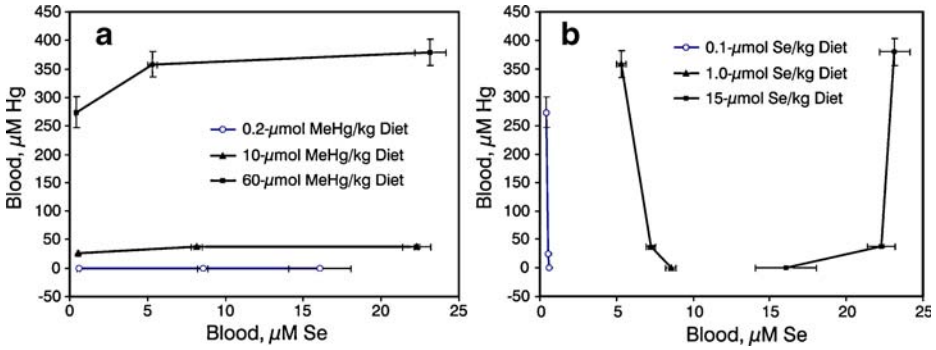

Fig. 2 Effect of MeHg (**a**) and Se (**b**) dietary treatment on the distribution of Hg and Se into the blood

Rats fed the low-Se diets were unable to maintain normal brain Se concentrations when challenged with high dietary MeHg. Low-Se rats fed 10 μmol MeHg/kg had slightly diminished brain Se contents, but when fed 60 μmol MeHg/kg, their brain Se dropped to approximately 70% of the normal ($p<0.01$). This is comparable to the lowest level of brain Se ever achieved in Se deficiency studies. In contrast, rats fed adequate-Se diets were able to maintain their brain Se concentrations at near-normal levels regardless of how much MeHg was present in their diets. The brain Se contents of rats fed rich-Se diets doubled when they were fed 10 μmol MeHg/kg and were approximately four times greater than normal when they were fed 60 μmol MeHg diets.

The influence of dietary Se on Hg accumulation in the brain was also dramatic. Brains of rats fed diets containing 10 μmol MeHg/g were 1.12±0.39 μmol Hg/kg in rats fed low-Se diets, but almost twice as high when rats were fed adequate-Se and four times greater when fed rich-Se diets ($p<0.01$). Mercury contents in brains of rats fed diets containing 60 μmol MeHg/g were 20.55±4.82 μmol Hg/kg in rats fed low-Se diets, and did not increase when rats were fed adequate-Se diets. However, brain Hg contents almost doubled among rats fed rich-Se diets ($p<0.01$).

Blood Hg to Se ratios closely resembled the MeHg to Se ratios of the diets, but brain Hg to Se ratios were greatly attenuated, often by at least a log order. Disparities in Hg to Se ratios of blood and brain were greatest in rats fed low-Se with moderate MeHg, and almost as great in rats fed low-Se diets with high-MeHg. In rats fed adequate- or rich-Se diets, the differences in Hg to Se ratios of the blood and brain were greatest in the high dietary MeHg groups.

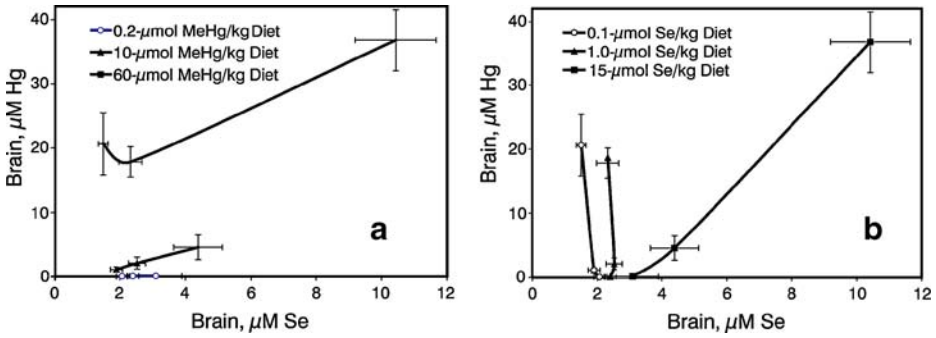

Fig. 3 Effect of MeHg (**a**) and Se (**b**) dietary treatment on the distribution of Hg and Se into the brain

Discussion

The results of this study showed that increasing Se intakes through the normal dietary range from 0.1 to 15 μmol Se/kg were directly associated with resistance to growth impairment resulting from MeHg toxicity. Growth impairments among rats fed high-MeHg diets were severe when rats were fed low-Se diets, less severe among those fed adequate-Se diets, and undetectable in rats fed rich-Se diets. These results indicate that the level of MeHg exposure associated with toxic effects is highly dependent on concurrent dietary Se intake.

The conventional hypothesis of selenium's protective effect against Hg toxicity is that supplemental Se diminishes Hg's negative effects because of Se's ability to sequester Hg. However, examining the molar Hg to Se ratios in the brains of rats provides a different insight into the nature of the Se protective effect against Hg toxicity. The Hg to Se ratio in the brains of rats fed low-Se diets with high dietary MeHg was approximately 14. In brains of rats fed high dietary MeHg along with adequate-Se or rich-Se diets, the brain Hg to Se ratios were approximately 8 and 4, respectively. As none of these ratios approach the 1:1 stoichiometry, the available Se could not sequester more than a fraction of the Hg present, yet growth in MeHg-exposed groups improved dramatically with increasing Se intake. Amounts of Hg in the blood and brain tissues of rats fed high-MeHg diets were inversely related to growth impairment from MeHg toxicity. Blood and brain Hg concentrations were proportional to MeHg exposure, but MeHg exposure was not proportional to risk of MeHg toxicity. In contrast, Hg to Se molar ratios in the blood and brain were directly proportional to toxicity.

The observation that Se protects against MeHg toxicity when Se is not in sufficient molar abundance to Hg suggests that the mechanism of protection is not because of Se-dependent sequestration of Hg. Instead, the protective effect may reflect effects of Hg on Se physiology. In addition, the great increases in both Hg and Se concentrations in the brains of rats fed high concentrations of MeHg and Se suggest HgSe formation may be occurring.

The high molar ratio of Hg to Se in low-Se-fed rats was associated with growth inhibition and diminished brain selenium. In low-Se rats that were not challenged with dietary MeHg, the body was able to mobilize and deliver sufficient Se from somatic stores to the brain to maintain near-normal Se concentrations. However, when challenged with dietary MeHg, Se levels were reduced to 60% of the normal, suggesting that mobilization and delivery of Se to the brain was impaired. In addition, the rats provided rich-Se and high-MeHg diets displayed an unexpected increase in the accumulation of Hg and Se in their brains, suggesting the formation and retention of HgSe.

The results of this study indicate the molecular mechanism responsible for Se-dependent protection against MeHg toxicity and the mechanism of Hg toxicity itself converge on Hg's effects on Se physiology. Although various physiological consequences and manifestations of MeHg toxicity have been described [2, 46–52], the direct molecular mechanisms responsible for the various physiological perturbations reported have not been well-defined. However, it has been recognized for more than 50 years that Se protects against Hg toxicity [5], and it has been known for more than 30 years that physiological consequences of MeHg exposure may relate to impairments of selenoenzyme synthesis or activities [35, 36]. This alternative "Se sequestration" hypothesis of Hg toxicity is depicted in Fig. 4.

Evidence in support of the Se sequestration hypothesis of Hg toxicity occurring as a result of Hg-dependent selenoenzyme inhibition is growing. For instance, although lipid peroxidation has long been known to accompany Hg toxicity, it has only recently been recognized that Hg does not promote the direct nonenzymatic lipid peroxidation [53]. Instead, inorganic Hg^{2+}, but not MeHg, inhibits Se-dependent glutathione peroxidase activity, particularly when Se availability is low. In the absence of glutathione peroxidase

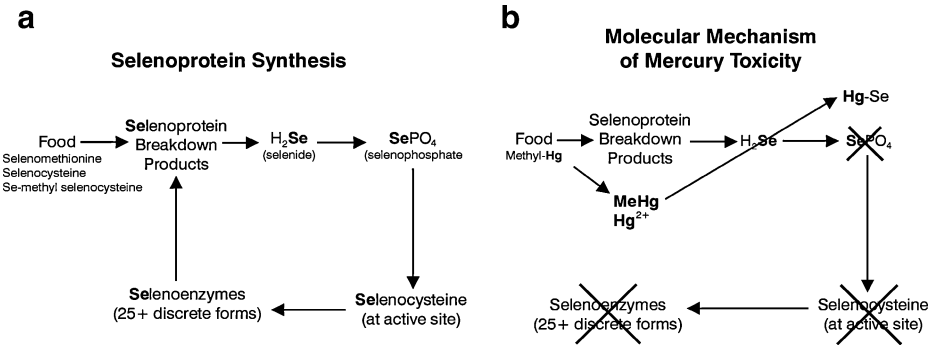

Fig. 4 Depiction of normal selenoprotein cycling (**a**) and the postulated "Se sequestration" mechanism of MeHg toxicity (**b**)

activity, increased levels of hydrogen peroxide accumulated, causing damage to lipids. As many of the selenoenzymes are active in protection against or reversal of oxidative damage, it is not surprising that oxidative damage would occur if their presence and activities diminished as a result of MeHg exposure. As incorporated MeHg is gradually demethylated to release intracellular Hg^{2+} that has high binding affinities for Se, the Se sequestration hypothesis may also provide an explanation for the latency period that often accompanies exposure to toxic amounts of MeHg [54]. This mechanism could also explain why MeHg exposure severely compromised selenoenzyme activities in tissues of neonatal experimental animals whose dams were fed low dietary Se, but not in tissues of neonates whose dams were fed Se-rich diets [10, 55].

MeHg not only has the ability to cross the placental and blood–brain barrier, but its high affinity for Se enables it to specifically sequester Se in the brain, thereby diminishing synthesis of selenoproteins, which participate in brain development and other important biological functions [30, 31, 56–58]. In this regard, it appears that MeHg must specifically deplete Se enzyme activity in specific cell populations of the brain in order for neurotoxicity to occur. The three main families of selenoproteins—deiodinases, thioredoxin reductases, and glutathione peroxidases—all have critical roles in fetal development, including cell growth and survival, free radical detoxification, and thyroid hormone metabolic regulation [33]. Therefore, the loss of these selenoenzymes and their functions may explain many of the pathological effects of Hg toxicity, including peroxidative damage, altered glutathione metabolism, and disruptions in regulatory signal transduction processes.

Increased Se accumulation has been noted in association with high Hg in the brain tissues of victims of the MeHg-poisoning events in Japan [59] and in the brains of Slovenian Hg miners analyzed upon autopsy [60, 61]. In the Slovenian miners, relatively high and prolonged Hg exposures below the toxic threshold resulted in extraordinarily high coaccumulation of Hg and Se that tended to approach but not exceed the 1:1 molar ratios. This suggests that HgSe is the predominant form of Hg that is accumulated, which was also recently shown in whale brains [Huggins and Ralston, in preparation]. However, bioavailable Se was uniformly present in excess of Hg and maintained at remarkably well-regulated molar concentrations, which is indicative of the actively cycling pool of Se involved in selenoenzyme synthesis [61]. The sustained availability of Se may explain why no symptoms of Hg toxicity were apparent in the retired Slovenian Hg miners. Brains of animals experimentally subjected to MeHg also demonstrate elevated Hg accumulation when increased dietary Se is provided, and elevated Se accumulation when MeHg is present in diets [10, 12, 37, 62].

Conclusions

Current assessments of risk from MeHg exposure are exclusively based on Hg concentrations in the fish in relation to total Hg in the blood and hair. However, the results of this study showed Se intakes through the normal dietary range from 0.1 to 15 μmol Se/kg resulted in increasing resistance to MeHg toxicity. As the rich-Se level used in this study is similar to typical concentrations of Se in ocean fish, these results directly pertain to the seafood safety issue. Ocean fish are among the richest dietary sources of Se. Among 11,000+ foods evaluated for Se content by the U.S. Department of Agriculture, ocean fish comprised 17 of the top 25 sources [63]. It is important to note that the high-MeHg concentrations fed in this study were approximately 20-fold higher than the amounts typically present in ocean fish. Thus, the Se present in ocean fish is more than sufficient to protect against the Hg that may also be present. Based on the abundance of Se and its favorable molar excess over Hg, ocean fish appear more likely to protect against Hg toxicity than to contribute to causing it. Several studies have demonstrated that Se from yellowfin tuna [7, 17], menhaden [18], swordfish [19], and rockfish [20] counteract the adverse impacts of Hg exposure, further supporting the likelihood that Se from fish provides significant natural protection. However, risks associated with consumption of certain seafoods that contain high and disproportionate amounts of Hg in molar excess of Se are expected to be directly related to their Hg to Se ratios.

In summation, the toxic effects displayed by rats exposed to identical dietary MeHg concentrations were inversely related to dietary Se consumption. High-MeHg exposure accentuated Se retention in blood and diminished Se delivery to brain when dietary MeHg to Se molar ratios were high. Conversely, increasing dietary Se resulted in greater total Hg retention in blood and brain, possibly as HgSe conjugates. Although Se binds to Hg in the blood and brain compartments, it does not appear that Hg sequestration is the mechanism of Se-dependent protection against Hg toxicity. Instead, it appears that sustained Se sequestration by Hg diminishes Se bioavailability, eventually resulting in the inhibition of synthesis and activities of selenoenzymes. Therefore, it follows that the mechanism of Se-dependent protection against Hg toxicity may be the result of supplemental Se replacing intracellular Se lost to HgSe formation, thereby maintaining normal synthesis and activities of essential selenoenzymes. The results of the current study indicate that seafood safety evaluations of health risks associated with Hg exposure would be improved by concurrent assessments of Se availability. Criteria that incorporate Hg to Se molar relationships will provide more comprehensive evaluations that will enhance environmental health assessments and improve public safety.

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