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Mercury bioaccumulation and its toxic effects in rats fed with methylmercury polluted rice

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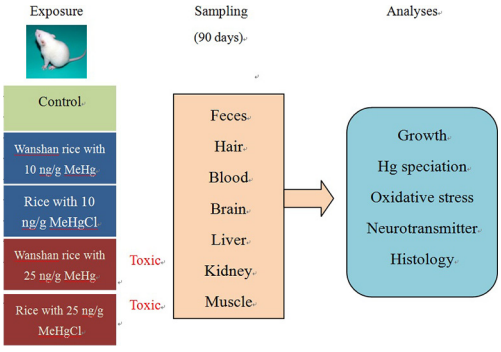
HIGHLIGHTS

- Long-term exposure to spiked MeHgCl inhibited the growth in female rats.
- Significant differences in accumulation of THg and MeHg among different groups and organs in rats.
- Exposure to rice containing 25 ng/g MeHg decreased antioxidant function and damaged the nervous system in rats.
- MeHgCys in rice is less toxic than spiked MeHgCl to rats.

GRAPHICAL ABSTRACT



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ABSTRACT

Recent evidence indicated that methylmercury (MeHg) contaminated rice can be a significant source of MeHg human exposure, but the health implications are not known. The objective of this study was to study the kinetics, speciation, and effects of MeHg contaminated rice using a rat model. Five groups of adult Sprague-Dawley rats ($n = 10$ in each group) were fed control rice, low (10 ng/g MeHg) and high (25 ng/g MeHg) MeHg contaminated rice. Two groups of the positive control were fed control rice spiked with the same levels of MeHgCl. Short-term exposure to low level of spiked MeHgCl stimulated the growth of male rats while long-term exposure to spiked MeHgCl inhibited the growth in female rats. There was no temporal variation of total mercury (THg) concentrations in the rat fecal samples from each group, and the THg concentrations significantly correlated with the inorganic Hg concentrations in the feeding rice. There were significant differences in the accumulation of THg and MeHg among different groups and different organs. THg and MeHg concentrations in the kidney were the highest among the organs examined. The blood and brain had high percentages of THg as MeHg, which indicates that MeHg can easily pass through the blood-brain barrier and has a high affinity for brain tissue. Exposure to rice containing 25 ng/g MeHg decreased antioxidant function and damaged the nervous system in rats, but no significant effects were found in the group fed with rice containing 10 ng/g MeHg. MeHgCys in rice is less toxic than spiked MeHgCl to rats. The toxicity of MeHg both decided by its concentration and speciation.

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1. Introduction

Methylmercury (MeHg) is a pollutant well-known for its neurotoxicity (Mergler et al., 2007). MeHg exposure in the general population occurs mainly via the consumption of fish and marine mammals (Driscoll et al., 2013). MeHg contamination in fish poses a particular challenge to public health because of the nutritional benefits for human health from the consumption of fish (Mahaffey et al., 2008; Ginsberg and Toal, 2009). MeHg in fish tissue has been identified to bind to the thiol group of the cysteine amino acids in fish protein (Harris et al., 2003) and cannot be removed and destroyed by any cooking or cleaning processes. The maximum allowed or recommended levels of MeHg in fish recommended by the Joint FAO/WHO Food Standards Programme CODEX Committee on Contaminants in Foods (JFFSP, 2011) are 1.0 μg/g for predatory fish and 0.5 μg/g for other fishery products (wet weight); this guideline has been adopted by most countries.

Recent studies highlighted that rice can accumulate high levels of MeHg in mercury (Hg) mining areas Guizhou and Southwestern China (Horvat et al., 2003; Zhang et al., 2010a). The paddy field can be considered as an ephemeral wetland, which provides an environment that is suitable for the bacterial methylation of Hg in the soil. MeHg in the soil is first absorbed by the roots, then translocated to the aboveground parts (leaf and stalk), and finally accumulated in the rice grain (Meng et al., 2011). Furthermore, rice, rather than fish, is the main route of human MeHg exposure in the Wanshan Hg mining area (Feng et al., 2008), Guizhou Province (Zhang et al., 2010b), and inland area of southern China (Li et al., 2012). MeHg in uncooked rice occurs predominantly as MeHg-L-cysteinate, which will convert to other forms of MeHg after cooking; however, its identity and toxicity remain unknown (Li et al., 2010). The toxicokinetic model based on fish consumption underestimated the risks caused by MeHg exposure from rice consumption (Li et al., 2015).

There are reports from animal studies showing that consumption of Hg contaminated rice caused significant decreases in the antioxidant enzymatic activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), decreased the concentration of serum nitric oxide (NO), and increased the content of reduced glutathione (GSH) and malonyldialdehyde (MDA) in rat serum and liver (Ji et al., 2006, 2007). The neurotransmitters in the rat brain, including acetylcholine (Ach), acetylcholinesterase (AchE), nitric oxide, and nitric oxide synthase (NOS) were also significantly affected (Cheng et al., 2005a). Additionally, the expression levels of c-jun and c-fos genes in the rat brain were significantly induced by MeHg polluted rice (Cheng et al., 2005b, 2006a, 2006b). However, these studies did not consider the speciation of Hg in the rice and how it affects the internal dose and species in the target organs. Moreover, they were conducted using rice with very high THg concentrations and MeHg concentrations (e.g., 133 ng/g THg and 33 ng/g MeHg; Cheng et al., 2005a, 2006b). In comparison, the MeHg concentrations in rice grains in the Wanshan Hg mining area generally range from 10 to 20 ng/g (Feng et al., 2008; Qiu et al., 2008; Zhang et al., 2010a).

The overall goal of this study is to understand the bioaccumulation of different Hg species and their toxic effects in rats fed with low-level MeHg polluted rice. We used contaminated rice with two environmentally relevant concentrations as well as two spiked rice samples with MeHgCl matching MeHg concentrations to evaluate the bioaccumulation of different Hg species and their health effects in rats in a subchronic exposure of 90 days. The hypothesis is that the MeHg in contaminated rice is bioavailable and can cause adverse effects in rats at a dosing rate similar to that of human consumption.

2. Materials and methods

2.1. Rat feeding experiments

The animal experimental protocol of this study is approved by the Institute of Geochemistry, Chinese Academy of Sciences.

Sprague-Dawley rats weighing 70–80 g were purchased from the Vital River Laboratories (Beijing, China) and were housed individually at the Pathogen Free Rat Breeding Unit at Harbin Medical University. The temperature was maintained at 22 ± 2 °C with a 12-h light/dark cycle and free access to food and water. Rats were raised for 1 week before the experiments. The rats were divided into five groups: a control group (A, fed with market rice containing a low MeHg concentration), a low MeHg dose group (B, fed with rice containing 10 ng/g MeHg from the Wanshan Hg mining area), a high MeHg dose group (C, fed with rice containing 25 ng/g MeHg from the Wanshan Hg mining area), a simulated low MeHg dose group (D, fed with market rice spiked with MeHgCl to reach 10 ng/g MeHg), a simulated high MeHg dose group (E, fed with rice spiked with MeHgCl to reach 25 ng/g MeHg). The USEPA set the limit of 0.1 μg/kg/day as the reference dose (RfD) for MeHg (USEPA, 1997) and the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) for MeHg at 0.23 μg/kg/d (JECFA, 2003). For rural residents in Hg mining area in Guizhou Province (such as Wanshan area), rice is the staple food for local residents and the average rice ingestion rate was 600 g/day. Considering the body weight of 60 kg, these two international guidelines correspond to rice MeHg levels of 10 and 23 ng/g, respectively. Therefore, we set these two rice MeHg levels to test the toxicity of MeHg to humans.

The feed was composed of 77.5% rice powder, 14.0% casein, 4.0% soybean oil, 3.5% mineral mix (AIN-93 M-MX), and 1% vitamin mix (AIN-93-VX). Each group consisted of half male and half female rats for comparison of sex differences. The detailed information of Hg concentrations in the feed for the rat experiments is shown in Supplementary Information (SI) Table S1. Because rice samples from the Wanshan Hg mining area can also bio-accumulate selenium (Zhang et al., 2012), which is known to protect against MeHg neurotoxicity (Sakamoto et al., 2013), total selenium (TSe) concentrations in the rice samples were also measured and found to have no significant difference among the groups (Table S1).

During the experiments, data regarding the amount of food consumed and the body weights of rats were recorded weekly. Rat fecal samples were also collected for total Hg (THg) analysis. After feeding for 90 days, the rats were sacrificed. Blood, brain, liver, kidney, muscle, and hair samples were collected to determine THg and MeHg concentrations. The brain and liver tissues were homogenized after the addition of 4.5 mL of a 10.0 mM Tris buffer (pH 7.5) for detection of enzyme activities. The brain tissues were treated with formalin for histopathological study.

2.2. Analytical methods

The TSe concentrations in the rice samples were digested with HNO_3 + HCl and determined by hydride generation atomic fluorescence spectrometry (Zhu et al., 2008). The fecal and organ samples were freeze dried for Hg speciation analysis. THg concentrations in solid samples (fecal, brain, liver, kidney, muscle, and hair, dry weight) were determined by the RA-915+ Hg analyzer coupled with the PYRO-915+ attachment (Lumex, Russia), which is based on thermal decomposition and Zeeman atomic absorption spectrometry detection. Whole blood samples were digested with a mixture of nitric and sulfuric acid (v/v 4:1) at 95 °C for 3 h. All digests were determined by BrCl oxidation, $SnCl_2$ reduction, purge, gold trap, and cold vapor atomic fluorescence spectrometry (Yan et al., 2005). For MeHg analysis, brain, liver, kidney, muscle, hair, and blood samples were digested using the KOH-methanol/solvent extraction technique (Liang et al., 1996) and then measured using aqueous ethylation, purge, trap, and GC-CVAFS detection (Brooks Rand MERX).

Activities of GSH-Px and GSH contents in the brain, liver, and serum were determined by the 5,5′-dithio-bis-(2-nitrobenzoicacid) (DTNB) photometric method using a T6 UV-VIS spectrophotometer (Puxi, Beijing, China) (Deng et al., 2000). The activity of AChE in brain samples

was measured by determining the amount of thiocholine, which formed via the hydrolysis of acetylthiocholine iodide by AChE in tissue samples, using the DTNB photometric method. Acetylcholine (ACh) concentrations in brain tissues were determined using an acholine/acetylcholine quantification kit (Nanjing Jiancheng Bioengineering Institute). To use the kit, free choline is oxidized to form betaine via the intermediate betaine aldehyde. The reaction generates products that react with the choline probe to generate color, which is measured at 550 nm. Protein concentrations in tissue homogenates were determined by the Bradford method using bovine serum albumin as the standard. All enzyme activities were expressed in the unit of mg per protein. To correct for spontaneous reactions in the biochemical analysis, blanks were run without sample and then blank values were subtracted from the determined values. Brain tissues were homogenized in a 10% neutral formalin buffer. Tissue sections (5 μm thick) were cut and stained with hematoxylin and eosin for histopathological studies.

2.3. Quality control

A quality-control system consisted of method blanks, blank spikes, matrix spikes, certified reference material (CRM), and blind duplicates. Recoveries of CRMs were satisfactory. Detailed results are listed in SI Table S2. The percentage of recoveries on spiked samples ranged from 85% to 120% for MeHg in blood and tissue samples. The relative percentage difference was lower than 10% for Hg speciation in biological duplicate samples.

2.4. Statistical analysis

All data were analyzed using IBM SPSS 19 for Windows. The characteristics of the data are described in Mean ± Standard Deviation (SD) for descriptive statistics. Mean values of biochemical parameters for the different groups were compared using analysis of variance (ANOVA). Relationships between different factors were analyzed using the Pearson correlation analysis. Results of statistical tests were considered statistically significant if $p < 0.05$.

3. Results and discussion

3.1. Effects on growth

Body weight is an important and sensitive index to evaluate physical growth and nutritional status. Temporal changes of rat body weights are shown in SI Table S3. During the experiments, the body weights of all the rats continued to rise. From 7 to 28 days, the body weights of the male rats in group D were significantly higher than those in the control group ($p < 0.05$). This observation was similar to a finding by Cheng et al. (2005a) that stated that a low dose of Hg could accelerate the growth of rats. After 35 days, the body weights of the female rats in groups D and E were significantly lower than those in the control group ($p < 0.05$). Additionally, after 35 days, the body weights of the female rats in groups B and C were lower than those in the control group, though not statistically significant. There was no effect on the growth of the male rats by the end of the experiments. The body weights of the male rats were significantly higher than those of the female rats (544 g compared with 318 g on average) by the end of the experiments (90 days). The results indicated that short-term exposure to low level of spiked MeHgCl stimulated the growth of male rats while long-term exposure to spiked MeHgCl inhibited the growth in female rats.

Organ weight and coefficient data (ratio of organ weight to body weight) of experimental animals are important indexes in biomedical research. Not only can these data provide information on the physiological status of animals, feeding, and management, but they are also an important reference index for clinical research. Effects of MeHg exposure on organ coefficients for the different groups of rats are shown in SI Table S4. Generally, there were no significant differences between the exposed and control groups on organ coefficients in both male and female rats, which indicated that low-level MeHg exposure had no effect on organ development in the rats.

3.2. Hg bioaccumulation and distribution

Feces can be a good biomarker of inorganic (IHg) exposure and accounts for 90% of total Hg elimination in humans and other mammals after exposure to MeHg (WHO, 1990). The conversion of MeHg to IHg maybe a key step in the processes of excretion (Feng et al., 2015). Temporal variations of THg concentrations in rat fecal samples during the experiments are shown in SI Fig. S1. Overall, THg concentrations in the rat fecal samples were mostly stable with time (SI Fig. S1). The coefficients of variation at different times were <16%. Compared to the control group, THg concentrations in rat fecal samples of the exposed group were significantly elevated. Interestingly, a significant positive correlation ($r^2 = 0.95$, $p < 0.001$) was observed between THg in the rat fecal samples and IHg in the corresponding rice fed to each group (SI Fig. S2). This result indicates that feces can be utilized as a good biomarker for IHg exposure, which can reflect body loading of dietary IHg intake.

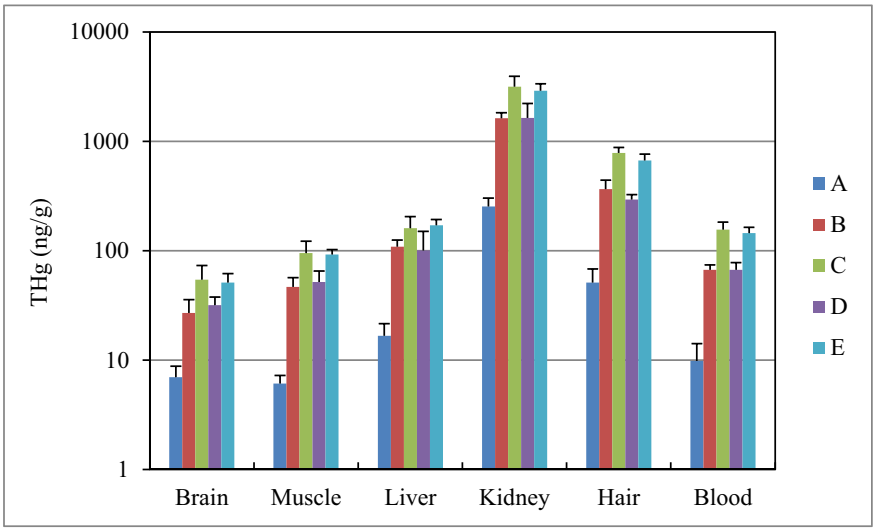


Fig. 1. THg concentrations in different organs of the rats at day 90 (4 male and 4 female rats for each group; for brain, muscle, liver, and kidney, dry weight).

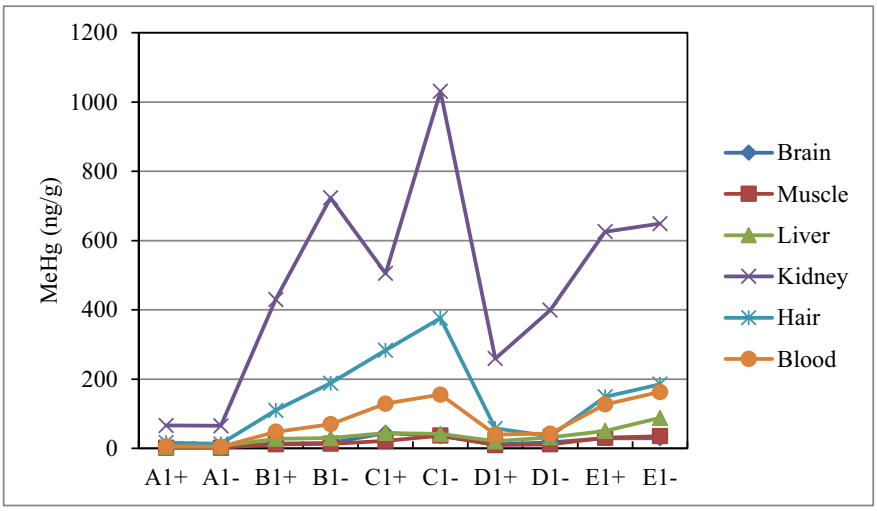


Fig. 2. MeHg concentrations in different organs of the rats (+ male; − female; for brain, muscle, liver, and kidney, dry weight).

THg burdens in all organs of the rats increased in a dose-dependent manner, and significant increases were found in exposed groups compared with the control group (Fig. 1). For the exposed groups, there were no significant THg concentration differences in the organs between groups B and D or between groups C and E. Regarding THg concentration differences in the organs, the highest accumulations of THg were observed in the kidney, followed by the hair, liver, blood, muscle, and brain. In terms of the MeHg accumulation pattern in organs, there were similar trends to that of THg (Fig. 2); kidney and hair revealed the highest accumulations of MeHg. Sex differences were observed in the MeHg concentrations in the organs, while male rats had lower MeHg concentrations (Fig. 2). According to the rice daily intake and body weight, we calculated the Probable Daily Intake (PDI) of MeHg for rat C1(+) and C1(−) at two typical time to reveal the sex difference (SI Table S5). Even lower daily rice intake, the female rat has significant higher PDIs (20–25%) than male due to lower body weight. The dilution effect of body weight was the main factor to control the sex difference of organ MeHg concentrations in rats.

If taking into consideration the portion of MeHg found in the THg, the blood and brain showed relatively high ratios, with averages of 89.8 ± 10.1% and 67.0 ± 16.9%, respectively (Fig. 3). Muscle, liver, kidney, and hair showed relatively low ratios around 30% (Fig. 3). The absorption rate of MeHg in food is about 95% and that of IHg is only 8% (WHO, 1990, 1991), which may be the cause of the high ratio of MeHg in the blood samples. The brain is a target organ of MeHg, and MeHg can cross the blood-brain barrier without hindrance to reach its principal target tissue (Ji et al., 2007; Hu et al., 2010). The brain-to-blood ratios of MeHg concentrations in the rats in the exposed groups ranged from 0.18 to 0.42, which is much higher than the result of 0.06, as reported by Magos (1987). The liver and kidney are target organs of IHg and are actively involved in the detoxification of heavy metals. This resulted in IHg deposition in the tissues and relatively low ratios of MeHg.

3.3. Toxic effects

Effects of MeHg exposure on oxidative stress and neurotransmitters in rat tissue are shown in Table 1. Activity levels of GSH-Px, GSH, and ACh contents in the brain, liver, and serum from groups C and E decreased significantly compared to those of the control group ($p < 0.01$). These biochemical parameters in groups B and D also decreased compared to those of the control group but were not statistically significant. Activities of AChE in brain tissue from groups C, D, and E increased significantly compared with the control group ($p < 0.01$). Activities of AChE in brain tissue from group B also decreased compared to those of the control group but were not statistically significant.

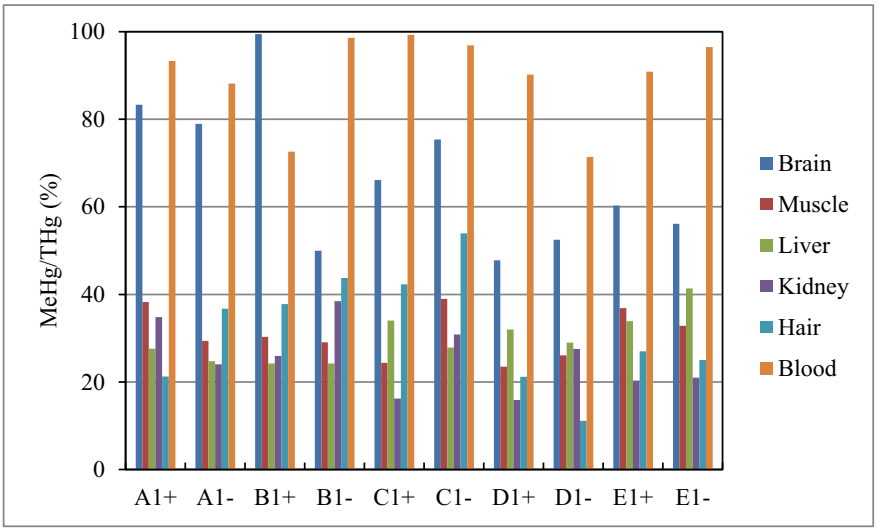


Fig. 3. The percentage of THg as MeHg in different organs of the rats (+ male; − female).

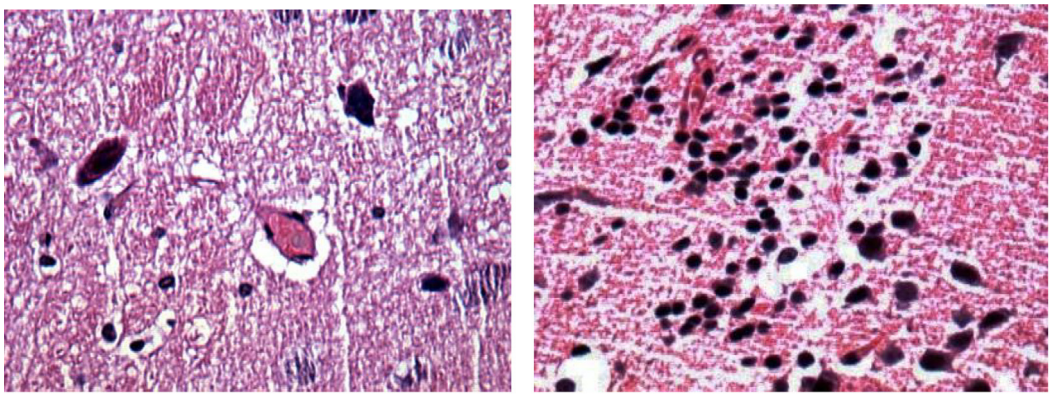
Table 1
Effects of MeHg exposure on oxidative stress and neurotransmitters in tissues of rats ($n = 8$ for each group, Mean ± SD).

Group	GSH-px			GSH		ACh	AChE
	Brain	Liver	Serum	Brain	Liver	Brain	Brain
	(U/mg pro)	(U/mg pro)	(U/mL)	(mg/g pro)	(mg/g pro)	(μg/mg pro)	(U/mg pro)
A	37.8 ± 1.46	708 ± 16.3	1103 ± 72.1	9.91 ± 0.24	8.84 ± 0.20	30.2 ± 1.36	0.376 ± 0.014
B	37.4 ± 1.03	702 ± 17.6	1050 ± 56.7	9.87 ± 0.34	8.68 ± 0.16	29.3 ± 1.47	0.392 ± 0.013
C	36.1 ± 1.63[a]	657 ± 25.7[a]	1023 ± 60.8[a]	9.56 ± 0.17[a]	8.30 ± 0.12[a]	24.9 ± 1.39[a]	0.560 ± 0.037[a]
D	37.2 ± 1.14	688 ± 17.4	1035 ± 85.6	9.84 ± 0.30	8.64 ± 0.28	28.3 ± 1.12[a]	0.493 ± 0.024[a]
E	34.2 ± 1.30[a]	593 ± 23.6[a,b]	939 ± 71.9[a]	8.71 ± 0.18[a,b]	7.05 ± 0.33[a,b]	22.5 ± 0.95[a,b]	0.631 ± 0.023[a,b]

[a] $p < 0.01$, compared with group A.
[b] $p < 0.01$, compared with group C.

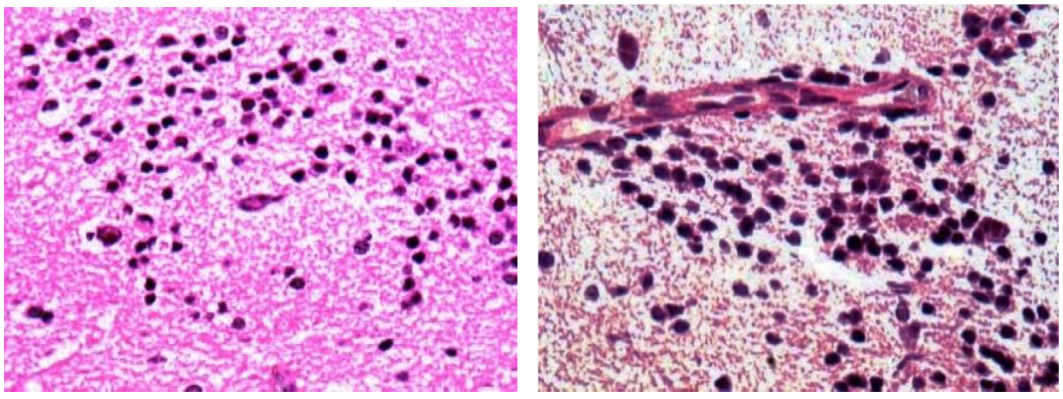
The GSH redox cycle is one of the most important intracellular antioxidant systems for scavenging free radicals and other reactive oxygen species (Forman et al., 2009). GSH-Px is an important target and major cellular defense against MeHg-induced neurotoxicity (Franco et al., 2009). The present results provided evidence that activities of GSH-px were significantly decreased in exposed groups, which indicated that the scavenging ability of GSH-px was inhibited in rats exposed to MeHg for 90 days. Similar phenomena were observed in activities of GSH-px in the liver and serum of rats fed MeHg-contaminated rice for 90 days (Ji et al., 2006, 2007).

Although the neurotoxic effects of MeHg are mediated by multiple mechanisms, previous studies have indicated that MeHg exposure can affect cholinergic neurotransmission in many species (Basu et al., 2005, 2006). In the brains of mink, MeHg exposure can increase mACh receptor levels and AChE activity (Basu et al., 2006). In this study, ACh concentrations in the exposed groups were significantly decreased compared to those of the control group. Activities of AChE were also significantly elevated compared to those of the control group. These results indicate that long-term MeHg stimulation will cause a decline in cholinergic neurotransmitter function and will finally result in general adverse effects on the normal functions of the central and peripheral nervous systems. Ji et al. (2006) observed temporal variation in the contents of Ach and activity levels of AChE in rat brains after MeHg exposure. MeHg exposure significantly increased the contents of ACh in rat brains after 7 days and were maintained at a high level up to 30 days, but decreased significantly at 90 days. The changes in the activity levels of



a. Pyramidal cell in rats of Group A (×400)

b. Midbrain in rats of Group C (×400)



c. Midbrain in rats of Group D (×400)

d. Midbrain in rats of Group E (×400)

Fig. 4. Histopathology images of brain tissues from the rats in each exposed group.

AChE represented an inverse trend compared to those of Ach. Low-dose MeHg exposure will stimulate Ach, but long-term stimulation will result in a decline in the function of the neurotransmitter.

Significant differences ($p < 0.01$) of activity levels of GSH-Px in liver, GSH contents in the brain and liver, ACh contents in liver, and activities of AChE in brain were observed between Group C and E (Table 1). Rats in Group E (spiked MeHgCl, 25 ng/g) exhibited higher oxidative stress and damage of neurotransmitter compared with Group C (MeHg contaminated rice). Coupled high performance liquid chromatography with inductively coupled plasma mass spectrometry, Li et al. (2010) found that MeHg in raw rice collected from Wanshan Hg mine is present almost exclusively as MeHg-L-cysteinate (MeHgCys), which can transfer of MeHg across the blood-brain and placental barriers. Based on technology of X-ray absorption near-edge spectroscopy, Meng et al. (2014) also confirmed this phenomenon. MeHgCys is proved to be much less toxic than MeHgCl, and zebrafish larvae can tolerate 20-fold concentration of MeHgCys than MeHgCl (Harris et al., 2003). We proposed that the difference of toxicity of MeHgCys and MeHgCl resulted in the results of oxidative stress and neurotransmitter between Group C and E both in MeHg level of 25 ng/g.

As observed with microscopy, the morphology of neurons remained normal in the different regions of the brain in the control group. The structure of the pyramidal cells is clear, and the Nissl bodies are visible in the cytoplasm; glial cells are evenly distributed; capillary lumens are relative; and neurons and capillaries are surrounded with gaps (Fig. 4a). However, significant pathological alterations were observed in the midbrain of the exposed groups. Hyperplastic nodules in glial cells were observed around the small vasculature in the midbrains of rats in groups C, D, and E (Fig. 4b, c, d), and cell necrosis was observed in the nodules in groups D and E (Fig. 4c, d). The hemorrhages occurred around small vessels in the midbrains of the rats in group E (Fig. 4d). The results indicated significant damage to the nervous system in rats in groups D and E.

4. Conclusions

Short-term MeHg exposures stimulated the growth of male rats while long-term MeHg exposure inhibited the growth rate in female rats. MeHg exposures via rice with 10 and 25 ng/g levels had no effect on organ development in rats. There were large differences in the accumulation of THg and MeHg among the different exposed groups and different organs. MeHg exposure to rice containing 25 ng/g MeHg decreased antioxidant function and damaged the nervous system in rats. MeHgCys in rice is less toxic than spiked MeHgCl to rats. The toxicity of MeHg both decided by its concentration and speciation.

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Appendix A. Supplementary Information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.03.185.

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