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Mercury pollution in two typical areas in Guizhou province, China and its neurotoxic effects in the brains of rats fed with local polluted rice

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Abstract

Guizhou province, which located in southwestern of China, is an important mercury (Hg) production center. This study was to investigate the environmental levels and ecological effects of mercury in two typical Hg polluted areas in Guizhou province. In addition, to improve the understanding of the neurotoxic effects of Hg, a rats based laboratory study was also carried out in this study. Samples of water, soil, plants, crops and animals collected from Wanshan mercury mine area, Guzhou province, were analyzed by mercury analyzer. The effects of Hg contaminated rice on the expression of c-jun mRNA in rat's brain and the expression of c-JUN protein in cortex, hippocampus were observed using reverse transcription polymerase chain reaction (RT-PCR) and immunocytochemical methods. The results showed that the mercury contents in most environmental samples of aquatics, soil, atmosphere and the biomass of corn, plant and animals, were higher than the national standard and the corresponding data from unpolluted area. It was found mercury pollutions were significant in soil and air. In the laboratory study, the expression of c-jun mRNA and its protein was significantly induced by Hg polluted rice collected from local area. Selenium could reduce the Hg accumulation in the body and had antagonist effect on Hg in terms of the expression of c-jun mRNA and c-JUN protein. The environmental data and Hg levels in different creatures collected in this study will facilitate the environmental and ecological risk assessment of Hg in the polluted areas. It was urged to be alert of mental health problem in human beings when any kind of Hg-polluted food was taken. More efforts should be performed to protect the local ecosystem and human health in the mercury polluted area of Wanshan, Guizhou province of China.

Introduction

Mercury (Hg) is a silvery, liquid metal at room temperature. It is harmful to human beings and advanced creatures. In addition, methylmercury is a neurotoxic compound and capable of inducing cancer, deformity and mutation (Pamphlett & Kum-Jew 2001; Karimi *et al.* 2002). Concerns about mercury are based on its effects on both ecosystem and human health. China is one of the countries with the high amount of mercury producer and consumer in the world. Guizhou province, which located in Southwestern China (N24°30′–29°13′, E103°1′–109°30′, 1100 m above sea level, subtropical humid climate), is currently one of the most important mercury production areas in the world. Hg mining in China approximately started from 3,000 years ago. Guizhou has a history of Hg production about 600 years. The cinnabar deposit reaches to about 80,000 tons in

Guizhou province and occupies 70% of the total deposits in China. The total output was reported to be 26,000 tons from 1949 to 1981. The peak annual mercury emission from mining and refining to the atmosphere reached 11 tons (Xiao *et al.* 1998; Tan *et al.* 2000; Horvat *et al.* 2003). In addition, Guizhou province is one of the major coal production provinces in China. Combustion of coal contributes about 80% of the total energy consumption in a population of 34 million people. Currently, about 40 million ton per annum of coal were combusted for both industrial and domestic purposes in Guizhou province. The average mercury concentration in coals from Guizhou coal mines was 0.55 mg g^{-1}. Thus, about 20 t of mercury was released into the environment from coal combustion process in Guizhou province in 1955 (Feng & Hong 1999). Another source of Hg in the environment of Guizhou province is the emission from chemical industries in which Hg is used as a catalyst for the production of acetaldehyde and other organic compounds (Horvat *et al.* 2003). Hg is also used in agriculture, medicine and the electrical industry, etc. Emissions of mercury from the province to the global atmosphere have been estimated to be around 12% of the world total emissions (Tan *et al.* 2000). The local population exposure to mercury may occur due to inhalation of mercury in air and consumption of mercury pollution food and water.

In this study, two typical areas in Guizhou province, i.e. WS district (Wanshan mercury mine area) and QZ district (Qingzhen chemical plant area), were selected to investigate the environmental behaviors, distributions and ecological effects of Hg in these areas. The basic information collected in this study will facilitate the environmental and ecological risk assessment of Hg in the aforecited areas. Additionally, to better understand the toxicological effects of Hg and its possible adverse effects on human beings, a rats based laboratory study was also carried out in this study. As the neurotoxic effect of Hg is one of the most seriously concerned aspects, the laboratory study is to observe the expression of c-jun in the brains of rats fed with local polluted rice. The c-jun gene is a member of immediate early genes. Expression of the c-JUN protein product has been used as a marker of the induced neuronal activation. The laboratory results obtained in this study will enhance the understanding of neurotoxic effects induced by Hg and provide the basic information of exposure and the risk assessment for human beings.

Materials and methods

Behaviors of mercury in the environment and food chain in WS area

Sampling

WS sampling stations were located in the valley of the Dashuixi River, into which waters from mining and ignition residue disposal sites are leached. All samples were collected in the beginning of November 2002. Soil (3–10 cm from the surface), rice and crop samples were taken from the fields along the river. Water samples were collected from the river. Two pigs, which were fed with local rice and crops for 10 months, were collected. Fish were caught from the Dashuixi River by a local fisherman. Seven ducks were bred freely in the river for 8 months before collection. Anther rice samples were taken from a field near QZ district where Hg is used as a catalyst for the production of acetaldehyde. QZ district is located in the vicinity of the city of Qingzhen. It discharges wastewater into the channel and irrigation areas for the sampling field.

Sample preparation

All precautions were taken in order to avoid any contamination during sampling. Samples were stored in a refrigerator before further processing. All the results are expressed on a dry weight basis.

Rice samples were divided into two parts, i.e. with and without hull. The hull removed part stands for white rice, which was used as feed for the later laboratory study.

Bigger particles in soil samples, e.g. stones and plant residues were removed. Soil samples were then ground to pass a 100-mesh sieve. During the whole sample preparation procedure, special precautions were necessary to avoid cross-contamination of samples, including the sequence of sample treatment. Thus, homogenization process was conducted in the following order, control samples, less contaminated samples and more contaminated samples. Homogenized samples were collected using plastic containers, which were sealed by polyethylene bags. The bags were then stored in a refrigerator until further processing.

Tissue samples from animals were carefully collected from the fish ($n=7$), ducks ($n=7$) and pigs ($n=2$). They were put into polyethylene vials and then stored in deep frozen (−70 °C). Before sample aliquots were taken for analysis, tissues were cut using a clean scalpel and macerated in the sterilized glass plates. A homogeneous mass was obtained and aliquots of the mass were sampled for total Hg determination. Laboratory tools and ware needed for samples preparation were washed with detergent solution, soaked in hot nitric acid, rinsed with double distilled Hg-free water and dried before use. To avoid changes of water contents in the tissues, the processing of tissues homogenization was carried out immediately after maceration.

Water samples were collected at the water body surface (20 cm depth) and put into seven acid-cleaned Teflon bottles. Samples were stored at 4 °C until further processing in the laboratory.

Analytical method for mercury and selenium

Total mercury (T–Hg) was determined by AMA-254 liquid/solid mercury analyzer (Milestone, Italy). Selenium (Se) was determined using a Hitachi Fluorescence Spectrophotometer F-450 (Hitachi, Japan).

Effect of Hg-contaminated rice on c-jun expression in the brains of rats

Animals and experimental procedures

Sprague–Dawley rats (purchased from Shanghai Animal Experimental Center of China Science Institute, weighted of 135–140 g) were housed separately and maintained on a 12 h light/12 h dark cycle. Ambient temperature was maintained at 22 °C with free access to food and water. Rats were raised for several days before experiment. Either too active rats or retarded ones were removed. The rats were then divided into six groups, i.e. control group (SHC), QZ group (QZP), WS group (WSM), methylmercury group (MMG), Se–Hg group (SMG), $HgCl_2$ group (MCG). The concentrations of total mercury, methyl mercury and selenium in rice were showed in Table 1. Each group had seven rats (the males were 4, the females were 3). SHC group were fed with the rice which was purchased in Shanghai market. QZP and WSM groups were fed with the rice collected from QZ and WS, respectively. MMG were fed with the Shanghai rice amalgamated with MeHg. To simulate the rice produced in the QZ, the amount of MeHg was added as 0.16 mgHg kg^{-1}. Similarly, SMG were fed on the Shanghai rice amalgamated with Na_2SeO_4 and $HgCl_2$ to simulate the rice produced in the WS. The added amount of Hg and Se were 0.13 mgHg kg^{-1} and 0.80 mgSe kg^{-1}, respectively. MCG were fed with the Shanghai rice but only amalgamated with $HgCl_2$ to observe the influence of Se when comparing with SMG. Vitamin mix, Corn oil and Minerals were added to all diet in order to ensure the reasonable nutrition (Domene *et al.* 2001). Every rat was fed on 50 g d^{-1} of rice (it was enough). Rats were weighted at 9.00 a.m every other day. After being fed for 20 days, rats were anaesthetized with 10% ketamine clorhydrate (0.005 ml g^{-1} weight) before perfusion via the ascending aorta with 0.1 M phosphate buffered saline. The brains were quickly dissected out of the skull. One part of tissue was post-fixed in the buffer of 4% paraformaldehyde solution for 24 h, washed in water, dehydrated and then embedded in paraffin. They were cut in arrowy aspect (the thickness was 5 μm). Five sections per rat (five rats per treatment) were used to examine the c-JUN expression. Another part of tissue was rapidly removed and immediately frozen by immersion in liquid-nitrogen cooled and stored at −70 °C refrigerator until later assay.

Reverse transcriptase polymerase chain reaction

The tissue sample was picked up from storage and placed on a clean surface. It was cut and weighted before use. Homogenization was performed using a suitable vessel. Total RNA was isolated using the RNeasy protocol (Qiagen, Germany). The concentration of RNA in each sample was determined by photo spectroscopy. An aliquot of total RNA (0.5 μg) from each sample was used for cDNA synthesis. RT-PCR was performed using Qiagen onestep RT-PCR kit (Qiagen, Germany).

Table 1. The Se and Hg content of rice in different areas.

Concentrations (mg kg^{-1})	Shanghai	WS	QZ
Total mercury	0.004	0.133	0.155
Organic mercury	~	0.033	0.130
Selenium	~	0.800	0.075

~ Below the limit of detection.

It was carried out on a Touchgene Gradient PCR system (Touchgene Gradien, England) . PCR cycles consisted of denaturing at 94 °C for 30 s, annealing at 60 °C for 50 s, and extension at 72 °C for 60 s, number of cycles 30. The sequences of primers (Qiagen Germany) used for analysis were listed in Table 2. Samples (10 μl) of PCR products were separated on a 2% agarose gel containing ethidium bromide using a DNA molecular weight marker for comparison purposes. After electrophoresis, the expression of c-jun and GADPH mRNA was indicated by measuring the density of the respective specific bands using the Electrophoresis Documentation and Analysis System along with the Tanon (Shanghai, China) Image Analysis Software program (Ver.3.61).The control was set as 1.0. The amount of mRNA expression was determined by dividing the densitometry value of the mRNA RT-PCR product by that of the GAPDH product. (Ho *et al.* 2002; Regalia *et al.* 2002).

Immunocytochemistry

Five μm-thickness paraffin sections were made from tissues. They were deparafinized and dehydrated as required. The sections were subjected to an immunohistochemical staining procedure on microscopic slides and the results were visualized using the avidin-biotin-peroxidase method (Gibson & Clowry 2001; Willaime-Morawek *et al.* 2003). In brief, the sections were incubated for 20 min with 1–3 drops of serum block; after aspirated serum from slides, immediately added 1–3 drops of pre-diluted primary antibody (Santa Cruz Biotechnology, santa Cruz), incubated for 2 h; rinsed with PBS then washed in PBS twice for 2 min each on a stir plate, aspirated excess liquid from slides, incubated for 30 min with 1–3 drops of biotinylated secondary antibody (Santa Cruz Biotechnology, santa Cruz); washed as above, incubated for 30 min with 1–3 drops of HRP-streptavidin complex; washed as above, added 1–3 drops HRP substrate mixture, developed for 30 s–10 min, or until desired stain intensity obtained; rinsed with demonized H_2O and transferred to a demonized H_2O for 2 min on a stir plate; counterstained, dehydrated and mounted slides. An addition set of control slides was tested to ensure the specificity of the primary antibody. These slides were included in every step of the staining protocol with the following exception: during the primary antibody incubation, a mixture containing only TBS, NGS, and 10% Trition was applied. Later analysis of these slides revealed virtually no staining. Labeled sections were examined using bright field microscopy throughout the rostracaudal extent of the striatum from each animal. Digitized brightfield images were obtained with a video camera attached to Microscope and analyzed with Tanon (Shanghai, China) Image Analysis Software program (Ver. 3.61). The program was used to measure the number of labeled cells.

Statistical analysis

Statistical comparisons between exposed groups and control groups were made with paired Student's *t*-test. All results were expressed as means ±S.D. All significance testing took place at 0.05 level. (Jiang *et al.* 2002).

Results

Behaviors of Hg in the environment and food chain in WS area

The concentration levels of Hg in environmental samples and animal tissues were summarized in Table 3. To evaluate the data, critical values required in GB (National Standard of China) were also list in the same table. It was noted that concentrations for the total Hg exceed the values set by GB at almost all sampling stations. As water

Table 2. Nucleotide sequence and size of the expected PCR products for oligonucleotide primers used for RT-PCR.

Gene	Sequence	PCR product (bp)
GAPDH	5′-ATGGAAGAAGAAATCGCCGC-3′ 5′-ACACGCAGCTCGTTGTAGAA-3′	287
c-jun	5′-ATGACTGCAAAGATGGAAAC-3′ 5′-TTGAAGTTGCTGAGGTTGGC-3′	530

samples are greatly affected by seasons and rainfall, the data obtained from one collection only reflects the specific hydrological condition of that time. The rice sample was divided into two portions. The hull was removed from the grain to obtain white rice. Rice grains with hull were also analyzed in order to examine surface contamination. The results showed there was no significant difference of Hg concentration between rice with and without hull. This phenomenon might probably indicate that atmospheric (or external) sources of mercury are minimal. The sources of Hg in rice are possibly from both the soil and atmosphere (through absorption by plant leaves) in these areas. It is difficult to interpret the relationship between Hg concentrations in rice and that in soil. There is no correlation between them. It was noted that the uptake and retention of Hg in rice were influenced by a number of factors. The concentration of total Hg in soil might play a less important role for that. On the other hand, the concentration of total Hg in fish was very high. It was indicated that Hg could be easily bioaccumulated in the tissues of creatures. Similarly, the data obtained from ducks and pigs were consistent with the indication.

Effect of Hg-contaminated rice on c-jun expression in brain of rats

The mercury concentrations in rat brain

Concentrations of total mercury, organic mercury and selenium in rice were presented in Table 1. Evidently, in terms of absolute values, the concentrations of total mercury in Shanghai samples were much lower than that in WS and QZ samples. Total mercury in rat brains was showed in Table 4. The decreasing order of mercury concentration was MMG > MCG > WSM > QZP > SMG > SHC. The accumulations of mercury in the brains of rats, which were in the groups of MMG, MCG, WSM and QZP, were significantly different from that in the brains of control rats(SHC) ($p < 0.01$). SMG had no significant influence on the total mercury concentrations in brains comparing with the SHC ($p > 0.05$). However, significant lower Hg concentration was observed than that of MCG ($p < 0.05$). The content of total mercury in MMG increased obviously and have significant difference compared with its simulating group QZP ($p < 0.01$). Contrary to WSM, the concentration of mercury in its simulating group SMG was significantly decreased ($p < 0.05$).

Table 3. Mercury content in samples from WS area.

Sampling	Description	Total Hg mg Kg^{-1}	GB (National standard of China)
Soils (A) ($n=7$)	From a rice field along the river Dashuixi.	24.31 ± 2.5	GB(mg Kg^{-1}): pH < 6.5;Hg ≤ 0.3; pH > 7.5;Hg ≤ 1.0; 7.5 > pH > 6.5, Hg ≤ 1.5
Rice with hull ($n=7$)	From above the same rice fields	0.142 ± 0.015	GB2762–81–94: ≤0.020 mg Kg^{-1}
Rice without hull ($n=7$)	From above the same rice fields	0.133 ± 0.02	GB2762–81–94: ≤0.020 mg Kg^{-1}
Soil (B) ($n=7$)	From a vegetable field along the river Dashuixi	19.08 ± 2.6	GB(mg Kg^{-1}): pH < 6.5;Hg ≤ 0.3; pH > 7.5;Hg ≤ 1.0; 7.5 > pH > 6.5, Hg ≤ 1.5
Capsicum ($n=7$)	From above the same vegetable fields	0.18 ± 0.05	GB2762–81–94: ≤0.020 mg Kg^{-1}
Potato ($n=7$)	From above the same vegetable fields	0.018 ± 0.008	GB2762–81–94: ≤0.010 mg Kg^{-1}
Corn ($n=7$)	From above the same vegetable fields	0.065 ± 0.006	GB2762–81–94: ≤0.020 mg Kg^{-1}
Fish muscle ($n=7$)	From the river Dashuixi.	0.13 ± 0.01	GB2762–81–94: ≤0.030 mg kg^{-1}
Water	From the river Dashuixi	0.17 ± 0.07 μg L^{-1}	GB/T14848–93 ≤0.1 μg L^{-1}
Duck brain ($n=7$)	Raised freely in river Dashuixi	0.073 ± 0.009	GB2762–81–94: ≤0.050 mg kg^{-1}
Duck liver ($n=7$)	Raised freely in rive Dashuixi	4.47 ± 1.51	GB2762–81–94: ≤0.050 mg kg^{-1}
Pig brain ($n=2$)	Rasied using rice and crop	0.017 ± 0.001	GB2762–81–94: ≤0.050 mg kg^{-1}

Mean ± S.D; Mean standard deviation of at least 5 determinations.

Table 4. The mercury concentrations in rat brain.

Group	SHC	WSM	SMG	QZP	MMG	MCG
Total mercury $\times 10^{-3}$ mg Kg^{-1}	4.7 ± 0.4	24.0 ± 3.6^{++}	5.38 ± 0.5^{++}	13.7 ± 0.3^{++}	480.8 ± 70.0^{++}	37.5 ± 0.5^{++}

$^{++}p < 0.01$, compared to control group, $n=7$ rats in each group.

c-jun mRNA expression

Figure 1 showed that the expression of c-jun mRNA. The control group (SHC) was so weak that it could not show the light strip while that of the other groups had different extent of light strip. The relative expression level of c-jun mRNA was showed in Figure 2. It was noted that the expressions of c-jun mRNA in brains of exposure groups were significantly different from that of control group ($p < 0.01$). The expression of c-jun mRNA in QZP was slightly higher than that in WSM, although there was no significant difference between them ($p > 0.05$). The expression of c-jun mRNA in MMG was obviously increased and had significant difference comparing with its simulating group QZP and its border upon SMG($p < 0.01$). In addition, the expressions of c-jun in WSM and MCG were significantly higher than that in SMG ($p < 0.05$).

c-JUN protein expression

Numbers of c-JUN positive cells in rat hippocampus and cortex were showed in Figure 3. It was observed that the c-JUN positive cells in hippocampus and cortex of exposure groups were significant different from that of control group. In all exposure groups, the expressions of c-JUN protein in hippocampus were increased more obviously than that in cortex. The expression of c-JUN between WSM and QZP had no significant difference. The expressions of c-JUN protein in hippocampus and cortex of WSM were slightly higher than that of SMG, while no significant difference was observed between them ($p > 0.05$). The expressions of c-JUN protein in QZP were decreased obviously and had significant difference with that in its simulating group MMG. The expressions of c-JUN in hippocampus and cortex of MCG were increased significantly ($p < 0.01$), comparing to that of its simulating group SMG.

Discussion

In the two selected Hg pollution areas, rice is the main food and provides the major caloricity for

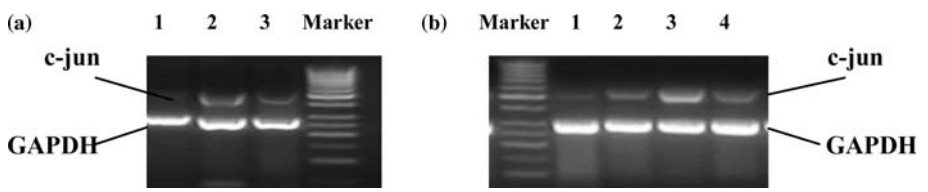

Fig. 1. RT-PCR products of c-jun and GAPDH on a 2.0% agarose gels stained with ethidium bromide. (a) SHC, QZP and WSM rats (lanes 1, 2 ,3 respectively) . (b) SHC, MCG, MMG and SMG rats (lanes 1, 2 ,3 respectively). DNA Marker (Songon, China) sizes were 50, 100, 150, 200, 250, 300,350, 400, 450, 500, 550, 600, 650, 700, 750-bp from bottom to top.

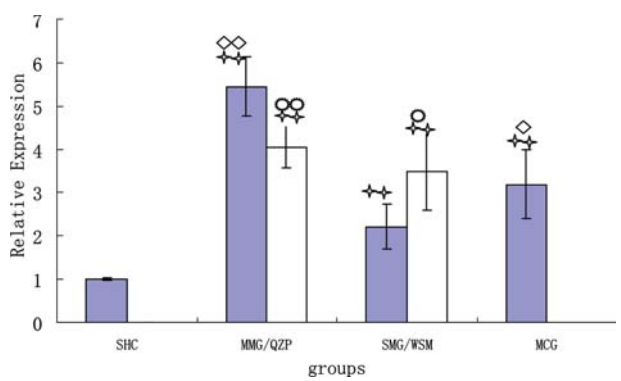

Fig.2. Densitometric analysis of RT-PCR products. Each value of c-jun mRNA level was normalized with GAPDH mRNA level. Each column and bar represents the mean ± S.D. $N = 7$ rats in each group at each time point. $p < 0.05$ compared to control, $p < 0.01$ compared to control. $p < 0.01$, $p < 0.5$, compared between border upon two groups. compared between QZ or WS groups to its simulation group, $p < 0.01$, $p < 0.5$.

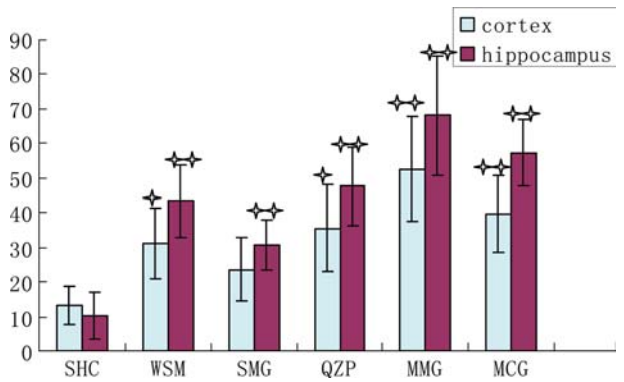

Fig.3. Number of c-JUN positive cells in rat hippocampus and cortex. Each column and bar represents the mean ± S.D. $N = 7$ rats in each group at each time point. $p < 0.05$ compared to control, $p < 0.01$ compared to control.

the local residents. Hg Exposure of the local population may occur due to inhalation and consumption of Hg contaminated food and water. In the air, the major form of Hg is volatile elemental Hg. It was reported that the highest value of Hg in air in the vicinity of the smelting furnace is close to 1.0 μg m^{-3} (Tan *et al.* 1997). These values considerably exceed over the USA EPA Reference Concentration (RfC) for chronic Hg exposure (0.0004 mg m^{-3}). Concentrations of Hg in air fluctuated dramatically and depended on the temperature, wind direction, humidity, vicinity of the sources of Hg, etc. Tan *et al.* (1997) found mercury was about 1000 ng m^{-3} in air near smelting furnace. Wang *et al.* (1995) investigated urine mercury and hair mercury of workers in Wanshan mercury mine. Very high Hg contents were found in both urine and hair.

The accumulations of mercury in rats brain of exposure groups were very significant different from that of control group (Table 4). The level of mercury contents in the brains of rats, which exposed to rice of WS (WSM) for 20 days, was about 1.75 times higher than that of rats exposed to rice of QZ (QZP). The accumulation process depends on the Hg species. In the groups of WSM, SMG, QZP and MCG, the process was slower than that of MMG. The uptake of MeHg by brain was easier than that of $HgCl_2$. It was probably due to the higher affinity of MeHg. Selenium (Se) showed an antagonist effect on the Hg accumulation. The atomic weights of mercury and selenium are 200.59 and 78.96, respectively. The molar ratio of selenium/mercury was calculated for the different areas. In QZ, the molar ratios of Se/Hg ranged from 1.19 to 1. In WS, however, selenium was present in a substantial surplus and with the Se/Hg ratio of 15.28 (Table 1). The simultaneous intaking of selenium may have protective effect on the Hg accumulation in body due to the interactive role of Se in mercury metabolism. Previous studies suggested that the formation of 1:1 Hg–Se compounds may explain Se detoxification effects on Hg (Falnoga *et al.* 2000).

We determined the levels of both c-jun mRNA and c-JUN protein in brains of rats exposed to different rice. It was found that polluted rice could induce the expression of c-jun gene (Figures 2, and 3). The increase of c-jun mRNA and its protein product were observed when rats had been exposed for 3 days (data not shown). It was found that the c-JUN protein plays a causal role in the activation of apoptosis. It was reported that the administration of cadmium could induce apoptotic cell death in the proximal tubules, testis and liver of experimental animals. In cultured LLC-PK1 renal epithelial cells, it was also found that incubation with cadmium results in DNA fragmentation (Habeebu *et al.* 1998; Masato *et al.* 2000). Expression of c-jun is part of a mitogenic response that is required for cell proliferation. Transcription of the c-jun gene is regulated in part by cAMP response element and serum response element. Increased Ca^{2+} can activate c-jun transcription through the latter mechanism while a rise in nuclear Ca^{2+} can activate c-jun transcription through CREB phosphorylation (Misra *et al.* 2002). The increase of c-jun gene expression level in hippocampus had a great link with the impairment of learning

and memory induced by mercury in rats. It may be one of the molecule mechanisms of mercury-induced impairment of learning and memory. JNK (c-JUN *N*-terminal kinase) may be an important signal for the cell death caused by Hg (Matsuoka *et al*. 1997). Hg can induce c-jun expression and phosphorylate JNK to a higher extent than other heavy metals do (Matsuoka *et al*. 1997). The possible role of c-jun in mercury-induced apoptosis is still not clear. To reveal the relationship between c-jun induction and apoptosis, further examinations on susceptible tissues in c-jun knockout rat exposed to mercury are required. In view of above, it was suggested to be alert of mental health problem in human beings when Hg-polluted food was taken.

In addition, it was noted that induced c-jun expression took place much earlier than apparent cell apoptosis when the rat was exposed to Hg (Habeebu *et al*. 1998; Masato *et al*. 2000). Thus, the expression change of immediately early gene (IEG) c-jun in rats' brain is possible to be applied as the early warning for the neuotoxicity of mercury. The expression intensities of c-JUN protein among the different regions such as hippocampus, cortex and ependyma, were compared. It was found that more sensitive expression was occurred in hippocampus and cortex. This observation supports that hippocampus and cortex could be selected as the checking regions for the early warning on mercury toxicity.

In 1997, the US Environmental Protection Agency (US EPA) set a new guideline for methylmercury in the diet (0.1 μg kg^{-1} day^{-1}) (US EPA 1997). It is much more stringent than that of the World Health Organization (WHO 1996) (0.47 μg kg^{-1} day^{-1}). The average concentration of organic mercury in rice of Guizhou chemical plant and Guizhou mercury mine was about 0.085 mg kg^{-1} (Tables 1 and 3). In practical terms, it means that a common person with the weight of 60 kg could only consume about 12 g of rice per day in the aforecited areas. Selenium has been found to play a role in the metabolism of mercury in human beings. Mercury and selenium accumulations in humans are already known. The results from different population groups, such as miners, dentists, and non-occupationally burdened individuals, suggest the formation of 1:1 Hg–Se compounds may explain Se detoxification effect on Hg (Falnoga *et al*. 2000). It should be pointed out, however, that the rice is not the only source of Se for the local population. Further studies are needed to address and investigate the role of Se. According to the well known antagonistic effects of co-accumulated Se in mercury exposed populations (Falnoga *et al*. 2000), Se in rice and probably in other foods may play an important protective role against Hg toxicity in local populations. In addition, much more efforts should be performed to protect the local ecosystem and human health in the mercury polluted area of WS, Guizhou province of China.

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