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Neurotransmitter level changes in domestic ducks(Shaoxing duck) growing up in typical mercury contaminated area in China

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Abstract: The neurotransmitter level changes of ducks exposed 8-month in a mercury-polluted site(Wanshan, China) and a reference site (Shanghai, China) were examined. Chemical analyses showed both higher mercury and selenium concentrations in the organ of Wanshan ducks. An increased content of acetylcholine(ACh) in brain and blood and a decreased activity of acetylcholinesterase(AChE) in blood were observed. Moreover, there was an increasing trend for nitric oxide synthase(NOS) activity and nitric oxide(NO) production in duck brain, but a reduction of NOS activity in duck serum. The possible explanations were due to the interactive effect of selenium accumulation and the sublethal exposure level of mercury in Wanshan area. The present study showed that AChE and NOS were sensitive to mercury contamination of real circumstance, suggesting that these two indexes have the potential to be biomarkers in assessment of health effects by mercury contamination.

Keywords: mercury; neurotransmitter; long-term exposure; Wanshan area

Introduction

Wanshan mercury mine, called "Mercury Capital" in China, is the largest cinnabar deposit in Guizhou. The total output was reported to be 26000 tons during 1949 to 1981, and the peak annual mercury emission from mining and refining to the atmosphere has reached 11 tons(Tan, 1997a; 1997b). Exploration of mercury in Wanshan has brought out serious environmental pollution and deteriorated local ecosystems severely. Although numerous studies have been carried out in Wanshan mercury mine, few attempts have been taken to study effects of mercury pollution on public health by examining lives living under natural conditions in Wanshan area.

Concerns about mercury are mainly based on its effects on ecosystems and in particular on public health. It is generally accepted that the nervous system is a main target for mercury. A number of studies have been confirmed that mercury can easily pass the blood-brain barrier, causing destruction of visual and hippocampal cells, and disrupts the neurons of the peripheral nervous system(Chang, 1979). Toxic effects include functional and structural damages are influenced by neurotransmitters (Weber, 1994), making toxicant-neurotransmitter interaction an important field of study.

Although researchers have examined the effect of organic and inorganic mercury pollutants on neurotransmitters, few were performed under nature conditions. In Wanshan mercury mining area, selenium is another important element that is coexisting with mercury. Selenium is an antagonistic element to mercury, and may play an interactive role in mercury metabolism. It makes the ecotoxic effects of Wanshan mercury contamination much more complex than that of single toxic element in laboratory. Therefore, we investigated animals growing up under natural conditions in Wanshan mining area in relation to their neurotransmitter level changes. Specifically, nitric oxide(NO), acetylcholine(ACh), nitric oxide synthase(NOS) and acetylcholinesterase(AChE) were measured. NO is a neurotransmitter/neuromodulator besides being a potent vasodilator(Bredt, 1994; Griffiths, 1998; Moncada, 1991). The ability of NO to penetrate cell membranes gives it a unique role in CNS which separates it from classical neurotransmitters, while the NOS is usually used to study the biological activity of NO. Acetylcholine(ACh) is involved with locomotion, conditioned responses, and feeding, and AChE activity is a commonly used biomarker of pollution stress. This study may provide some consults for public health risk assessment of mercury contamination in Wanshan mining area.

1 Methods and materials

1.1 Samples collecting and preparation

Samples of fish, rice, duck and pig were collected around Wanshan mercury mining area. Rice samples were taken from cultivated land, and re-cultivated areas. Fishes were collected from Dashuixi Stream—a stream flowing through Wanshan mercury mine. Eight-month-old Domestic ducks(Shaoxing duck) and ten-month-old pigs were bought from local peasants. The ducks were herded in natural situation, took crops as food and sometimes preyed on fish and shrimp in Dashuixi Stream, but the pigs were bred up with crops only. Control samples for fishes, rice, pigs and domestic ducks(Shaoxing duck) were collected from Shanghai—a relatively lower mercury contaminated area. Animals from experimental and control groups were sacrificed and brains and blood were collected. Duck brains and pig brains were stored at −20℃ for chemical analyses with rice and fish samples. Blood and brain of ducks were collected for biochemical analyses.

1.2 Analysis of mercury and selenium

Mercury analysis was carried out by an AMA254 solid/liquid mercury analyzer (Milestone, Italy) with an absolute detection limit of 0.01 ng. Every sample was analyzed three times, and got the mean value. The accuracy of procedure was monitored by analysis of reference materials BCR(No. 40 and No. 143) from Commission of the European Communities.

Selenium was determined by hydride generation-atomic fluorescence spectrometry technique (Titan, China). A selenate standard solution (SB010115 from the Shanghai Institute of Measurement and Testing Technology) was used to prepare the corresponding calibration solutions from 1 μg/L to 20.0 μg/L. Analyses of samples in duplicate yielded coefficients of variation of 8% for selenium.

1.3 Biochemical analysis

The activity of AChE and the ACh content of samples were determined using regular method(Xue, 1991; Lu, 1992). NO amount was determined by enzymatic reduction assay, and total NOS enzyme activity was measured by the L-arginine to L-citrulline conversion assay. Both of the measurements of NO and NOS were processed according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, China), followed by spectrophotometric analysis.

1.4 Statistical analysis

All results were expressed as means ± S.D. Statistical analysis was performed using SPSS v. 11.0(SPSS). All significance testing took

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place at 0.05 level.

2 Results

2.1 Mercury and selenium in samples

Concentrations of mercury in samples from Wanshan mining area are presented in Fig.1. It shows that mercury content in samples from Wanshan was much higher than that of Shanghai samples. This result confirmed the heavy contamination of Hg in Wanshan mining area. The highest accumulation of mercury occurred in rice and fish samples, which amounted to 0.137 mg/kg and 0.134 mg/kg, followed by duck brain and swine brain. High accumulation of mercury should be resulted from the high mercury background in Wanshan area. Our previous study (Ding, 2004) showed that mercury contents in soil samples from Wanshan are 24.31—347.52 mg/kg, which are about 2—3 magnitudes higher than the average value of China.

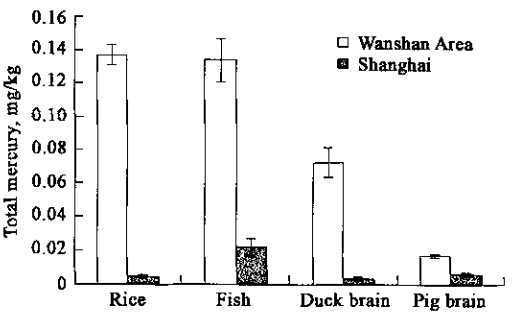

Fig.1 Comparison of total mercury in Wanshan samples and the controls
rice, $n = 2$; fish, $n = 5$; duck, $n = 7$; pig, $n = 2$.

The accumulation of selenium in samples is shown in Fig.2. Selenium has been found to play a role in the metabolism of mercury in man. The presence of accumulated endogenous Se can protect against harmful effects of accumulated and retained Hg by forming 1∶1 Hg-Se compounds. In Wanshan mercury area, selenium is a coexisting element with mercury. Horvat *et al.* (Horvat, 2003) reported that the concentrations of selenium could reach up to 16 mg/kg in soil and up to 1 mg/kg in rice. In the present study, the accumulation of mercury was obviously accompanied with an accumulation of selenium in Wanshan samples with respect to the control, showing that Se most probably plays a role in mercury metabolism and toxicity.

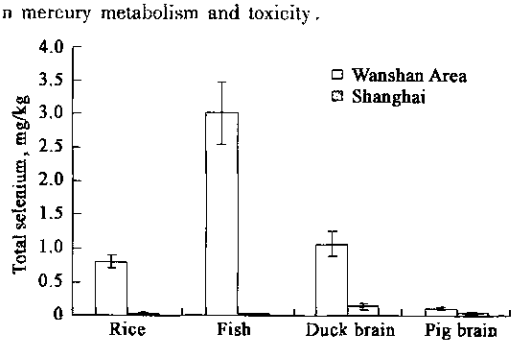

Fig.2 Comparison of total selenium in Wanshan samples and the controls
rice, $n = 2$; fish, $n = 5$; duck, $n = 7$; pig, $n = 2$

2.2 Neurotransmitter level changes

The contents of ACh, NO and enzymic activities of AChE and NOS on Wanshan and control groups are listed in Table 1. The content of ACh was clearly increased in the brain and blood of Wanshan ducks compared to the control, while the activity of AChE was decreased about 75% in blood of Wanshan duck. The level of NO and NOS increased significantly in brain of Wanshan ducks, but the activity of NOS decreased about 20% in blood of Wanshan ducks, compared with the control.

3 Discussion

Chemical analysis confirmed heavy contamination of Hg in Wanshan mining area. However, the accumulation of mercury was accompanied with an accumulation of selenium, showing that Se most probably plays a role in mercury metabolism and toxicity. In the biochemical analysis, we found significant differences in content of ACh in ducks from Wanshan area. This change paralleled changes observed in decreased activity of AChE in duck brain. The changes in ACh and the activity of AChE should be associated with the accumulation of mercury in Wanshan ducks. Mercury acts at the neuromuscular junction and the synaptic cleft, producing a weakened postsynaptic potentiation. Previous studies showed that lower mercury concentration could facilitate the release of ACh, while higher mercury concentrations may induce inhibition of ACh release (Mirzoian, 2002; Suresh, 1992). In our present study, the possible explanations of the increased ACh were due to the simultaneous accumulation of selenium, which alleviated the toxicity of mercury by mercury binding, and the sublethal exposure level of mercury that was not high enough to cause mercury intoxication. The significant decrease of AChE activity in blood may be resulted from a conformational change brought about by the binding of Hg to a sulfhydryl group on the cholinesterase. Another explanation could be the destruction of anticholinergic cells (Grippo, 2003). The significantly decreased AChE activity in blood suggested the use of AChE measurement as a regular monitoring protacol in assessment of the mercury pollution in Wanshan area.

Table 1 Levels of ACh, AChE, NO and of NOS in the organs of Wanshan ducks and the control

Sample	Control	Wanshan	Control	Wanshan
	Brain of ducks	Brain of ducks	Blood of ducks	Blood of ducks
ACh, μg/ml	238.61 ± 29.51	272.59 ± 15.56*	166.67 ± 14.05	196.89 ± 17.26*
AChE, u	0.51 ± 0.09	0.63 ± 0.24	1.81 ± 0.12	0.46 ± 0.07**
NO, μmol/L	5.98 ± 1.18	7.62 ± 1.45*	22.2 ± 14.48	23.57 ± 4.21
NOS, u/ml	9.56 ± 0.19	20.97 ± 0.99**	20.48 ± 3.43	16.09 ± 0.73*

Notes: The superscripts indicate significant differences between Wanshan samples and control; * $P < 0.05$; ** $P < 0.01$; every value represented as mean values ± SD ($n = 7$)

After 8-month exposure, NO concentration and NOS activity in duck brain increased significantly, while NOS activity in blood decrease compared with the control. NO is a new discovered messenger molecular, and has been found to act as an unconventional type of neurotransmitter, which plays an important role in regulating physiological functions of endocrine, immunity, and nerve systems in organisms. NO signalling forms the basis of functional cell responses to accommodate changes in the cellular microenvironment. NOS plays an important role in the production of NO. As NO is not stable in organisms, NOS is usually used to study the biological activity of NO. Changes in NO and NOS might indirectly indicate the effects of mercury on normal physiological functions (endocrine, immunity, reproduction, etc.) of organisms.

Prior studies observed variations in NO and NOS levels under laboratory exposure to mercury contaminants, and the responses were variable for different subjects and different organs. According to Jiunn-Jye Chuu *et al.* (Jiunn-Jye, 2001), excessive NO was found in brainstem for mice treated with MeHg and HgS. Ikeda M *et al.* (Ikeda, 1999) showed that subcutaneous administration of MeHg chloride to mice, 10 mg/(kg·d) for 9 d, could increase calcium-dependent NOS activity to 60% more than the controls in the cerebellum. However, Sang Hyun Kim *et al.* (Sang, 2002) studied the effects of low-dose inorganic mercury on the production of NO in murine macrophages, and detected mercury dose-dependently decreased expression of NO and iNOS in LPS-stimulated cells. Hiroyuki Yanagisawa *et al.* (Hiroyuki, 2002) showed in their study that mercury chloride induced decreased

expression of bNOS in the renal cortex of rats, and presumed the decrease in bNOS expression may be involoved in the $HgCl_2$-induced acute renal failure. Li Jianping *et al*. (Li, 2000) indicated the exposure of humans to mercury depressed formation of NO in the serum significantly, which is related to a high urine mercury accumulation. Thus, the increased production of NOS and NO in duck brain is proposed to be involved in the progression of mercury-induced cerebella degeneration. Whereas the decreased activity of NOS in duck serum may be in part contribute to the progression of mercury induced renal failure, or the result of macrophage injury.

4 Conclusions

In the present study, the result confirmed the heavy contamination of Hg in Wanshan mining area. It can be concluded that the local population is mostly at risk of mercury by consumption of mercury-contaminated food. Changes of neurotransmitter indexes showed that mercury contamination has exerted neurotoxicity on duck central nerve system. However, the increased ACh neurotransmitter level indicated that the contamination in Wanshan area was not severe enough to induce functional exhaustion of cholinergic neurons. This result was consistent with the undetected pathological changes in Wanshan area. A possible reason was due to the exposure level and duration of ducks in Wanshan mercury mining area was not high enough to cause mercury intoxication. Also, the simultaneous accumulation of selenium had alleviated the toxicity of mercury by mercury binding. Our present study showed that all of the four checked indexes were sensitive to mercury contamination of real circumstance, especially AChE and NOS, suggesting that these two indexes have the potential to be biomakers in assessment of health effects by mercury contamination.

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