High Exposure of Chinese Mercury Mine Workers to Elemental Mercury Vapor and Increased Methylmercury Levels in Their Hair

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Abstract

Objective: The aim of this study was to determine the level of exposure of mercury (Hg) miners and smelter workers to elemental mercury (Hg⁰) vapor in China, who work in Hg mines without using protective equipment against Hg⁰ vapor. In addition, the level of methylmercury (MeHg) intake by the workers was estimated from the MeHg concentration in their hair.

Methods: Urinary total mercury (THg) and hair THg and MeHg concentrations were measured in 26 Hg miners and smelter workers (i.e., exposed group), and 48 unexposed people (unexposed group).

Results: The exposed group showed high geometric mean THg concentrations in urine (258 ng/ml, 226 µg/g creatinine) and hair (20.0 µg/g). The urinary THg concentration of the smelter workers in particular was extremely high (338 µg/g creatinine in urine). The highest urine THg concentration reached 4577 µg/g creatinine. THg concentrations in urine and hair showed a significant correlation in the exposed group ($r=0.62$), indicating the adhesion of Hg⁰ vapor to hair. However, no such significant correlation was found in the unexposed group. Hair MeHg concentration in the exposed group (1.97 µg/g) was about threefold higher than that in the unexposed group (0.60 µg/g).

Conclusions: This study shows that smelter workers in a Chinese Hg mine are exposed to extremely high levels of Hg⁰ vapor, and that Hg miners are exposed to higher levels of MeHg than the unexposed subjects. Further study is needed to determine the cause of the higher hair MeHg concentration in the exposed group.

Key words: mercury mine, elemental mercury, hair, urine, methylmercury

Introduction

Guizhou Province in Southwestern China has been one of the world's most Hg-rich areas. Although Hg mining has been officially banned by local authorities in the province, some people continue to work illegally in the mercury (Hg) mine. These Hg mine workers are at a high risk of exposure to elemental mercury (Hg⁰) vapor, particularly those engaged in smelting among the miners (1–8). Moreover, such Hg mining operations is one of the primary source of environmental Hg

pollution (2, 4).

When Hg⁰ vapor is inhaled, it easily passes through the pulmonary alveolar membranes and enters the circulation (6). The high lipophilicity of Hg⁰ in solution in the body allows it to readily cross both the blood-brain and blood-placental barriers (6). Hg⁰ is easily oxidized and changed to divalent Hg in the human body, then it accumulates in the kidneys (6). Therefore, the central nervous system (CNS) and kidneys are the two target organs of Hg⁰ exposure (6).

For the assessment of the level of human exposure to Hg⁰ vapor, urine and serum total mercury (THg) concentrations are used as biomarkers (6, 9, 10). Many studies have shown a significant correlation between Hg concentrations in urine and serum and the Hg⁰ concentration in air when exposure levels are high (6, 11–13). In this study, THg concentration in urine was measured to assess the level of exposure to Hg⁰ vapor. THg concentration in hair was also measured to evaluate

Received Oct. 18, 2006/Accepted Dec. 12, 2006

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the adhesion level of Hg^0 vapor. Usually, most methylmercury (MeHg) is passed to humans from ingesting a fish and seafood. However, recent studies (2, 4) have shown that in a Hg-contaminated area in China, MeHg concentration in rice crops is high, reaching up to 145 ng/g. Furthermore, the percentage of MeHg in THg in rice grains varied from 5 to 83%. High MeHg concentrations in the soil were also reported in their studies (2, 4). Although people living in a mining area seldom eat fish, they might be exposed to MeHg through rice consumption. Therefore, an assessment of the MeHg exposure level of people living in a Hg-contaminated area is also important. We measured MeHg concentration in hair to evaluate the MeHg exposure level of the mine workers.

The aim of this study was to determine the level of Hg miners and smelter workers to Hg^0 vapor in China, who work in mines without using with protective equipment against Hg^0 vapor. In addition, the level of MeHg intake by the workers was estimated from MeHg concentration in their hair.

Materials and Methods

Twenty-six Hg miners and smelter workers (i.e., exposed group) in Guizhou Province in South western China and 48 unexposed people (unexposed group) living in an agricultural village more than 200 km away from the Hg mining area in the same province gave their informed consent to participate in the study. Sixteen of the mine workers were engaged in cinnabar smelting. For the measurement of THg concentration in urine, and THg and MeHg concentrations in hair, 2 ml of urine and 40 to 50 strands of hair from the root were collected from June 23 to 27, 2005. To preserve the urine samples for the measurement of THg concentration, 1 ml of nitric acid was added to each 2 ml urine sample immediately after sampling. This protocol of this study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD).

A mixture of 2 ml of a urine sample and 1 ml of nitric acid was used for THg concentration measurement. Whole-length hair samples were placed in a screw-capped and the vial was filled with water and neutral soap and shaken vigorously to remove the surface dust from the hair samples, then the hair samples were washed with distilled water. The hair samples

were then rinsed with acetone to remove the excess distilled water and cut into fine pieces with scissors. About 10 mg of hair samples was prepared for THg and MeHg concentration measurements. THg concentration in the hair and urine samples was determined by cold vapor atomic absorption spectrophotometry (CVAAS) according to the method of Akagi et al. (14), which involves sample digestion with HNO_3 , HClO_4 and H_2SO_4 (1+1+5), followed by reduction of divalent Hg to Hg^0 vapor with SnCl_2 . The detection limit was 0.01 ng/g. MeHg concentration in the hair samples was determined using a gas chromatography with electron capture detection (GC-ECD) system according to the method of Akagi et al. (14), which involves the immersion of hair samples in 2 N HCl and heating at 100°C for 5 minutes to leach out MeHg from the samples. MeHg was then extracted into toluene and analyzed using the GC-ECD system. The detection limit was 0.01 ng/g. Creatinine concentration in the urine sample was analyzed using a HITACHI 7170A automatic analyzer at the Guizhou Provincial People's hospital.

The differences in THg and MeHg concentrations between the unexposed and exposed groups were analyzed by Student's *t* test. The correlations among THg concentrations in urine and those among THg or MeHg concentrations in hair of each group were analyzed by Pearson and Spearman's correlation analysis. A *p* value less than or equal to 0.01 was considered statistically significant.

Results

Table 1 shows the geometric mean THg concentration in urine and THg and MeHg concentrations in hair in the exposed group (miners and smelter workers), and those in the unexposed group. In the unexposed group, the geometric mean urine THg, and hair THg and MeHg concentrations were 2.08 ng/ml (2.48 µg/g creatinine in urine), and 0.71 and 0.60 µg/g, respectively. On the other hand, the exposed group showed high geometric mean THg concentrations in urine (258 ng/ml, 226 µg/g creatinine) and hair (20.0 µg/g). The geometric mean urine THg concentration in the smelter workers in particular was extremely high (320 ng/ml, 338 µg/g creatinine in urine). The highest urine THg concentration among the smelter workers reached 3662 ng/ml (4577 µg/g creatinine). The geometric mean

Table 1 Geometric means and ranges of total mercury (THg) concentration in urine and THg and methylmercury (MeHg) concentrations in hair of exposed (mercury miners and smelter workers) and unexposed groups

		Urine		Hair		
		µg Hg/L	µg Hg/g Cr	THg (µg/g)	MeHg (µg/g)	(MeHg %)
Exposed group	Miners	182**	119**	11.0**	2.31**	21.0**
	n=10	(42.6–1113)	(26.6–569)	(3.36–57.7)	(1.01–3.61)	(2.21–82.0)
	Smelter workers	320**	338**	29.1**	1.78**	6.12**
	n=16	(9.52–3662)	(6.35–4577)	(4.32–213)	(0.72–3.91)	(0.65–45.6)
	Sum	258**	226**	20.0**	1.97**	9.83**
	n=26	(9.52–3662)	(6.35–4577)	(4.32–213)	(0.72–3.91)	(0.65–82.0)
Unexposed group		2.08	2.48	0.71	0.60	84.8
	n=48	(1.18–3.59)	(1.01–17.4)	(0.25–1.80)	(0.22–1.80)	(39.1–100)

Cr: creatinine.

** *p*<0.01, when compared with unexposed group.

Table 2 Correlation coefficients of logarithmic total mercury (THg) concentration in urine and THg and methylmercury (MeHg) concentrations in hair of exposed (mercury miners and smelter workers) and unexposed groups

	r	Urine	Hair	
		µg Hg/g Cr	THg (µg/g)	MeHg (µg/g)
Exposed group n=26	Urine THg	1.000		
	Hair THg	0.624**	1.000	
	Hair MeHg	0.056	−0.234	1.000
Unexposed group n=48	Urine THg	1.000		
	Hair THg	−0.047	1.000	
	Hair MeHg	−0.030	0.856**	1.000

Cr: creatinine.

** $p < 0.01$. **Fig. 1** Correlation between urine and hair mercury concentrations in exposed group (mercury miners and smelter workers).

Cr: creatinine.

hair THg concentration was also very high in the smelter workers (29.1 µg/g). The geometric mean hair MeHg concentration in the exposed group (1.97 µg/g) was about threefold higher than that in the unexposed group (0.60 µg/g). The percentages of hair MeHg concentration in the smelter workers, miners and unexposed group were about 6%, 20%, and 85%, respectively. The highest hair MeHg concentration in the exposed group was 3.91 µg/g. As shown in Table 2 and Figure 1, THg concentrations in the urine and hair samples showed a significant correlation in the exposed group ($r=0.62$; $p<0.05$), but not in the unexposed group. Moreover, about 85% of hair THg in the unexposed group was MeHg, and the hair THg and MeHg concentrations in the unexposed group showed a significant correlation ($r=0.86$), which was not found in the exposed group.

Discussion

Although the Hg exposure level is relatively low, the general population is primarily exposed to Hg⁰ vapor through dental amalgam (6). On the other hand, a high exposure to Hg⁰ vapor is reported chloralkali plant and gold and Hg mining workers (6). In particular, smelter workers who are engaged in heating cinnabar to release Hg⁰ vapor from the compound

without using proper protective equipment would highly be exposed to Hg⁰ vapor (1–4). In this study, we measured urine THg concentration to estimate the Hg⁰ vapor level of Hg mine workers in Guizhou Province, China. Hair THg and MeHg concentrations were also measured to differentiate the adhesion level of Hg⁰ vapor in the hair from the MeHg intake levels of the mine workers.

Urine and serum or plasma THg concentrations are good biomarkers of level of exposure to Hg⁰ vapor or divalent Hg (6, 9, 10). In this study, THg concentration in urine was measured to assess the level of exposure of mine workers to Hg⁰ vapor, because urine samples from the mine workers were easy to obtain. Hair Hg is predominantly composed of MeHg, and hair THg or MeHg is a good biomarker of MeHg exposure level in the general population (15, 16). The concentrations of urine and hair THg in an unexposed population are generally expected to be 4 µg/L and 2 µg/g (6, 10, 17), respectively. The geometric mean Hg concentration of urine in the unexposed group was 2.1 µg/L. Hair THg and MeHg concentrations in the unexposed group were also low (0.71 and 0.60 µg/g, respectively) indicating that their levels of exposure of the unexposed group to Hg⁰ vapor or MeHg were even lower than the general population. On the other hand, the exposed group showed very high THg concentrations in urine and hair. In particular, mine workers engaged in smelting showed extremely high geometric mean THg levels in their urine (338 µg/g creatinine) and hair (29.1 µg/g). The highest urine THg concentration reached 3662 µg/L (4577 µg/g creatinine). To our knowledge, this is the highest Hg concentration in urine ever reported, being about 90-fold higher than the health-based occupational exposure limit level (50 µg/g creatinine) set by WHO (6). From the previous studies, the ratio between Hg concentration in urine (µg/L or µg/g creatinine) and Hg concentration in air (µg/m³) was 1–3 (11, 18–21). From this ratio, the Hg level in the air of the working environment was estimated to range from about 3 hundreds to 1 thousands µg/m³ in average. In the present study groups, we also examined the relationship between Hg⁰ vapor exposure and neuromotor function (22). The results suggested that postural sway, as well as hand tremor, might be caused by Hg⁰ vapor exposure. Therefore, further health surveys and provisions for adequate protection of mine workers from the present exposure level must be given top priority.

The distribution of Hg in blood and hair is well established in a group exposed to MeHg through fish consumption (15).

Under a steady state condition, the hair to blood Hg concentration ratio is about 250–300. However, when other groups such as dentists and gold miners are exposed to Hg⁰ vapor (23, 24), the ratio becomes markedly high because of external Hg contamination. This prompted us to measure both THg and MeHg concentration in hair to differentiate the MeHg intake level and the level of external exposure to Hg⁰ vapor. In a report on the biological monitoring of toxic metals, Elinder et al. (25) concluded that hair is not a suitable indicator medium for monitoring the level of exposure to Hg⁰ vapor, thus confirming that hair Hg concentration in people who are mainly exposed to Hg⁰ vapor does not express the actual internal Hg exposure level. However, our results showed that Hg concentrations in urine and hair had a significant correlation among the exposed group. Even though most of the Hg compounds in hair are from the external adhesion of Hg, hair Hg concentration levels remains useful indicator for screening the Hg⁰ exposure level of individuals, similar to a film badge used for monitoring the level of exposure to radioisotopes or air pollutants, particularly for the screening of high Hg⁰ exposure level in remote areas. The MeHg percentages in hair THg were about 6% for smelter workers, 20% for miners, and 85% for the unexposed group. Hair Hg is predominantly composed of MeHg, and hair THg or MeHg is a good biomarker of MeHg exposure level in the general population (15, 16) as previously mentioned. The low MeHg percentages in the former groups indicate that these groups had high level of external exposure to Hg⁰ vapor. Most of the external hair Hg compounds at Hg concentration measurement may be divalent Hg compounds because Hg⁰ might have been washed off from the hair samples during the washing

with neutral soap and rinsing with acetone before Hg concentration measurement.

The high MeHg concentration in the hair samples from the exposed group was another interesting finding, even though the percentage of MeHg with respect to THg in this group was very low. People living in or nearby mining areas seldom eat fish of any kind, because these areas are mountainous, have no rivers with fish, and are distant from the sea. The *in vivo* methylation of inorganic Hg in humans is not yet established (6, 26). Therefore, the high MeHg level in rice crops grown in a heavily polluted Hg mine environment can be the source (2, 4) of the MeHg exposure of the mine workers because rice is their staple food. The families living with in the vicinity of the mines also use the heat of the smelting process for cooking. The methylation of Hg by intestinal or oral bacteria (27, 28) may also be a source of MeHg for them. The average MeHg exposure level in the exposed group was estimated to be about 0.2 µg/kg/day, which is twofold higher than the reference dose level established by the United States Environmental Protection Agency (US EPA 2001). To our knowledge, this is the first report showing an increased MeHg level in people who are not exposed to MeHg through fish consumption. Increased MeHg levels will be another subject to be investigated in relation to the health of people living in Hg mining areas.

Acknowledgements

This research was supported by the Chinese Academy of Sciences through an innovation project (KZCX3-SW-443) and by the Natural Science Foundation (40532014).

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