クについて関連性を明らかにできるものと期待される。これまで海外で報告されてきた調査事例を参考にしつつ，今後とも調査研究を進めていきたい。

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Short Communication

Effects of Hair Treatment on Hair Mercury—The Best Biomarker of Methylmercury Exposure?

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Abstract

Objectives: Exposure misclassification is a major obstacle to obtain accurate dose-response relationships. In order to solve this problem, the impact of hair treatment on total mercury in hair was assessed in Japanese women.

Methods: A cross-sectional study was carried out among 327 women at age 24–49 years to determine hair mercury levels and estimate daily mercury intakes from seafood by using a food frequency questionnaire.

Results: Hair mercury levels in the women and daily mercury intake ranged from 0.11 to 6.86 (median 1.63) μg/g and from 0.77 to 144.9 (median 15.0) μg/day, respectively. The hair mercury was positively correlated with the daily mercury intake ($p<0.001$). When the women were divided into two subgroups based on artificial hair-waving, hair coloring/dyeing, residence (non-fishing and fishing areas), and working status, a significant difference in the hair mercury level was observed between the women with and without artificial hair-waving only ($p<0.001$). The multiple regression analysis showed that the log-transformed hair mercury level was significantly related to the log-transformed daily mercury intake (standardized regression coefficient $\beta s=0.307$) and artificial hair-waving ($\beta s=-0.276$); but not to hair coloring/dyeing, residence, working status or age. Permanent hair treatment was estimated to reduce total mercury in hair by approximately 30%, after adjusting for daily mercury intake and other possible factors.

Conclusions: These findings suggest that hair mercury is not the best biomarker of methylmercury exposure when a study population includes women with artificial hair-waving.

Key words: hair mercury, daily mercury intake, permanent hair treatment, exposure biomarker, Japanese women

Introduction

The total mercury concentration in hair has been reported to be affected by various preanalytical factors besides analytical imprecision, for instance, adhesion of environmental mercury vapor (1), permanent hair treatment (2–4), and hair color (5), although hair mercury is believed to reflect the average methylmercury concentrations circulating in the blood (6, 7) and it is frequently used as the biomarker of individual exposures to methylmercury. If any preanalytical factors exist in a study population, a dose response (or effect) relationship obtained from a study based on hair mercury may be overlooked or underestimated (8), because the effects of such factors on hair mercury do not seem to have been explored in detail. In fact, neither the Faroese birth cohort study nor the Seychelles child development study provided information on hair treatment in the Materials and Methods sections (9–11). The degree of exposure misclassification in hair mercury may be inferred from comparisons between exposure indicators. In this study, we determined hair mercury levels in Japanese mothers and estimated daily mercury intakes from seafood by using a food frequency questionnaire (FFQ), in order to evaluate the effects of preanalytical factors, especially hair

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treatment of artificial waving and coloring/dyeing, on hair mercury.

Materials and Methods

Subjects

The nature of the procedures used in this study was explained to parents with a first grader (7-year-old child), of 28 different elementary schools in Akita and Tottori Prefectures, Japan, 14 of which were located near a fishing harbor (i.e., fishing areas). In Japan, there were many mines and smelters 30 years ago, and it is probable that soil or water was contaminated by lead, cadmium, mercury vapor, etc; therefore, the study population did not include people who came from such areas. Finally, 327 mothers participated (12). This study was carried out with the approval of the ethical review committee at the Akita University School of Medicine.

Methods

Hair samples were collected by cutting strands of hair close to the scalp from the occipital area in all mothers. The hair length ranged from 5 to 30 (mean 10) cm. Total mercury in aliquots of dried hair samples (15 to 20 mg), which were cut into small pieces (<2 mm) with scissors after being washed well with detergent and rinsed two times with acetone, was determined by the cold vapor atomic absorption spectrophotometry method at the National Institute for Minamata Disease (13, 14).

A detailed survey of the frequency and volume of seafood ingested in a year was conducted by a trained interviewer at the schools or civic centers, showing 25 kinds of full-scale pictures including fish, shellfish and seaweed items (e.g., tuna, swordfish, skipjack tuna, codfish, flatfish, mackerel, sardine, sea bream, whale, salmon, eel, crab, prawn, octopus, squid, oyster, sea urchin, fish paste, shellfish, seaweed, etc.) to each mother, based upon the FFQ (4, 15), i.e., a modified version of Date et al. (16). Then, the total mercury intake from seafood (μg/year) was estimated on the basis of the previous references on mercury concentrations in seafood (17, 18), and daily mercury intake (μg/day) was calculated dividing by 365 days. Moreover, questionnaires on artificial hair-waving and hair coloring/dyeing were collected from the mothers, and a medical doctor confirmed them, together with working status, using the interview method.

Statistical analysis

The relationships between the hair mercury level and daily mercury intake were analyzed by the Spearman rank correlation coefficient (r_s). The Mann-Whitney U test was used to compare the two subgroups divided on the basis of artificial hair-waving, hair coloring/dyeing, residence, and working status. Logarithmic transformation ($\log_{10}$) of the hair mercury concentration and daily mercury intake was used because of skewed distributions. The relation of the daily mercury intake, artificial hair-waving, hair coloring/dyeing, residence, working status, and age to the hair mercury level was examined by the multiple regression analysis. Artificial hair-waving, hair coloring/dyeing, and working status were scored as "absence"=0 and "presence"=1; also, residence was scored as "non-fishing area"=0 and "fishing area"=1. Also, the analysis of covariance was used to compare hair mercury concentrations in mothers with and without artificial hair-waving (or hair coloring/dyeing) after adjustment for daily mercury intake, residence, working status, age, and hair coloring/dyeing (or artificial hair-waving). All analyses, with two-sided p values, were performed using the Statistical Package for the Biosciences (19).

Results

The hair mercury concentrations in the 327 Japanese mothers at 24–49 (mean 36) years of age ranged from 0.11 to 6.86 (median 1.63) μg/g, and the daily mercury intakes, calculated from the FFQ data, were between 0.77 and 144.9 (median 15.0) μg/day. Among the mothers, there was a significant correlation between the hair mercury and daily mercury intake (Fig. 1). As the average value of body weight was 54.6 kg in 16,353 women aged 30–44 years, residing in Akita Prefecture (2002's data of the Akita Prefectural Center of Health Care), body weight of 55 kg for mothers was used to convert daily ingested dose (μg/day) to that per body weight (μg/kg body weight per day). Assuming the methylmercury content of 93% in seafood mercury (20), the mothers were suspected of having ingested methylmercury at a geometric mean of 0.25 μg/kg body weight per day, as shown in Table 1.

When the 327 mothers were divided into two subgroups based on artificial hair-waving, hair coloring/dyeing, residence, and working status (Table 2), there was only a significant differ-

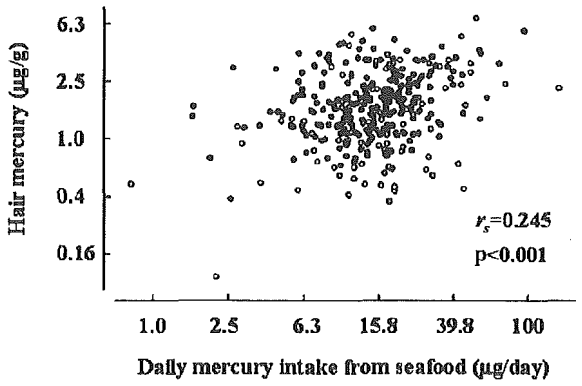

Fig. 1 Relationship between daily mercury intake and hair mercury level in 327 Japanese mothers. r_s, open circle, and closed circle indicate the Spearman rank correlation coefficient, and mothers with and without artificial hair-waving, respectively.

Table 1 Distribution of daily methylmercury intakes (μg/kg body weight per day), estimated from 327 Japanese mothers, under the assumption that body weight of mother was 55 kg, and methylmercury-mercury ratio in seafood was 0.93

Daily intake	Number of mothers	Proportion (%)
≤0.1	27	8.3
≤0.2	89	27.2
≤0.3	86	26.3
≤0.4	60	18.3
≤0.5	27	8.3
>0.5	38	11.6

Table 2 Mercury in hair and daily mercury intake in two subgroups divided according to artificial hair-waving, hair coloring/dyeing, residence, and working status in Japanese mothers

	Median (range)	Median (range)	p*
Artificial hair-waving:	*Absence* (N=219)	*Presence* (N=108)	
Hair mercury (μg/g)	1.81 (0.39~5.83)	1.31 (0.11~6.86)	<0.0001
Daily mercury intake (μg/day)	14.5 (1.61~94.1)	16.5 (0.77~144.9)	0.11
Hair coloring/dyeing:	*Absence* (N=69)	*Presence* (N=258)	
Hair mercury (μg/g)	1.74 (0.64~6.86)	1.58 (0.11~5.83)	0.08
Daily mercury intake (μg/day)	16.3 (2.65~74.5)	14.7 (0.77~144.9)	0.68
Residence:	*Non-fishing areas* (N=127)	*Fishing areas* (N=200)	
Hair mercury (μg/g)	1.84 (0.48~4.79)	1.55 (0.11~6.86)	0.08
Daily mercury intake (μg/day)	15.0 (0.77~74.5)	16.6 (1.63~144.9)	0.94
Working status:	*Without job* (N=120)	*With job* (N=207)	
Hair mercury (μg/g)	1.64 (0.42~4.86)	1.62 (0.11~6.86)	0.63
Daily mercury intake (μg/day)	14.9 (2.80~144.9)	15.0 (0.77~94.1)	0.83

* Mann-Whitney U test.

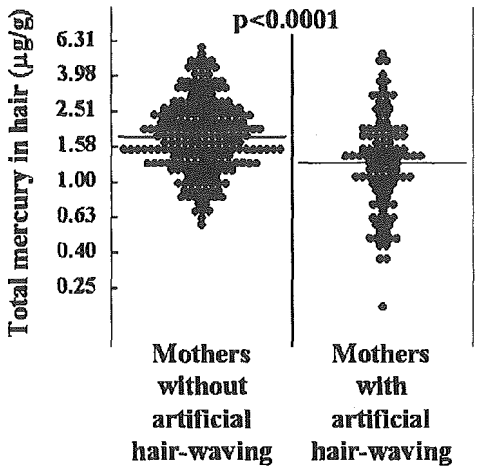

Fig. 2 Hair mercury concentrations in 219 mothers without and 108 mothers with artificial hair-waving after the adjustment for daily mercury intake, hair coloring/dyeing, residence, working status, and age: results of analysis of covariance.

ence in the hair mercury level between the mothers with and without artificial hair-waving. Using the multiple regression analysis, the log-transformed hair mercury level was significantly related to the log-transformed daily mercury intake (standardized regression coefficient βs=0.307, p<0.001) and artificial hair-waving (βs=−0.276, p<0.001); but, not to hair coloring/dyeing (βs=−0.065, p=0.21), residence (βs=−0.070, p=0.18), working status (βs=−0.012, p=0.81) or age (βs=0.046, p=0.37). The hair mercury concentrations adjusted by daily mercury intake and the above factors were significantly higher in the mothers without artificial hair-waving (geometric mean, 1.81 μg/g) than in the mothers with (1.29 μg/g), as shown in Fig. 2; but, no significant difference in the hair mercury was seen between the mothers without hair coloring/dyeing (1.74 μg/g) and with (1.59 μg/g) (p=0.21).

Discussion

In a previous study, we examined the accuracy of daily mercury intake estimated from the FFQ data of 154 mothers residing in Akita, Japan (4). Also, another study of the FFQ with 122 food items reported that the correlation coefficients between nutrients estimated by the first and second tests conducted at an interval of one week (i.e., reproducibility) ranged from 0.64 for vegetable protein to 0.78 for calcium (16). In this study using the FFQ, a large number of mothers including those who had different food habits in Japan were investigated, and they were suspected of ingesting mercury amounting to median 15 μg/day from seafood and freshwater fish, which was similar to the level of mothers residing in Akita (4). Since the daily mercury intake in our study was significantly correlated with hair mercury levels, daily mercury intake from seafood could reflect the individual methylmercury exposure to some extent.

The principal findings in 327 Japanese mothers of this study were that artificial hair-waving was associated with current total mercury levels in hair, and that permanent hair treatment reduced total mercury in hair by approximately 30% as a mean value, even after adjusting for daily mercury intake, hair coloring/dyeing, and other possible factors (Fig. 2). We used aliquots of hair samples corresponding to the duration of a mean of ten months for the determination of total mercury, whereas we did not have accurate information on when the mothers got their hair permed. In fact, the distributions of hair mercury in the mothers seemed to differ; i.e., open circles indicating a mother with artificial hair-waving, as shown in Fig. 1, were somewhat skewed to the downward direction. Yamamoto and Suzuki (2) explained that thioglycolate in artificial waving lotion removed hair mercury effectively. Likewise, one experiment by Yasutake et al. (3) reported that more than 30% of the hair mercury in four women was removed by a single treatment of the above lotions, and repeated treatments further removed the hair mercury. This error is unmeasurably bigger than analytical imprecisions in the laboratory; the latter has been estimated to be less than 5% (5, 8). For that reason, the myth that mercury concentration in hair reflects the methylmercury concentration circulating in the blood (6, 7) may collapse if a study population includes subjects with such hair treatment.

In the present study, hair coloring/dyeing was not signifi-

cantly associated with current hair mercury levels, and no significant difference in the hair mercury level was observed between the mothers with and without hair coloring/dyeing, although approximately 10% of hair mercury in the mothers with hair coloring/dyeing seemed to be removed when compared with the mothers with natural hair. On the contrary, a preliminary study has reported that the overall average mercury concentration in four subjects was 14.2 μg/g for white hair and 15.3 μg/g for pigmented hair (5); in another report, total and organic mercury concentrations in Japanese elderly men and women were significantly higher in naturally grey hair than in dark hair (21). Thus, it is likely that when women conceal grey hair by coloring or dyeing, the hair treatment may cause possible differences in the hair mercury level. Further research is necessary to explore whether mercury concentrations in naturally grey hair or in pigmented hair are higher, as well as whether the ratio of mercury concentrations in naturally grey hair and pigmented hair differs with respect to race.

Neither daily mercury intakes nor hair mercury levels in Japanese mothers differed significantly according to residence (Table 2). A similar finding has been observed both between fishing and non-fishing areas and between cities and towns in Akita Prefecture (4). Also, working status was not relevant to the daily mercury intake or hair mercury level. Since no mothers residing near the areas where a mine/smelter existed in the past were included, the possibility of environmental mercury vapor binding to the hair was minimal. In this way, the impacts of preanalytical factors except the above hair treatment on hair mercury levels appear to be omitted in the current study.

In environmental epidemiological research, it is essentially impossible to obtain an error-free measurement of exposure. When unavoidable measurement error, i.e. artificial hair-waving, is ignored, it is probable that the estimation of the exposure effects is biased toward the null hypothesis (22, 23). In interpreting epidemiological studies based on hair mercury only, attention should be directed toward the consequences of exposure misclassification. Rather, mercury in blood or methylmercury in cord tissue, together with hair mercury, may be recommended in such studies on risk assessment (9, 12, 24), because the usefulness of the mercury concentration in cord blood has been emphasized as a main risk indicator (25).

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SPECIAL ISSUE PAPER

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Metal components analysis of metallothionein-III in the brain sections of metallothionein-I and metallothionein-II null mice exposed to mercury vapor with HPLC/ICP-MS

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Abstract Mercury vapor is effectively absorbed via inhalation and easily passes through the blood–brain barrier; therefore, mercury poisoning with primarily central nervous system symptoms occurs. Metallothionein (MT) is a cysteine-rich metal-binding protein and plays a protective role in heavy-metal poisoning and it is associated with the metabolism of trace elements. Two MT isoforms, MT-I and MT-II, are expressed coordinately in all mammalian tissues, whereas MT-III is a brain-specific member of the MT family. MT-III binds zinc and copper physiologically and is seemed to have important neurophysiological and neuromodulatory functions. The MT functions and metal components of MTs in the brain after mercury vapor exposure are of much interest; however, until now they have not been fully examined. In this study, the influences of the lack of MT-I and MT-II on mercury accumulation in the brain and the changes of zinc and copper concentrations and metal components of MTs were examined after mercury vapor exposure by using MT-I, II null mice and 129/Sv (wild-type) mice as experimental animals. MT-I, II null mice and wild-type mice were exposed to mercury vapor or an air stream for 2 h and were killed 24 h later. The brain was dissected into the cerebral cortex, the cerebellum, and the hippocampus. The concentrations of mercury in each brain section were determined by cold vapor atomic absorption spectrometry. The concentrations of mercury, copper, and zinc in each brain section were determined by inductively coupled plasma mass spectrometry (ICP-MS). The mercury accumulated in brains after mercury vapor exposure for MT-I, II null mice and wild-type mice. The mercury levels of MT-I, II null mice in each brain section were significantly higher than those of wild-type mice after mercury vapor exposure. A significant change of zinc concentrations with the following mercury vapor exposure for MT-I, II null mice was observed only in the cerebellum analyzed by two-way analysis of variance. As for zinc, the copper concentrations only changed significantly in the cerebellum. Metal components of metal-binding proteins of soluble fractions in the brain sections were analyzed by size-exclusion high-performance liquid chromatography (HPLC) connected with ICP-MS. From the results of HPLC/ICP-MS analyses, it was concluded that the mercury components of MT-III and high molecular weight metal-binding proteins in the cerebellum of MT-I, II null mice were much higher than those of wild-type mice. It was suggested that MT-III is associated with the storage of mercury in conditions lacking MT-I, and MT-II. It was also suggested that the physiological role of MT-III and some kind of high molecular weight proteins might be impaired by exposure to mercury vapor and lack of MT-I and MT-II.

Keywords Mercury vapor · Metallothionein · Brain · ICP-MS · Zinc · Copper

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Introduction

Mercury is a serious environmental pollutant with toxic effects on all living organisms. Mercury exists in various chemical forms; the major physical forms of mercury to which humans are exposed are elemental mercury and methylmercury compounds. Elemental mercury (Hg^0; mercury vapor) has a high vapor pressure and thus the vapor causes a number of cases of poisoning via inhalation. Human exposure to mercury vapor is from dental amalgam and industries using mercury. The health effects of mercury vapor have been known since ancient times. Severe exposure results in a triad of symptoms,

erethism, tremor, and gingivitis. Today, we are concerned with subtler effects such as behavioral and cognitive changes associated with effects on the central nervous system [1, 2]. Mercury vapor easily passes through the blood–brain barrier. Therefore, even though elemental mercury is classified as inorganic, mercury poisoning with primarily central nervous system symptoms occurs. After being absorbed into the body, mercury vapor is oxidized into the divalent mercury ion catalyzed mainly by catalase. This leads to strong protein binding and, thus, mercury is retained in the tissue [3–6].

Metallothionein (MT) is a cysteine-rich metal-binding protein and is known to play a protective role in heavy-metal poisoning. In 1957, Margoshes and Vallee [7] reported the isolation of a protein from horse kidney which showed a high affinity for cadmium. This protein was subsequently characterized biochemically by Kägi and Vallee, and, owing to its high content of metals and cysteine residues, it was named MT [8–10]. Mammalian MTs are composed of four major isoforms designated MT-I through MT-IV. MT-I and MT-II are expressed in most tissues, including the brain, whereas MT-III (also called growth inhibitory factor) and MT-IV are expressed predominantly in the central nervous system and in keratinizing epithelia, respectively. MT-III was discovered unexpectedly while pursuing the hypothesis that in the brains of patients with Alzheimer's disease in which a loss of growth inhibitory factors occurs an unbalanced total neurotrophic activity of the brain results [11–13]. It was also shown that MTs have an important role in trace element homeostasis and regulation [14]. Although the exact function of MT is still a matter of controversy, it is known that MT synthesis can be induced by some metal ions [15]. In mammalian MTs, mainly Zn, Cu, Cd, and Hg are natural parts of the protein structure. Two MT isoforms, MT-I and MT-II, are expressed coordinately in all mammalian tissues. MT-III is a brain-specific member of the MT family, binds Zn and Cu physiologically, and might have important neurophysiological and neuromodulatory functions [16, 17].

In previous studies, it was reported that the levels of Cu and Zn were changed in the brain after Hg vapor exposure [18–21]. However, the association between Hg vapor exposure and lack of MT-I and MT-II on the components of trace elements in MTs has not been thoroughly elucidated. High-performance liquid chromatography (HPLC) followed by metal-specific detection with inductively coupled plasma mass spectrometry (ICP-MS) has been widely applied to the detection and determination of metal-binding proteins and MTs in mammalian tissues [22–27]. In this study, the influences of Hg vapor exposure and the lack of MT-I, and MT-II on the accumulations of mercury, the levels of the trace elements, and metal components of MTs were analyzed by HPLC/ICP-MS in MT-I, II null mice and wild-type mice.

Materials and methods

Animals

MT-I, II null mice and 129/Sv (wild-type) mice were purchased from Jackson Laboratory. They were housed in plastic cages with free access to food and water. The light cycle was 12:12 h and the room temperature was maintained at 21–23 °C. The entire procedure was reviewed and approved by the Committee of Animal Experimentation of Tohoku University School of Medicine.

Chemicals

Mercuric chloride and metal standard solutions were products of Wako Pure Chemical Industries, Osaka, Japan. Ultrapure nitric acid was purchased from Kanto Chemicals, Tokyo, Japan. All other chemicals used in this experiment were commercially available analytical grade products.

Mercury vapor exposure

MT-I, II null mice and wild-type mice were exposed to mercury vapor (Hg°) or an air stream for 2 h by the following procedure and were decapitated 24 h later. Hg° was generated by the method of Kim et al. [28], slightly modified from the original method of Sugata et al. [29]. Solutions of 1 mM $HgCl_2$ and 5% $NaBH_4$ (in 0.01 N NaOH) were introduced into the reaction vessel separately, at a rate of 30 ml/h. The Hg° was generated and supplied to mice which were placed in an exposure chamber for 2 h. Air samples were taken every 30 min with a gas-tight syringe and bubbled into an acidified $KMnO_4$ solution for Hg analysis. The concentration of Hg° in the exposure chamber was approximately 6.0 mg/m^3.

Determination of mercury

The tissues were weighed and wet-ashed in a Pyrex tube with a mixture of nitrate/sulfate/perchloric acid (1:4:1 v/v) at 160 °C for 30 min. The concentrations of mercury (as total mercury) in the tissues were determined by cold vapor atomic absorption spectrometry [30]. To assure the accuracy of the determination, BCR no. 185 (bovine liver) and IAEA MA-A-2 (fish flesh) were used as reference materials. The values obtained fell within the range of the certified value.

Determination of trace elements

The tissues were weighed and digested using an MLS-1200 MEGA microwave oven (Milestone, Bergamo,

Italy) containing ten PTFE-TFM vessels with 20 smaller PTFE-PFA vials (Tuf-tainer vial, GL Science Co., Tokyo, Japan) with ultrapure nitric acid [31]. The concentrations of trace elements in the tissues were determined with ICP-MS (Elan 5000, PerkinElmer, Toronto, Canada) by using the standard addition method [31–33]. In order to evaluate the determination, NIST 1577b (bovine liver) and IAEA MA-A-2 (fish flesh) was analyzed as standard reference materials. The values obtained fell within the range of the certified value.

Analysis of metal-binding protein by HPLC/ICP-MS

A size-exclusion column, TSK gel G2000 SW XL PEEK (Tosoh, Tokyo, Japan) HPLC system, NANOSPACE SI-2 (Shiseido, Tokyo, Japan), was connected to the ICP-MS apparatus. Samples of brain sections were homogenized in 4 vol 25 mM tris(hydroxymethyl)aminomethane (Tris)–12.5 mM HCl buffer and centrifuged at 13,000 rpm for 1 h. Supernatant was applied to the size-exclusion column of the HPLC system equilibrated with 25 mM Tris–12.5 mM HCl (containing 20 mM KCl) and elution was performed with same buffer at a flow rate of 1 ml/min. Metal components of metal-binding proteins eluted from the HPLC system were detected by ICP-MS. All of the connections were made of PEEK (polyetheretherketone polymer) tubing. Isotopes ^{65}Cu, ^{66}Zn and ^{202}Hg were monitored.

Statistical analyses

All data are represented as the mean ± the standard deviation. Statistical analyses were performed by two-way analysis of variance with the mice (MT-I, II null or wild-type) and exposure (to mercury vapor or air) as two factors.

Results

Mercury levels in brain sections

The total mercury concentrations of each brain section (cerebral cortex, cerebellum, and hippocampus) in MT-I, II null mice and wild-type mice after treatment with an air stream as a control or mercury vapor exposure are shown in Table 1. The effects of MT-I, II null and mercury vapor exposure on mercury accumulation in the brain sections were analyzed. The mercury accumulated after mercury vapor exposure in the brains of MT-I, II null mice and wild-type mice ($p < 0.0001$). The mercury levels of MT-I, II null mice in each brain section were significantly higher than those of wild-type mice for mercury vapor exposure. The Hg levels in the hippocampus were approximately 1.4–1.9 times higher than those in the cerebral cortex or cerebellum of MT-I, II null mice and wild-type mice.

Table 1 Concentrations of mercury in the cerebral cortex, the cerebellum, and the hippocampus of wild-type (129/Sv) mice and metallothionein I (MT-I) and metallothionein II (MT-II) null mice exposed to an air stream as a control or mercury vapor

Hg concentration (μg/g/tissue)			
Mice and treatment	Cerebral cortex	Cerebellum	Hippocampus
129/Sv (air)	ND	ND	ND
129/Sv (Hg°)	0.81 ± 0.15	0.94 ± 0.36	1.58 ± 0.72
MT-I, II null (air)	ND	ND	ND
MT-I, II null (Hg°)	2.03 ± 0.39	2.16 ± 0.27	3.06 ± 0.95
Two-way ANOVA			
Mice (MT-I, II null/wild-type)	****	****	**
Exposure (Hg° /air)	****	****	****
Mice × exposure	****	****	**

Each value represents the mean ± the standard deviation (*SD*) ($n = 6$). The effects of MT-I and MT-II null and Hg vapor exposure were analyzed by two-way analysis of variance (*ANOVA*)
ND not detected
$^{**}p < 0.01$; $^{****}p < 0.0001$

Trace element levels in brain sections

The zinc concentrations in each brain section (cerebral cortex, cerebellum, and hippocampus) in MT-I, II null mice and wild-type mice after air exposure as a control or mercury vapor exposure are shown in Table 2. The Zn levels in the hippocampus were approximately 1.1–1.6 times higher than those in the cerebral cortex or the cerebellum of MT-I, II null mice and wild-type mice. The effects of MT-I and MT-II null and mercury vapor exposure on Zn concentrations in the brain sections were analyzed. A significant change of the Zn concentrations was observed only in the cerebellum for different mice and for the combination of different mice (MT-I, II null or wild-type) and exposure (to mercury vapor or air).

Table 2 Concentrations of zinc in the cerebral cortex, the cerebellum, and the hippocampus of wild-type (129/Sv) mice and MT-I, II null mice exposed to an air stream as a control or mercury vapor

Zn concentration (μg/g/tissue)			
Mice and treatment	Cerebral cortex	Cerebellum	Hippocampus
129/Sv (air)	13.65 ± 5.15	13.48 ± 2.94	17.30 ± 4.62
129/Sv (Hg°)	13.26 ± 4.19	11.90 ± 1.32	14.34 ± 4.41
MT-I, II null (air)	12.80 ± 1.51	10.20 ± 1.46	16.38 ± 3.66
MT-I, II null (Hg°)	13.07 ± 2.91	10.85 ± 0.33	15.98 ± 3.52
Two-way ANOVA			
Mice (MT-I, II null/wild-type)	NS	*	NS
Exposure (Hg° /air)	NS	NS	NS
Mice × exposure	NS	*	NS

Each value represents the mean ± the SD ($n = 6$). The effects of MT-I and MT-II null and Hg vapor exposure were analyzed by two-way ANOVA.
NS not significant
$^{*}p < 0.05$

Table 3 Concentrations of copper in the cerebral cortex, the cerebellum, and the hippocampus of wild-type (129/Sv) mice and MT-I, II null mice exposed to an air stream as a control or mercury vapor

Cu concentration (μg/g/tissue)			
Mice and treatment	Cerebral cortex	Cerebellum	Hippocampus
129/Sv (air)	2.73 ± 1.31	4.35 ± 0.20	3.58 ± 0.13
129/Sv (Hg^0)	2.57 ± 0.88	4.11 ± 0.41	3.44 ± 0.71
MT-I, II null (air)	2.63 ± 0.46	3.55 ± 0.34	3.43 ± 0.48
MT-I, II null (Hg^0)	3.05 ± 0.53	3.85 ± 0.34	3.26 ± 0.45
Two-way ANOVA			
Mice (MT-I, II null/wild-type)	NS	*	NS
Exposure (Hg^0/air)	NS	NS	NS
Mice × exposure	NS	*	NS

Each value represents the mean ± the SD ($n = 6$). The effects of MT-I and MT-II null and Hg vapor exposure were analyzed by two-way ANOVA.
*$p < 0.05$

The copper concentrations in brain sections are shown in Table 3. The Cu concentrations in each brain section were not so different. As for Zn, the Cu concentrations in the cerebellum were only changed significantly for different mice and the combination of different mice (MT-I, II null or wild-type) and exposure (to mercury vapor or air).

HPLC/ICP-MS analyses

Metal components of metal-binding proteins such as MTs of soluble fractions in the brain sections were analyzed by size-exclusion HPLC connected with ICP-MS. Chromatograms of HPLC/ICP-MS analyses of the cerebellum of the wild-type mice and the MT-I, II null mice are shown in Figs. 1 and 2, respectively. An MT-III peak was observed in each chromatogram of HPLC/ICP-MS of the cerebellum of the MT-I, II null mice and the wild-type mice after air exposure as control groups. MT-III binds mainly to Cu and Zn in this condition (Figs. 1a, 2a). After mercury vapor exposure, mercury seemed to be slightly incorporated into the MT-III, MT-I, MT-II, and high molecular weight proteins in the wild-type mice (Fig. 1b), whereas, mercury seemed to be greatly incorporated into the MT-III and high molecular

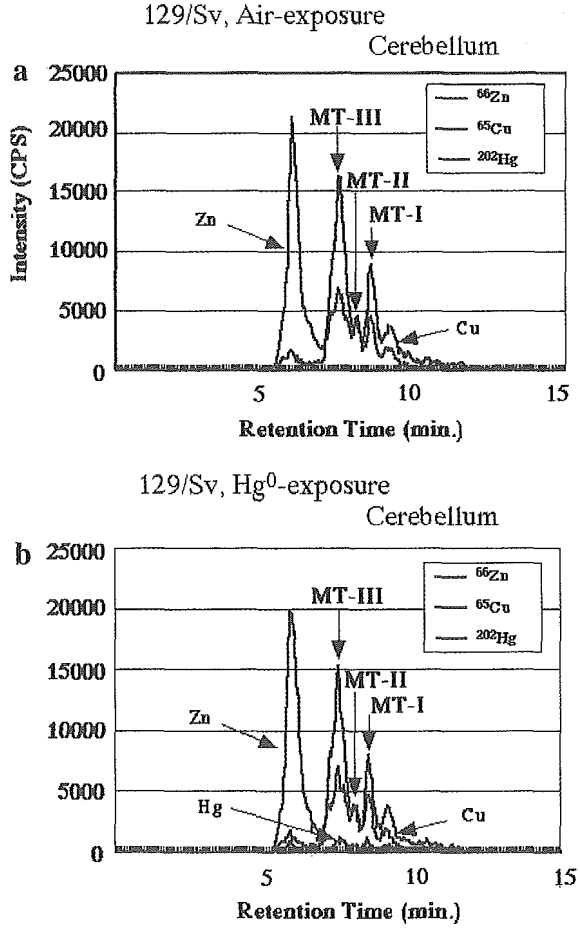

Fig. 1 Metal components analysis of the supernatant from cerebellum homogenate by high-performace liquid chromatography (*HPLC*)/inductively coupled plasma mass spectrometry (*ICP-MS*) of wild-type (129/Sv) mice exposed to **a** air and **b** mercury vapor. Isotopes ^{65}Cu, ^{66}Zn and ^{202}Hg were monitored

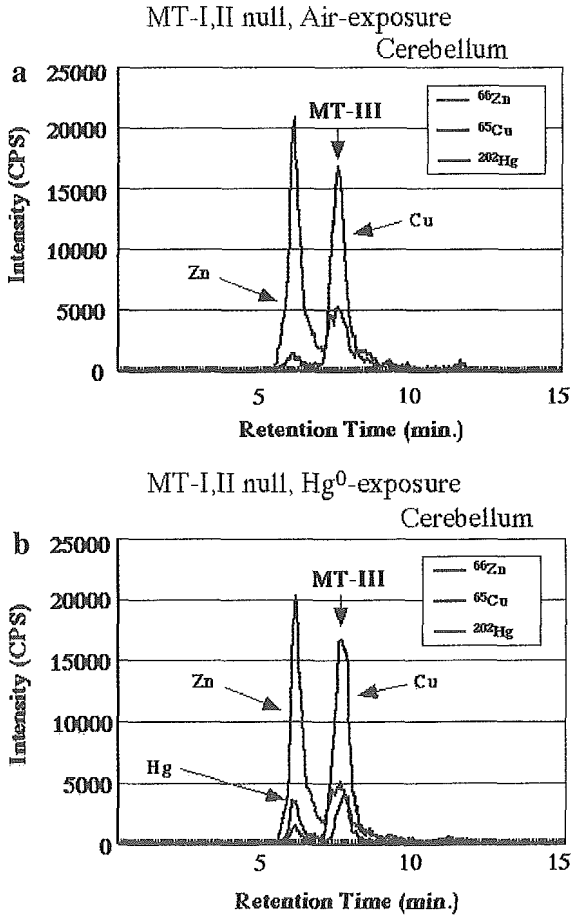

Fig. 2 Metal components analysis of the supernatant from cerebellum homogenate by HPLC/ICP-MS of metallothionein I (*MT-I*) and metallothionein II (*MT-II*) null mice exposed to **a** air and **b** mercury vapor. Isotopes ^{65}Cu, ^{66}Zn and ^{202}Hg were monitored

weight proteins in the MT-I, II null mice (Fig. 2b). The Cu and Zn of MT-III were partially replaced with Hg in each of the brain sections of the wild-type mice and the MT-I, II null mice. HPLC/ICP-MS analyses revealed that the levels of the Hg component in MT-III and high molecular weight proteins in the cerebellum of the MT-I, II null mice were higher than those of the wild-type mice after mercury vapor exposure (Fig. 2b).

Discussion

In this study, it was observed that mercury was accumulated in the brain sections of MT-I, II null mice and wild-type mice. As shown in Table 1, the total mercury concentrations of each brain section (cerebral cortex, cerebellum, and hippocampus) in MT-I, II null mice were significantly higher than those of wild-type mice after Hg° exposure. In previous studies, accumulation of mercury in the brain of MT-I, II null mice was quite similar to that of the wild-type mice after Hg° exposure [20, 21, 27, 34]. Because of the lower accumulation of mercury in wild-type mice, it is suggested that it might be difficult to incorporate mercury, or oxidation from Hg° to Hg^{2+} might be inhibited in the brain, or more mercury might be excreted in MT-I, II null mice; however, the exact reason for the lower accumulation of mercury in wild-type mice is unclear. In order to elucidate the accumulation of mercury, further investigations are necessary. In our previous studies, it was found that MT-I and MT-II were induced in the kidney of wild-type mice after mercury vapor exposure: mercury binds strongly to MT-I and MT-II in the kidney [27, 34]. MT-I and MT-II may play an important role in the accumulation of mercury in the liver and the kidney; however, in the brain, MT-III might be associated with the storage of mercury.

In this study, HPLC/ICP-MS analyses revealed that the levels of the mercury component of MT-III and high molecular weight proteins in each brain section of the MT-I, II null mice were much higher than those of wild-type mice. From these results, it is thought that MT-III is associated with the storage of mercury in conditions lacking MT-I and MT-II. It is also suggested that the physiological role of MT-III and some kind of high molecular weight proteins might be impaired by exposure to mercury vapor and the lack of MT-I and MT-II.

Conclusions

The Hg levels of MT-I, II null mice in each brain section were significantly higher than those of wild-type mice after mercury vapor exposure. A significant change of Zn concentrations was observed only in the cerebellum. As for Zn, the Cu concentrations only changed significantly in the cerebellum. From the results of HPLC/ICP-MS analyses, it was concluded that the mercury components of MT-III and high molecular weight metal-binding proteins in the cerebellum of MT-I, II null mice were much higher than those of wild-type mice. It is suggested that MT-III is associated with the storage of mercury in conditions lacking MT-I and MT-II. It is also suggested that the physiological role of MT-III and some kind of high molecular weight proteins might be impaired by exposure to mercury vapor and lack of MT-I and MT-II.

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Difference in Methylmercury Exposure to Fetus and Breast-feeding Offspring: A Mini-Review

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Abstract : The purpose of this paper was to concisely review the practical changes in MeHg concentrations in fetus and offspring throughout gestation and suckling from our recent animal and human studies. In the animal study, adult female rats were given a diet containing 5 μg/g Hg (as MeHg) for 8 weeks. Then they were mated and subsequently given the same diet throughout gestation and suckling. On embryonic days 18, 20, 22 and at parturition, the concentrations of Hg in the brains of fetus were approximately 1.5-2.0 times higher than those in the mothers. However, during the suckling period Hg concentrations in the brain rapidly declined to about 1/10 of that during late pregnancy. Hg concentrations in blood also decreased rapidly after birth. In human study, Hg concentrations in red blood cells (RBC-Hg) in 16 pairs of maternal and umbilical cord blood samples were compared at birth and 3 months of age after parturition. RBC-Hg in the umbilical cords was about 1.6 times higher than those in the mothers at parturition. However, all the infants showed declines in Hg concentrations throughout the breast-feeding period. RBC-Hg at 3 months of age was about half that at birth. Both the animal and human studies indicated that MeHg exposure to the fetus might be especially high but it dramatically decreases during the suckling period. Therefore, close attention should be paid to the gestation rather than the breast-feeding period to avoid the risk of MeHg to human infants.

Keywords : methylmercury, fetus, pregnant, breast-feeding

Introduction

Methylmercury (MeHg) is a well-known and widespread environmental neurotoxicant. In the natural course of events, most humans are exposed to MeHg through fish and sea mammal consumption. Generally, the larger fish and sea mammals at the top of the food chain, such as whale, shark, sword fish, and tuna, contain higher levels of MeHg than smaller ones (NRC, 2000). Susceptibility of the developing brain during both gestation and suckling might be high (Sakamoto *et al.*, 2002a; Rice and Barone, 2000; Sakamoto *et al.*, 1998; WHO, 1991; Burbacher *et al.*, 1990; Choi, 1989). MeHg can be transferred from mothers to offspring through breast milk, in addition to its passage through the placenta during intrauterine life (Sakamoto *et al.*, 2004; Grandjean, 1994; Kosta *et al.*, 1982; Skerfving, 1988; Amin-Zaki *et al.*, 1974) according to their nutrition demands. Therefore, the effects of MeHg exposure on pregnant and breast-feeding women remain an important issue for elucidation, especially those of continuous uptake in high-fish-consumption populations (NRC, 2000; WHO, 1990; Galster, 1976). Some countries, such as Australia, Canada, Norway, Sweden, the United Kingdom, the United States and Japan, have issued fish consumption advisories to pregnant women and/or women of child-bearing ages concerning the fish and sea mammals that are known to contain high MeHg concentrations (UNEP, 2002; Japanese Government, 2003). Even though the Hg concentration in breast milk is known to be very low (Sakamoto, 2002; Okarsson, 1996; Okarsson, 1995; Skerfving, 1988), some recent studies have still suggested that infants reared on breast milk for a long period might have increased risk (Nunes and Ferreira, 2000; Grandjean *et al.*, 1994). The higher MeHg accumulation at parturition in the fetal brain than that in the mother

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is well established (Sakamoto, 2004; Sakamoto, 2002; WHO, 1990). However, to what extent MeHg in breast milk contributes to the child MeHg concentration is not clear. Therefore, the difference in MeHg exposure to fetus and offspring throughout gestation and suckling must be established under the natural course of MeHg exposure to mother.

Though the MeHg exposure is through fish, an important source of protein especially for Japanese and Asian people, fish consumption also derives some other nutrients (Furst A, 2002; Clarkson and Strain, 2003) and n-3 polyunsaturated fatty acids, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are important for human health and normal brain development and function. Breast-feeding has many benefits and breast milk is the best nutrition source for infants. Therefore, we need an appropriate evaluation of MeHg exposure to fetus and infant through the mother during gestation and lactation to prevent excess avoidance of fish consumption and breast-feeding. The purpose of this paper was to emphasize the difference of methylmercury exposure to offspring during gestation and lactation by showing our own animal and human studies (Pan *et al.*, 2004; Sakamoto *et al.*, 2002b) at the same time. The sample number increased to 16 in comparison with 7 in the last paper (Sakamoto *et al.*, 2002b).

Materials and Methods

Animal Study

Animals and Administrative Procedure: Thirty 4-week-old female Wistar rats were supplied by CLEA Japan, and housed in a room under a 12-h light/12-h dark cycle at 23°C. The rats were maintained with free access to γ-ray-sterilized CE-2 laboratory powdered chow (CLEA Japan) containing MeHg (5 μg/g of Hg). This level of MeHg caused no decrease in body weight or apparent toxicity symptoms in our previous experiment (Eto *et al.*, 1997). In the previous experiment, the daily uptake of the diet was restricted to 16 g per rat until mating, but this time we maintained the rats with free access to the diet. The MeHg exposure level was monitored by measuring Hg concentration in blood (described in the next subsection) and, when the level had almost reached a plateau, the females were mated with males. The pregnant females were then continued on the same diet, with access *ad libitum*, throughout the gestation and suckling periods until postnatal day 20 (P20). The MeHg exposure level in the mothers and offspring were also monitored throughout late gestation and after parturition by measuring Hg concentrations in the blood and brain.

Samples: About 5-10 μl of blood was withdrawn from the tail vein of 4 randomly-chosen females from 30, fed on the MeHg-containing diet every two weeks until mating, to monitor the MeHg exposure level at various stages before mating, during gestation and late gestation. On embryonic days 18, 20, 22 (E18, E20, E22), 3 mothers and 6 fetuses (two fetuses from each litter) were randomly chosen to be sacrificed to determine the tissue concentration of Hg. Another randomly-chosen group of 3 mothers and 6 infants (2 females and 2 males each from each litter) were sacrificed on the day of parturition and postnatal days 10 and 20 (P10, P20). On postnatal days 5 and 15 (P5, P15), 8 infants (4 male and 4 female offspring) were also sacrificed to determine tissue Hg concentrations. For tissue sampling, rats were deeply anesthetized by an intraperitoneal injection of pentobarbital. Blood samples were collected by cardiac puncture, and the rats were then killed by transcardiac perfusion with physiological saline for 5 min to flush out blood from the brain. Hg concentrations in the samples were measured according to the method described elsewhere (Sakamoto, 2002a).

Human Study

Subjects: In the previous study (Sakamoto *et al.*, 2002b) the number of the subjects was 7. We added another 9 subjects. In total sixteen healthy Japanese pregnant women, ranging in age from 22 to 36 yr (average 30.4 ± 4.3 yr), planning to deliver in Munakata Suikokai General Hospital, Munakata City, Fukuoka, Japan, gave informed consent to take part in the present trial. Among all infants, five were males. The average body weights at birth and the age of 3 months were 3.3 ± 0.32 and 6.36 ± 0.62 kg, respectively. During the study, five mothers had delivered their first child, three their second, and eight their third. Two mothers consumed fish everyday, and the others two or three times

per week. Fifteen of the infants were reared on breast milk. Only one was reared mainly on breast milk and additional milk formula beginning at 4 and 6 weeks of age.

Samples: Blood samples were collected from sixteen pairs of mothers and infants. The samples included 13 m*l* of venous umbilical cord blood at birth and 10 m*l* of venous maternal blood 1 day after parturition before breakfast, and 2 m*l* of each infant's blood at 3 months of age. All blood samples were obtained by venipuncture with a small amount of heparin-Na and centrifuged at 3000 rpm for 10 min to separate into red blood cells (RBC). Samples were stored at −80°C until analysis. Hg concentrations in the samples were measured according to the method described elsewhere (Sakamoto, 2002b).

Ethics and Informed Consent: Human study was approved by the Ethics Committee of NIMD (National Institute for Minamata Disease). Sixteen normal Japanese pregnant women without any special exposure to mercury, and living in Munakata City, Fukuoka, Japan, gave their informed consent to take part in the trial.

Statistical Analysis

Hg concentrations were represented by means ± SD. The differences between samples were determined by paired *t*-test. The association between the samples was studied by Pearson and Spearman correlation analysis. A *P* value less than or equal to 0.01 was considered to demonstrate statistical significance.

Result

Animal Study

Hg Concentrations in Blood: The time-course changes in Hg concentrations measured in whole blood of the females before pregnancy and after parturition, as well as of the delivered offspring, are depicted in Fig. 1. The Hg concentration of the females increased with the duration of administration, and reached a near plateau after 8 weeks. The Hg concentration of the mothers decreased throughout gestation, and at parturition fell to approximately 50% of that in the mating period. After 20 days of lactation, the Hg concentration of mothers resembled that in the mating period. However, the time-course changes in Hg concentration of the offspring showed a pattern different from that of the mothers. On the day of birth, the concentration in blood was significantly (p<0.01 by paired t-test) higher, i.e., approximately 2 times that of their mothers on that same day. However, that concentration rapidly decreased throughout their suckling period. All offspring grew up without any physical signs of typical MeHg poisoning, such as ataxia or hind-limb crossing.

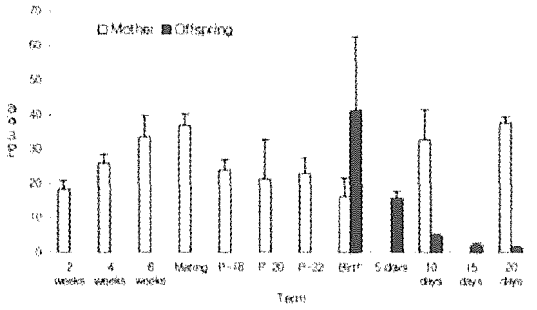

Fig. 1. Time-course changes in Hg concentrations in the whole blood of female rats during 8 weeks before and during pregnancy as well as after parturition and those in their offspring at birth and during suckling. For 8 weeks, female rats were fed a MeHg-containing diet (5 μg/g Hg as MeHg). They were then mated and continuously fed this diet during the gestation and lactation periods. The offspring are fed on mother's milk until weaning on postnatal day 20. Data represent means ± SD for mothers (n = 3-4) and infants (n = 4-6).

Hg Concentrations in the Brain: We also measured the changes in Hg concentration in the brain

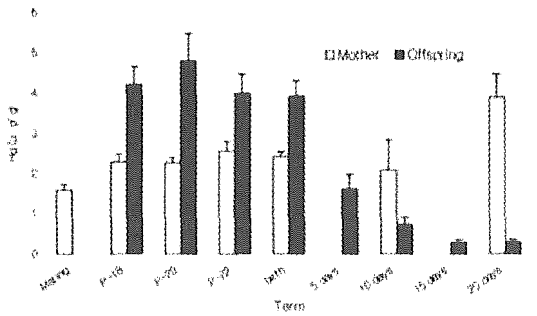

Fig. 2. Time-course changes in Hg concentrations in the brain of maternal rats during late gestation and suckling and those in offspring at during late gestation and during suckling. Data represent means ± SD for mothers (n = 3) and infants (n = 6).

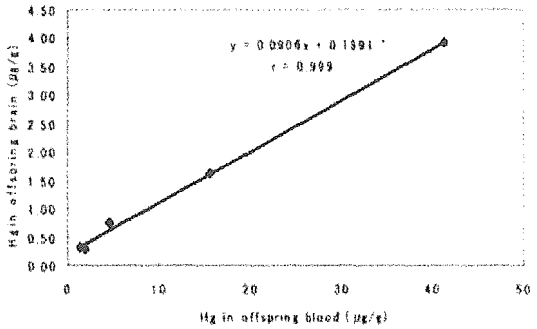

Fig. 3. Correlation between Hg concentrations in blood and brain at birth, days 5, 10, 15 and 20.
A strong correlation was observed in Hg between blood and the brain ($r = 0.999$, $p < 0.01$).

tissue of both mothers and offspring (Fig. 2). The patterns of time-course changes in the brain were similar to those in the blood. The average concentrations in the brain on fetal days E18, E20, E22 and at birth were about 4-4.5 μg/g, which was about 1.5 to 2 times higher than those in the brain of the mothers ($p<0.01$ by Student's *t*-test). Average concentrations of offspring rapidly decreased during the suckling period down to about 1/10 of that at parturition. Correlation between Hg concentrations in blood and the brain at birth, days 5, 10, 15 and 20 is shown in Fig. 3. A strong correlation was observed in Hg concentrations between blood and the brain ($r = 0.999$, $p<0.01$).

Human Study

RBC-Hg in Infants at Birth and 3 Months of Age: At birth, RBC-Hg in umbilical cords were higher than those in mothers in all sixteen cases. The mean RBC-Hg in umbilical cord was 13.0 ng/g, which was significantly higher than in mothers (8.19 ng/g) by paired *t*-test ($p<0.01$; Fig. 3). A strong correlation was observed in RBC-Hg in mothers and umbilical cords at parturition ($r = 0.96$, $p<0.01$, Fig. 4).

Although most of the mothers said that the amount and the species of fish consumed did not change during the lactation period, in all sixteen infants the Hg concentrations decreased throughout this period. The mean RBC-Hg in infants at 3 months of age was 6.87 ng/g, significantly lower than that at birth (13.0 ng/g) by paired *t* test ($p<0.01$; Fig. 5).

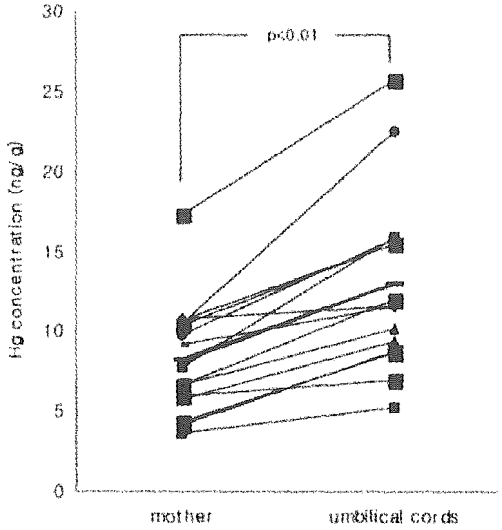

Fig. 4. Comparison of Hg concentrations in maternal and umbilical cord RBC. Fetal RBC-Hg level was significantly higher than that of maternal at birth by paired *t*-test ($p<0.01$). Horizontal lines indicate the means.

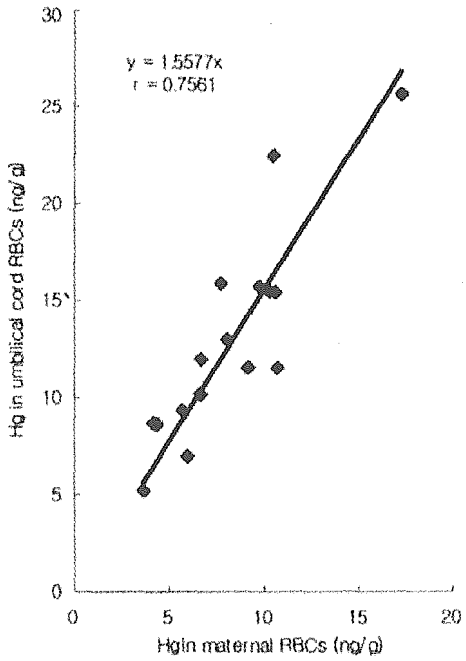

Fig. 5. Correlation between maternal and umbilical cord Hg concentrations in RBC in 16 maternal-fetal pairs. In all 16 cases umbilical cord RBC-Hg levels were higher than maternal levels. A strong correlation was observed in RBCs-Hg between mothers and umbilical cord ($r = 0.76$, $p < 0.01$).

Discussion

Animal Study

This animal study was designed so that fetuses conceived in females that had been exposed to a constant and consecutive dose of MeHg before and throughout gestation were exposed to MeHg transplacentally throughout the entire gestational period, followed by post-partum exposure through contaminated milk. This was considered to simulate the natural course of fetal and infant exposure to MeHg among people who commonly consume much fish and sea mammals, and to reveal the difference in the risk of MeHg to fetus and infants. The concentrations of Hg in the brain of fetus were 1.5 to 2 times higher than those in their mothers throughout late gestation. This is in accordance with the proposal that MeHg is actively transferred to the fetus across the placenta via neutral amino acids carriers (Ashner and Clarkson, 1988; Kajiwara *et al.*, 1996) throughout the late gestation period. The decrease in Hg concentrations in maternal blood during the gestation period partly explains the accelerated MeHg transfer to the fetus according to the demand of amino acids to promote fetal growth. It is known that the developing brain is most vulnerable the toxic effect of MeHg during the third-trimester (Rice and Barone, 2000; Takeuchi, 1982). Our results indicate that the Hg concentrations in offspring brain were higher than that in maternal brain not only at parturition but also throughout the late gestation period when the human brain is most vulnerable. We also demonstrated that Hg accumulations in offspring differ significantly between periods of gestation and suckling. During the 20 days after birth, the offspring concentrations in the blood and brain decreased dramatically as demonstrated in a previous animal experiment (Sakamoto *et al.*, 2002a; Oliveira *et al.*, 2001, Newland *et al.*, 1999; Sundberg *et al.*,1999). This will be explained by limited MeHg transfer from milk (Sundberg *et al.*, 1999; Sundberg *et al.*, 1991; Yoshida *et al.*, 1994) and rapid increase in the organ and body volume. Further, the increase in blood Hg concentrations in maternal blood during suckling can be partly explained by the diminished MeHg transfer to the fetus compared with that during the gestation period. However, our animal study indicated that Hg concentration in blood and brain in offspring increased after weaning when they started to eat MeHg contaminated diet (Sakamoto, 2002a). Therefore, attention should be paid to MeHg exposure through fish and sea mammals as baby food during breast-feeding period and after weaning. The strong correlation in Hg concentrations between blood and the brain also revealed that the Hg concentration in blood reflect the changes in the brain. In human also correlation coefficients for infant blood and the six brain regions were 0.4-0.8 in a population exposed to MeHg in fish (Cernichiari *et al.*, 1995). These results strongly suggest that fetal blood Hg concentration are very good biomarker to now the concentration in the brain which is the primary target organ for MeHg exposure.

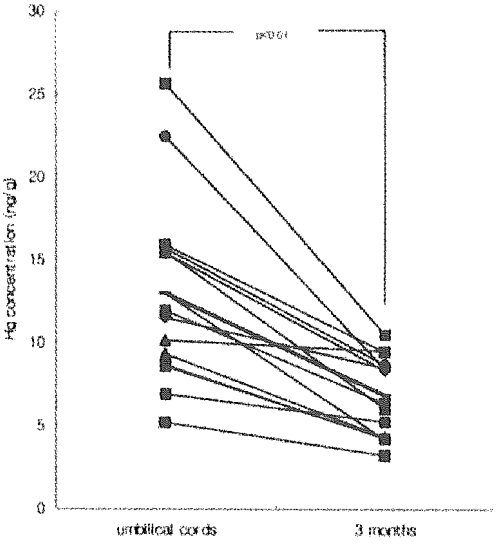

Fig. 6. Changes in Hg concentration in RBC from umbilical cord to those in infants at 3 months of age. The infant RBC-Hg level at 3 months of age was significantly lower than that at birth by paired *t* test ($p < 0.01$). The horizontal lines indicate the means.

Human Study

The present study was designed mainly to determine the changes in MeHg levels in infants after parturition, followed by further MeHg exposure through milk after birth. The primary target organ for MeHg exposure is the brain and blood Hg concentration reflects the concentration in the organ as mentioned in the previous animal study discussion. Not only

blood but also RBC can be used as a biomarker of MeHg exposure (Svensson, 1992; WHO, 1990; Swedish Expert Group, 1971). It is known that the RBC to plasma ratio of Hg concentration is approximately 1:1 in non fish-consuming populations and after exposure to Hg^0 vapor (Svensson *et al.*, 1992; HO, 1990). However, in general the higher the fish consumption (MeHg exposure) the higher the RBC to plasma ratio, which reaches approximately 8-9:1 in populations that consume a higher amount of fish (Sakamoto *et al.*, 2002b; Svensson *et al.*, 1992; WHO, 1990; Hansen *et al.*, 1990; Suzuki *et al.*, 1971). Additionally, more than 80% of Hg in the total blood (Akagi *et al.*, 1998, Kershaw, 1980) and more than 90% of that in RBC is known to be in the methyl form (Kershaw, 1980) in high fish-consumption, indicating that the Hg source was predominantly MeHg from fish. RBC-Hg is one of the best biomarkers to determine MeHg exposure level (Sakamoto *et al.*, 2002b; Oddy, 2001). Therefore, the changes in MeHg level infants during breast-feeding were investigated using the total Hg concentrations in RBCs in our present and previous studies (Sakamoto *et al.*, 2002b; Sakamoto *et al.*, 1993a; Sakamoto *et al.*, 1991) studies. RBC-Hg level in umbilical cords were about 1.6 times higher than that in the mothers, and there was a strong correlation between them at birth. This result was similar to the previous animal study suggesting that MeHg actively transfers to the fetus across the placenta via neutral amino acids carriers (Kajiwara *et al.*, 1996) as mentioned in the animal study. After 3 months of breast-feeding the RBC-Hg in infants dramatically declined to 53% of that at birth as was observed in animal study. During this period, the average body weight of infants quickly increased and became about 1.9 times that at birth. Consequently, the average body volume and the limited Hg transfer from breast milk might have caused the dilution in RBC-Hg levels during this period.

Conclusion

The higher Hg accumulation in fetus than mother was demonstrated in both the animals and humans studies. It is known that the susceptibility of the developing brain in the late gestation period is high (WHO, 1990). Thus, the risk of exposure of the fetus to MeHg must be very high. However, the Hg levels in the blood in infant decreased drastically during breast feeding in both the animals and humans studies. The brain Hg concentration also dramatically decreased during breast feeding. The contribution of breast milk to MeHg transfer to infants seems limited, as was recently suggested by Sandborgh-Englund *et al.* (2001) and Sakamoto *et al.* (2002b) However, from some viewpoints, the rate of MeHg excretion is thought to be low, and the biological half-time of MeHg in lactating women is shorter than in non-lactating women (WHO, 1990). Suckling mice are incapable of excreting MeHg (WHO, 1990). In Iraq, human milk was suspected source of MeHg exposure of poisoned infants (Amin-Zaki *et al.*, 1981). These phenomena may lead to the consideration that infants at breast-feeding also seem to be at high risk for MeHg. However, as were demonstrated by our animal and human studies, once neonates are separated from the active intrauterine amino acid transport system, Hg transfer depends on the milk, in which the Hg concentration is low. Offspring body and their brain grow rapidly after birth. The growth may dilute the Hg concentrations in the body and brain. Grandjean *et al.* (1994) mentioned that human milk seemed to be an important source of MeHg exposure in infants because hair Hg at approximately 12 months of age increased with the length of the nursing period. However, the same authors mentioned the concentration in the child's hair at 1 year was only about 25% of that of the mother at the time of delivery. Also, in MeHg poisoning in Iraq, both the infants' and mothers' clearance half-time was approximately 50 days. However, maternal MeHg can be transferred to infants through breast milk following exposure through the placenta during their intrauterine life. The seeming contradiction that MeHg half-life in infant was about 50 days in the case, even though excretion of MeHg in infants was thought to be much lower than in adults, can be explained by the rapid body and organ growth during the period.

In conclusion, the risk to offspring might be especially high throughout the gestation period but rapidly decreases during suckling under the practical and natural course of MeHg exposure from daily fish consumption. Thus, sufficient attention should

be paid to gestation rather than the breast-feeding period to avoid dangers of MeHg to human infants. Further, if exposure levels are constant and low enough not to cause adverse effects on fetuses during gestation, mothers need not worry about breast-feeding. The benefits of breast-feeding (Oddy, 2001; Kunz *et al.*, 1999) may well outright the possible adverse effects of MeHg in breast milk under these conditions. However, if mothers were exposed to high MeHg levels during the suckling period, due caution is recommended concerning breast-feeding as the possible poisoning was reported in Iraq (Amin-Zaki *et al.*, 1981). In addition, attention should be paid to MeHg exposure through fish and sea mammals as baby food during breast-feeding period and after weaning.

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