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Methylmercury in the breast milk of Japanese mothers and lactational exposure of their infants

Miyuki Iwai-Shimada [a,b,*], Hiroshi Satoh [a,1], Kunihiko Nakai [c], Nozomi Tatsuta [c], Katsuyuki Murata [d], Hirokatsu Akagi [e]

[a] Environmental Health Sciences, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
[b] Research Fellow of the Japan Society for the Promotion of Science, Chiyoda-ku, Tokyo, Japan
[c] Department of Development and Environmental Medicine, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan
[d] Environmental Health Sciences, Akita University Graduate School of Medicine, Akita, Japan
[e] International Mercury Laboratory Inc., Minamata, Kumamoto, Japan

HIGHLIGHTS

- Exposure of infants to methylmercury (MeHg) via milk has not been well examined.
- One half or more of total mercury in milk was comprised of MeHg in Japanese.
- Milk MeHg was associated with the internal accumulation of MeHg and milk lipid.

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ABSTRACT

The human fetus is known to be exposed to methylmercury (MeHg), but little is known about the risk of infant exposure via breast milk. To evaluate the lactational exposure to MeHg via breast milk in Japanese infants, the levels of total mercury (THg) and MeHg were determined in breast milk and maternal blood using samples from a birth cohort study at the Tohoku Study of Child Development. Maternal blood and breast milk were collected one day postpartum and one month after delivery, respectively. The median THg (and MeHg) concentrations in maternal RBCs, plasma and breast milk were 17.8 ng g^{-1} (17.8 ng g^{-1}), 1.51 ng g^{-1} (1.33 ng g^{-1}) and 0.81 ng g^{-1} (0.45 ng g^{-1}), respectively ($n = 27$). The median percentage of MeHg in THg was 54% in breast milk. Breast milk contained substantial amounts of MeHg, which was strongly associated with the internal accumulation of MeHg and the lipid content of the milk ($r = 0.684$). The range of lipid contents in milk varied widely from 0.50 to 6.60 g/100 g of milk, with a median of 3.60 g/100 g. The median (range) weekly average intake of MeHg via breast milk was estimated to be 0.63 μg kg^{-1} (0.08–1.68 μg kg^{-1}) BW/week. Because the MeHg and lipid contents in milk substantially fluctuate, an investigation of the variations of MeHg and lipid content in breast milk may be required for a more precise risk assessment.

1. Introduction

The human fetus is known to be exposed to for methylmercury (MeHg) (IPCS, 1990), but little is known about the risk of infant exposure via breast milk. Breastfeeding has many benefits, and breast milk is the best source of nutrition for infants (Gartner et al., 2005) and is an important factor in the initiation, development and/or composition of the neonatal gut microflora (Caicedo et al., 2005). However, breast milk may also be a source of environmental contaminants, such as polychlorinated biphenyls (PCBs), methylmercury (MeHg) and lead (Anderson and Wolff, 2000;

Abbreviations: THg, total mercury; MeHg, methylmercury; IHg, inorganic mercury; Me/T, percentage of MeHg in THg; BW, body weight; PCB, polychlorinated biphenyls; TSCD, Tohoku Study of Child Development; FFQ, food frequency questionnaire; CVAAS, cold vapor atomic absorption spectrometry; GC-ECD, gas chromatography with electron capture detection; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; PUFAs, polyunsaturated fatty acids; BMI, body mass index; TWI, tolerable weekly intake; RfD, reference dose.

* Corresponding author. Present address: Laboratory of Molecular and Biochemical Toxicology, Graduate School of Pharmaceutical Sciences, Tohoku University, 6-3 Aramakiazaaoba, Aoba-ku, Sendai, Miyagi 9808578, Japan. Tel.: +81 22 795 6871; fax: +81 22 795 6872.
E-mail address: miyuki@m.tohoku.ac.jp (M. Iwai-Shimada).
[1] Present address: Professor Emeritus, Tohoku University, Sendai, Miyagi, Japan.

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Dórea, 2004). Infants nourished with breast milk for a long period might be at increased risk for mercury exposure (Grandjean et al., 1994).

Populations that consume fish and fish products are exposed to naturally occurring MeHg. Recently, certain studies described MeHg concentrations in breast milk from fish-eating populations in Europe (Valent et al., 2011; Miklavčič et al., 2011, 2013), and inconsistent results regarding the correlation between MeHg in breast milk and fish consumption were obtained.

Inorganic mercury (IHg) is excreted into breast milk (Sundberg et al., 1999; Vahter et al., 2000). Correlations between the total mercury (THg) levels in milk and plasma (primarily consisting of IHg) (Skerfving, 1988) and between the IHg levels in milk and whole blood (Oskarsson et al., 1996) have been reported. THg in breast milk is correlated with the number of amalgam fillings but not with fish consumption (Oskarsson et al., 1996). However, IHg is poorly absorbed in the gastrointestinal tract at a level averaging less than 10% (IPCS, 1991), whereas intestinal absorption of MeHg is nearly complete. Thus, MeHg in breast milk may have toxicological significance in infants nursed with breast milk.

Because fish and fish products are staple foods of the Japanese people, in the present study, we determined the THg and MeHg concentrations in breast milk of lactating Japanese women. We assumed that certain nutritional factors, such as lipids and proteins, in breast milk might contribute to the mercury concentrations in the milk. For the MeHg analysis of breast milk, the analytical method conventionally used to analyze biological samples, such as blood and tissues, was applied with slight modifications in the pretreatment processes. Because breast milk contains various nutrients, such as casein, lactose and lipids, it is easily carbonized. Moreover, the emulsion formed during pretreatment gives low MeHg concentrations and inaccurate measurements with a large distribution. Finally, we attempted to estimate MeHg intake via breast milk to evaluate the transfer of MeHg from the breast milk to the infants.

2. Materials and methods

2.1. Study design, subjects and sampling

We have been conducting a birth cohort study called the Tohoku Study of Child Development (TSCD). The study protocol of the TSCD has been described elsewhere (Nakai et al., 2004). The medical ethics committee of the Tohoku University Graduate School of Medicine approved the study protocol. The research was conducted in two areas, an urban area and a coastal area (Suzuki et al., 2010; Tatsuta et al., 2012). In this study, the samples from the coastal area were used. We enrolled 884 pregnant women who gave their written informed consent to participate in this study, and 749 mother–child pairs were registered according to the eligibility criteria. However, because MeHg analysis requires a long pretreatment process and is laborious, only 27 subjects were selected for determination of MeHg in breast milk. To ensure that the MeHg exposure of the subjects was representative of the full range of the registered mother–child pairs, we randomly selected five to six subjects from each of the five categories of maternal hair THg using statistical software. All of the subjects had complete data sets, sufficient volumes (>20 mL) of breast milk, and hair without permanent waving or straightening (Ohba et al., 2008).

Information on demographics and dental fillings was obtained through an interview and questionnaire. Information on the delivery conditions and neonatal characteristics was obtained from hospital medical records. Fish intake during pregnancy was estimated from a food frequency questionnaire (FFQ) administered four days after delivery (Yaginuma-Sakurai et al., 2009).

Maternal blood was collected one day postpartum by venipuncture into a tube containing heparin. Maternal hair and breast milk were collected two days and one month after delivery, respectively. RBC, plasma and breast milk samples were stored at −80 °C until the analyses.

2.2. Analytical methods

2.2.1. Determination of THg

The THg levels in maternal hair, maternal RBCs, plasma and breast milk were measured using cold vapor atomic absorption spectrometry (CVAAS, HG-201, Sanso Seisakusho Co. Ltd., Tokyo, Japan). The analytical method of CVAAS has been fully described elsewhere (Ministry of the Environment, 2004).

2.2.2. Determination of MeHg

The MeHg levels in the blood samples were measured using gas chromatography with electron capture detection (GC-ECD) (Akagi et al., 2000; Ministry of the Environment, 2004). To accurately analyze the MeHg in breast milk, the method was modified from the referenced procedure (see Supplemental material, Fig. S1). To avoid carbonization, the length of the heating period was shortened based on trial results. The formation of an emulsion during pretreatment was minimized by washing the organic layer with 1N NaOH twice without adding Na_2SO_4 to the aqueous layer.

2.3. Analytical quality control

Sample analysis was performed in triplicate and was repeated when the coefficient of variation (CV) of the triplicate analyses was more than 5%. Accuracy was ensured using a certified reference material (DOLT-3: dogfish liver, NRC, Canada) for quality control. The average of the duplicate THg (or MeHg) determinations was 3.288 μg g^{-1} (1.454 μg g^{-1}), and the certified value was 3.37 ± 0.14 μg g^{-1} (1.59 ± 0.12 μg g^{-1}). We also used another certified reference material (TORT-2: Lobster hepatopancreas, NRC, Canada), for which the average of the duplicate THg (MeHg) determinations was 0.269 μg g^{-1} (0.146 μg g^{-1}) and the recommended value was 0.27 ± 0.06 μg g^{-1} (0.152 ± 0.013 μg g^{-1}). We confirmed the analytical precision using the standard addition method. Reproducibility was tested by repeated measurements of a pooled sample of breast milk, resulting in a value of 1.136 ± 0.004 ng g^{-1} (n = 6; CV = 0.39%).

2.4. Other assays

The protein concentrations of the breast milk samples were determined according to the Bradford Coomassie blue dye-binding method (Bio-Rad, Richmond, CA). The lipid content in the breast milk was determined according to the Röse-Gottlieb method and was performed at the Japan Food Research Laboratories (Tokyo, Japan). To evaluate fish consumption, we transferred the analyses of maternal plasma levels of EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) to SRL, Inc. (Tokyo, Japan).

2.5. Data analysis

The concentration of IHg was calculated as the difference between THg and MeHg. Milk IHg was logarithmically transformed because of the apparently skewed distribution. These data were analyzed using the Pearson product-moment correlation coefficient (r). Multiple regression analysis was used to investigate factors affecting the mercury levels in breast milk. The independent variables were RBCs and plasma Hg (as indicators of internal mercury accumulation), milk protein, milk lipid, and BMI before pregnancy (Model 1). Then, unrelated variables were excluded from the