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Implications of mercury concentrations in umbilical cord tissue in relation to maternal hair segments as biomarkers for prenatal exposure to methylmercury

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ABSTRACT

In this study, we investigated how mercury (Hg) concentrations in umbilical cord tissue are correlated with those in biomarkers for prenatal exposure to methylmercury (MeHg). Total Hg (T-Hg) concentrations were measured in 54 mother–child paired samples of maternal blood, umbilical cord tissue, cord blood, and maternal hair segments (1–cm incremental segments from the scalp) collected at parturition. MeHg concentrations were also measured in the cord tissue. Median T-Hg and MeHg concentrations in cord tissue on a dry-weight basis (d.w.) were 62.2 ng/g and 56.7 ng/g, respectively. Proportions of MeHg to T-Hg were approximately 95%. Both T-Hg and MeHg in cord tissue (d.w.) showed better correlations with T-Hg in cord blood than did T-Hg in cord tissue on a wet-weight basis (w.w.). Median T-Hg concentrations in maternal blood, cord blood, and maternal hair (0–1 cm from the scalp) were 3.79 ng/g, 7.26 ng/g, and 1.35 μg/g, respectively. Median T-Hg concentration in cord blood was 1.92 times higher than that in maternal blood. T-Hg in cord tissue (d.w.) showed a strong correlation with that in cord blood ($r=0.912$, $p<0.01$). Among the hair segments, T-Hg in cord tissue (d.w.) showed the strongest correlation ($r=0.854$, $p<0.01$) with that in maternal hair at 0–1 cm from the scalp, reflecting growth for approximately 1 month before parturition. Based on the present results, T-Hg and MeHg concentrations in cord tissue may be useful biomarkers for prenatal MeHg exposure of the fetus, especially reflecting the maternal MeHg body burden during late gestation. The conversion factors for T-Hg and MeHg concentrations in cord tissue (d.w.) to T-Hg concentrations in maternal hair (0–1 cm from the scalp) were calculated to be 22.37 and 24.09, respectively. This information will be useful for evaluating maternal MeHg exposure levels in retrospective studies using preserved umbilical cord tissue.

1. Introduction

Minamata disease was the first outbreak of severe methylmercury (MeHg) poisoning caused by man-made environmental pollution (Irukayama and Kondo, 1966). Its key feature was neurological disorders caused by MeHg that penetrated into the central nervous system through the blood–brain barrier (Takeuchi et al., 1962). In addition, patients with fetal-type Minamata disease, who were exposed to MeHg during the gestation period, displayed severe cerebral palsy-like symptoms, while their mothers had mild or no manifestations of poisoning (Harada, 1978). This landmark disease was the first event to bring worldwide attention to the high risk to fetuses imposed by MeHg exposure. In addition to the high vulnerability of the developing brain, MeHg accumulates at higher concentrations in fetuses than in mothers, as we previously reported in human and animal studies (Sakamoto et al., 2002a, 2004, 2006, 2010a, 2013).

After the disaster that caused fetal-type MeHg intoxication in Minamata, many cohort studies have been conducted to address the neurodevelopmental effects of MeHg exposure during gestation. Birth cohort studies in the Faroe Islands (Grandjean et al., 1997; Grandjean et al., 1999; Grandjean et al., 2005) and

Abbreviations: Hg, Mercury; MeHg, methylmercury; d.w., dry-weight basis; w.w., wet-weight basis; GC-ECD, gas chromatography with electron capture detection

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Seychelles (Davidson et al., 1998; Myers et al., 2003) have especially attracted the attention of many researchers.

The governing council of the United Nations Environmental Programme (UNEP) started a global mercury (Hg) assessment in 2003 (UNEP, 2008). Global Hg levels were estimated to be increasing since the industrial revolution, reflecting increases in anthropogenic Hg emissions. As a result, UNEP began to develop a legally-binding global instrument on mercury in January 2013 (UNEP, 2013). Increased delivery of Hg to ecosystems usually results in increased levels of MeHg in fish and marine mammals. Human exposure to MeHg mainly occurs through consumption of such fish and marine mammals. However, the adverse effects of MeHg exposure on pregnant women and their fetuses remain unclear, especially in populations who consume large amounts of fish and marine mammals (UNEP, 2013).

Hg concentrations in biomarkers such as maternal hair at parturition and cord blood were used to predict the effects of fetal MeHg exposure in the above-mentioned birth cohort studies in the Faroe Islands and Seychelles (Grandjean et al., 2001; Myers et al., 1995). Although hair Hg analysis involves a number of variables, such as hair growth rate, density, color, waving, external contamination, and permanent treatment (WHO, 1990), segmental analysis of maternal hair that grows during gestation will provide time-course data (Björnberg et al., 2005; Morrissette et al., 2004; Sakamoto et al., 2008), because the average hair growth rate is commonly assumed to be approximately 1 cm per month (Boischio and Cernichiari, 1998; Cernichiari et al., 1995; Grandjean et al., 1992). Meanwhile, cord blood represents the fetal blood circulating in the fetal body, suggesting that the cord blood can directly reflect the fetal MeHg body burden at late gestation (Cernichiari et al., 1995; NRC, 2000). In turn, umbilical cord tissue is a fetus-side tissue, and its usefulness as a predictor of fetal exposure to MeHg was shown by Grandjean et al. (2005).

Even today, Japanese people have the custom of preserving the umbilical cord as a memento of birth (Sakamoto et al., 2010b). The MeHg concentration in preserved umbilical cord has been recognized as an effective biomarker for retrospective determination of MeHg levels in the fetus (Akagi et al., 1998; Harada et al., 1999; Nishigaki and Harada, 1975; Sakamoto et al., 2006). In Japan, preserved umbilical cords are traditionally stored in a chest placed inside a small paulownia wood or plastic box. MeHg in the cord tissue should not be decreased by microorganisms, because it is completely dried. However, preserved umbilical cords are often treated with mercurochrome upon removal at parturition, and mercurochrome can easily dissociate into Hg ions in solution, acting as a disinfectant. Thus, treatment with mercurochrome can affect Hg concentrations when total Hg (T-Hg) is measured. Accordingly, MeHg must be measured in traditionally preserved cord tissue. Temporal changes in the MeHg pollution in the Minamata area of Japan have been reported using MeHg concentrations in traditionally preserved umbilical cord tissue (Nishigaki and Harada, 1975; Sakamoto et al., 2010b).

However, studies on the period when the maternal MeHg body burden reflects the Hg concentrations in cord tissue have not been conducted. Furthermore, the conversion factor that can be used to estimate maternal hair Hg using MeHg concentrations in cord tissue has not been well studied. This conversion factor is especially important for evaluating maternal MeHg exposure levels in retrospective cohort studies using traditionally preserved umbilical cord tissue.

In the present study, we investigated the relationships between Hg concentrations in cord tissue and those in maternal hair segments, maternal blood, and cord blood as biomarkers that are useful for predicting fetal exposure to MeHg.

2. Materials and methods

2.1. Subjects and sample collection

Fifty-four healthy Japanese pregnant women without any occupational exposure to Hg compounds, and planning to deliver their babies at Fukuda Hospital, Kumamoto, Japan from 2006 to 2007 provided informed consent at their first pregnancy check-up to participate in this study. Their ages ranged from 21 to 41 years (mean: 29.7 years).

None of the women had undergone permanent hair treatments for at least 6 months prior to participation in the survey, having been asked not to do such treatments during gestation. Approximately 50 full-length strands of maternal hair were collected at parturition by cutting the strands close to the scalp in the occipital area. Each maternal hair strand was cut into 1-cm incremental segments from the scalp end toward the tip.

Fasting venous blood samples were collected from the mothers at 1 day after parturition. Umbilical cord venous blood and umbilical cord tissue (length: 5 cm on the fetus side) were collected at parturition. Blood samples were obtained by venipuncture and collected into heparin-Na-containing vacutainer tubes. The samples were stored at −80 °C until analysis. The umbilical cord tissue was rinsed five times with 0.9% saline and pressed using paper towels each time to remove blood from the blood vessels. Approximately 0.5 g of umbilical cord tissue was cut into fine pieces, freeze-dried for 1 day, and subsequently kept in a desiccator with silica gel for 2 days. T-Hg and MeHg concentrations in the cord tissue were calculated on a wet-weight basis (w.w.) and dry-weight basis (d.w.).

The conversion factors from dried cord T-Hg and MeHg concentrations (ng/g) to T-Hg concentrations (μg/g) in maternal hair (0–1 cm from the scalp), maternal blood, and cord blood were calculated from the individual ratios of the Hg concentrations in the paired samples and expressed as means ± SD.

In the present study, traditionally preserved umbilical cord samples were also collected in Taiji (14 samples) and Nachikatsuura (27 samples), Wakayama, Japan. Taiji is a whaling town and its inhabitants have a custom of consuming toothed whales (Nakamura et al., 2014). Nachikatsuura is a neighboring town to Taiji and is famous for its fish market, especially for tuna (Endo and Haraguchi, 2010). The preserved cord tissues were collected and analyzed for their MeHg concentrations as a pilot study to estimate maternal hair Hg levels at parturition. We collected these samples for a planned retrospective cohort study on the effects of MeHg exposure during gestation in these towns using MeHg concentrations in preserved cord tissue. The preserved cord tissue samples were provided by mothers whose children were 6–7-year-old primary school students and who were born between 2006 and 2009. To measure MeHg concentrations in the preserved umbilical cord tissue samples, part of the tissue was crushed using a plastic hammer. The remaining dried blood in the vessels was removed from the tissue with tweezers.

The study protocol was reviewed and approved by the Ethics Committee of the National Institute for Minamata Disease, Minamata, Japan.

2.2. T-Hg and MeHg analysis methods

T-Hg concentrations in approximately 0.5 mL of blood, 5–10 mg of hair, and 100 mg of umbilical cord tissue (d.w.) were determined by cold vapor atomic absorption spectrophotometry according to a previously described method (Akagi et al., 2000) using a mercury analyzer (Model Hg-201; Sanso Seisakusho Co. Ltd., Tokyo, Japan). The method involved sample digestion with HNO_3, $HClO_4$, and H_2SO_4, followed by reduction to Hg^0 by $SnCl_2$. The

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