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Relationships between trace element concentrations in chorionic tissue of placenta and umbilical cord tissue: Potential use as indicators for prenatal exposure

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ABSTRACT

The role of the placenta was assessed by comparing the profiles of methylmercury (MeHg), inorganic mercury (I-Hg), lead (Pb), cadmium (Cd), selenium (Se), zinc (Zn), and copper (Cu) in freeze-dried chorionic tissue of the placenta and umbilical cord tissue. The significance of the placenta and cord tissue as predictors of prenatal exposure to these trace elements in pregnant women and newborns was also examined by comparing the element profiles among placenta and cord tissue, and maternal and cord blood red blood cells (RBCs). The samples were collected from 48 mother–child pairs at birth in the general population of Japanese. The concentrations of all elements, except for MeHg, were significantly higher in placenta than in cord tissue. In particular, the Cd showed the highest placenta vs. cord tissue ratio (59:1), followed by I-Hg (2.4:1), indicating that the placental barrier works most strongly against Cd among the examined toxic elements. Contrary to the other elements, the MeHg concentration in cord tissue was significantly higher (1.6 times) than that in placenta, indicating its exceptionally high placental transfer. The MeHg in placenta showed significant correlations with total mercury (T-Hg) in maternal and cord RBCs ($r_s = 0.80$ and 0.91, respectively). The MeHg in cord tissue also showed significant correlations with T-Hg in maternal and cord RBCs ($r_s = 0.75$ and 0.85, respectively). Therefore, both placenta and cord tissue are useful for predicting maternal and fetal exposure to MeHg. The Se concentration in placenta showed significant but moderate correlations with that in maternal and cord RBCs ($r_s = 0.38$ and 0.57, respectively). The Pb, Zn, and Cu concentrations in placenta and cord tissue showed no significant correlations with those in maternal and cord RBCs. As an exception, the Cd concentration in placenta showed a moderate but significant correlation ($r_s = 0.41$) with that in maternal RBCs, suggesting that the placenta is useful for predicting maternal exposure to Cd during gestation.

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1. Introduction

Fetuses depend on their mothers for nutrition, including essential elements such as selenium (Se), zinc (Zn), and copper (Cu). However, they are also exposed through their mothers to toxic elements such as methylmercury (MeHg), inorganic mercury (I-Hg), lead (Pb), and cadmium (Cd). The transfers of these toxic metals from mother to fetus have mainly been studied by comparing the concentrations of the elements in maternal and cord blood or red blood cells (RBCs) (Butler Walker et al., 2006; Miklavcic et al., 2013; Sakamoto et al., 2012; Truska et al., 1989). To date, however, simultaneous comparisons of trace elements among placenta, cord tissue, maternal blood/RBCs, and cord blood/RBCs have not been well investigated. To examine the role of the placenta in this study, we compared the concentrations of the above-mentioned toxic and essential elements between chorionic tissue of the placenta and umbilical cord tissue in 48 mother–child pairs in the general Japanese population. In addition, we assessed the usefulness of the placenta and cord tissue as predictors of maternal and fetal exposure to these trace elements.

Among the analyzed toxic elements, mercury (Hg), especially MeHg, has attracted much attention because several man-made pollution incidences and animal studies have indicated that the developing brain during the prenatal stage is vulnerable to MeHg exposure (Choi, 1989; NRC, 2000; WHO, 1990). In the severe MeHg pollution incident in Minamata, Japan, more than 20 infants exposed to MeHg through

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their mothers showed a severe cerebral palsy like-syndrome, while their mothers had mild or no manifestations of poisoning (Harada, 1978; Takeuchi et al., 1962). Although the results of the Seychelles child development study and the Faroese birth cohort study did not reach the same conclusion (NRC, 2000), the global adverse effects of MeHg exposure on pregnant women, especially those consuming large amounts of fish and seafood, remain to be elucidated. The total mercury (T-Hg) concentration in blood/RBCs is known to be a good biomarker of MeHg exposure in humans (Svensson et al., 1995; WHO, 1990). The T-Hg concentration in umbilical cord blood has been used as an effective biomarker of fetal MeHg exposure (Grandjean et al., 1999). Umbilical cord tissue has also been used to determine fetal MeHg exposure in some studies (Akagi et al., 1998; Grandjean et al., 2005; Nishigaki and Harada, 1975; Sakamoto et al., 2010).

In addition to MeHg, mercury vapor (Hg^0), a neurotoxic agent, easily crosses the blood–brain barrier and causes damage to the brain (WHO, 1991). Furthermore, Hg^0 can transfer from mother to fetus through the placenta (Yoshida, 2002). In contrast to MeHg or Hg^0, the intestinal absorption, brain uptake, and placental transfer of divalent mercury (Hg^{2+}) are known to be limited (WHO, 1991). A comparison of I-Hg concentrations in the placenta and cord tissue may explain the limited Hg^{2+} transfer through the placenta.

With respect to other trace elements, the neurobehavioral effects of Pb, especially in children, are well documented (Liu et al., 2013; Wright et al., 2008). The Cd is also an important toxic element whose main target organ is the kidney. However, a cross-sectional epidemiological study revealed neurological effects resulting from occupational exposure to Cd (Viaene et al., 2000). A study of American children showed a negative association between Cd levels and neurodevelopmental outcomes (Ciesielski et al., 2012). Meanwhile, another cross-sectional study failed to find any neuropsychological effects of Cd (Wright et al., 2006). Consequently, investigations of the placental transfer of the above-mentioned toxic elements are of considerable interest.

The Se, an essential nutrient, plays an important role in antioxidant enzymes such as glutathione peroxidase and thioredoxin reductase (Tapiero et al., 2003). It is also well established that Se can counteract the toxicity of various elements in animals, especially that of Hg^{2+} and MeHg (Beyrouty and Chan, 2006; El-Begearmi et al., 1982; Fredriksson et al., 1993; Ganther et al., 1972; Satoh et al., 1985). Moreover, Se is known to coexist with Hg in fish and sea mammals (Burger and Gochfeld, 2007; Burger et al., 2007; Cabanero et al., 2005), and may play a role in protecting against MeHg toxicity. We recently demonstrated that selenomethionine, a food-derived Se, directly protected against neuronal degeneration caused by MeHg in the developing rat cerebrum (Sakamoto et al., 2013).

The main objectives of the present study were: 1) to investigate the role of the placenta in the transfer of various trace elements from mother to fetus during gestation, by comparing the element concentrations in chorionic tissue of placenta and cord tissue; and 2) to assess the potential use of trace element concentrations in placenta and cord tissue for predicting their body burden in mothers and newborns during gestation, by studying the relationships of the element concentrations among chorionic tissue of placenta and cord tissue as well as maternal and cord RBCs.

2. Material and methods

2.1. Subjects

Approximately 1 week before parturition, a total of 48 healthy Japanese pregnant women without any known exposure to heavy metals provided written informed consent to participate in the study. The women were aged between 21 and 41 years (mean age: 29.3 ± 4.2 years), and resided in Munakata City, Fukuoka, Japan. The babies were born healthy after full-term pregnancies (37–41 months), and comprised 25 males and 23 females. The study was approved by the Ethics Committee of the National Institute for Minamata Disease (NIMD).

2.2. Sampling

Placenta, umbilical cord tissue for 5 cm on the fetus side, and venous umbilical cord blood (13 mL) were collected from the 48 pairs of mothers and infants at parturition between May and December 2002. Venous maternal blood (10 mL) was collected before breakfast on the first day after parturition. The blood samples were obtained by venipuncture and collected in heparin-containing vacutainer tubes. RBCs were obtained by centrifugation at 3000 rpm for 10 min. All samples were stored at −80 °C until analysis.

Chorionic tissue of the placenta was separated from the placenta using scissors. The chorionic tissue of the placenta and cord tissue were rinsed five times with 0.9% saline and pressed using paper towels each time to remove the blood, and then freeze-dried. The reason for using freeze-dried cord tissue was that 48 paires of T-Hg in cord RBCs showed a better correlation coefficient with T-Hg in freeze-dried cord tissue ($r_s = 0.85$) than with T-Hg in wet cord tissue ($r_s = 0.82$) in a preliminary experiment. Grandjean et al. (2005) also previously recommended the use of freeze-dried cord tissue rather than wet cord tissue as a biomarker of fetal exposure to MeHg.

2.3. Metal analysis

The T-Hg concentrations were determined by cold vapor atomic absorption spectrophotometry using a mercury analyzer Model Hg-201 (Sanso Seisakusho Co. Ltd., Tokyo, Japan) according to the method of Akagi et al. (2000), which involved sample digestion with HNO_3, $HClO_4$, and H_2SO_4, followed by reduction to Hg^0 by $SnCl_2$. The method detection limit was 0.01 ng/g. A blood reference material, Level 2, MR9067 (Nycomed Co., Oslo, Norway) was used to check the accuracy of the results. The average Hg concentration measured in the reference material was 7.5 μg/L (recommended range: 6.8–8.5 μg/L). For selective quantification of I-Hg, MeHg in the acidified sample homogenate was removed by toluene as much as possible (5 times) using a previously reported procedure (Yasutake and Hirayama, 1990), and the Hg concentrations were determined using an oxygen combustion-gold amalgamation method and an atomic absorption mercury detector (MD-A; Nippon Instruments Co. Ltd., Tokyo, Japan). The method detection limit was 0.01 ng/g. The MeHg was calculated as T-Hg minus I-Hg.

Analyses of the remaining trace elements in RBCs, placenta, and cord tissue were carried out by IDEA Consultants Inc. (Shizuoka, Japan). The RBC samples (about 200 mg) were precisely weighed. Freeze-dried placenta and cord tissue (about 20 mg) were precisely weighed. Samples were diluted to 2 mL with a matrix solution containing 0.05 mL of concentrated ammonia, 1 mL of 0.01 M disodium ethylenediaminetetraacetate, 0.7 mL of Triton X-100, and 20 mL of butanol per liter. The diluted samples were analyzed by a standard addition analysis technique using a 7500c ICP-MS system (Agilent Technologies, Santa Clara, CA). Accuracy was checked by measuring a reference blood material, Level 1, MR4206 (Nycomed Co.). The average values measured in the reference blood and the recommended values were as follows: 27.3 and 27.6 ± 1.4 ng/mL for Pb; 0.74 and 0.74 ± 0.06 ng/mL for Cd; 72.3 and 79.8 ± 5.4 ng/L for Se; 5330 and 5550 ± 300 ng/mL for Zn; and 552 and 564 ± 33 ng/mL for Cu. The detection limits were 0.4 ng/mL for Pb, 0.08 ng/mL for Cd, 2 ng/mL for Se, 4 ng/mL for Zn, and 1 ng/mL for Cu.

2.4. Statistical analysis

Differences in trace element concentrations between placenta and cord tissue were analyzed by a paired *t*-test. Associations among elements in the samples were tested using Spearman rank correlation coefficient. Values of $P < 0.05$ were considered to indicate statistical significance.

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