CHARACTERIZATION OF DIOXINS IN SETS OF PLACENTAL TISSUE, MATERNAL BLOOD AND UMBILICAL CORD BLOOD SAMPLES COLLECTED FROM JAPANESE PREGNANT WOMEN

Hori T[1]*, Takahashi K[1], Kajiwara J[1], Morokuma S[2], Otera Y[2], Yamamoto T[3], Kawamoto T[4], Hirata T[1], Furue M[2], Tsukimori K[5]

[1]Fukuoka Institute of Health and Environmental Sciences, 39 Mukaizano Dazaifu-shi, Fukuoka, Japan; [2]Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi Higashi-ku, Fukuoka, Japan; [3]Kitakyushu Life Science Center, 1-4 Nakabaru shinmachi Tobata-ku, Kitakyushu, Japan; [4]University of Occupational and Environmental Health, 1-1 Iseigaoka Yahatanishi-Ku, Kitakyushu, Japan; [5]Fukuoka Children's Hospital and Medical Center for Infectious Diseases, 2-5-1 Tojinmachi Chuo-ku, Fukuoka, Japan.

Introduction

The nationwide investigation regarding the mean dietary intake of dioxins began in 1998 using the total diet study method. As part of the latest 2010 investigation, eight sets of total diet study samples were prepared in seven different regions of Japan. Based on that investigation, the average intake was estimated to be 0.81 pg-TEQ/kg b.w./day, with a range of 0.43 – 1.61 pg-TEQ/kg b.w./day. The recent Japanese dietary intake of dioxins is estimated to be below the TDI, 4 pg-TEQ/kg b.w./day, and the estimated value has been gradually decreasing for the last decade[1),2)]. The health risk caused by dioxins exposure from food is considered to not be serious for Japanese adults at present, but there is little information regarding the risk during growing periods of small infants and fetuses. Dioxins that have accumulated in the maternal body as a result of long-term exposure to the chemicals can be transferred to the fetus via the placenta or to nursing infants via breast milk, potentially causing developmental health problems in children. In this study, we determined dioxin concentrations in human biological tissue samples collected to assess pediatric health risks from exposure of mothers and children to dioxins.

Materials and methods

We began recruiting pregnant women at Kyushu University Hospital in October 2009 to collect sets of tissue samples, that is, placenta, amniotic fluid, maternal blood, umbilical cord venous blood, umbilical cord arterial blood, umbilical cord, maternal fat, meconium, and other biological tissues. To date, we have accumulated 217 individual samples from 29 donors, 19 of whom had normal pregnancy deliveries and 10 of whom had fetal growth restriction (FGR) deliveries. All samples were frozen below –20 °C in glass containers until the chemical analysis. Among these collected samples, we decided to analyze three tissues first, that is, placenta, maternal blood, and umbilical cord blood, from the perspective of maternal-fetus transfer of dioxins.

Our analytical method for detecting dioxins in biological samples consists of both efficient extraction using an accelerated solvent extractor instead of the usual liquid-liquid extraction and sensitive detection using HRGC/HRMS equipped with a large-volume injection system. Automated extraction was performed using an ASE-350 (Dionex) under the conditions of 150 °C, 1500 psi. Before lipid extraction, weighed biological samples were dehydrated in an AdVantage freeze-dryer (VirTis). As a result, homogeneous lipid solutions were obtained without any emulsion formations, which often appear with the usual shaking extraction using saturated ammonium sulfate and ethanol/hexane. Dioxins were analyzed using a model 7890A gas chromatograph (Agilent Technologies) coupled to a model Autospec-Premier mass spectrometer (Waters). We employed a VF-5ms (0.25 mm x 30 m) capillary column (Agilent Technologies) on an LVI-S200 high-volume injection system (AiSTI SCIENCE) to determine PCDD/Fs and non-*ortho* PCBs.

Detection limit values on a lipid-weight basis were as follows: PCDD/Fs, 0.3-2 pg/g lipid for placenta and cord venous blood, 1-4 pg/g lipid for maternal blood; non-*ortho* PCBs, 0.3-0.6 pg/g lipid for placenta, 0.3-1 pg/g lipid for cord venous blood, 10 pg/g lipid for maternal blood. All TEQ values were calculated using WHO-TEF (2005), and concentration values below the detection limit were taken to be zero. Determination of 8 kinds of mono-*ortho* PCBs is underway for the entire collection of sets of samples, so these congeners were excluded from

the TEQ evaluations for the present. Other details of analytical procedures have been described in our previous reports[3), 4)].

Results and discussion

We compared dioxin concentrations of umbilical cord venous blood with those of umbilical cord arterial blood obtained from 11 donors, 3 of whom had normal pregnancy deliveries and 8 of whom had fetal growth restriction (FGR) deliveries. No clear differences were observed between cord venous and arterial blood (Table 2).

Sets of total TEQ concentrations on a lipid weight basis in the placenta, maternal blood, and umbilical cord venous blood from 15 normal pregnancies were compared. As shown in Table 2, the total dioxin concentrations in maternal blood samples were 6.5-44 pg-TEQ/g lipid with a mean of 16 pg-TEQ/g lipid, while total dioxin concentrations were 1.9-18 pg-TEQ/g lipid with a mean of 5.8 pg-TEQ/g lipid in cord venous blood samples. In placental tissue samples, total dioxin concentrations were 5.2-36 pg-TEQ/g lipid with a mean of 13 pg-TEQ/g lipid. The mean total TEQ concentrations in cord venous blood were approximately 60% lower than those in maternal blood samples, and this tendency was in agreement with that reported in other studies[5), 6)].

As shown in Fig. 1, there was a positive linear relationship between total TEQ concentrations in placenta and those in maternal blood (r^2=0.821). The individual total TEQ concentrations in maternal blood were higher than those in placenta, excepting for four donors. In these four donors, small differences were observed in TEQ concentrations between the specimens. These results were similar to the results from the analysis of five sets of placenta and maternal blood collected in the United States, in which the dioxin concentrations in maternal blood were higher than those in placenta, excepting for one donor[6)]. In all 15 pregnant women, individual total TEQ concentrations in maternal blood were higher than those in cord venous blood. A positive linear relationship (r^2=0.905) was observed between the concentrations in maternal blood and those in cord venous blood (Fig. 1).

We found that the mean total TEQ concentrations for three kinds of specimens were on the order of maternal blood (1.0) > placenta (0.82) > cord blood (0.37); relative concentration ratios are shown in parentheses based on maternal blood. The downtrends in the lipid weight-based dioxin concentrations suggest that the placental tissue suppresses the transfer of dioxin molecules to cord blood and fetuses.

Table 1 Comparison of dioxin concentrations in cord venous blood with those of cord arterial blood obtained from 11 donors.

	Total dioxins (pg-TEQ/g lipid)										
	Normal			FGR							
	N17	N18	N19	F02	F03	F04	F05	F06	F07	F09	F10
Venous blood	3.3	8.5	5.4	4.9	5.9	7.3	5.3	10	2.7	4.9	9.6
Arterial blood	2.3	10	1.9	4.2	6.2	8.2	4.3	12	3.9	7.3	6.7

Table 2 Summary of 15 sets of maternal blood, cord venous blood and placental tissue analysis from normal pregnancies.

	Total dioxins (pg-TEQ/g lipid)																	
	N01	N02	N03	N04	N05	N06	N07	N08	N09	N10	N11	N12	N13	N14	N15	min.	max.	mean
Maternal blood	25	24	14	6.6	6.8	15	6.9	8.2	12	11	15	26	44	13	6.5	6.5	44	16 (100)
Placenta	14	21	12	7.1	6.6	18	5.4	5.2	10	7.3	10	15	36	17	9.1	5.2	36	13 (82)
Cord venous blood	7.1	7.4	4.2	3.2	3.3	5.0	3.1	1.9	5.6	4.7	6.7	11	18	3.5	3.4	1.9	18	5.8 (37)

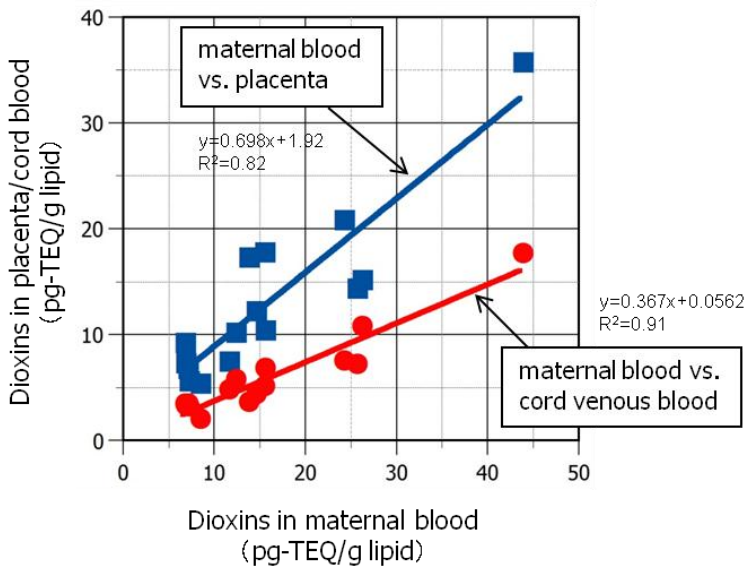

Fig.1 The relationship of total dioxins concentration between maternal blood and placenta, and also between maternal blood and cord venous blood.

Figure 2 summarizes the distribution of 1,2,3,7,8-pentaCDD, PCB169 and OctaCDD in three different specimens from 15 normal pregnancies. Generally, the concentrations of 1,2,3,7,8-pentaCDD in placenta were higher than those in both maternal and cord venous blood. These results suggest that the congener has a tendency to accumulate in placental tissue. On the other hand, the other two kinds of congeners have different distribution profiles, with the concentrations of each congener in placenta being lower than those in both maternal and cord venous blood. It is especially notable that OctaCDD concentrations in placenta were clearly lower than those in cord venous blood. These findings suggest that 1,2,3,7,8-pentaCDD, which has the highest TEF value among the three congeners, tends to accumulate in placental tissue, while the congeners with lower TEF values are easily transferred to the fetus. These results might be explained by each dioxin molecule's affinity to the Ah-receptor present in placental tissue.

Acknowledgements

This study was supported by the Environment Research and Technology Development Fund (ERTDF) of the Ministry of the Environment (MOE) of Japan (C-0903). This study was also supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor, and Welfare, Japan.

References:

1. Tsutsumi T, Yanagi T, Nakamura M, Kono Y, Uchibe H, Iida T, Hori T, Nakagawa R, Tobiishi K, Matsuda R, Sasaki K, Toyoda M. (2001) *Chemosphere* 45: 1129-1137
2. Ministry of the Environment, Japan. The Accumulation of Dioxins in the Japanese People (2008)
3. Takahashi K, Hori T, Yasutake D, Morokuma S, Otera Y, Fukushima K, Yamamoto T, Kajiwara J, Kawamoto T, Hirata T, Tsukimori K, Furue M. (2011) *Organohalogen Compounds* 73: 645-648
4. Hori T, Takahashi K, Yasutake D, Morokuma S, Otera Y, Todaka T, Yamamoto T, Hirakawa H, Kajiwara J, Kawamoto T, Hirata T, Tsukimori K. (2011) *Organohalogen Compounds* 73: 1600-1603
5. Nakamura T, Nakai K, Matsumura T, Suzuki S, Saito Y, Satoh H. (2008); *Sci Total Environ*. 394: 39-51
6. Schecter A, Kassis I, Päpke O. (1998); *Chemosphere*. 37: 1817-1823

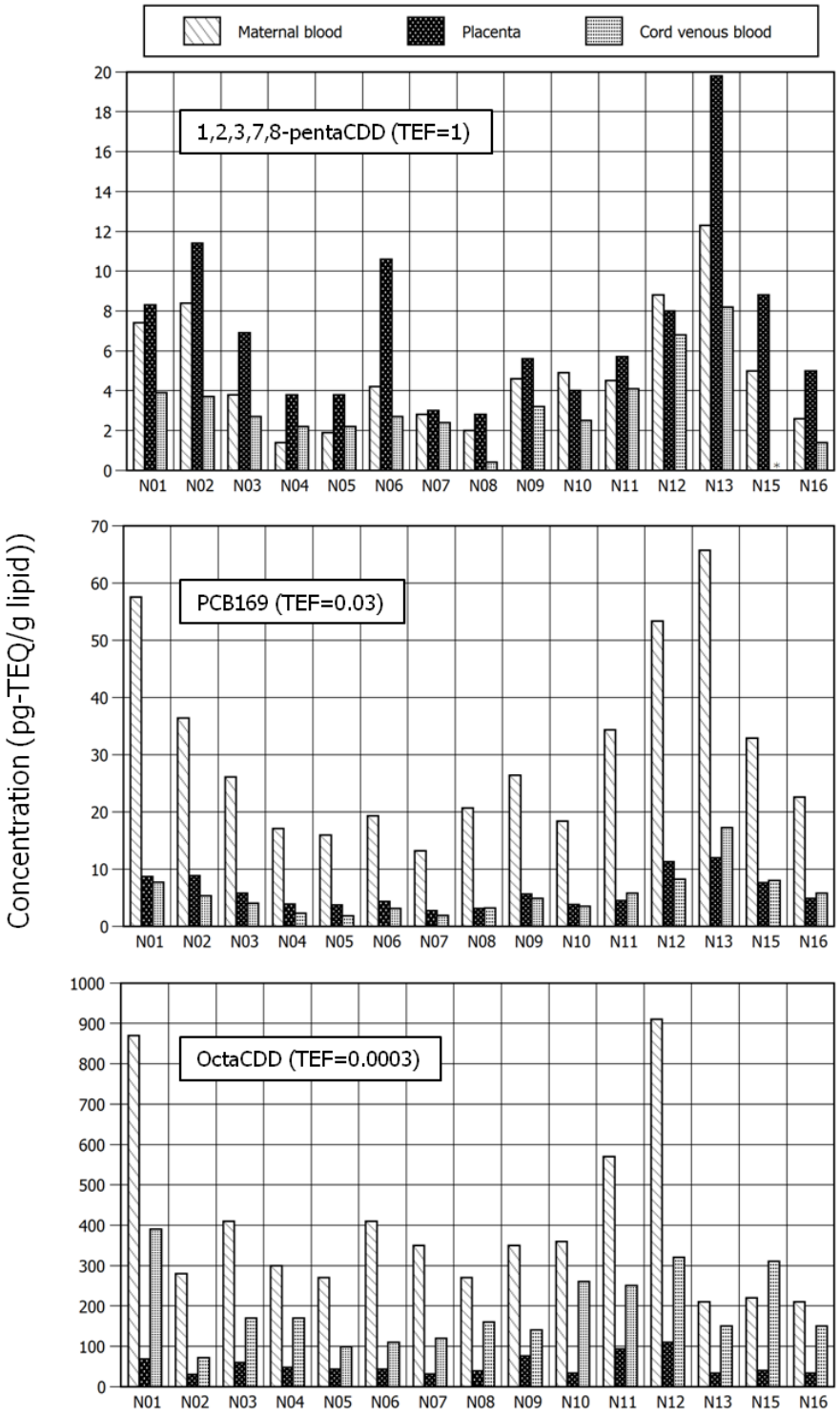

Fig. 2 Comparison of concentrations of 1,2,3,7,8-pentaCDD, PCB169, and OctaCDD in three different specimens from 15 normal pregnancies.