DIOXIN LEVELS IN HUMAN BILE, BLOOD AND LIVER: EFFECT OF AGE

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Introduction

The toxicity of dioxins is extremely high and, moreover, their half-life in humans is generally very long. We have studied daily intake from meals and their daily excretion in feces and sebum, and demonstrated that about 50% of daily intake in TEQ is excreted from feces and sebum at about 22% and 29%, respectively.[1)] Another study demonstrated that excretion in feces of most of the dioxins examined exceeded the daily intake[2)]. There is a possibility that dioxins are subjected to enterohepatic circulation, although there is no solid evidence yet indicating all dioxin congeners are subjected to enterohepatic circulation. It has been reported that 2,3,7,8-TCDD is absent in the bile of guinea pigs[3)] and excretion of 2,3,7,8-TCDD in feces is a result of direct excretion from the intestine. However, it is not known whether this is the cases for human or not.

We have studied dioxin levels in the bile, blood and liver of 27 autopsy cases (Kitamura et al., in preparation), and clarified that all 20 dioxin congeners, 7 PCDDs, 10 PCDFs and 3 Co-PCBs, are present in bile, and it was clarified that the total TEQ level in bile is almost the same as in blood, and it is three time lower than in the liver, in terms of per g lipid. However, the ratio of the level of each congener in bile to blood is not necessarily the same, ranging from 0.1 to 1.5.

In this study, we studied the effect of age on the levels of dioxins in bile, blood and liver and further examined the effect of age on the levels of 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF, 3,3',4,4',5-PeCB, 2,3,7,8-TCDD and 1,2,5,6,7,8-HxCDD, which contribute the most to the total, more than 80%, in all these three organs.

Materials and Methods

Chemicals

Authentic standards of native PCDDs, PCDFs and Co-PCBs, and $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$- Co-PCBs were purchased from Wellington Laboratories (Ontario, Canada). Active carbon-impregnated silica gel of dioxin-analysis grade was purchased from Wako Pure Chemicals (Osaka, Japan). All solvents used were of dioxin-analysis grade. Ultra-pure water was supplied from a Milli-Q SP TOC system from Japan Millipore (Tokyo, Japan).

Human sample

Twenty seven autopsy cases were provided. All cases were autopsied within 2 hours after death. About 50 ml, 50 ml and 5g of gallbladder bile, cardiac blood and liver, respectively, were

stored for measurement of dioxins levels in deep freeze until analysis. Permission for analyzing dioxins was obtained from the bereaved families.

Preparation of samples for analysis

The above samples, 40 g, 50 g and 1.5 g of bile, blood and liver, respectively, from each case were used. Samples were spiked with $^{13}C_{12}$-PCDDs, $^{13}C_{12}$-PCDFs and $^{13}C_{12}$-Co-PCBs, as internal standards. Bile was extracted with about 160 ml of acetone/hexane (2:1). Blood was extracted by the $(NH_4)_2SO_4$-ethanol/hexane method[4]. Liver was homogenized in a mortar in the presence of Na_2SO_4[5] and extracted with 50 ml acetone/hexane (2:1)[6]. These extracts were washed with ultra-pure water. The *n*-hexane layers were dried over anhydrous sodium sulfate, evaporated to dryness, and the lipid residues were weighed. The residues were dissolved into two ml of *n*-hexane and applied to a multi-layer column composed of 10% silver nitrate-silica gel, 22% sulfuric acid-silica gel, 44% sulfuric acid-silica gel, and 2% potassium hydroxide-silica gel [7]. 150 ml of *n*-hexane was passed through the column and the effluent was evaporated. The concentrate was applied to an active carbon-impregnated silica gel column[8], washed with 200ml of 25% (v/v) dichloromethane/ *n*-hexane, then eluted with 200 ml of toluene. The eluent, toluene was evaporated to 2 to 3 ml by a rotary evaporator and then spontaneously at room temperature to almost empty in a vessel. Five *u*l of *n*-nonane containing $^{13}C_{12}$-1,2,3,4-TCDD and $^{13}C_{12}$-1,2,3,7,8,9- HxCDD spiking substances were added to this vessel.

Analysis

A GC/MS, which consisted of a Finnigan MAT-95S mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) and a HP-6890A gas chromatograph (Hewllet-Packard, Palo Alto, California, U.S.A.) was used. The column used was a DB-5MS fused silica capillary column, 0.25 mm i.d. ×60m, with 0.25 *u*m film thickness (J&W Scientific, Folsom, California, U.S.A.). The column temperature was maintained at 140 ℃ for 1 min, heated to 220 ℃ at a rate of 17℃ /min, heated to 310℃ at a rate of 3℃/min, and maintained at 310℃ for 4 min. The injection temperature was 260℃, ion source temperature was maintained at 250℃, and the carrier gas (helium) rate was 1.2 ml/min. The ionizing current, ionizing energy and accelerating voltage were 1 mA, 60 eV and 5 kV, respectively. The resolution of the mass spectrometer was maintained at about 10,000 throughout the work, and analysis was carried out according to an SIM using 50 selected ions.

Results and Discussion

The ages of the 27 subjects studied ranged from 21 to 85, and the total TEQ of bile ranged from 1.6 to 104.8 pg TEQ /g lipid , with an average of 43.2±30.9. Total TEQ of blood ranged from 10.4 to 72.4 pg TEQ/g lipid, with an average of 43.1±24.2, and that of liver from 17.1 to 287.3 pg TEQ/g lipid, with an average of 127.8±57.4. The congener of the highest TEQ value was 3,3',4,4',5-PeCB in these three organs, followed by 1,2,3,7,8-PeCDD and then 2,3,4,7,8-PeCDF in the bile and blood, and 2,3,4,7,8-PeCDFand then 1,2,3,7,8-PeCDD in the liver. The contribution of these three congeners to the total TEQ was more than 70% in bile, blood and liver.

The relationship between age and total TEQ was examined in bile, blood and liver, by

regression analysis and it was demonstrated that there were positive correlations in all these three organs, with correlation coefficients of 0.520 ($p<0.01$), 0.473 ($p<0.05$) and 0.539 ($p<0.01$), respectively, as shown in Fig 1. Regression equations for bile, blood and liver were $y = 0.99x - 20.87$, $y = 0.70x - 2.37$ and $y = 1.91x + 4.36$, respectively, where y indicates total TEQ/g lipid and x age. The level of each of the major three congeners in bile, blood and liver also showed good correlation between age, except for 3,3',4,4',5-PeCB in blood and 1,2,3,7,8-PeCDD in liver. These results indicate that the accumulation rate of dioxins is about two times higher in the liver than in blood or bile.

Sielken[9] reported an increase of 2,3,7,8-TCDD levels with an increase of age in human adipose tissue and Schuhmacher *et al.* [10] an increase of total TEQ levels derived from PCDDs and PCDFs with an increase of age, in human serum. In our case, a significant increase of 2,3,7,8-TCDD levels with an increase of age was also observed in bile, blood and liver. This kind of information could be useful to analyze the harmful effects of dioxins and to devise ways to reduce body burden from toxic dioxins.

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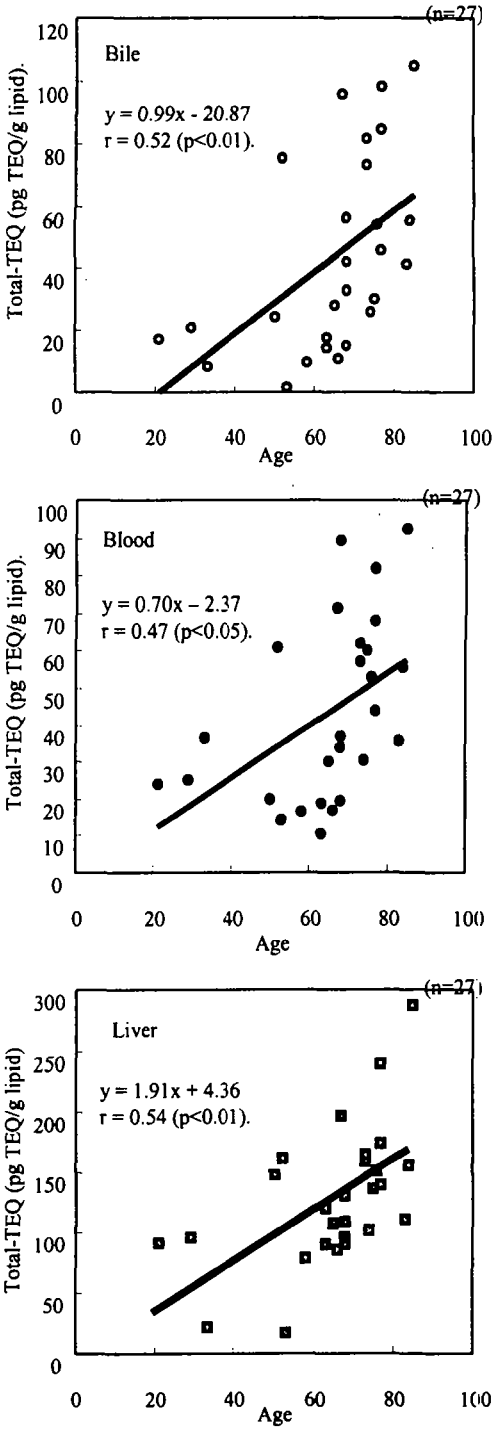

Fig.1. Relationship between age and total TEQ in bile, blood and liver