Comparison of dioxin concentrations in various human biological fluids

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Introduction

Dioxins have generated growing scientific concern and public debate over their potential adverse effects that may result from their exposure. Substantial evidence has been accumulated that point to their potential to alter the normal function of the endocrine system in wildlife and humans (1). Since studies that clearly address human exposure may be the most valuable means of assessing the impact of dioxins on human health, we measured dioxin levels in various human fluids employing the gas chromatography-mass spectometry analysis. Our results indicate that not only fetus but oocytes may be contaminated with dioxins and that large amounts of dioxins may be present in the hepato-intestinal circulation.

Materials and methods

Human fluids: Serum samples were collected from health pregnant women at term and from the cord of their new born babies at the time of c-sections. Ovarian follicular fluid was aspirated from follicles of 5 women during oocyte collection for in vitro fertilization procedure. Human bile fluid was obtained from healthy living-related liver transplantation (LRLT) donors (n=11). They was centrifuged at 2,000 g for 10 min and all the samples were stored at -20℃ until use. Informed consent was obtained from all subjects in the present studies and the studies were approved by the Research and Ethics Committee of theUniversity of Tokyo.

Determination of dioxins: About 50 g of wet sample was spiked 13C labeled standard mixture and digested in 2 mol/L-KOHaq (30 ml) at room temperature for one night. The sample, after addition of methylalcohol (20 ml) was shaken. Ten ml of hexane and the solution were put in a separatory funnel and s haken for 10 minutes. The solution was extracted 2 more times with hexane using 10 ml each time. The combined hexane extracts were rinsed with about 50 ml of water twice and dried by passing through a layer of anhydrous sodium sulfate in a glass funnel. The extract was concentrated up to about 50 ml on a rotary evaporator and cleaned up by participation with concentratec sulfuric acid for 3 to 5 times. The hexane layer was rinsed with water 3 times then dried by passing through anhydrous sodium sulfate in a glass funnel. The solution was concentrated and subjected to silica gel and activated carbon column cleanup. The sample was finally dissolved in 20 µl of toluene.The gas chromatography -mass spectometry analysis was performed on a JEOL JMS -700 high performance double focusing mass spectrometer. An aliquot (1 µl) of a sample was injected into a Hewlett Packard 6890 gas chromatograph equipped with a Spelco PTE-5 column (30 m x 0.25 mm i.d., film thickness 0.25 µm). The area of the mass profile peaks of the quantificatior ions was used for the quantitative analysis. Quantified values were calculated by the internal standard methods for PCDDs and PCDFs. They were presented as dioxin toxic equivalents (TEQ) and the data were given as mean±SD.

Results and discussion

We have shown that dioxins are present not only in human maternal serum but also cord serum showing dioxins pas through the placenta (Fig.1). There was positive correlation between maternal and fetal concentrations of dioxins. It was of interest to note t hat dioxin concentrations in both maternal and fetal serum were decreased as their parity (number of previous delivery of child) increased (data not shown). Dioxins were also detected in the ovarian follicular fluids suggesting t hat oocytes may be contaminated by dioxins. Contamination of dioxins in reproductive fluids indicates that more studies t hat clearly address exposure-outcome relationships may be the most valuable means of assessing the impact of dioxins on human health.

Here, we have for the first time determined the concentrations of dioxins in human bile fluid obtained from healthy living-related liver transplantation (LRLT) donors (Fig 1). The concentrations of dioxins (PCDDs + PCDFs) were significantly higher in the bile fluids obtained from the gallbladder (mean 0.370 ±0.071 pg dioxin toxic equivalents (TEQ)/ml) t han in maternal serum (0.084 ±0.014 pg TEQ/ml; $p<0.001$) (Fig. 1), showing t hat dioxins are excreted in the bile into the intestinal tract, and are then probably reabsorbed into the body through the liver.

Assuming a daily volume of bile of 1 liter, we estimate that approximately 0.4 ng of dioxins are circulating between the intestine and hepato-biliary tract each day in healthy humans. It has been reported that Ukranian president had a serum dioxin concentration of 100 ng/g-fat, a 6000-fold increase over normal background levels which suggest that up to 2.4 µg or 30 ng/kg/day of dioxins may be excreted and reabsorbed in the president, which may be deleterious for liver function. Notably, dioxin at a dose of 10ng/kg/day caused hepatocyte carcinoma in rats (3). Therefore, care should be taken because he may be at high risk of ongoing health problems, that would include cancer.

Among the dioxins there are 75 congeners of polychlorodibenzo-dioxins (PCDDs) and 135 congeners of polychlorodibenzo-furans (PCDFs), however, only 7 PCDDs and 10 PCDFs are generally found in humans. The concentrations of these 7 PCDD and 10 PCDF congeners were determined by gas chromatography and mass. They were presented as dioxin toxic equivalents (TEQ) and the data were given as mean±SD.

References

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Figure 1. Concentrations of dioxins in maternal and fetal serum and follicular fluids and bile.