COMPARISON OF CONCENTRATIONS OF PCDDs, PCDFs AND COPLANAR PCBs IN BREAST MILK OF YUSHO PATIENTS AND NORMAL CONTROLS

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ABSTRACT

To assess the potential effects of chlorinated dioxins and related compounds on breast-feeding babies of Yusho patients, we analyzed breast milk obtained from two Yusho patients and nine normal subjects for concentrations of PCDDs, PCDFs and coplanar PCBs using high-resolution gas chromatography/high resolution mass spectrometry. The concentrations of PCDDs, PCDFs and coplanar PCBs in breast milk of respective Yusho patients were 29.6 and 18.8, 417.3 and 168.2, and 55.1 and 23.8 pg Toxic Equivalents (TEQs)/g fat, respectively, and mean concentrations were 8.1, 4.8 and 21.8 pg TEQs/g fat, respectively, in normal controls. The results also indicated that there was a significant difference between Yusho patients and normal controls in breast milk concentrations of certain isomers of PCDDs, PCDFs and coplanar PCBs. Daily intakes of TCDD equivalent values were estimated to be 519 and 2216 pg TEQs/kg/day for breast-feeding babies of Yusho patients, and to be 170.2 pg TEQs/kg/day for normal subjects.

KEYWORDS: Polychlorinated dibenzo-p-dioxins; Polychlorinated dibenzofurans; Coplanar PCBs; Human breast milk; Yusho; Risk evaluation

INTRODUCTION

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar polychlorinated biphenyls (PCBs) have been detected in environmental and biological samples, including human adipose tissue[1], human breast milk[2, 3], fish, and marine and terrestrial mammalian wildlife. These organochlorinated compounds cause toxic symptoms, including a wasting syndrome, hepatic damage, reproductive toxicity, and immunotoxicity. According to a recent survey on the levels of 2,3,7,8-TCDD Toxic Equivalents (TEQs) in milk fat, a breast-feeding baby consumes between 50 and 200 pg TEQs per kg of body weight daily, which values several times more than the Acceptable Daily Intake (ADI)[4]. However, the World Health Organization and the UK Department of Health have both issued statements to the effect that there is no apparent cause for concern. Even though there is a great deal of evidence pointing to the extreme toxic potential of these compounds, very limited information on their concentrations in the breast milk of Yusho patients is available. In order to evaluate the health consequences of these chemicals for nursing infants, in this study we collected samples of breast milk from two Yusho patients who had ingested contaminated rice oil in 1968 as well as from normal controls, and analyzed

the concentrations of PCDDs, PCDFs and coplanar PCBs in detail.

EXPERIMENTAL

Human breast milk samples: Control milk samples (100 to 250 ml) were collected from 9 female volunteers who had all delivered in the same hospital between April, 1991 and June, 1991. Milk samples (50 to 100 ml) of two Yusho patients, who had ingested rice oil contaminated with PCBs, PCDFs and PCDDs in 1968, were collected consecutive 5 and 7 days after delivery in April 1989 and May 1990, respectively. The average age of controls was 30 years, and the Yusho patients were 31 and 32 years old, respectively.

Chemicals: All solvents were of pesticide reagent grade. ^{13}C-labelled 2,3,7,8-substituted PCDDs/PCDFs, 3,3',4,4'-TeCB, 3,3',4,4',5-PeCB and 3,3',4,4',5,5'-HxCB (Cambridge Isotope Laboratories, Inc., USA) were used.

Procedure: Breast milk samples (50 to 250 g) were fortified with ^{13}C-labeled PCDDs/PCDFs and three ^{13}C-labeled coplanar PCBs internal quantitation standards. The milk samples were mixed with sodium oxalate and ethanol, and then extracted with a mixed solvent of diethyl other and petroleum ether. The extracts were washed with disilled water, and then dried with anhydrous sodium sulfate. Each extract was then concentrated to constant weight, and the percent lipid was determined gravimetrically. The lipid residue was diluted in hexane and washed with concentrated sulfuric acid. The extract was purified on a $AgNO_3$-Silica gel column and charcoal column, then separated into coplanar PCBs and PCDDs/PCDFs on Florisil column chromatography. The details of this procedure have been reported elsewhere[5].

Gas chromatography/mass spectrometry (GC/MS): The PCDDs/PCDFs and coplanar PCBs were analyzed by GC/MS using a Finnigan MAT-90 mass spectrometer (Finnigan MAT, Bremen, Germany) directly interfaced with a Varian Model 3400 gas chromatogrph. The gas chromatograph was equipped with a splitless injector and a capillary column bonded with 50 % methyl phenylsilicon, OV-17 (0.25mm × 25m; film thickness, 0.1 μm) for coplanar PCBs analysis. The gas chromatograph column temperature was maintained at 120 ℃ for 1 min, then programmed to 170 ℃ at the rate of 10 ℃/min, and followed by programmed to 260 ℃ at the rate of 20℃/min. For the analysis of PCDDs/PCDFs, SP-2331 capillary column was used. The mass resolution (5 % valley) was 7000 to 10000. Two ions of molecular cluster were recorded.

RESULTS AND DISCUSSION

Table 1 shows the concentrations of PCDDs, PCDFs and coplanar PCBs detected in the human breast milk samples on a lipid basis. The TEQs of PCDDs and PCDFs were calculated based on the Toxic Equivalency Factors (TEFs) proposed by the NATO Committee on Challenge to Modern Society, and those of the coplanar PCBs were calculated using the data reported by Safe (1990)[6]. The concentration of PCDDs in the breast milk obtained from the respective Yusho patients was 404 and 444.6 pg/g fat and the mean concentration in the normal controls was 222.3 pg/g. The concentration of all PCDDs except 1,2,3,6,7,8-HxCDD in the Yusho patients was somewhat higher than that in normal controls, 1,2,3,6,7,8-HxCDD concentration was 3.9 and 7.8 times higher than the mean concentration in the normal controls. The concentration of PCDFs in the breast milk of the respective Yusho patients was 448.7 and 1223 pg/g, and the mean concentration in the normal subjects was 20.7 pg/g. The total concentration of PCDFs in the breast milk of Yusho patients was 21.6 and 59.1 times higher than the mean concentration in the normal controls, in particular the concentrations of 2,3,4,7,8-PeCDF, 1,2,3,4,7,8-HxCDF, and 1,2,3,6,7,8-HxCDF, so-called 'Yusho isomers' were much higher than those in the normal controls. The concentra-

Table 1 Leveles of PCDDs, PCDFs and coplanar PCBs in human milk as pg/g fat

Samples	Yusho patient-1 (n=5)	Yusho patient-2 (n=7)	Normal subjects (n=9)
2,3,7,8-TCDD	2.0	1.7	1.4
1,2,3,7,8-PeCDD	15.1	11.3	5.7
1,2,3,4,7,8-HxCDD	3.6	2.4	4.4
1,2,3,6,7,8-HxCDD	189.5	95.4	24.2
1,2,3,7,8,9-HxCDD	4.2	10.1	4.9
1,2,3,4,6,7,8-HpCDD	17.8	32.1	36.4
OCDD	171.8	293.6	145.3
Total PCDDs	404.0	446.6	222.3
2,3,7,8-TCDF	7.0	5.4	2.1
1,2,3,7,8-PeCDF	<1.0	<1.0	<1.0
2,3,4,7,8-PeCDF	745.9	309.6	8.5
1,2,3,4,7,8-HxCDF	322.7	94.9	3.3
1,2,3,6,7,8-HxCDF	117.6	38.4	2.3
1,2,3,4,6,7,8-HpCDF	29.1	5.8	1.7
1,2,3,4,7,8,9-HpCDF	<1.0	<1.0	<1.0
OCDF	1.1	<5.0	2.8
Total PCDFs	1223.4	448.7	20.7
3,3',4,4'-TeCB	11.5	13.1	12.4
3,3',4,4',5-PeCB	160.1	111.6	183.4
3,3',4,4',5,5'-HxCB	778.8	249.7	65.7
Total coplanar PCBs	950.4	374.4	261.5
Total PCDDs (TEQs)	29.6 (5.9%)	18.8 (8.9%)	8.1 (23.4%)
Total PCDFs (TEQs)	417.3 (83.1%)	168.2 (79.8%)	4.8 (13.8%)
Total coplanar PCBs (TEQs)	55.1 (11.0%)	23.8 (11.3%)	21.8 (62.8%)
Total TEQs	502.0	210.8	34.6
Lipid content (%)	3.68	2.05	4.10

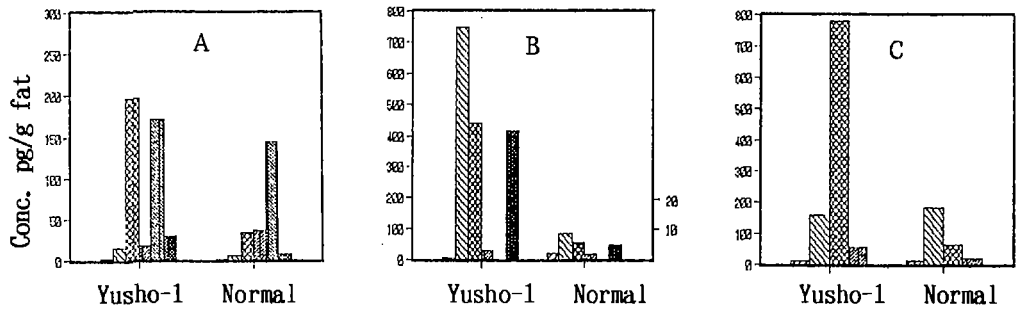

Fig.1 Congener pattern of PCDDs, PCDFs and coplanar PCBs in human breast milk of Yusho patient and normal control

A ▨:TeCDD ▧:PeCDD ▩:HxCDD ▩:HpCDD ▧:OCDD ■:Total-TEQs
B ▨:TeCDF ▧:PeCDF ▩:HxCDF ▩:HpCDF ▧:OCDF ■:Total-TEQs
C ▨:3,3',4,4'-TeCB ▧: 3,3',4,4',5-PeCB ▩:3,3',4,4',5,5'-HxCB ▨:Total-TEQs

tions of 3,3',4,4'-TeCB and 3,3',4,4',5-PeCB in the breast milk obtained from the Yusho patients were almost the same as those from the normal subjects. However, the respective concentrations of 3,3',4,4',5,5'-HxCB were 3.8 and 11.9 times higher than the mean concentration in the normal controls. The concentrations in terms of TEQs of PCDDs, PCDFs, and coplanar PCBs in the breast milk of the respective Yusho patients were 29.6 and 18.8, 417.3 and 168.2, 55.1 and 23.8 pg/g, respectively, while mean concentrations were 8.1, 4.8, and 21.3 pg/g, respectively, in that of the normal controls. The concentration of PCDDs and coplanar PCBs in the milk of the respective Yusho patients was 2.3 and 3.6 times higher than that of the normal controls, and the PCDFs concentration was 35 and 87 times higher than that of the normal controls. The total TEQs level of the respective Yusho patients was 6 and 14.5 times higher than that of the normal controls. Fig.1 shows congener pattern of PCDDs, PCDFs and coplanar PCBs. The results indicate that there is a marked difference between the Yusho patients and the normal subjects in breast milk concentrations of certain isomers of PCDDs, PCDFs and coplanar PCBs. In the breast milk of Yusho patient, the toxic contribution of PCDFs (87.1 and 89.9 %, respectively) was higher than that of PCDDs or coplanar PCBs . However, the estimated toxic contribution of coplanar PCBs (63%) to nursing infant ingestion was relatively greater than that estimated for infants of normal controls. Masuda[7] have estimated that the smallest TCDD equivalent ingestion level which elicits Yusho-like symptoms is 30 ng/kg/day. A baby nursing from a Yusho patient, is estimated to ingest more than 2.2 pg/kg /day in terms of TEQs via the breast milk. This level is about tenfold less than that of which elicits Yusho-like symptoms, but 2000 times more than the ADI of 1 ng/kg /day proposed by ATSDR[4]. On the other hand, babies who are nursing from normal mothers ingest still 170 pg/kg/day, which is 170 times more than the ADI . In conclusion, PCDFs , PCDFs and coplanar PCBs are excreted in the breast milk of Yusho mothers at much higher concentrations than in normal mothers, and nursing Yusho patients should be cautioned regarding the possible health effects on nursing of higher concentrations.

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