DISTRIBUTION OF DIOXIN CONGENERS IN VARIOUS COMPONENTS OF HUMAN BLOOD

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Introduction

In our previous studies, we developed a measurement method at ultra-trace quantitative determinations for polychlorodibenzo-p-dioxins (PCDDs),polychlorodibenzofurans (PCDFs) and coplanar polychlorobiphenyls (Co-PCBs) in several human samples (blood, serum, plasma, red blood cell and breast milk).[1] By using this method, we canexactly evaluate human exposure of dioxins. It is important to elucidate the circulationand elimination pathway of dioxins in the human body. It was reported that the distribution of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the plasma and lipoproteins of human and various animals, the consideration of binding abilities between TDCC and lipoproteins.[2-4]

In this report, wedescribe the concentrationsand distribution of PCDDs, PCDFs and Co-PCBs in whole blood, plasma andred blood cell (RBC) fraction in healthy male volunteers. Furthermore, we determined the concentration of dioxins in different plasma lipoproteins, such as very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) andhigh-density lipoproteins (LDL) from the same subject. The relationship between the differentcomponent and the concentration of dioxin congeners was examined.

Methods

Blood samples were obtained from fourhealthy male volunteers. Their ages were32, 35, 45, 47 years old, respectively. Lipoproteins were separated from fresh plasma to VLDL ($d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml) and HDL ($1.063 < d < 1.21$ g/ml) by ultracentrifugation .[5] Finally Lipoprotein free plasma were obtained.

Cholesterol (Cho).free cholesterol (FC). triglyceride (TG) and phospholipid (PL) levels were separately measured. Plasma lipid levels were spectro-photometrically measured by the diagnostic kits using the enzymatic method (Kyowa Medix Co., Tokyo). Metabolic pathway of the plasma lipoproteins in the human body was shown in Fig.1.

All samples were storedat -20°C until analysis. Fifteen kinds of ^{13}C-labeled PCDDs/PCDFs and eight kinds of ^{13}C-labeled Co-PCBs were added to all samples as internal standards for checking recoveries of PCDDs/PCDFs and Co-PCBs throughout the analytical procedures.

PCDDs/PCDFs and Co-PCBs were analyzed by the HRGC/HRMS technique using a Micromass Autospec-Ultima mass spectrometer (Micromass,UK) directly interfaced with a Hewlett Packard 6890 Series gas chromatograph.

The target compounds of PCDDs/PCDFs and Co-PCBs were measured with BPX-5 capillary column (I.D.: 0.22 mm; length:50m; film thickness: 0.25micrometer) and HT-8 capillary column (I.D.: 0.22 mm; length: 50m; film thickness: 0.25 micrometer), respectively. The mass resolution (5 % valley) was about 10000, respectively. Details of the sample treatment (extraction and purification) procedures are shown in Fig.2.

Results

Table 1 shows determined each plasma lipid level in lipoprotein fractions, whole blood and RBC fractions.

Plasma lipids levels (Table 1)

In all subjects,lipid concentrations in the lipoprotein fractions showed the same pattern. That is, VLDL is TG-rich fraction, LDL is cholesterol-rich fraction and HDL is PL-rich fraction. Lipoprotein fractionation yielded high recovery rate,because the total lipid concentrations of each lipoprotein fractions (in VLDV, LDL and HDL) were equal to lipid concentration in the plasma.

Lipid levels using liquid-liquid extraction from whole blood, plasma and RBC fraction in the Table.

Dioxin levels at several matrixes (Table 2)

The concentration of dioxins in the whole blood, plasma and RBC fraction was determined on the same subjects. The result of statistical analysis of several concentrations from four subjects is shown in table. Dioxins concentrations in plasma is higher than that in whole blood and RBC fraction. This phenomenon is common in all subjects. Small quantity of lipids is extracted from RBC fraction. Dioxins values per lipid levels from plasma and RBC fraction were different from those extracted from whole blood,because the extraction efficiency for lipids was different at individual material.

Conclusion

There were great differences in the dioxin congener concentrations that rectified by pe extracted lipid weight among those from whole blood, plasma and RBC fraction. The concentration of dioxins (Mean of Total values of PCDDs, PCDFs and Co-PCBs) in plasma were more than twice of those in the whole blood. In other words, almost all dioxins in blood are solved into plasma. It was based on the amount of lipids extracted by the organic solvents. On the other hand, dioxins level in RBC fraction is quite few. Because the organic solvents extraction method had the possibility to extract other material

We investigated the levels of PCDDs, PCDFs and Co-PCBs in the several lipoprotein fractions, and the correlation factor was calculated from the findings. The concentration of PCDDs and PCDFs (PCDDs/DFs) congeners had relatively good correlation to lipid levels of TG ($r>0.824$). But, in Cholesterol, all congeners had good negative correlation to lipid levels. Therefore, we suggest that the congeners of PCDDs/DFs may be behaved with TG in circulation pathway of dioxins within the human body.

The dioxins congener concentrations in each plasma lipoprotein fractions shall be shown.

Reference

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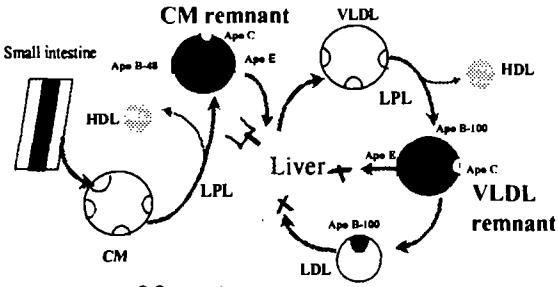
Fig. 1 Metabolic Pathway of Plasma Lipoproteins



CM @:@Chylomicron
VLDL :@very Low-density Lipoproteins
LDL @:@Low-density Lipoproteins
HDL @:@High-density Lipoproteins
LPL @:@Lipoprotein Lipase

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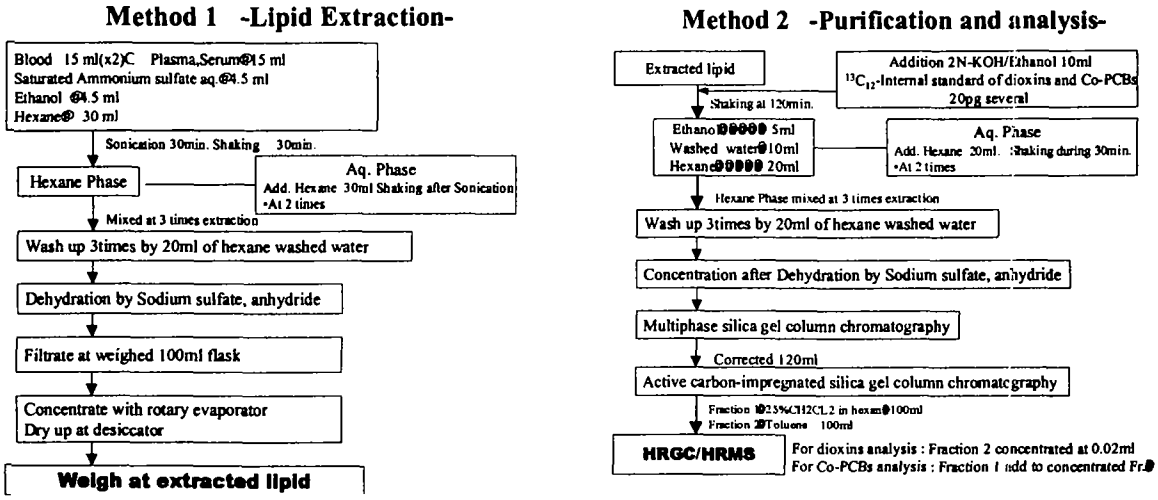

Fig. 2. Procedure of Lipids Extraction and Purification

Table 2. Distribution of Dioxins Congeners in Four Subjects

	Congener	Concentration in Whole Blood, Plasma and Red Blood Cell (pg/g-lipid)								
		*Blood (127.3mg+/-125.8)			*Plasma (51.3mg+/-7.0)			*RBC (31.9mg+/-6.6)		
		Mean	Median	S.D.	Mean	Median	S.D.	Mean	Median	S.D.
PCDFs	2,3,7,8-TCDF	1.6	1.1	1.5	2.6	2.4	2.0	0.6	0.5	0.3
	1,2,3,7,8-PeCDF	1.0	0.8	0.7	2.1	1.8	1.5	1.2	0.7	1.1
	2,3,4,7,8-PeCDF	9.5	10.3	3.6	22.8	26.1	7.7	3.7	2.9	3.6
	1,2,3,4,7,8-HxCDF	3.0	3.3	1.2	8.1	8.6	3.6	1.7	1.7	1.4
	1,2,3,6,7,8-HxCDF	5.1	5.3	1.2	10.3	11.3	4.3	1.9	1.9	1.7
	2,3,4,6,7,8-HxCDF	2.7	2.5	1.2	5.6	5.4	3.3	0.7	0.5	0.3
	1,2,3,7,8,9-HxCDF	0.5	0.5	0.0	5.0	5.0	0.0	0.5	0.5	0.0
	1,2,3,4,6,7,8-HpCDF	5.0	5.0	1.6	8.2	8.2	4.0	3.9	2.1	5.0
	1,2,3,4,7,8,9-HpCDF	0.5	0.5	0.0	0.5	0.5	0.0	0.5	0.5	0.0
	OCDF	0.8	0.5	0.5	0.5	0.5	0.0	0.5	0.5	0.0
PCDDs	2,3,7,8-TCDD	1.6	1.6	0.3	2.6	2.6	1.0	3.7	4.1	1.5
	1,2,3,7,8-PeCDD	3.9	3.5	1.8	12.4	13.7	4.6	3.0	2.9	2.9
	1,2,3,4,7,8-HxCDD	2.2	2.3	1.3	5.4	5.4	0.6	1.2	0.5	1.4
	1,2,3,6,7,8-HxCDD	32.3	35.4	12.4	74.4	68.4	29.0	18.3	17.5	15.8
	1,2,3,7,8,9-HxCDD	3.6	2.8	3.8	12.2	10.1	6.0	3.0	2.0	3.3
	1,2,3,4,6,7,8-HpCDD	21.6	23.2	6.7	41.8	48.9	22.8	5.3	3.1	6.6
	OCDD	153.0	151.1	36.8	308.6	343.5	104.0	98.8	85.3	38.1
Co-PCBs	3,4,4',5-TeCB (#81)	21.0	21.6	14.5	52.5	55.3	30.8	12.8	10.3	11.2
	3,3',4,4'-TeCB (#77)	46.8	46.0	14.6	131.9	110.3	85.6	39.7	36.0	17.7
	2,3,4,4',5-PeCB (#123)	226.5	269.8	132.2	412.4	478.6	249.8	89.1	85.0	64.3
	2,3,4,4',5-PeCB (#118)	12298.6	14773.9	7094.0	23984.1	27769.8	14730.4	4379.9	4173.9	3048.5
	2,3,4,4',5-PeCB (#114)	738.5	877.3	328.7	1435.4	1517.1	804.9	340.3	294.1	225.1
	2,3,3',4,4'-PeCB (#105)	2274.7	2840.2	1218.2	4851.2	5686.3	2810.8	1137.7	1153.2	701.9
	3,3',4,4',5-PeCB (#126)	58.8	70.0	33.3	133.2	159.4	67.5	13.5	9.8	12.9
	2,3,4,4',5,5'-HxCB (#167)	2590.8	2952.5	1608.4	4667.1	4286.8	3468.8	696.1	541.8	580.3
	2,3,3',4,4',5-HxCB (#156)	4983.9	4915.7	2661.0	11831.1	13134.5	5107.6	6241.8	3068.1	7690.9
	2,3,3',4,4',5-HxCB (#157)	1421.9	1593.2	548.1	2649.5	2672.4	1251.1	531.0	470.2	270.3
	3,3',4,4',5,5'-HxCB (#169)	61.2	67.5	32.3	265.4	156.9	294.3	21.4	3.8	37.8
	2,3,3',4,4'5,5'-HpCB (#189)	582.1	615.5	277.9	1136.9	1133.1	534.9	208.8	153.2	144.8
Total-PCDFs		28.2	30.2	7.8	60.0	67.9	24.2	12.0	10.7	7.3
Total-PCDDs		217.7	231.6	38.4	457.3	490.9	147.0	129.6	131.5	42.1
Total Co-PCBs		25304.8	28986.6	13347.4	51550.7	58206.7	28651.0	13712.1	14011.2	9093.6

*: Weight of extracted lipid from 30ml whole blood in parenthesis (Mean+/-S.D.).
Harf value of detectin limits (1pg/g-lipid) was substituted ND value.

Table 1. Plasma Lipid Levels at Lipoprotein Fractions and Extracted Lipid Weight on Individual Subjects (mg/ml)

Subject A	Cho	TG	FC	PL	Total lipid	Extracted Weight(mg)
VLDL	0.233	0.630	0.094	0.297	1.254	***
LDL	0.725	0.169	0.209	0.518	1.621	***
HDL	0.592	0.116	0.116	1.145	1.969	***
Plasma	1.566	0.765	0.379	2.065	4.775	57.2
Whole Blood						142.0
Red Blood Cell Fraction						38.2

Subject B	Cho	TG	FC	PL	Total lipid	Extracted Weight(mg)
VLDL	0.056	0.349	0.030	0.123	0.558	***
LDL	1.951	0.210	0.552	1.229	3.942	***
HDL	0.531	0.032	0.086	0.846	1.495	***
Plasma	2.536	0.661	0.691	2.299	6.187	53.9
Whole Blood						108.2
Red Blood Cell Fraction						35.1

Subject C	Cho	TG	FC	PL	Total lipid	Extracted Weight(mg)
VLDL	0.321	1.663	0.146	0.539	2.669	***
LDL	1.402	0.244	0.323	0.913	2.882	***
HDL	0.453	0.102	0.068	0.804	1.427	***
Plasma	2.110	1.657	0.432	2.354	6.553	53.6
Whole Blood						149.5
Red Blood Cell Fraction						31.7

Subject D	Cho	TG	FC	PL	Total lipid	Extracted Weight(mg)
VLDL	0.242	1.414	0.107	0.431	2.194	***
LDL	1.467	0.186	0.359	0.917	2.929	***
HDL	0.422	0.087	0.067	0.738	1.314	***
Plasma	2.200	1.799	0.593	2.259	6.851	41.1
Whole Blood						109.6
Red Blood Cell Fraction						22.8

Extracted weight: weight of extracted lipid by liquid-liquid extraction from 30ml whole blood.