THE KINETICS OF DIOXIN CONGENERS IN HUMAN BODY

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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) as dioxins in human blood sample.[1)] The human blood is used as a typical sample for extensive investigations into human exposure to dioxins. 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, according to the consideration of binding abilities between TCDD and lipoproteins.[2-4)]

In this report, we describe the kinetics of PCDDs, PCDFs and Co-PCBs in whole blood in healthy volunteers after eating the meal that specified the contents. Furthermore, we report the kinetics and the correlation of concentration between dioxins and these lipoproteins, such as High Density Lipoprotein-Cholesterol (HDL-Cho), Low Density Lipoprotein-Cholesterol (HDL-Cho), Total-Cholesterol (T-Cho), Free-Cholesterol (F-Cho), and the correlation between dioxins and these plasma lipids, such as Total Lipid (TL), Triglyceride (TG) and Phospholipid (PL) levels and Extracted Lipid (EL) from whole blood by using organic solvents at procedure of dioxin analysis.

Methods

Blood samples were obtained from healthy six male volunteers and six female volunteers before and after eating the meal that specified the contents. Used specified meal was containing 100ml of milk, 50ml of fresh cream, 8g of butter and few cookies. Their ages were distributed from 25 to 39 years old. Blood collecting was performed at four times, at initial as before meal ingestion, after 30 min., 90 min., 180 min. and 300 min. Plasma sample was separated at quickly after blood collecting from whole blood. Collected whole blood and separated plasma samples were stored at -20ºC until analysis.

Plasma Lipid Analysis

High Density Lipoprotein-Cholesterol (HDL-Cho), Low Density Lipoprotein-Cholesterol (LDL-Cho), Total-Cholesterol (T-Cho), Free-Cholesterol (F-Cho), Total Lipid (TL), 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), respectively.

Dioxins Analysis in Whole blood

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.

All samples were stored at -20 ºC until analysis. The Extracted Lipids (EL) was extracted from 30ml of whole blood sample with 70ml of hexane, 9ml of ethanol and addition of 9ml of saturated ammonium sulfate. The EL level as weight was measured after dried up at over night. The EL was saponificated by mechanical shaking at room temperature for 2 hours with 2N KOH solution. The solution was extracted 2 times with 30mL of hexane. The hexane phase concentrated to 2mL was passed through a multi-layered silica gel column. The eluted solution was concentrated and loaded to an active carbon-dispersed silica gel column (0.5 g), and separated into two fractions. The first fraction with elution of 50 ml of hexane was discarded. The second fraction containing mono-ortho Co-PCBs were eluted with 40 mL of hexane containing 25 % CH_2Cl_2. PCDDs, PCDFs and none-ortho Co-PCBs were eluted with 200 mL of toluene as the third fraction.

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: 0.25 micrometer) and HT-8 capillary column (I.D.: 0.22 mm; length: 50 m; film thickness: 0.25 micrometer), respectively. The mass resolution (5 % valley) was about 10000, respectively.

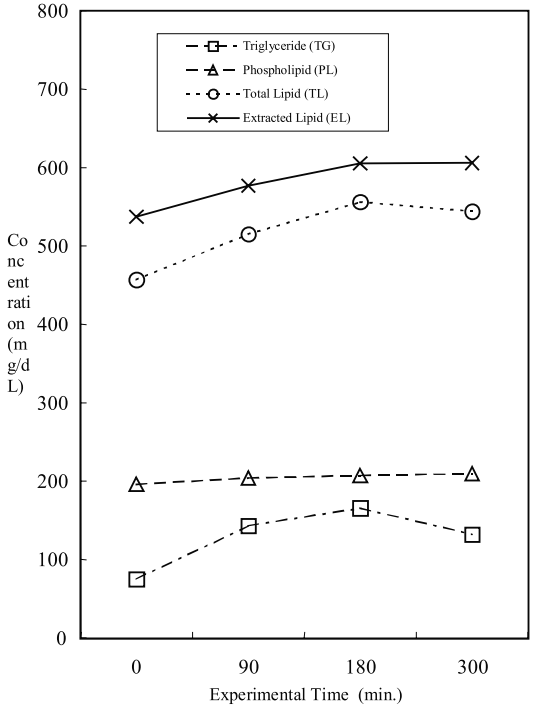

Fig.1. Time course change of average lipid level in the òÿÐÐú

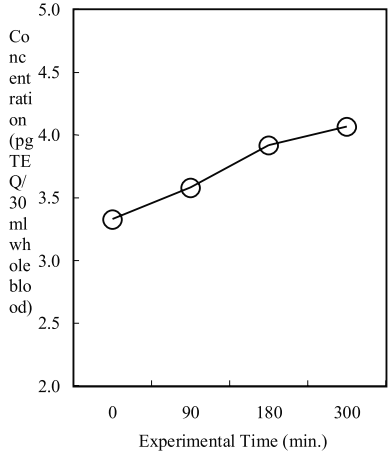

Fig.2'
Time course change of the îÆÍ ÿÛýÛÿ in the whole blood òÒÕÛ

Results

The Kinetics of Plasma lipids (Table 1)

The table 1 shown, the typical data of the kinetics as the time course of each plasma lipids and plasma lipoproteins. On the time course of plasma lipoproteins, the sum of total levels of LDL-, HDL- and Free-Cho was almost the same level as determined Total-Cho. The concentration of plasma lipoproteins showed the same level at all and their composition did not change through this experiment time. Therefore, the kinetics cannot be presumed.

While, on the time course of plasma lipids, the sum of levels of TG and PL was about 60% of TL level at several mean concentrations. The concentration of TG and TL changed at time depending. However, as well as the case of lipoprotein, the concentration of PL was almost constant through the experimental time. The time course as kinetics of plasma lipid levels with the mean value was shown in Fig.1

The Kinetics of Dioxins (Table 2)

Table 2. Shows the TEQ concentrations of PCDDs, PCDFs and Co-PCBs in the blood as the lipid base and the whole base. As shown in this table, the total TEQ level of dioxin analogues was almost

Table 1. The time course change of plasma lipids and lipoproteins

Plasama lipids and lipoproteins		Mean (mg/dL) ± S.D.			(n = 10, 12)
		0 min.	90 min.	180 min.	300 min.
Lipoprotein					
LDL-Cho[1]		111 ± 26	108 ± 26	105 ± 25	107 ± 23
HDL-Cho		61 ± 11	58 ± 10	56 ± 10	57 ± 9
Free-Cho		49 ± 5	49 ± 7	49 ± 5	50 ± 5
Total-Cho		188 ± 23	188 ± 29	185 ± 21	185 ± 20
Lipid					
Triglyceride	(TG)	75 ± 38	143 ± 54	166 ± 44	132 ± 36
Phospholipid	(PL)	196 ± 22	204 ± 26	207 ± 16	210 ± 19
Total Lipid	(TL)	457 ± 56	515 ± 72	556 ± 54	544 ± 58
Extracted Lipid	(EL)	537 ± 55	577 ± 76	605 ± 66	606 ± 83

1) These values were calculated from number of 10.

Table 2. The time course change of TEQ levels of dioxin analogues in the blood

Dioxin analogues	Mean ± S.D.			(n = 6)
	0 min.	90 min.	180 min.	300 min.
lipid base TEQ (pg TEQ/g-lipid)				
PCDDs	8.54 ± 1.83	8.57 ± 2.03	8.47 ± 1.77	8.64 ± 1.63
PCDFs	4.52 ± 1.54	4.25 ± 1.00	4.48 ± 1.53	4.58 ± 1.48
Co-PCBs	7.80 ± 2.59	7.80 ± 2.40	8.35 ± 2.65	8.45 ± 2.63
Total	20.85 ± 4.99	20.62 ± 3.99	21.30 ± 4.20	21.68 ± 4.65
whole blood baseTEQ (pgTEQ/30ml whole blood)				
PCDDs	1.35 ± 0.33	1.48 ± 0.48	1.55 ± 0.42	1.61 ± 0.44
PCDFs	0.71 ± 0.24	0.72 ± 0.19	0.81 ± 0.27	0.85 ± 0.30
Co-PCBs	1.26 ± 0.55	1.38 ± 0.66	1.55 ± 0.64	1.60 ± 0.68
Total	3.33 ± 1.01	3.58 ± 1.20	3.92 ± 1.11	4.07 ± 1.29

stable in the lipid base through the whole experimental time, but increased in the whole base with passing experimental time. The results of the time course as kinetics for dioxins in blood were shown in Fig.2. In this figure, the average of whole based TEQ level at experimental time was shown.

Conclusion

Our aim in this experiment was to reveal a correlation between the time alternation on the blood levels of lipids and dioxin analogues in the process of passing time after eating specified meal. Consequently, it was revealed that the total TEQ of dioxin analogues in the lipid base was almost constant through the whole experimental time of 300min. after meal eating. The similar kinetics was also seen in the cases of all plasma lipoproteins. These phenomena, however, was surmised to have no relationship with eating the meal, because we had already confirmed that the concentrations of dioxin congeners had a good correlation ($r>0.824$) with TG in the lipid base, suggesting the dioxin congeners to have the same behavior to TG in the circulation pathway of human body. [6)]

On the other hand, there was observed the time alteration of concentrations as kinetics on some plasma lipids and the whole base dioxin analogues. The whole base TEQ level increased with an increase of experimental time. Some lipids such as TG, TL and EL showed the similar kinetics. Therefore, from these results, it seems that dioxin analogues may behave with some lipids such as TG, TL and EL at a limited time depending in the circulation pathway of the human body. However, there was a discrepancy in the time alterations of the total TEQ level and the TG level. Therefore, we consider that the kinetics of dioxin analogues might closely related to lipids, especially TG level, but that the detail mechanism is ambiguous. Now, we try to solve the problem.

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