TISSUE DISTRIBUTION OF 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN PREGNANT RHESUS MONKEYS AND THEIR FETUSES FOLLOWING A SUBCUTANEOUS SINGLE INJECTION

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Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of dioxins which are environmentally and biologically stable. Reports concerning highly exposed human populations indicate that dioxins cause immunological dysfunctions, carcinogenesis, and developmental and reproductive dysfunctions. Assessment of TCDD exposure on human health including reproductive functions requires more information concerning the distribution of TCDD in target organs, and toxicity of TCDD in non-human primates exposed to TCDD. Although a lot of information is available from studies in rodents [1-4], not much is known of the pharmocokinetics of TCDD in non-human primates[5]. Considering the pronounced species differences observed in some studies on TCDD, pharmacokinetic studies using primates are needed for assessment of TCDD exposure on human health. In the present study TCDD was subcutaneously administered to female rhesus monkeys on Day 140 of gestation. Distribution of TCDD in tissues of mothers, and the transfer of TCDD to fetal tissues via the placenta were investigated by measurement of TCDD concentrations in different tissues of mothers and fetuses.

Materials and Methods

Chemicals. ^{3}H-2,3,7,8-TCDD (3.84 GBq/mg) dissolved in toluene and DMSO (1:2, v/v) was purchased from Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan).

Animals. Rhesus monkeys were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China), The monkeys (5-7 years old and 5.3-6.7 kg in body

weight) were kept in Shin Nippon Biomedical Laboratories, Ltd (Kagoshima, Japan). The breeding conditions, mating and diagnosis of pregnancy were described previously[6]. ^{3}H-2,3,7,8-TCDD (30 or 300 ng/kg body weight) was subcutaneously administered to two groups of two pregnant monkeys on day 140 of gestation. After 7 days the radioactivity in the blood and tissues was counted.

Measurement of TCDD in blood and tissues. The concentration of TCDD in the blood, plasma, mammary gland, placenta, liver, and fetal ovary or testis was measured. Two hundred ul of plasma samples were used for analysis. Tissues obtained from mother and fetus were homogenized and solubilized. After the addition of 10 ml of scintillation cocktail, samples were analyzed by scintillation counting. The experiments in this study were performed using ^{3}H-2,3,7,8-TCDD. Because TCDD is metabolically stable, the radioactivity measured represented the unchanged substance.

Results and Discussion

The results from this study are shown in Tables 1 and 2. The concentrations of TCDD are expressed as pg eq/ml or g, and tissue/plasma ratio (T/P ratio). Table 1 summarizes the concentrations of TCDD in the blood and plasma of mothers and fetuses 7 days after subcutaneous administration of ^{3}H-2,3,7,8-TCDD (30 ng/kg or 300 ng/kg body weight) to female rhesus monkeys on Day 140 of gestation. In group 1 (1G) administered 30 ng/kg TCDD, the highest and lowest concentrations were observed in fetal blood, and cord plasma, respectively. In group 2 (2G) administered 300 ng/kg TCDD, the highest concentrations were observed in mother and fetal blood. The highest T/P ratios in groups 1 and 2 were observed in fetal blood and mother blood, respectively. The ratios of radioactivity in the plasma of mothers and fetuses were 147.9 and 108.4, respectively. The results suggest that the concentration of TCDD in plasma was not dose-dependent. The T/P ratios in group 1 were higher than those observed in group 2. The data indicate that in group 2 clearance rate reached saturation level 7 days after TCDD administration.

Table 2 summarizes the concentration of TCDD in different tissues of mothers and fetuses. In group 1 the highest and lowest concentrations of TCDD were observed in the mammary gland of mothers and the blood of mothers, respectively. In fetal tissues the highest level was observed in liver. In group 2 the highest and lowest concentrations of TCDD were observed in fetal livers and fetal blood, respectively. The results suggests a persistent transfer of TCDD to fetal livers via the placenta possibly due to a higher level of TCDD in blood of mothers. The highest T/P ratios in groups 1 and 2 were observed in the mammary gland of mothers and fetal livers, respectively. Comparing the radioactivity in tissues of groups 1 and 2 the level of TCDD in fetal tissues was higher than that observed in mothers. The data indicate that clearance rate of TCDD via the placenta from fetuses decreased.

This study is ongoing. To obtain more information on the toxicokinetic properties of TCDD in non-human primates we plan to perform a pharmacokinetic experiment in which TCDD is administered to mothers 30 days after delivery. We also examine the effects of TCDD on pathological and functional changes in target organs.

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Table 1 Radioactivity concentrations in Plasma and Blood at 7 days after subcutaneous administration of 3H-TCDD to female rhesus monkeys on Day 140 of gestation

	Radioactivity concentration of 3H-TCDD (pg eq./mL) 30ng/kg (1G)					
Tissue	No.1	(T/P ratio)	No.2	(T/P ratio)	mean	(T/P ratio)
Blood	1.19	1187	1.64	37.25	1.41	61.43
Plasma	0.001	1	0.04	1	0.02	1
Cord blood	2.51	2512	1.66	37.63	2.08	90.61
Cord plasma	n.d.	(-)	n.d.	(-)	(-)	(-)
Fetal blood	2.53	2530	3.92	89.16	3.23	140.30
Fetal plasma	0.02	17	n.d.	(-)	0.02	0.74

	Radioactivity concentration of 3H-TCDD (pg eq./mL) 300ng/kg (2G)						
Tissue	No.3	(T/P ratio)	No.4	(T/P ratio)	mean	(T/P ratio)	2G/1G
Blood	5.08	2.02	6.325	1.450	5.71	1.68	4.04
Plasma	2.52	1.0	4.28	1.0	3.40	1.0	147.9
Cord blood	4.32	1.71	5.74	1.34	5.03	1.48	2.42
Cord plasma	1.75	0.69	1.52	0.35	1.63	0.48	-
Fetal blood	3.16	1.25	7.76	1.81	5.46	1.61	1.69
Fetal plasma	1.47	0.58	2.22	0.52	1.84	0.54	108.4

-: no sample (-) : not calculated

n.d. : not detected (T/P ratio) : Tissue/Plasma ratio

Table 2 Radioactivity concentrations in tissues 7 days after subcutaneous administration of 3H-TCDD to female rhesus monkeys on Day 140 of gestation

Tissue	Radioactivity concentration of 3H-TCDD (pg eq./g or mL) 30 ng/kg (1G)					
	No.1	(T/P ratio)	No.2	(T/P ratio)	Mean	(T/P ratio)
Plasma	0.001	1	0.044	1	0.023	1
Blood	1.19	1187	1.64	37.25	1.41	61.43
Liver	8.92	8918	8.09	183.84	8.50	369.74
Mammary gland	25.25	25247	28.06	637.77	26.66	1158.9
Fat	21.10	21096	12.30	279.59	16.70	726.0
Placenta	21.27	21266	15.63	355.18	18.45	802.0
Fetus	(Female)		(Male)			
Fetal blood	2.53	2530	3.92	89.16	3.23	140.30
Fetal liver	22.89	22888	5.75	130.72	14.32	622.61
Fetal fat	2.62	2619	-	(-)	2.62	113.87
Fetal ovary or testes	n.d.	(-)	1.45	33.02	1.45	63.17

Tissue	Radioactivity concentration of 3H-TCDD (pg eq./g or mL) 300 ng/kg (2G)						
	No.3	(T/P ratio)	No.4	(T/P ratio)	Mean	(T/P ratio)	2G/1G
Plasma	2.52	1	4.28	1	3.4	1	147.82
Blood	5.08	2.02	6.33	1.48	5.7	1.68	4.03
Liver	123.76	49.05	118.43	27.67	121.09	35.61	14.24
Mammary gland	63.24	25.07	38.70	9.04	50.97	14.99	1.91
Fat	86.80	34.40	243.29	56.84	165.04	48.54	9.88
Placenta	277.98	110.18	120.07	28.05	199.02	58.54	10.79
Fetus	(Female)		(Female)				
Fetal blood	3.16	1.25	7.76	1.814	5.46	1.61	1.69
Fetal liver	427.6	169.5	275.21	64.30	351.4	103.4	24.54
Fetal fat	164.93	65.37	128.14	29.94	146.54	43.1	55.95
Fetal ovary or testes	79.72	31.60	41.56	9.71	60.64	17.83	41.73

- : no sample
(-) : not calculated
n.d. : not detected
(T/P ratio) : Tissue/Plasma ratio