表 4. F1のリンパ球表面抗原の割合

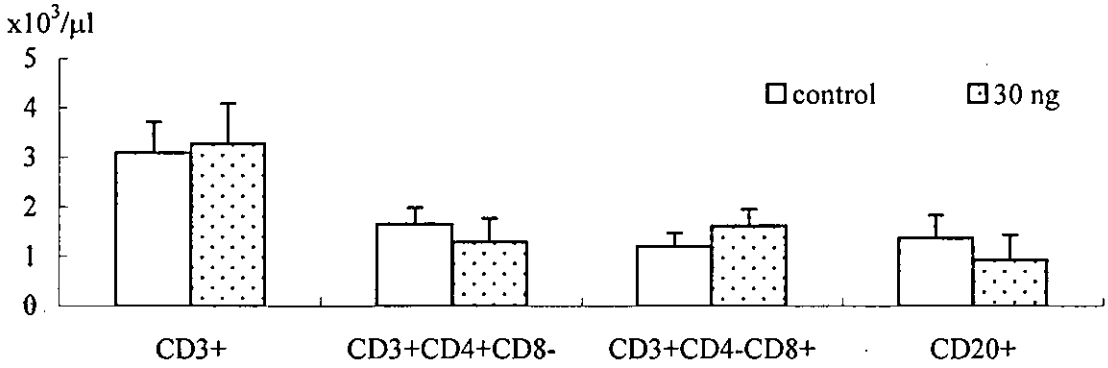
初産児(F1a)		control			30 ng				300 ng		
$CD3^+$											
	♀	78.19	±	2.09	74.18	±	3.45		70.64	±	4.29
	♂	76.18	±	4.23	66.20	±	5.21	**	58.28	±	2.76
$CD3^+CD4^+CD8^-$											
	♀	54.93	±	3.32	49.80	±	5.61		58.49	±	10.18
	♂	57.61	±	3.38	57.91	±	4.39		56.48	±	2.94
$CD3^+CD4^-CD8^+$											
	♀	36.08	±	3.45	43.33	±	5.61		33.23	±	7.75
	♂	32.30	±	1.97	32.83	±	3.92		32.08	±	3.61
CD4/CD8											
	♀	1.66	±	0.24	1.31	±	0.27		2.08	±	0.71
	♂	1.83	±	0.20	2.20	±	0.44		1.94	±	0.41
$CD20^+$											
	♀	15.20	±	1.73	18.14	±	1.80		20.97	±	5.82
	♂	17.26	±	2.01	23.14	±	3.05	**	30.77	±	2.75

第二児 (F1b) (♀のみ)	control				30 ng				300 ng		
$CD3^+$	59.10	±	1.43		66.20	±	3.00	**	68.66	±	0.47
$CD3^+CD4^+CD8^-$	52.56	±	2.86		61.35	±	4.24		53.13	±	2.10
$CD3^+CD4^-CD8^+$	37.51	±	1.87	*	28.51	±	3.29		35.41	±	1.40
CD4/CD8	1.44	±	0.16		2.24	±	0.37		1.51	±	0.11
$CD20^+$	23.58	±	2.54		19.54	±	2.14		15.64	±	1.79

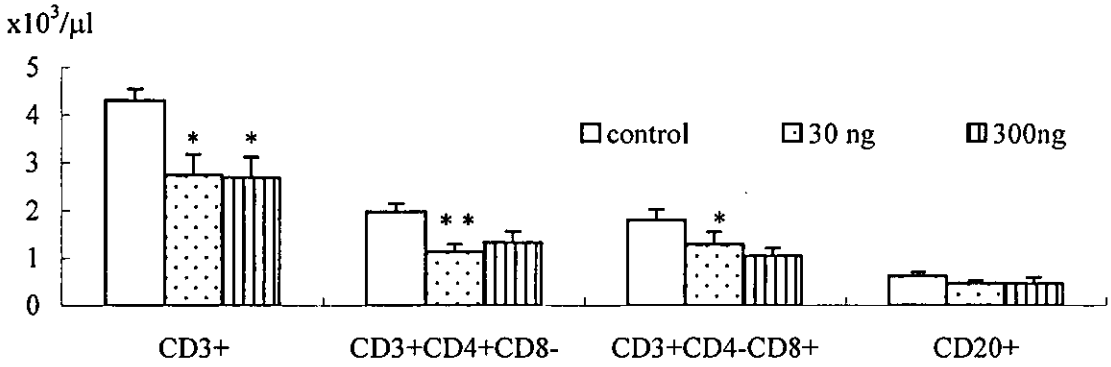
データは平均値 ± SEで、対照群との有意差は、*: P<0.05、**: <0.01で示す。

図1. F0のリンパ球各サブセット数

初産のみのF0



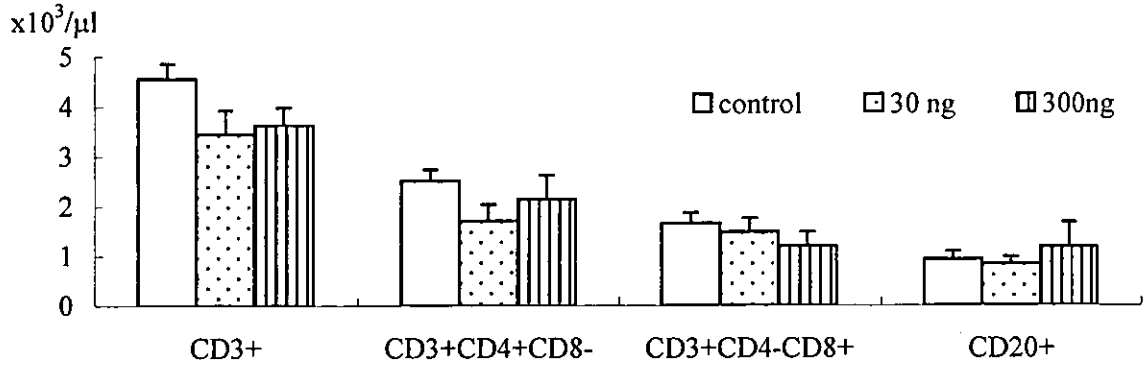
第二児を出産したF0



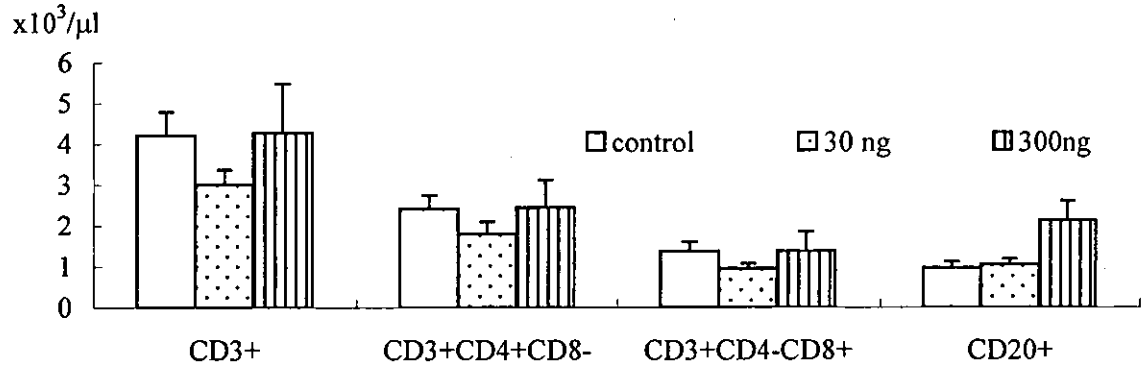
データは平均値 ± SEで示す。対照群との有意差は、*: P<0.05、**: <0.01で示す。
第二児を出産したTCDD投与群ではCD3+リンパ球(T細胞)が有意に減少している。
30 ng投与群では特にCD4+T細胞が減少している。

図2. F1のリンパ球各サブセット数

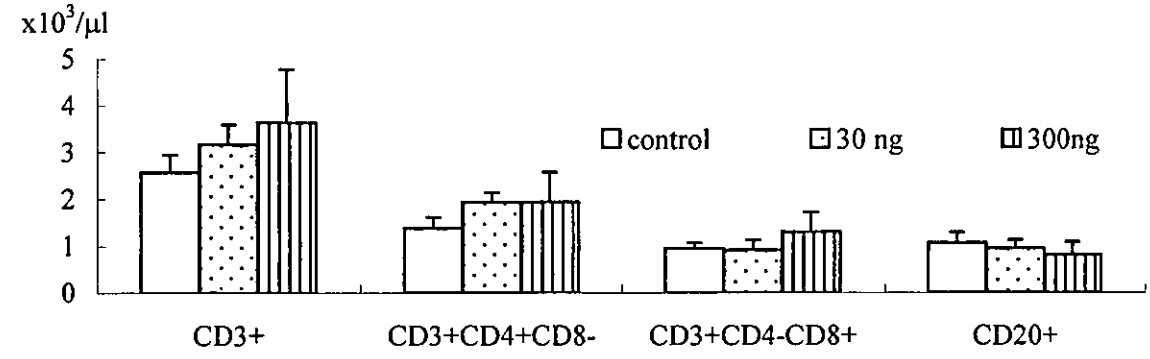
初産児 (F1a)♀



初産児 (F1a)♂

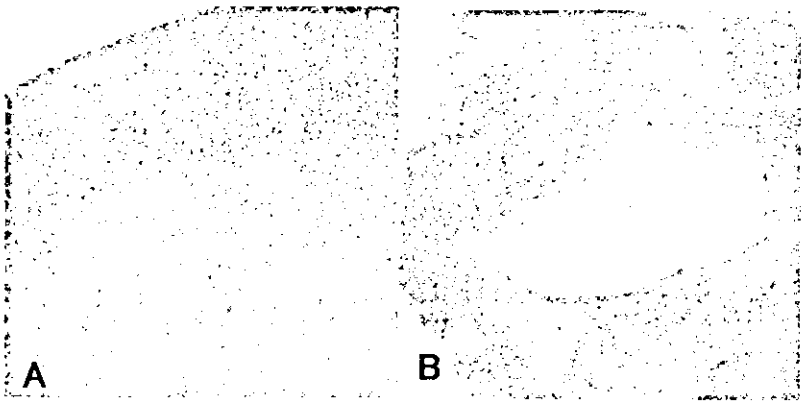


第二児(F1b)♀



データは平均値±SEで示す。
F1aでは投与群でT細胞数が減少する傾向が見られたが、有意差はない。

図3. 胸腺組織像



A: 正常胸腺。皮質・髄質の境界がはっきりしており、ハッサル小体が散見される。
B:F1胸腺の一例。髄質の中に腺様(のう胞様)の構造が見られる。

研究成果刊行一覧

発表者氏名	論文タイトル	雑誌名	巻	ページ	出版年
Mari,Ohta., Satoshi,Akema., Masami,Tsuzuki., Tatsumi, Korenaga., Toshio,Fukusato., Kazuo,Asaoka., Nobuo,Murata., Motoyoshi,Nomizu., Akihiro,Arima., Shunichiro,Kubota.	Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on signal transduction pathway-related protein expression in liver and cerebrum of rhesus monkey.	Organohalogen Compounds	66	3299-3304	2004
Korenaga,Tatsumi., Shunichiro,Kubota., Mari,Ohta., Kazuo,Asaoka., Nobuo,Murata., Motoyoshi,Nomizu., Akihiro,Arima., Toshio,Fukusato.	Liver injury in rhesus monkeys subcutaneously injected with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.	Organohalogen Compounds	66	3315-3320	2004
Mari,Ohta.,.Satoshi,Akema., Masami,Tsuzuki., Tatsumi ,Korenaga., Toshio, Fukusato., Kazuo, Asaoka., Nobuo, Murata., Akihiro, Arima., Shunichiro, Kubota.	Long-term Effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on signal transduction pathway-related Protein Expression in Precentral gyrus, amygdaroid body andliver of rhesus monkey.	Chemosphere submitted			2005
Tatsumi,Korenaga., Toshio, Fukusato., Mari, Ohta., Kazuo, Asaoka., Nobuo, Murata., Akihiro, Arima., Shunichiro, Kubota.	Long-term effects of subcutaneously injected 2,3,7,8-tetrachlorodibenzo-p-dioxin on the liver of rhesus monkeys.	Chemosphere submitted			2005
Yasuda I, Yasuda M, Sumida H, Tsusaki H, Arima A,Ihara T, Kubota S, Asaoka K, Tsuga K, Akagawa Y	*In utero* and lactational exposure to 2,3,7,8-tetrachloro-dibenzo- *p*-dioxin (TCDD) affects tooth development in rhesus monkeys.	Reprod Toxicol	20	21-30	2005
Yasuda I, Yasuda M, Sumida H, Arima A,Ihara T, Kubota S, Asaoka K, Takasuga T, Tsuga K, Akagawa Y	*In utero* and lactational exposure to 2,3,7,8-tetrachloro-dibenzo- *p*-dioxin (TCDD) affects tooth development in rhesus monkeys.	Organohalogen Compounds	66	3321-3325	2004

発表者氏名	論文タイトル	雑誌名	巻	ページ	出版年
Asaoka, K., Iida, H., Watanabe, K., Miyaji, K., Goda, H., Ihara, T., Yasuda, M., Kubota, S.	Contamination of dioxins in free ranging and breeding monkeys in Japan and relationship analysis between limb malformations and administration with 2,3,7,8- tetrachlorodibenzo-p-dioxin(TCDD)on macaque monkeys.	Chemosphere submitted			2005
Ohta, M., Akema , S., Tsuzuki , M., Korenaga, T., Fukusato, T., Asaoka, K., Murata, N., Nomizu, M., Arima, A., Kubota, S.	Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on signal transduction pathway-related protein expression in liver and cerebrum of rhesus monkey.	Organohalogen Compounds	66	3299-3304	2004
Tatsumi, K., Kubota, S., Ohta, M., Asaoka, K., Murata, N., Nomizu, M., Arima, A., Fukusato, T.	Liver injury in Rhesus monkeys subcutaneously injected with 2,3,7,8-tetrachlorodibenzo-p-dioxin	Organohalogen Compounds	66	3315-3320	2004
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In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects tooth development in rhesus monkeys

Iku Yasuda[1], Yasuda Mineo[2], Sumida Hiroshi[2], Arima Akihiro[3], Ihara Toshio[3], Kubota Shunichiro[4], Asaoka Kazuo[5], Takasuga Takumi[6], Tsuga Kazuhiro[1], Akagawa Yasumasa[1]

[1]Hiroshima University, Hiroshima
[2]Hiroshima International University, Hiroshima
[3]Shin Nippon Biomedical Laboratories, Ltd., Kagoshima
[4]University of Tokyo, Tokyo
[5]Primate Research Institute, Kyoto University, Inuyama
[6]Shimadzu Techno-Research Inc., Kyoto

Introduction

The current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg TEQ/kg/day in Japan. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.* reported that a single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the offspring[1]. The maternal body burden at this dose was measured to be 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. An uncertainty factor of 10 was applied to this value, and the human TDI was established. However, due to great differences in the biological half life of TCDD between human and rodents, the validity of this calculation is questioned. To obtain more reliable LOAEL in the second generation, we initiated a long-term study in rhesus monkeys in 1999.

In rodents, teeth are known to be targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[2,3]. In humans also tooth abnormalities were reported among populations exposed to dioxins[4]. In our monkey experiment, some young were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate young exposed to TCDD *in utero* and lactationally. By macroscopic observation we found some tooth abnormalities among died young exposed to TCDD[5]. This prompted us to examine surviving young by radiography. This is an interim report of our findings in these young.

Methods and Materials

Animals: Adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijin, China) were used. Details of breeding conditions were given elsewhere[6]. Female monkeys were allowed to cohabit with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period was disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups each consisting of approximately 20 animals and allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PD0).

Administration of TCDD: TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy and lactation until day 90 after birth.

Measurement of TCDD in maternal serum: Approximately 20 ml of blood was taken from the femoral vein of the dams on day 80 of pregnancy, and centrifuged. The obtained serum was subjected to high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) by the method of Patterson *et al.*[7]

Observation of teeth of the young: Stillborn and postnatally died young were autopsied, and the upper and lower jaws were dissected for detailed observation. Surviving young were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

Pregnancy outcome and postnatal development of the young: Table 1 summarizes the pregnancy outcome and postnatal mortality of the young. Abortions, stillborns, and postnatal deaths occurred fairly frequently even in the control group. To increase the number of surviving young in the 300 ng/kg, we added 9 dams to the group approximately 2 years after the initiation of the experiment. However, only two young survived more than a year due to a high incidence of abortions. No significant differences were noted in the gestation length and birth weight among the control and TCDD-treated groups, indicating the body burden of TCDD at 300 ng/kg did not affected general growth of the young.

Table 1: Pregnancy outcome and postnatal mortality of rhesus monkeys exposed to TCDD.

Group	No. of dams	No. of abortions	No. of stillborns	No. of live borns	No. of postnatal deaths	Gestation length (days)	Birth weight (g)
Control	23	2	3	18	5	161.8±7.8	426.1±58.6
30 ng/kg	20	0	5	15	3	163.8±5.9	426.8±56.9
300 ng/kg	20	2	2	16	8	164.9±9.7	408.6±63.7
300 ng/kg[1)]	9	5	1	3	1	165.0±3.0	466.0±87.1

1) Newly added group

Tooth abnormalities in the young: The incidence of tooth abnormalities in the young was shown in Table 2. Tooth abnormalities in the stillborn and postnatally died young were described previously[5]. No abnormalities were detected in the control and 30 ng/kg groups, whereas more than half of the young in the 300 ng/kg had tooth abnormalities as listed in Table 3. The upper permanent lateral incisors were most frequently affected. In contrast, among the deciduous teeth, the central incisors seemed to be most sensitive targets of developmental toxicity of TCDD. The permanent premolars were also affected frequently, while the canine and the first molar were resistant to the adverse effect of TCDD. Probably these larger teeth have become resistant to odontotoxic chemicals during the course of evolution.

Table 2: Incidence of tooth abnormalities among F1 exposed to TCDD.

Group	Stillborns and postnatally died young			Surviving young		
	No. of specimens	No. of specimens with tooth abnormalities (%)		No. of young	No. of young with tooth abnormalities (%)	
Control	4	0	(0)	13	0	(0)
30 ng/kg	5	0	(0)	12	0	(0)
300 ng/kg	8	3	(38)	8	6	(75)
300 ng/kg[1)]	2	0	(0)	2	1	(50)

1) Newly added group

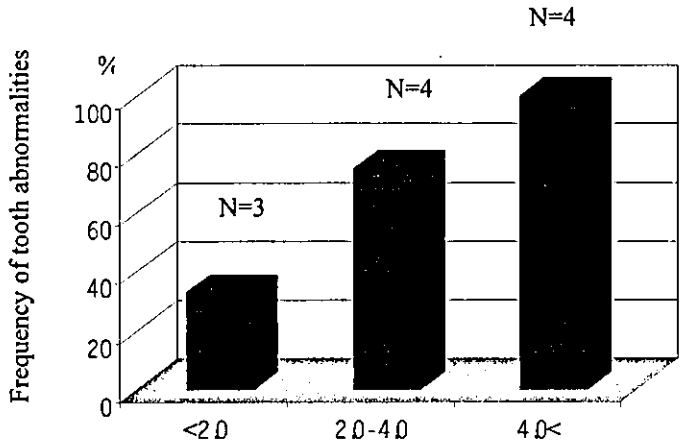
Relationship between maternal serum TCDD concentration and occurrence of tooth abnormalities: In the control maternal serum, the TCDD levels were below the detection limit. In the 30 ng/kg group, the levels were fairly constant, ranging from 0.19 to 0.21 pg/g wet weight. In contrast, the levels varied largely in the 300 ng/kg group, ranging from 1.1 to 8.9 pg/g wet weight. The average of those without tooth abnormalities in their young was 1.4 ± 0.6 pg/g wet weight, whereas that with tooth abnormalities was 4.3 ± 2.4 pg/g wet weight. The concentration-response relationship is shown in Fig. 1.

Table 3: Tooth abnormalities detected in the young exposed to TCDD at 300 ng/kg.

Young	Sex	Age (days)[2]	Abnormal Findings
31	♀	1430	542|24 missing |5 conical
39	♂	1410	542|245 missing
42	♀	1415	5 |5 missing |4 conical
44	♂	1415	54 |45 missing |5 conical
60	♂	1388	542|245 5|5 missing
66	♂	1338	52|2 1|1 missing
			54 |45 malaligned |45 conical
106[1]	♀	688	A |A 4 |24 missing

1) Newly added group
2) Age at X-ray examination

Figure 1: Maternal serum concentration of TCDD and the incidence of tooth abnormalities.



Maternal serum concentration of TCDD (pg/g wet weight)
N = number of specimens in each concentration category

Validity of the current TDI: The above results indicate that the LOAEL body burden for induction of tooth abnormalities in the rhesus monkey is at a certain level between 30 ng/kg and 300 ng/kg, probably not much different from the LOAEL body burden for rodents, 86 ng/kg. Hence it is reasonable to conclude that the current TDI of dioxins in Japan needs no immediate modification.

Acknowledgements
This study was supported by Health Labour Science Research Grants for Research on Chemical Risk from the Ministry of Health Labour and Welfare of Japan.

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Effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on signal transduction pathway-related protein expression in liver and cerebrum of rhesus monkey

Mari Ohta[1], Satoshi Akema[1], Masami Tsuzuki[1], Tatsumi Korenaga[2], Toshio Fukusato[2], Kazuo Asaoka[3], Nobuo Murata[4], Motoyoshi Nomizu[5], Akihiro Arima[6], Shunichiro Kubota[1]

[1]The University of Tokyo, Tokyo
[2]Teikyo University of School of Medicine, Tokyo
[3]Kyoto University, Kyoto
[4]Teikyo University of School of Medicine, Kawasaki
[5]Hokkaido University, Sapporo
[6]Shin Nippon Biomedical Laboratories, Ltd., Kagoshima

Introduction

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is known to produce a wide range of toxic and biochemical effects in experimental animals, including immunological dysfunctions, chloracne, tetragenecity and carcinogenesis[1-3]. Recently, the potential impact of dioxins on neurological disorders with particular focus on attention deficit hyperactivity disorder (ADHD) are concerned. Although a lot of information is available from studies in rodents[4-6], not much is known of the low dose effects of TCDD in non-human primates[7]. In higher animals, dioxins are metabolized slowly, as evidenced by the estimated TCDD half-life of 5.8 to 14.1 years[8]. Therefore, it is necessary to investigate the long-term effects of TCDD on human health. Considering the pronounced species differences observed in some studies of TCDD, the studies using primates are needed for assessment of TCDD exposure on human health. We have been studying the metabolism and the effects of single administration of TCDD on pregnant monkey (F0) and F1 rhesus monkey[9-11]. The focus of the present study is to study the effects of TCDD on signal transduction pathway-related protein levels in various organs, especially in liver and brain of F0 monkeys.

Methods and Materials

Chemicals and antibodies: 2,3,7,8-TCDD dissolved in toluene and DMSO (1:2, v/v) were purchased from Kanto Chemicals Co. Ltd. (Tokyo, Japan). Anti-aromatic (aryl) hydrocarbon receptor (Ah-R, 5579), anti-Ah-R nuclear translocator proteins (Arntl, 8076), anti-Akt1/2 (8312), anti-phospho-Akt1/2/3 (7985), anti-EGFR (03), anti-VE-cadherin (6458), anti-Bad (8044), anti-caspase3 (7148), anti-caspase8 (7890), and anti-beta-actin (1615) antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-rabbit (7074) and mouse (7076) IgG, horseradish peroxidase-linked antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). An anti-cytochrome P450 1A1 (CYP1A1, 299124) was purchased by Daiichi Pure Chemicals Co., Ltd. (Tokyo, Japan). Anti-goat IgG, horseradish peroxidase-linked antibody was obtained from Vector Laboratories, Inc. (Burlingame, CA, USA).

Animals: Rhesus monkeys were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). All procedures involving animal care were in accord with the institutional guidelines in compliance with national laws. Monkeys (5-6 years old and 5.3-6.7 kg of body weight) were kept in Shin Nippon Biomedical Laboratories, Ltd. (Kagoshima, Japan). 2,3,7,8-TCDD (0, 30 and 300 ng/kg of body weight) was subcutaneously administered to pregnant monkeys (F0). The detailed breeding condition was described previously[12]. After delivery F0 monkeys have been observed for 3-4 years and sacrificed for analysis of protein and gene expression. In this study, the liver and cerebrum were obtained from 2 monkeys from three groups (0, 30 ng/kg TCDD, 300 ng/kg TCDD) which were observed for more than 3 years after TCDD administration.

Western blotting: The protein levels of various signaling transduction-related proteins were analyzed using western blotting. The proteins were visualized using Phototope®-HRP Western Blot detection system (7071, Cell Signaling Technology, Beverly, MA, USA). The level of proteins determined average of spot intensity per each proteins by using Chemidoc XRS system and Quantity One® image analysis software (Bio-Rad Laboratories, Inc., USA).

Results and Discussion

Effects of TCDD on protein levels: We observed alterations of signal transduction-related protein levels at more than 3 years after a single administration of low dose TCDD (0, 30 and 300 ng/kg) in liver and cerebrum of rhesus monkey (F0). The results analyzed by western blotting are expressed as an average of two monkeys, and summarized in Table 1 and Figure 1. Though TCDD did not alter the levels of Ah-R and Arntl in liver, TCDD (30 and 300 ng/kg) increased the level of CYP1A1 in the liver. In the cerebrum, there is no significant difference of CYP1A1 protein levels among control, 30 and 300 ng/kg of TCDD groups.

There were significant decrease of VE-cadherin protein levels in liver and cerebrum of TCDD-treated monkeys. As compared to untreated controls, VE-cadherin protein levels in liver were decreased 0.38-fold, and 0.46-fold in 30 ng of TCDD/kg group, and 300 ng of TCDD/kg group, respectively. In cerebrum, 300 ng of TCDD/kg decreased VE-cadherin protein level 0.45-fold, compared to the control group.

Epidermal growth factor receptor (EGFR) protein levels in liver and cerebrum were increased in 30 ng TCDD/kg-treated monkeys. There was no difference between the control and the 300 ng of TCDD/kg groups. As TCDD was reported to cause alterations in the growth factor signal transduction pathways in endocervical cells from single exposure to TCDD (2-4 μg/kg) in monkey[13], it is possible that 300 ng TCDD/kg caused down-regulation of EGFR protein levels.

The increase of Akt protein level, and phosphorylation of Akt, in liver and cerebrum were observed in 300 ng TCDD/kg-treated monkeys. The protein levels of Bad were significantly increased 2.7-fold in the liver and 1.8-fold in the cerebrum of TCDD (300 ng/kg)-treated groups. Akt plays a critical role in controlling the balance between survival and apoptosis[14], and Akt promotes cell survival by inhibiting apoptosis through inactivation of Bad[15]. In the liver of 300 ng of TCDD/kg -treated monkeys, caspase 8 protein levels were increased. Caspase 3 was also increased in cerebrum of 300 ng of TCDD/kg-treated monkeys. Bad, caspase 8 and caspase 3 are known to play roles in apoptosis signal transduction pathway

There are few reports which investigated the effect of TCDD on signal transduction-related protein levels in liver and cerebrum of rhesus monkeys. The

results in this study suggest that a single administration of low dose TCDD may induce apoptosis in liver and cerebrum. To elucidate whether TCDD induces apoptosis and carcinogenesis, we are currently studying the effects of TCDD at molecular level. This study is ongoing, and we will observe the effects of a single administration of low dose TCDD on hepatic dysfunctions and neurological abnormalities in rhesus monkeys.

Acknowledgements

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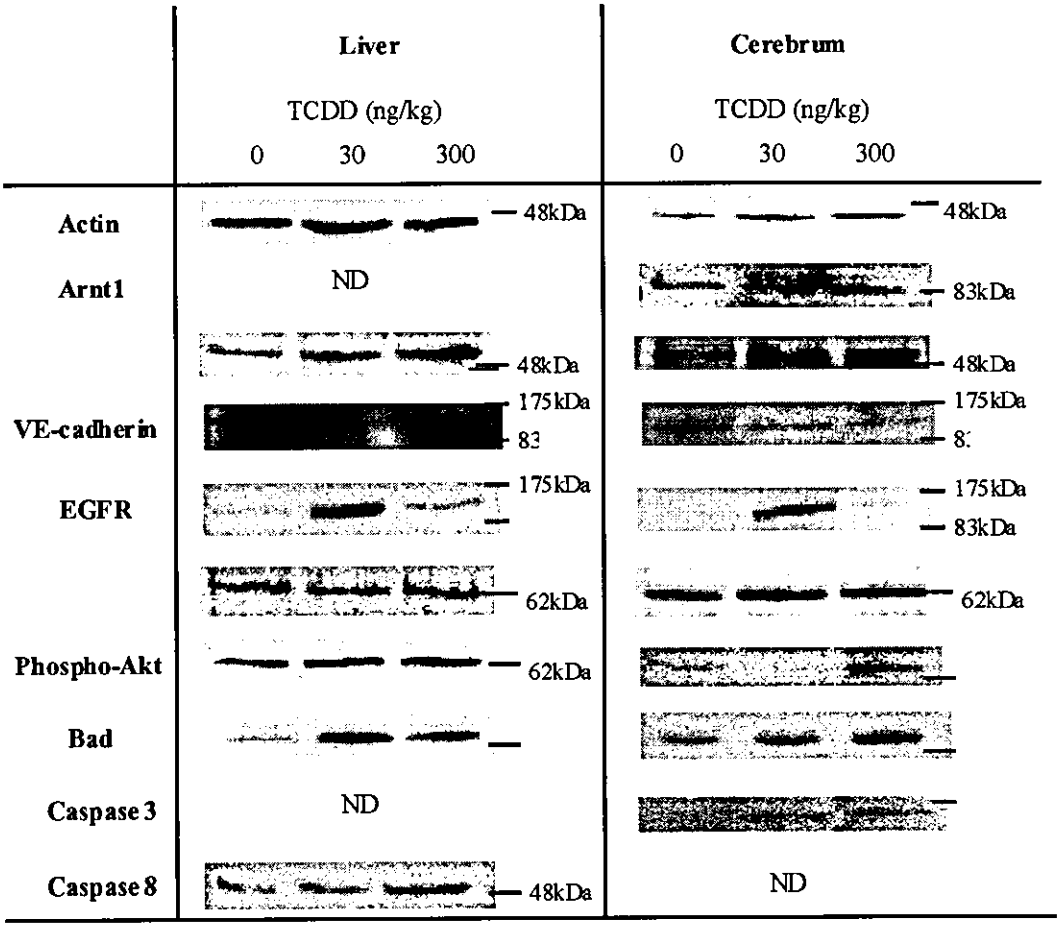
Table 1: Effects of TCDD on signal transduction pathway-related protein levels in liver and cerebrum of rhesus monkeys.

	Dose of TCDD (ng/kg)					
	0	30	300	0	30	300
	Liver			*Cerebrum*		
Ah-R		ND			ND	
Arnt1		ND		0.084	0.112	0.177
CYP1A1	0.265	1.181	1.812	0.097	0.144	0.098
VE-cadherin	0.242	0.093	0.111	0.031	0.030	0.014
EGFR	0.084	0.161	0.112	0.032	0.091	0.050
Akt1/2	0.161	0.161	0.160	0.109	0.125	0.110
Phospho-Akt	0.214	0.281	0.439	0.047	0.061	0.070
Bad	0.052	0.101	0.140	0.126	0.225	0.227
Caspase3		ND		0.013	0.010	0.043
Caspase8	0.031	0.029	0.059		ND	

The results are shown as an average from two monkeys of each group (0, 30 ng/kg, and 300 ng/kg). The intensity of each protein per spot intensity was quantitated using Chemidoc XRS system and Quantity One® image analysis software (Bio-Rad Laboratories, Inc., USA), and corrected using the intensity of beta-actin protein level. ND represents not detected.

Figure 1: Effect of 30 and 300 ng of TCDD/ kg on protein levels liver and cerebrum.of rhesus monkeys

The expression levels of proteins in liver and cerebrum of TCDD-treated rhesus monkeys were determined by Western blotting. The beta-actin protein levels were used as controls confirming that an equal amount of protein was loaded.



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Liver injury in Rhesus monkeys subcutaneously injected with 2.3.7.8-tetrachlorodibenzo-p-dioxin

Korenaga Tatsumi[1], Shunichiro Kubota[2], Mari Ohta[2], Kazuo Asaoka[3], Nobuo Murata [4], Motoyoshi Nomizu[5], Akihiro Arima[6], Toshio Fukusato[1]

[1]Teikyo University School of Medicine, Tokyo
[2]The University of Tokyo, Tokyo
[3]Kyoto University, Aichi
[4]Teikyo University School of Medicine, Mizonokuchi Hospital, Kawasaki
[5]Hokkaido University, Sapporo
[6]Shin Nippon Biomedical Laboratories, Ltd., Kagoshima

Introduction

2.3.7.8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of dioxins which are environmentally and biologically stable. Exposure to these compounds results in wide variety of effects including immunological dysfunction, tetragenecity and carcinogenesis[1-4]. The liver is one of the central organs in which TCDD metabolized after absorption into the human and animal bodies. In experiments using rodents, TCDD accumulats and remains stable in the fatty tissues and liver for a long time. Kinetic profile of TCDD in our experiments using rhesus monkeys demonstrated the higher concentrations of TCDD in the fat, liver, and mammary gland[5-7]. TCDD-induced liver injury in humans has been reported in Japan (PCB), Taiwan (PCB or PCDF), Italy (Sebeso, TCDD), and Vietnam (TCDD). Considerating the pronounced difference between species observed in some studies on non-human primates to assess effects of relatively low dose of TCDD, in the present study, liver injury in rhesus monkeys after a single subcutaneous administration of low dose of TCDD during pregnancy was investigated.

Materials and methods

Chemicals: 2,3,7,8-TCDD dissolved in toluene and DMSO (1:2, v/v) were purchased from Kanto Chemicals Co. Ltd. (Tokyo, Japan).

Animals: Rhesus monkeys were purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijing, China). All procedures involving animal care were in accord with the institutional guidelines in compliance with national laws. 30ng/kg or 300ng/kg of TCDD was subcutaneously administrated to female pregnant monkeys. Control monkeys were administered vehicle alone. Three years after administration, ten monkeys (3, 4, 3 in each group) were sacrified. Macroscopic and histological studies of the liver followed by electron microscopic examination were carried out.

Immunohistochemstry: Immunohistochemical staining for MIB-1 (Dako cytomation, Glostrup, Denmark) as a proliferating cell marker and alpha smooth muscle actin (SMA) (Dako Cytomation, Glostrup, Denmark) as satellite cell marker was carried out. Monkey liver was fixed in 10% neutral-buffered formalin and embedded in paraffin for histological analysis. Immunohistochemical staining was performed on paraffin-embedded tissues using LSAB Kit (Dako Cytomation, Glostrup, Denmark) and DAB substrate kit (Nichirei, Tokyo, Japan). Monkey tissue sections (4 μm) were deparaffinized and rehydrated, and antigen retrieval was performed by treatment of the slides in 0.01M citrate buffer (pH 6.0) for 15 minutes in the microwave oven. Thereafter the slides were cooled to room temperature and washed in the phosphate buffered saline (PBS). Slides were immersed in 1% hydrogen peroxide for 30 minutes to block endogenous peroxidase activity. After washing and blocking, the sections were incubated at 4°C overnight with anti-MIB-1 antibody (Dako Cytomation, Glostrup, Denmark), followed by a standard procedure using biotin-blocking kit (Dako Cytomation, Glostrup, Denmark), LSAB kit and DAB-substrate kit. In the case of immunohistochemical staining with anti-alpha-SMA antibody, pretreatment with microwave oven was omitted. Incubation with anti-alpha-SMA antibody was performed at room temperature for 1 hour.

Western blot analysis: Liver tissue samples for protein analysis were frozen at once and kept at –80°C until use. The cells and tissues were homogenized in the

PBS containing 1mM EDTA, 0.2 mM PMSF and 1 μM pepstein A. Protein extracts were subjected to sodium dodecyl sulfate-polyacrylamide gel (7.5%) electrophoresis (SDS-PAGE) and transferred to nitrocellulose membrane by electroblotting (150mA, at room temperature for 1h). Nonspecific binding of proteins was blocked by incubating the membranes in 5% non-fat milk in PBS containing 0.1% Tween-20. The membranes were incubated with anti-AhR (aryl hydrocarbon receptor) or anti-Arntl(aryl hydrocarbon receptor nuclear translocator 1) antibody (Santa Cruz Biotechnology, INC, CA) at 4°C overnight. The membranes were incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin antibody for 1 hour at room temperature. Detection of proteins which bind to primary antibody was performed with enhanced chemiluminscence reaction. (Amersham Biosciences Corp., Piscataway, NJ)

Results

Histopathological findings: Focal fatty change localized at the periphery and infarction with hemorrhage (Fig. 1) was found in 4 and 2 monkeys, respectively, to which TCDD was administrated (Table 1). Focal fatty change was nodular and simulated tumor. Coagulation necrosis or cytolytic change and hemorrhage were indicated in the infarction and infarctoid lesions of the liver. Parenchymal hemorrhage, sinusoidal ectasia and intrasinusoidal microthrombi-formation were also disclosed in 2, 5 and 4 monkeys, respectively. These abnormal histological findings were not found in control group of monkeys. Small cell hypercellularity of hepatocytes in the hepatic lobules was evident in 5 of 7 monkeys injected with TCDD.

Electron microscopic study Electron microscopic examination showed sinusoidal endothelical cell injury with degeneration and sinusoidal luminal stenosis.

Immunohistochemical staining of the liver: In control group, none has positive cells for alpha-SMA antibody. In contrast, intrasinusoidal alpha-SMA-positive cell hyperplasia was detected in most TCDD-administrated monkeys indicating satellite cell hyperplasia or transformation into the myofibroblast cells in TCDD-injected group. Small hepatocyte hypercellularity within hepatic lobules showed no labeling with MIB-1 antibody.