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

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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 (Arnt1, 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 Arnt1 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.

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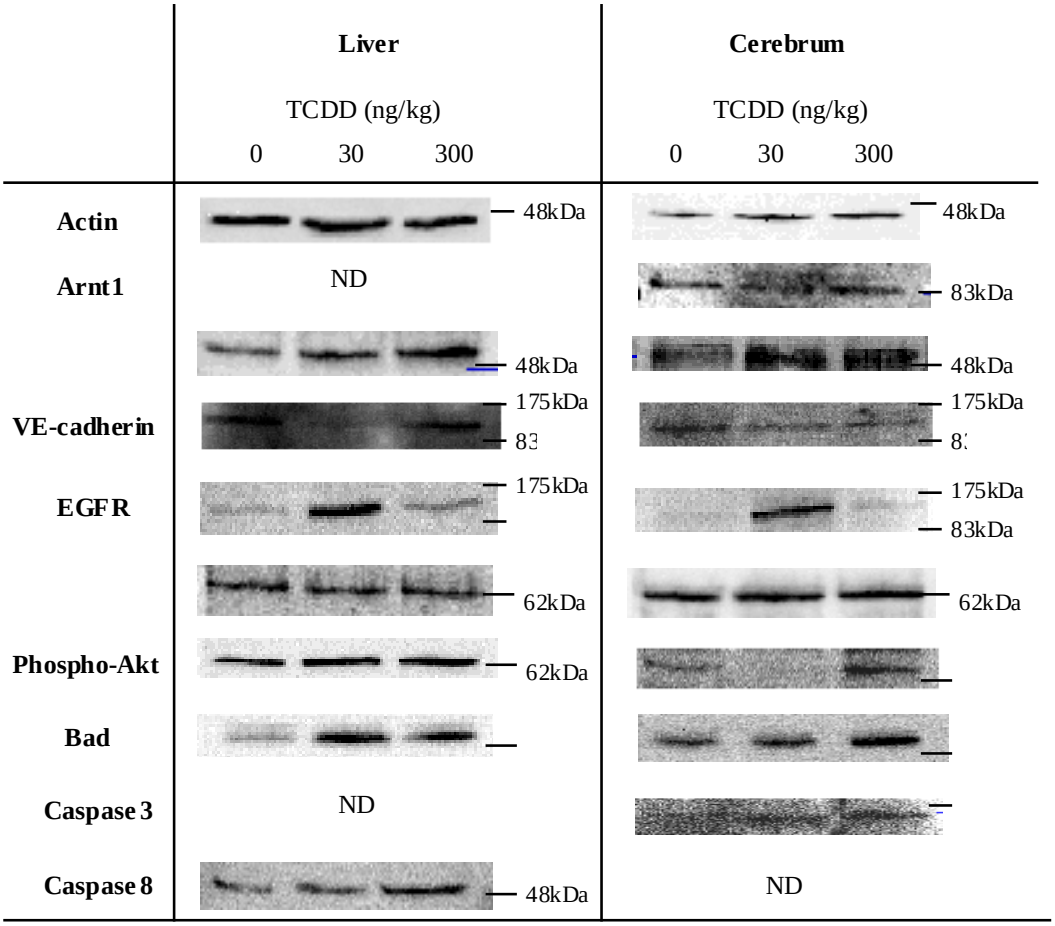
Table 1: Effects of TCDD on signal transduction pathway-related protein levels in liver and cerebrum of rhesus monkeys.

	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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