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

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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. 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-Arnt1(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 luninal 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.

Western blot analysis of liver tissues: Control monkey has positive band at 110kD which corresponds to molecular weight of AhR. TCDD-injected ones have no positive band at the same position. On the other hand, positive bands as Arnt1 were observed both control and TCDD groups.

Discussion

These histopathological findings found in the liver of monkeys which were administrated with TCDD suggest sinusoidal endothelical cell injury and impairment in intrasinusoidal microcirculation because infarction, focal fatty change, and microthrombi-formation, that are rare events in the liver[8], are considerated to be closely associated with intrahepatic circularatoy impairment. As the hepatic parenchyma is protected against ischemia by its double blood supply, hepatic infarction is an uncommon lesion and usually accompanied by impairment in hepatic arterial and portal blood supply. Focal fatty change[8] is also unusually identified lesions which are first described in human in 1980 and becomes increasingly recognized by imaging techniques. It is surprising that these rare lesions were thus frequently identified in the liver of TCDD-treated rhesus monkeys. It is possible that small hepatocyte hypercellularity and alpha-SMA-positive satellite cell hyperplasia or transformation result from intralobular circulatory disturbance with local hypoxia and ischemic injury. Increased number of alpha-SMA-positive cells may suggest perisinusoidal fibrosis in the liver. There is no previous report describing these finding in the liver after injection with TCDD into the animal models although it has been reported that TCDD induced endothelical cell injury. It remains unclear whether or how TCDD induced intrasinusoidal endothelical cell injury. Further studies are necessary to explore the mechanism.

Acknowledgment

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Figure 1. Hemorrhagic infarction of the liver detected in rhesus monkey injected with TCDD.

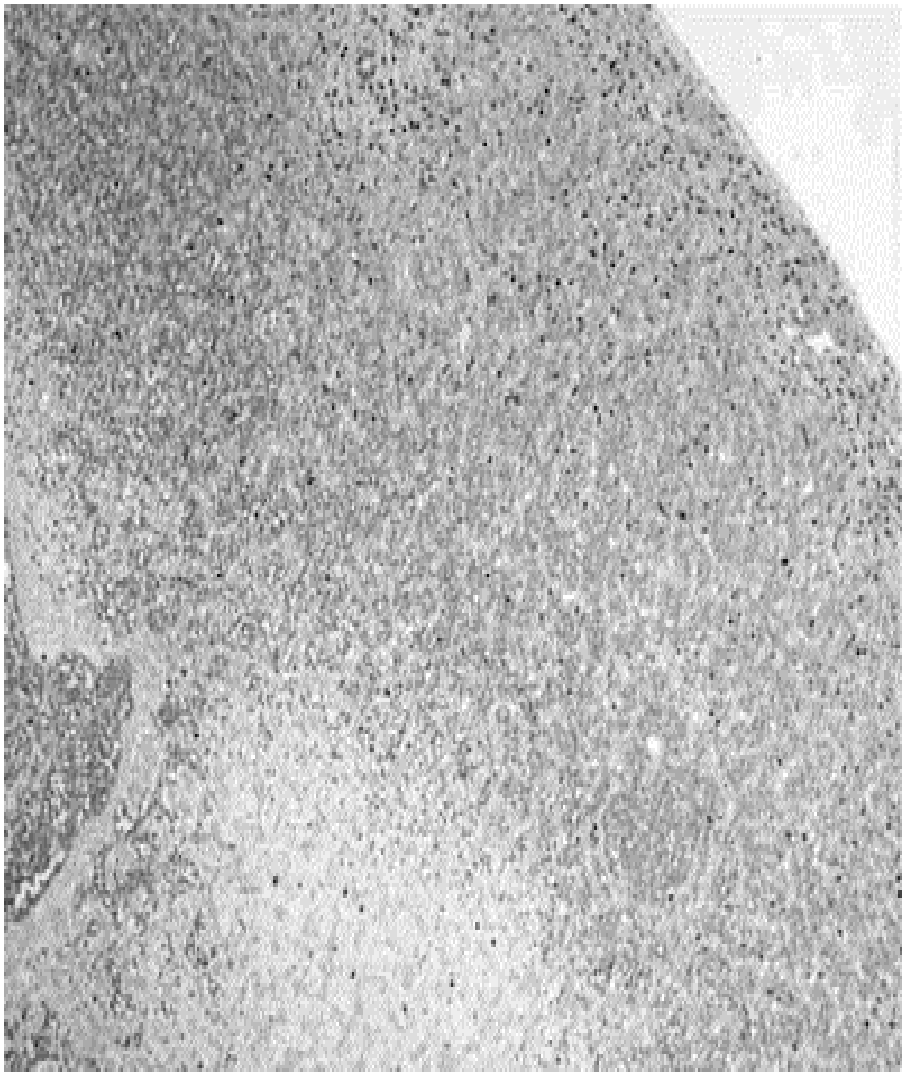

Table 1. Histopathological findings in the liver of TCDD- administrated rhesus monkeys.

	Positive /total number examined in monkeys injected with TCDD		
	Control	30ng/kg	300ng/kg
Focal fatty change	0/3	2/4	2/3
Fatty change	0/3	1/4	1/3
Infarction	0/3	1/4	1/3
Hemorrhage	0/3	1/4	1/3
Microthrombi	0/3	2/4	2/3
Sinusoidal ectasia	0/3	3/4	2/3
Small cell hypercellularity	0/3	2/4	3/3
Alpha-smooth muscle actin-Positive cell hyperplasia	0/3	3/4	3/3

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