2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN-MEDIATED OXIDATIVE STRESS IN THE OFFSPRING RATS FOLLOWING GESTATIONAL AND LACTATIONAL EXPOSURE

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most potent member of a large family of dioxin-like compounds that are ubiquitous environmental contaminants. Most of the biological adverse effects of TCDD are thought to be mediated by the aryl hydrocarbon receptor (AhR).[1,2] The activation of the AhR leads to an induction of drug-metabolizing enzymes, such as CYP1A subfamily.[2] Although induction of CYP1A does not directly manifest the toxicological endpoint of TCDD, it is an extremely sensitive marker for exposure and tissue responsiveness to TCDD. [2,3]

An additional response that has been under investigation in other laboratories is oxidative stress following exposure to TCDD[4-6.] Oxidative stress following acute exposure of high dose TCDD in laboratory animals has been demonstrated to increase the production of reactive oxygen species, lipid peroxidation, and DNA damage, although the mechanism of action is not completely understood. The mechanism of TCDD-mediated ROS production has been proposed to involve the cytochrome P450s[7]. Due to their membership in the Ah gene battery and induction following exposure to TCDD, CYP1A1/2 have been proposed to be associated with TCDD-mediated oxidative stress.

In utero and lactational exposure to TCDD has been reported in the fetus and neonate of experimental animals[8,9]. Neither of the reported studies, however, was CYP isozymes except CYP1A1 determined. The present study was designed to investigate oxidative stress and inducibility of hepatic CYP isozymes in the offspring of gestational and lactational rats exposed to low, nontoxic dose of TCDD.

Materials and Methods

Pregnant Sprague Dawley rats were orally exposed to TCDD (1, 10, and 100 ng/kg body weight/day) starting at Day 1 of gestation up to Day 20 of postpartum, respectively. Control animals received an equal volume of corn

oil. Lipid peroxidation was determined in the cytosolic fractions of rat liver by measuring the formation of thiobarbituric acid-reactive substances (TBARS), as previously described by Uchiyama and Mihara[10]. Malondialdehyde was used as a standard. The activity of EROD and induction of CYP isozymes were measured in the liver microsomes of fetuses from Day 20 of gestation, of neonates from Day 1 of postpartum, and of lactational offsprings 20 days after birth. Western blot analysis of microsomes was performed following SDS-PAGE as described previously[11]. Primary antibodies used for detection of CYP1A1/2, CYP2B, CYP3A, and CYP2E1 were kindly gifted from Dr. Sang-Shin Park. Monoclonal sheep anti-mouse IgG-horseradish peroxidase-conjugated secondary antibodies were used at 1:5000. Proteins were detected by enhanced chemiluminescence using a commercial kit (Amersham).

Results and Discussion

We measured lipid peroxidation to check the inducibility of oxidative stress in rat liver after *in vivo* exposure to TCDD. Hepatic lipid peroxidation in maternal rats was increased with increasing dose of TCDD as well as with increasing gestational period (Fig. 1A). However lipid peroxidation in offspring rats was markedly decreased with increasing gestational and lactational periods (Fig. 1B), suggesting that oxidative stress induced by TCDD in dams throughout gestational and lactational period completely does not transfer to their offsprings.

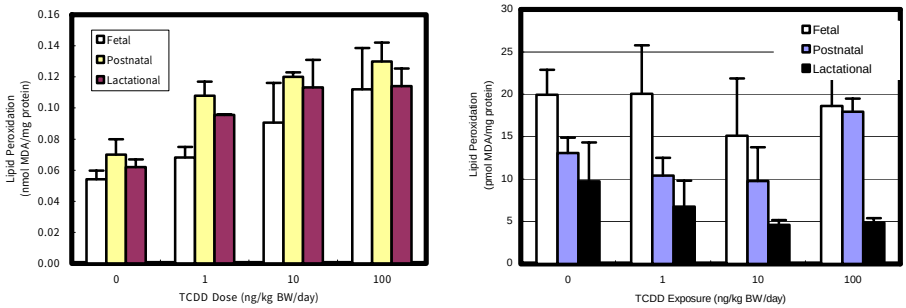

Fig. 1. The effects of subchronic treatment with various doses of TCDD on the production of lipid peroxidation (TBARS) in maternal (A) and offspring (B) rat liver cytosolic fractions. Three different doses (1, 10, and 100 ng/kg body weight/day) of TCDD were orally exposed to pregnant rats starting at Day 1 of gestation up to Day 20 of postpartum, respectively. Lipid peroxidation was measured in dams (A) and in fetal, postnatal, and lactational offspring rats (B).

We measured the activity of EROD and induction of CYP1A1/2 in the offspring liver microsomes of gestational and lactational rats exposed to TCDD. EROD activity in the liver microsomes of fetal, postnatal, and lactational offspring of TCDD-treated dams throughout pregnancy and lactation increased 0, 5, and 15-fold compared to control group, respectively (Fig. 2A). Western blot analysis shows that CYP1A1/2 was strongly induced in the liver of lactational offsprings of TCDD-treated dams by the gestational exposure to the LOEL (10ng/kg b.w/day) level of TCDD. CYP1A1/2 was also induced in the postnatal and lactational offspring rat livers by the

exposure to the NOAEL (1 ng/kg b.w/day), but not in the liver of fetal group (Fig. 2B).

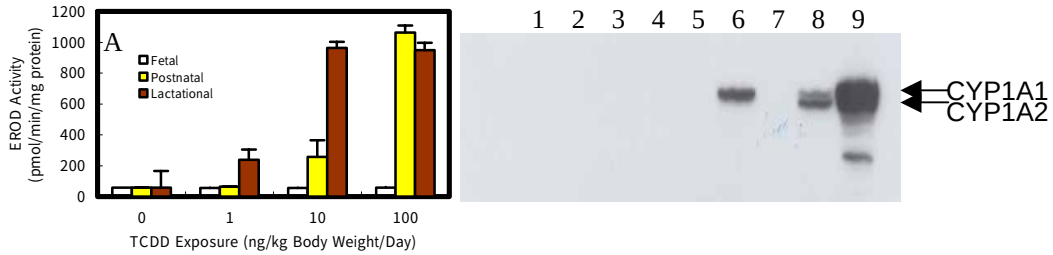

Fig. 2. EROD activity (A) and Western blot analysis (B) of CYP1A1/2 in the hepatic microsomes of fetal, postnatal, and lactational offspring rats after gestational exposure of dams to TCDD. Each lane in (B) contained 45 μg microsomal protein. TCDD exposures are as follows. Lanes 1-3. Fetal-0, 1, 10; Lanes 4-6, Postnatal-0, 1, 10; Lanes 7-9, lactational-0, 1, 10 ng TCDD/kg B.W/Day

We investigated whether TCDD exposed to dams during gestational and lactational periods is capable of inducing CYP2B, CYP3A and CYP2E1 in the livers of the offsprings. As shown in Fig. 3, TCDD exposed to dams throughout lactational period induced these three CYP isozymes in the liver of lactational offspring rats. However these three CYP isozymes were not induced in the liver of fetal and postnatal offsrings of TCDD-treated dams. These results suggest that, even though fetuses are protected from the TCDD exposure by the placenta, TCDD exposed to dams is directly transferred to their offsprings via lactation and induces CYP isozymes and oxidative stress with increasing dose of TCDD and gestation period.

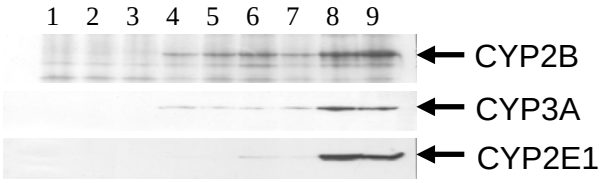

Fig. 3. Western blot analysis of CYP isozymes in the hepatic microsomes of fetal, postnatal, and lactational offsprings of dams gestationally and lactationally exposed to TCDD. Each lane contained 45 μg microsomal protein. Lanes corresponding to offsprings of TCDD-exposed dams and the levels of TCDD treated are as follows. Lanes 1-3. Fetal-0, 1, 10; Lanes 4-6, Postnatal-0, 1, 10; Lanes 7-9, lactational-0, 1, 10 ng TCDD/kg B.W/Day

We checked the cleavage of well-known substrate proteins of caspase-3, PARP and β-catenin. PARP was cleaved in the liver of offspring rats of TCDD-treated dams throughout postnatal and lactational periods, and the appearance of the 85-kDa, indicator fragment correlated well with the caspase-3 activation (Fig. 4A). This result provides new evidence that TCDD induces oxidative stress in the liver cells of postnatal and lactational offsprings of dams exposed to TCDD. β-Catenin was induced in the liver of offspring rats of TCDD-treated

dams throughout postnatal and lactational periods by unknown mechanisms (Fig. 4B), which are remained for further study.

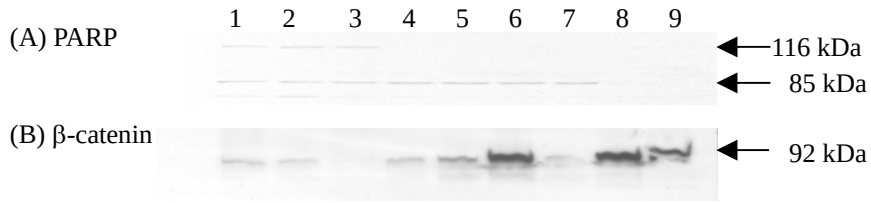

Fig. 4. Western blot analysis of PARP and β-catenin in the liver homogenates of fetal, postnatal, and lactational offsprings of dams gestationally and lactationally exposed to TCDD. Each lane contained 45 μg microsomal protein. Lanes corresponding to offsprings of TCDD-exposed dams and the levels of TCDD treated are as follows. Lanes 1-3. Fetal-0, 1, 10; Lanes 4-6, Postnatal-0, 1, 10; Lanes 7-9, lactational-0, 1, 10 ng TCDD/kg B.W/Day

Acknowledgments

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