HYDRONEPHROSIS AT WEANING, NOT DURING GESTATION, IS CAUSED BY LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN IN HOLTZMAN RATS

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Introduction

Dioxin and dioxin-like polychlorinated biphenyls (PCBs) are ubiquitously present in the environment, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic compound among this large group of dioxin congeners. TCDD induces a variety of toxic responses, including reproductive toxicity, teratogenicity, carcinogenicity and immunotoxicity (1, 2). Regarding teratogenicity, cleft palate and hydoronephrosis are the two major forms of abnormal morphogenesis that have been shown to occur in fetuses administered to a relatively high dose of TCDD (40 µg/kg TCDD) during pregnancy (3, 4). Using AhR-null mice Mimura and coworkers showed that the occurrence of cleft palate and hydronephrosis found in gestational day (GD) 12.5 of TCDD-exposed (40 µg/kg TCDD) dams depends, in complete and incomplete fashions, respectively, upon AhR-mediated mechanism (5). We have incidentally found that pups from Holtzman rats exposed to TCDD in utero and lactationally exhibited abnormally high incidence of hydronephrosis, not cleft palate, when they reached weaning. This observation prompted us to investigate the critical period of TCDD exposure for the occurrence of hydronephrosis in TCDD-exposed pups.

Adopting cross-fostering experimental protocol, we here report that lactational exposure to TCDD caused the hydronephrosis in the neonatal rat during organogenesis of the kidney. We also determine a possible involvement of the

arylhydrocarbon receptor (AhR) in the developmental of hydronephrosis that occurred at the weaning.

Materials and Methods

Animals and treatment:

In cross-fostering experiment, pregnant Holtzman rats (6 per group) were given an oral dose of 1000 ng TCDD/kg bw on GD 15, or an equivalent volume of corn oil as vehicle-control. Pregnant rats were allowed to deliver. Litters were then assigned to the following four groups on postnatal day (PND) 1: C/C (control), T/C (prenatal exposure only), C/T (postnatal exposure only) and T/T (perinatal exposure).

For AhR-null mouse experiment, male and female AhR heterozygous (AhR+/-) mice were mated to produce offspring having three AhR genotypes: wild-type (AhR+/+), AhR+/- and AhR-null (AhR-/-). Pregnant mice were dosed with 10 µg TCDD/kg bw by gavage on GD 12.

Sample collection and processing:

In the cross-fostering experiment, six rat pups (one per litter) of each group were randomly selected and used. On PND 21 body weight and organ weight of each pup were measured, followed by collection of sera and tissues under light ethyl ether anesthesia. Portions of the liver were immediately frozen in liquid N_2 and stored at -80°C until mRNA measurement. Liver and kidney were fixed in Zamboni's fixative and embedded in paraffin.

Immunohistochemical detection of CYP1A1:

CYP1A1 in the liver and kidney were visualized according to the method described in our previous paper (6).

RNA Extraction and RT-PCR:

Total RNAs in the tissues was extracted by Isogen (Nippon Gene, Tokyo, Japan). Expression of CYP1A1, CYP1A2, AhR, UGT1A6 and β-actin was determined by reverse transcription and polymerase chain reaction (RT-PCR) using PCR primers for amplification as previously described (7). PCR products were detected as a single band on 1.5% agarose gel in 1x TBE containing 2 µg/ml of ethidium bromide. Band intensity was quantified by EDAS120 system ver.2.02 (Kodak).

TCDD analysis:

Liver, kidney and serum from male pups (PND 21), and milk collected from the pup stomach (PND 1) were analyzed for TCDD concentration using high resolution GC/MS (8).

Results and Discussion

Incidence of hydronephrosis among the cross-fostered Holtzman rats was investigated in pups at the weaning (PND 21). Hydronephrosis was detected in the C/T, and T/T groups with high incidence, 25% and 33%, respectively, while no incidence of hydronephrosis was observed in pups from the C/C and T/C groups. In the cross-fostered pups, serum and liver in the C/T and T/T groups on PND 21 showed 10 to 20-fold, and 25 to 40-fold higher concentrations of TCDD, respectively as compared to the T/C group. Renal concentrations of TCDD exhibited almost the same tendency as those observed in the serum.

In order to study localization of TCDD within the renal tissues, we dissected the kidney into three portions, the cortex, outer medulla, and inner medulla, and analyzed them for TCDD amounts by GC/MS, which showed that TCDD distributed equally within the whole kidney. We also quantified the TCDD concentrations in milk remaining in the pup stomach on PND 1, and found that breast milk from the perinatal and postnatal exposure groups contained a large amount of TCDD compared to the control and prenatal exposure groups which contained trace amount of TCDD.

The RT-PCR analysis of the renal CYP1A1 mRNA showed that it was greatly induced in the C/T and T/T groups in contrast to nearly no expression in the C/C and T/C groups. Interestingly, immunohistochemical examinations revealed CYP1A1 was induced and localized in a very limited area of the kidney from the C/T and T/T groups: strong cytoplasmic immunostaining for CYP1A1 was found to localize only in the ascending Henle's loop in spite of equal distribution of TCDD in kidney as described above.

Investigating a possible involvement of the AhR in the production of hydronephrosis of the neonates, we confirmed that AhR-null mice pups did not show any incidence of hydronephrosis but that the corresponding values for AhR-heterozygotes (AhR+/-) was increased to 37.5 %.

The present results led us to conclude that a primary route of exposure to TCDD responsible for the renal developmental malformation in neonates is lactation, and that this teratogenicity is mediated by AhR.

Despite the even distribution of TCDD in the renal tissue, region-specific expression of AhR and CYP1A1 in the developing kidney may suggest that AhR-dependent genes or CYP1A1-related cellular events might be involved in the occurrence of hydronephrosis. How hydronephrosis is caused by TCDD and/or other yet-unknown factors warrants for further study.

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