ALTERATIONS OF GENE EXPRESSION DURING DEVELOPMENT OF HYDRONEPHROSIS CAUSED BY LACTATIONAL EXPOSURE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN THE NEONATE OF RATS

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Introduction

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces a variety of toxic responses, including reproductive toxicity, teratogenicity, carcinogenicity and immunotoxicity. Cleft palate and hydronephrosis are the two typical features of terata, and hydronephrosis is induced by approximately more than 10 times lower dose of TCDD than cleft palate and is a sensitive indicator of TCDD teratogenicity. Since investigations on hydronephrosis by TCDD were reported to initiate nearly two decades ago[1], the TCDD-induced hydronephrosis as well as other TCDD toxicities was found to depend upon aryl hydrocarbon receptor (AhR). There are conflicting results with regard to the etiology of TCDD-induced hydronephrosis. Abbot et al. [1,2] reported that TCDD-induced hydronephrosis in the fetal mouse kidney was accompanied with hyperplasia of the ureteric luminal epithelium as a consequence of the uncontrolled proliferation of the ureteric epithelial cells due to unbalanced expressed levels of epidermal growth factor and transforming growth factor, and speculated that occlusion of the ureteric lumen resulted in dilation of the renal pelvis due to obstructed outflow of urine. On the other hand, no microscopic difference in ureteral morphology such as ureteric epithelium has been shown following TCDD exposure[3]. Additionally, the molecular mechanism for pathogenesis of this disease condition is largely unknown. Thus, our objective was to evaluate whether hydronephrosis could be induced lactationally in a cross-fostering experiment. We also assessed alteration of gene expression during the development of hydronephrosis in TCDD-exposed rat kidney.

Materials and Methods

Animals and Treatments: By gavage, pregnant rats (six per group) were administered once with corn oil (vehicle) or 1.0 μg TCDD/kg bw on GD 15. The pups were divided into four experimental groups: (1) pups not exposed by either route (C/C group) , (2) pups exposed only *in utero* (T/C group), (3) pups exposed only lactationally (C/T group), and (4) pups exposed by both routes (T/T group). In groups (2) and (4), the initial TCDD exposure of pups occurred late in gestation and continued through weaning, and group (4) had an additional exposure to TCDD via lactation. On PND 21, liver and kidney tissues were excised from male and female pups anesthetized mildly with diethyl ether. For a comparative study, 10-week-old male Holtzman rats were administered TCDD at a single oral dose of 1.0 μg/kg bw and sacrificed under diethyl ether anesthesia for the collection of the liver and kidney tissues.

RNA Extraction and Microarray: For global gene expression analysis, kidneys (right) were collected from three male pups each of the C/C group and T/T group. The total RNA was subjected to global gene expression analysis by using Rat Oligo DNA Microarray (G4130A, Agilent Technologies). After hybridization, microarray was scanned by

DNA Microarray Scanner and the scanned data was analyzed with a software (Agilent Technologies). The relative expression level of the T/T group vs. the C/C group was calculated in the present study.

RNA extraction and semiquantitative reverse transcriptase–polymerase chain reaction: The sequences of PCR primers for the amplification of CYP1A1 and β-actin are described in our previous paper[4]. The semiquantitative RT-PCR analysis of CYP1A1 and β-actin in kidney tissues was performed by essentially the same method as that described in our previous paper[4].

Immunohistochemistry: CYP1A1 and PCNA in the liver and kidney was immunostained according to a method as described previously [4]. Negative controls, in which the primary antibody was replaced with normal rabbit IgG, did not show nonspecific staining.

TCDD Analysis: To determine TCDD in different parts of the kidney (cortex, the outer zone of the medulla and the inner zone of the medulla), kidney tissues were sliced into three parts using a razor knife. They were subjected to TCDD determination using a high-resolution gas-chromatograph equipped with a high-resolution mass-spectrometer as described in our previous paper[5].

Results

Incidence and histopathology of hydronephrosis: Whereas no signs of hydronephrosis were observed in the C/C or T/C groups, hydronephrosis with an advanced stage (Fig. 1) was found in the C/T and T/T groups of rats on PND21; that is, 32% (7 of 22 pups) and 30% (6 of 20 pups), respectively.

Immunostaining of CYP1A1 in kidney of pups on PND21: In the kidney of pups on PND21, almost no immunostaining of CYP1A1 was observed in the entire kidney of pups exposed to TCDD *in utero* only (Fig. 2B) and those of pups administered with corn oil (Fig. 2A). In contrast, the immunostaining of CYP1A1 was confined in the outer zone of the medulla from all the pups that were exposed to TCDD via lactation only (Fig. 2C) and via both *in utero* and lactation (Fig. 2D). All the tissue specimens, 12 each, from the two lactationally-exposed groups showed the immunostaining. The observation on the immunostaining of CYP1A1 in the kidney from the cross-fostered pups was consistent with the CYP1A1 mRNA level as determined by semiquantitative RT-PCR analysis. The two groups of both male and female pups exposed to via lactation only or both lactation and *in utero* showed markedly induced CYP1A1 mRNA, whereas no detectable levels of CYP1A1 mRNA were observed in pups exposed to TCDD *in utero* only and in those that were not experimentally exposed to TCDD. To answer the question of whether the occurrence of CYP1A1 in the kidney is developmental-stage-related, we examined the localization of CYP1A1 in 10-week-old rats 7 days after TCDD administration. Although the immunostaining of CYP1A1 was detected in the area around the centrilobular vein in the liver upon TCDD administration, it was not found in any region of the kidney.

PCNA immunostaining in the developing rat kidney on PND21: It has been established that the rodent kidney is at a developmental stage from the latter stage of gestation to the newborn stage. By the immunohistochemical localization of PCNA, we confirmed that PCNA was predominantly localized in the medulla of kidney from pups on PND21 regardless of TCDD treatment both *in utero* and via lactation.

Altered gene expressions in the kidney of pups exposed to TCDD: To obtain global gene expression profile for a screening purpose, we have performed microarray analysis on the kidney tissues of pups whose dams were exposed

to vehicle only or TCDD during gestation and lactation. Twenty five up-regulated genes were found to include xenobiotic enzymes (CYP1a1 and CYP2a10), inflammation (prostaglandin I2 synthase and coagulation factor 2), transporters (K+ voltage-gated channel, subfamily G, member 1channel mRNA and Vamp2), tooth morphogenesis (ameloblastin) and signal transductions (catechol-O-methyltransferase, serine/threonine phosphatase 7, FK-binding protein, uroplakin 3A, G-protein coupled receptor 65, ionotoropic glutamate receptor (AMPA3). Of 26 down-regulated genes, possible functions of 12 genes were assigned from the NCBI database. There are some transporter genes (nongastric type H+/K+ ATPase (Atp12a) and solute carrier family 7 (cationic amino acid transporter, y+ system) member 10 (Slc7a10)), signal transduction related proteins (galanin), immune and stress-related functions (2'-5' oligoadenylate synthetase 1I (Oas1) and interferon stimulated g (Isg12)), and cancer antigen (UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase 2).Functions of 14 genes did not have distinct known functions.

Distribution of TCDD in kidney of pups on PND21: We determined TCDD concentration by high-resolution gas chromatography in combination with high-resolution mass-spectroscopy (Table 1). No significant differences in TCDD concentration among the three regions of the kidney, namely, the cortex and the outer and inner zones of the medulla, were found in each of the three groups of pups.

Discussion

In the present study, we showed that lactational exposure to TCDD is primarily responsible for the occurrence of hydronephrosis in rat neonates, which is consistent with the results of a similar study in mice[3]. Based on the findings that TCDD exposure did not cause morphological abnormalities in the kidney, it seems unlikely that morphological abnormality of the ureter can account fully for TCDD-induced hydronephrosis of rat pups. However, the most intriguing aspect of the present study is that TCDD-inducible genes mediated via an AhR-dependent mechanism may be associated with the etiology of hydronephrosis in a particular region of the kidney. We found that the immunostaining of CYP1A1 was confined to the thick ascending limb of Henle's loop in the outer zone of the medulla of the kidney. One could easily speculate that TCDD is distributed unevenly in the kidney with the highest concentration in the outer zone of the medulla, but the finding of similar TCDD concentrations among the three different regions of the kidney precludes this possibility. Thus, it is considered that CYP1A1 induction by TCDD in the kidney is region-specific with the kidney development, and that the region that includes the thick ascending limb of Henle's loop may be very sensitive to TCDD exposure. The rodent kidney is still at the developmental stage from the latter stage of gestation to the neonatal stage, as clearly shown by PCNA staining particularly in the medulla. In the present microarray analysis, the ion channel-related genes were observed to be affected by TCDD during the development of hydronephrosis, suggesting a functional disorder of ion channel by TCDD could participate with development of hydronephrosis. As in support of our hypothesis, Takahashi *et al* showed that NKCC2 gene-deficient mice exhibited hydronephrosis and polyuria resulting from an impaired reabsorption of NaCl in the distal tubule[6]. Additionally, hydronephrosis characterized by an enoumously dialated renal pelvis was reported to occur in the ROMK-deficient mice[7]. Although the present microarray results may provide a clue to solve the etiology of TCDD-induced hydronephrosis, a more intensive studies remain to be performed in the future studies.

References

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Table1. TCDD concentrations in various parts of kidney of pups exposed to TCDD

Treatment Type *In utero*/ Lactation	Sex	TCDD Concentration (pg/g tissue)		
		Cortex	Medulla	
			Outer Zone	Inner Zone
Control/Control	Male	N.D.	N.D.	N.D.
	Female	N.D.	N.D.	N.D.
Control/TCDD	Male	4.6	5.2	N.D.
	Female	7.0	6.7	7.3
TCDD/Control	Male	42	47	51
	Female	36	29	45
TCDD/TCDD	Male	60	72	73
	Female	54	43	66

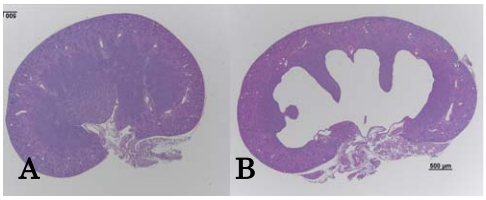

Fig.1. The morphological comparison between oil-(A) and TCDD-(B) treated rat kidneys on PND 21, showing a characteristic hydronephrosis with the pronounced pelvic dilation and the compressed renal parenchyma by TCDD exposure

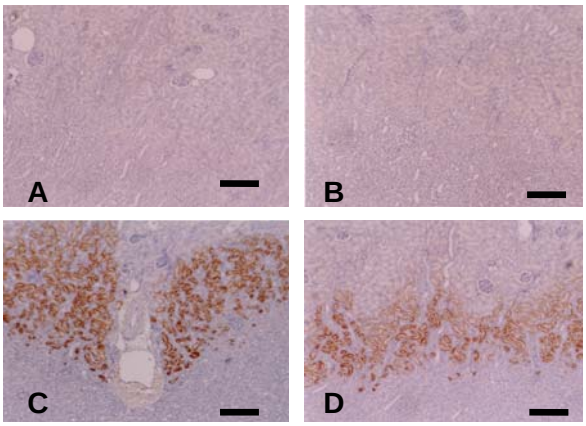

Fig. 2. Immunohistochemical localization of CYP1A1 in the kidney on postnatal day 21 from 4 groups of pups born to dams exposed to (A) corn oil (vehicle), or (B) TCDD via *in utero* only, (C) via lactation only and (D) via both *in utero* and lactation. Bar = 200 (m