STUDIES ON THE MOLECULAR MECHANISMS OF 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN–INDUCED MORPHOLOGICAL ABNORMALITIES IN THE DEVELOPING MOUSE KIDNEY

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Abstract

The developing rodent kidney is one of the most sensitive tissues to the toxic effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The aim of the present study was to evaluate the etiology of hydronephrosis by focusing on the mechanisms of TCDD-induced morphological abnormalities in mouse pup kidneys through measuring gene expression of several factors involved in nephrogenesis. Mouse neonates were exposed to TCDD through milk of dams that had received 15 μg TCDD/kg body weight orally after delivery. In this time-course study, the critical period of susceptibility for development of hydronephrosis was postnatal days (PND) 1–4. In the vehicle-treated mice, mRNAs for insulin-like growth factor (IGF)II, and transforming growth factor (TGF)-β were expressed at maximum levels for the first 2 days after birth and gradually decreased with advancing development. TCDD was found to cause up-regulation of TGF-β expression and down-regulation of IGFII in the early postnatal period. Furthermore, TCDD significantly up-regulated the cyclin-dependent kinase inhibitors $p27^{kip1}$ and $p57^{kip2}$ in developing kidneys. Studies using AhR-null mice showed that the up-regulation of $p57^{kip2}$ mRNA was AhR dependent. Taken together, these data suggest that the hydronephrotic lesion caused by TCDD could be mediated by TCDD-induced G_1 cell cycle arrest through mechanisms involving the AhR.

Introduction

Dioxins induce a wide variety of toxic, carcinogenic, and teratogenic effects mediated by the aryl hydrocarbon receptor (AhR), which is a transcriptional regulator with functions beyond xenobiotic detoxification[1]. The kidney is composed of two embryologically distinct tissues: the nephrons, which are derived from undifferentiated mesenchyme, and the collecting duct system, which is derived from the ureteric bud. Nephrogenesis proceeds by mesenchymal to epithelial transition (MET). AhR is involved in MET regulation during nephrogenesis and is an essential protein in renal development. Morphological and functional maturation of the kidney is thought to terminate during the first 2 weeks after birth in rodents. Many transcriptional and growth factors, cytokines, and signaling events are involved in this biological process. Hydronephrosis is one of the most sensitive indicators of TCDD teratogenicity in mice, and postnatal day

(PND) 1 is the peak postnatal period of susceptibility for development of hydronephrosis in mice lactationally exposed to TCDD[2]. A difference in growth rates between the renal papillae and the parenchyma is suggested to be responsible for hydronephrosis[3]. Based on the morphological features of hydronephrosis, with aberrant nephrogenesis characterized by marked pelvic dilation and progressive attenuation of the renal papilla and medulla, TCDD may act by disrupting the integration of apoptosis and cell cycle progression during nephrogenesis in the early postnatal period. The aim of the present study was to elucidate hydronephrotic response caused by TCDD at the molecular level by evaluating the expression of candidate genes involved in nephrogenesis.

Materials and Methods

Animals and treatments

C57 Bl/6J mice and AhR-heterozygous (AhR+/-) mice, a kind gift fron Prof. Yoshiaki Fujii-Kuriyama, University of Tsukuba, received food and distilled water *ad libitum* and were handled in accordance with the National Institute for Environmental Studies Animal Care Committee guidelines. After spontaneous delivery, dams were given 15 μg TCDD/kg body weight orally or an equivalent volume of corn oil as vehicle on PND 1, and pups were exposed to TCDD via lactation. Kidneys from pups were collected on PNDs 2, 3, 4, 5, 7, and 14.

Real-time reverse-transcriptase polymerase chain reaction (RT-PCR)

Total RNA was isolated from the mouse pup kidneys using TRIsol reagent, and cDNA was synthesized. The mRNA of various genes including insulin-like growth factor(IGF) II, Wilms' tumor suppressor (WT1), AhR, transforming growth factor-β (TGF-β), and cyclin-dependent kinase (CDK) inhibitors $p27^{kip1}$ and $p57^{kip2}$ was determined with a LightCycler (Roche Diagnostics, Mannheim, Germany) using Fast Real-Time SYBR Green PCR Kit (Qiagen, Valencia, CA) according to the manufacturers' instructions.

Immunohistochemistry of the kidney

Zamboni's solution–fixed and paraffin-embedded tissues were subjected to routine sectioning of 3 μm thickness for immunohistochemistry and histopathology. Single-stranded DNA, WT1, IGFII, TGF-β, $p27^{kip1}$, and $p57^{kip2}$ were each identified using polyclonal antibody. The antibody was then detected using biotinylated secondary reagents with standard avidin–biotin complex staining according to the method described by Nishimura et al.[4] Proliferating cell nuclear antigen (PCNA) in the kidney was stained in tissue sections by an indirect immunohistochemical technique described earlier[3].

Statistical analysis

StatView for Windows (Version 5.0, SAS Institute, Cary, NC, USA) was used for statistical analysis. Data are

expressed as mean ± SE. Differences in means among the groups were analyzed by one-way analysis of variance (ANOVA). P values less than 0.05 were considered statistically significant.

Results

Effects of TCDD on expression of TGF-β, IGF2, $p27^{kip1}$, and $p57^{kip2}$ in developing mouse kidneys

The time course of renal expression of TGF-β and IGFII mRNA levels in the control revealed the highest expression around the time of birth. Expression declined gradually as development proceeded. Exposure to TCDD caused a significant up-regulation of TGF-β as well as down-regulation of IGFII mRNA levels in the earlier period of development.

Whereas TCDD treatment induced significant up-regulation of the $p27^{kip1}$ mRNA level in mouse kidneys on PND 7, $p57^{kip2}$ mRNA was significantly up-regulated on PNDs 3 and 7 in TCDD-exposed fetuses compared to controls.

Immunohistologic examinations of the kidney

Immunohistochemical studies demonstrated strong immunoreactivity for TGF-β localized in both the outer medulla and the outer renal papilla during the first 2 days after birth in the vehicle-treated mouse kidneys. The TGF-β immunoreactivity was characterized by intense staining at basolateral sites of the distal tubular cells. This immunoreactivity became weaker with time and localization gradually proceeded in an ascending manner. Interestingly, among epithelial cells of cross-sections in the same tubule, stained and unstained cells co-existed. Intense immunoreactivity for IGFII was localized in the outer medulla and the outer papilla of control mice on PND 1 with staining at basolateral sites (similar to TGF-β) that rapidly declined as the organ matured. By PND 7, immunoreactivity remained only in the outermost part of the outer medulla close to the inner cortex. Numerous PCNA-positive nuclei were found in the outer medulla and the outer renal papilla, including mesenchymal cells, as well as in the cortical labyrinth during PND 1 to 4 in vehicle-treated mice. However, treatment with TCDD resulted in a decrease in both the number of stained cells and the IGFII staining intensity in contrast to an increase in both staining intensity and the number of TGF-β stained cells in the distal tubule kidney sections. The $p27^{kip1}$ and $p57^{kip2}$ positive cells were distributed in the outer medulla and the renal papilla. Treatment with TCDD significantly increased the number of stained cells as well as the intensity of the staining for the $p27^{kip1}$ and $p57^{kip2}$ CDK inhibitors.

Discussion

TCDD is well known to readily induce hydronephrosis in the rodent fetus or neonate by placental or lactational exposure. We showed that this hydronephrotic response via lactational exposure to TCDD developed during PND 1–4 and peaked on PND 1. Extensive loss of the medulla is a characteristic feature of hydronephrosis, suggesting that the medullary regions may be particularly susceptible to toxic insult by TCDD in the early

developmental period, and that inhibition of cell proliferation and differentiation in this region may be involved in the etiology of hydronephrosis. PCNA-positive nuclei were found in the outer medulla and the outer renal papilla, including mesenchymal cells, as well as in the cortical labyrinth during PND 1 to 4 in vehicle-treated mice. IGFII, the stimulator for cell proliferation, and TGF-β, the inhibitor for cell cycle, regulate morphogenesis, differentiation, proliferation, and adhesion. Proliferation requires normal progression through the cell cycle, and cell cycle progression is controlled by positive (cyclins and CDKs) and negative (CDK inhibitors) regulatory proteins. Since TCDD has been reported to inhibit proliferation and induce cell cycle arrest, we examined effects of TCDD on gene expression of TGF-β, IGFII, and CDK inhibitors $p27^{kip1}$ and $p57^{kip2}$ in the early postnatal period. Exposure to TCDD resulted in a marked up-regulation of $p57^{kip2}$ mRNA levels on PND 3 and 7 as well as $p27^{kip1}$ expression on PND 7. Furthermore, up-regulation of $p57^{kip2}$, but not $p27^{kip1}$, was confirmed to be AhR-mediated using AhR-null mice. TCDD treatment induced a marked increase in TGF-β expression as well as a significant down-regulation of IGF2 in the early developmental period. Immunohistochemical examination for those proteins supported the RT-PCR analysis. The present studies with RT-PCR analysis showed TCDD significantly up-regulated CDK inhibitors, especially $p57^{kip2}$ in the early postnatal period. Immunohistochemical examination for those proteins supported the RT-PCR analysis. Immunohistochemistry also showed strongly increased renal TGF-β mRNA abundance in the outer medulla and the outer renal papilla, which are main sites of cell proliferation and differentiation in the developing kidney. The present findings suggest that hydronephrosis caused by TCDD treatment is due to TCDD-induced G_1 cell cycle arrest via $p57^{kip2}$ up-regulation, which appears to be AhR-mediated.

References

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