UP-REGULATION OF 25-HYDROXYVITAMIN D_3 1α-HYDROXYLASE AND DISRUPTION OF CALCIUM METABOLISM BY 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN IN THE DEVELOPING MOUSE KIDNEY

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Abstract

The present studies were performed to elucidate the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on vitamin D metabolism and on calcium (Ca) homeostasis in developing mouse kidney. Mouse neonates were exposed to TCDD through milk of dams that were given 15 μg TCDD/kg body weight orally and humanely killed on postnatal day (PND) 7, 14, and 21. Reverse transcriptase-polymerase chain reaction revealed a marked induction of 25-hydroxyvitamin D_3 1α-hydroxylase (CYP 27b1) mRNA on PNDs 14 and 21 in mouse kidney by TCDD exposure. Immunohistochemical studies demonstrated that TCDD exposure resulted in an increased immunoreactivity of the CYP 27b1 protein both in the distal tubule and the proximal tubule. TCDD down-regulated calbindin protein and mRNA as well as decreased expression of Na^+-Ca^{2+} exchanger (NCX) mRNA in early postnatal days. Although urinary Ca excretion was significantly increased in mice exposed to TCDD, serum Ca concentrations were almost the same as those in controls. The findings illustrated that TCDD disrupts vitamin D metabolism and transport of Ca^{2+} in the developing mouse kidney.

Introduction

Dioxins are highly toxic and stable environmental pollutants known to induce a wide range of toxic effects in rodents. Bone toxicity is one of the most sensitive indicators for dioxin toxicity during development, including bone geometry, bone mineral density, and mechanical properties.[1] However, the molecular mechanisms underlying dioxin toxicity on bone are not fully understood. The secosteroid hormone 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) has a critical role in calcium (Ca) homeostasis and normal bone growth and is essential for cellular differentiation. Both the synthesis and degradation of 1,25-$(OH)_2D_3$ are tightly regulated by many factors such as Ca, hormones, and 1,25-$(OH)_2D_3$ itself to regulate mineral homeostasis.[2]

1,25-$(OH)_2D_3$ is the most active metabolite of vitamin D and is produced by two sequential hydroxylations of vitamin D by 25-hydroxylase in the liver and by 25-hydroxyvitamin D_3 1α-hydroxylase (CYP 27b1) in the

kidney. CYP 24a1 is responsible for the catabolic breakdown of 1,25-$(OH)_2D_3$. Intestine, bone, and kidney are the three major target tissues involved in the regulation of mineral homeostasis by 1,25-$(OH)_2D_3$. The important endocrine system regulating circulating Ca concentrations involves vitamin D. Despite numerous studies providing evidence for an important role of 1,25-$(OH)_2D_3$ in bone metabolism, no information is available on the effects of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) on vitamin D metabolism nor on Ca homeostasis in kidney. In this study, we investigated whether TCDD elicited disruptive effects on vitamin D metabolism and on Ca homeostasis in developing mouse kidney.

Materials and Methods

Animals and treatments

C57 Bl/6J mice received food and distilled water *ad libitum* and were handled with humane care under the guidelines for animal experiments at the National Institute for Environmental Studies (NIES; Tsukuba, Japan). After spontaneous delivery, dams were given 15 μg TCDD/kg body weight orally or an equivalent volume of corn oil as vehicle on postnatal day (PND) 1, and pups were exposed to TCDD via lactation. Serum and kidneys from pups were collected on PNDs 7, 14, and 21 and subjected to analyses.

Serum analysis

Serum concentrations of 1,25-$(OH)_2D_3$ were analyzed by enzyme immunoassay (Immundiagnostik, Bensheim, Germany) and phosphorus and Ca were measured by the Spotchem analyzer (Arkray, Kyoto, Japan) according to the manufacturer's instructions.

Urinalysis

Urine specimens were collected from TCDD-exposed 5-week-old mice housed in metabolic cages, and levels of Ca and phosphorus were determined by inductively coupled plasma-mass spectrometry (ICP-MS) according to the method described in our previous paper.[3]

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

Total RNA was isolated from the kidney using TRIsol reagent, and cDNA was synthesized. mRNA of various genes including calbindin, transient receptor potentiation cation channel subfamily V, member 5 (TRPV5), Na^+-Ca^{2+} exchanger (NCX1), plasma membrane calcium ATPase (PMCA1b), cytochrome P450 (CYP) 27b1, CYP 24 a1, CYP 1a1, and vitamin D receptor (VDR) were determined with a LightCycler (Roche Diagnostics, Mannheim, Germany) using Fast Real-Time SYBR Green PCR Kit (Qiagen, Valencia, CA) according to the manufacturer's 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. CYP 1A1, calbindin D, and CYP 27b1 proteins in kidney were identified using each polyclonal antibody. The antibody was then detected using biotinylated secondary reagents

with standard Avidin-biotin complex (ABC) staining according to the method as described earlier by Nishimura.[4]

Statistical analysis

StatView for Windows (Version 5.0, SAS Institute, Cary, NC, USA) was used for statistical analysis. Data are expressed as mean ± SD. Differences in means among the three groups were analyzed by one-way analysis of variance (ANOVA).

Results

Effects of TCDD on CYP 27b1 and CYP 24a1 mRNA expression levels of developing mouse kidneys

CYP 27b1 mRNA levels in oil-treated controls remained constant throughout the entire course of the experiment. Exposure to TCDD induced a dramatic up-regulation of CYP 27b1, 4- to 5-fold higher than in the corn oil control, in mouse kidney on PNDs 14 and 21. On the other hand, TCDD treatment resulted in up-regulation of CYP 24a1 mRNA significantly only on PND 14 compared with the corn oil control.

Effects of TCDD on the expression of genes involved in Ca transport of mouse kidneys

The active transcellular Ca transport in the kidney occurs through a three-step process consisting of Ca entry, intracellular Ca transport, and Ca extrusion. 1,25-$(OH)_2D_3$ stimulates the individual steps of transcellular Ca transport by increasing the expression levels of genes involved. Whereas TCDD did not affect PMCA1b and TRPV5 at mRNA levels at any time point examined after birth, TCDD significantly down-regulated gene expression of both calbindin D_{28K} and NCX1 on PND 7.

Ca levels in serum and urine

Five-week-old mice were housed in metabolic cages for 24 hours, and urine samples were collected. Despite the fact that Ca was significantly excreted into urine, the serum level of Ca^{2+} was unchanged by TCDD exposure.

Immunohistologic examinations of the kidney

Although weak immunoreactivity for CYP 27b1 was observed in both the proximal and distal tubular cells in oil-treated mouse kidneys, strongly increased immunoreactivity was observed in the proximal tubular cells and the distal tubules in mice exposed to TCDD. Calbindin is strongly expressed in renal tubular epithelial cells of the convoluted distal tubules in oil-treated mice. However, treatment with TCDD resulted in a decrease in the number of stained cells as well as the staining intensity for calbindin in the distal tubule kidney sections from 7-day-old pups.

Effect of TCDD on serum 1,25-$(OH)_2D_3$ levels

Circulating levels of serum 1,25-$(OH)_2D_3$ were determined in 21-day-old male pups. TCDD treatment induced a 2-fold increase in serum 1,25-$(OH)_2D_3$ levels compared to those of controls.

Discussion

The present studies with RT-PCR analysis clearly demonstrated that TCDD up-regulated CYP 27b1 mRNA

levels of kidneys in the early postnatal period. A marked increase in serum levels of 1,25-$(OH)_2D_3$ supported an enhancement of 1,25-$(OH)_2D_3$ synthesis in the kidney in response to TCDD. Immunohistochemical examination demonstrated that weak CYP 27b1 protein was widely, but weakly expressed both the proximal and distal tubules of control mouse kidneys. CYP 27b1 protein was strongly expressed in both the proximal and distal tubular cells within pup kidneys exposed to TCDD, suggesting these nephron segments that strongly expressed CYP 27b1 protein were responsible for the increased serum levels of 1,25-$(OH)_2D_3$ in TCDD-treated mice. The proximal tubules, but not in the distal tubules, was reported to be the principal site of synthesis of CYP 27b1 in vitamin D-deficient rats.[5] On the other hand, CYP 27b1 mRNA and protein were expressed strongly in the distal tubules in comparison with low-level expression in the proximal tubules in the normal human and mouse kidneys, indicating that the distal nephron is the predominant site of CYP 27b1 expression under conditions of vitamin D sufficiency.[6] The reasons for the apparent discrepancy in the site of synthesis of CYP 27b1 within the nephron segments between our studies and the earlier observations are unknown at this time. These discrepancies may have been caused by differences in responsiveness of kidneys to the toxicant. Nephron segment heterogeneity may also contribute to the disparity. Interestingly, TCDD exposure caused a significant increase in urinary excretion of Ca. Nevertheless, serum concentrations of Ca did not differ between TCDD-exposed and oil-treated control mice.

Based on the fact that TCDD showed an inhibitory effect on Ca reabsorption as indicated by down-regulation of expression of calbindin and NCX1, it is suggested that bone could, in part, be able to maintain circulating Ca concentration through bone resorption.

References

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