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Low-dose dioxins alter gene expression related to cholesterol biosynthesis, lipogenesis, and glucose metabolism through the aryl hydrocarbon receptor-mediated pathway in mouse liver

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a common environmental contaminant. TCDD binds and activates the transcription factor aryl hydrocarbon receptor (AHR), leading to adverse biological responses via the alteration of the expression of various AHR target genes. Although small amounts of TCDD are consumed via contaminated daily foodstuffs and environmental exposures, the effects of low-dose TCDD on gene expression in animal tissues have not been clarified, while a number of genes affected by high-dose TCDD were reported. In this study, we comprehensively analyzed gene expression profiles in livers of C57BL/6N mice that were orally administered relatively low doses of TCDD (5, 50, or 500 ng/kg body weight (bw) day^{-1}) for 18 days. The hepatic TCDD concentrations, measured by gas chromatography-mass spectrometry, were 1.2, 17, and 1063 pg toxicity equivalent quantity (TEQ)/g, respectively. The mRNA level of the cytochrome P450 CYP1A1 was significantly increased by treatment with only TCDD 500 ng/kg bw day^{-1}. DNA microarray and quantitative RT-PCR analyses revealed changes in the expression of genes involved in the circadian rhythm, cholesterol biosynthesis, fatty acid synthesis, and glucose metabolism in the liver with at all doses of TCDD employed. However, repression of expression of genes involved in energy metabolism was not observed in the livers of *Ahr*-null mice that were administered the same dose of TCDD. These results indicate that changes in gene expression by TCDD are mediated by AHR and that exposure to low-dose TCDD could affect energy metabolism via alterations of gene expression.

Keywords: Dioxin; Low dose; Mouse liver; DNA microarray

Introduction

Polycyclic aromatic hydrocarbons, including dioxins, benzo[*a*]pyrene, and coplanar polychlorinated biphenyls, are common environmental contaminants that elicit various adverse biological responses in humans and experimental animals. These compounds are ligands of the transcription factor aryl hydrocarbon receptor (AHR), which is a member of the basic helix-loop-helix (bHLH), Per-Arnt-Sim (PAS) family. In particular, as a result of chemical stability and high binding affinity, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), chronically modulates gene expression through AHR causing teratogenesis, immune suppression, reproductive disruption, hepatic injury, and carcinogenesis in experimental animals (Poland and Knutson, 1982; Hankinson,

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1995; Safe, 1995). Most of the TCDD-induced toxicity observed in wild-type mice is not observed in *Ahr*-null mice (Fernandez-Salguero et al., 1996; Mimura et al., 1997). Therefore, it appears that the first stage in the mechanism of TCDD-induced toxicity involves modulation of AHR-mediated gene expression.

The role of AHR in TCDD-induced gene expression was revealed in a study of the transcriptional activation of cytochrome P450 1A1 (*CYP1A1*), a gene encoding a phase I xenobiotic-metabolizing enzyme. AHR exists in the cytoplasm without a ligand and bound to chaperones such as HSP90 and ARA9. Upon binding with a ligand such as TCDD, AHR is translocated to the nucleus, where it dimerizes with its heterodimer partner, another PAS protein designated AHR nuclear transloactor (ARNT). The AHR-ARNT heterodimer binds to a DNA sequence termed XRE (xenobiotic response element) upstream of the *CYP1A1* gene (Fujisawa-Sehara et al., 1987; Denison et al., 1988, 1989; Hines et al., 1988) and induces gene transcription (Hankinson, 1995; Safe, 1995). In addition to transcriptional regulation via XRE, AHR interacts directly with transcription factors such as NF-κB (Tian et al., 1999; Kim et al., 2000) and estrogen receptor (Ohtake et al., 2003; Wormke et al., 2003) to stimulate or suppress the expression of target genes. Hence, liganded AHR directly or indirectly regulates the transcription of the many genes associated with xenobiotic metabolism, cell cycle control, development, and reproduction in an XRE-dependent or -independent manner.

A comprehensive analysis of gene expression has revealed various novel target genes of TCDD. Frueh et al. (2001) investigated approximately 1/3 of the genes expressed in human hepatoma HepG2 cells by cDNA microarray analysis and detected 112 genes that were up- or down-regulated after treatment with 10 nM TCDD for 18 h. In addition to experiments with cultured cells, novel target genes were screened using experimental animal models. Fletcher et al. (2005) analyzed the alteration of gene expression in rat liver at 6 h, 24 h, and 7 days after a single administration of 0.4 or 40 μg TCDD/kg body weight (bw). TCDD affected the expression of genes related to cholesterol metabolism and biosynthesis of bile acids. Boverhof et al. (2005) investigated TCDD-induced temporal and dose-response changes in hepatic gene expression and serum clinical chemistry. Through extensive gene expression analysis, they proposed a mechanism of hepatotoxicity that involves TCDD-modified fatty acid metabolism. In addition, a comprehensive study using serial analysis of gene expression revealed 346 transcripts that were altered in the mouse liver on the 7th day following a single dose of 20 μg TCDD/kg bw (Kurachi et al., 2002). These studies revealed that high TCDD doses affect the expression of a large number of the genes in cultured cells and liver of mice and rats. On the other hand, the effects of low doses of TCDD on gene expression in animal tissues have not been clarified although small amounts of TCDD are consumed via contaminated daily foodstuffs and environmental exposures.

In this study, to elucidate the effects of low-dose TCDD on gene expression, we investigated the alteration of gene expression in livers of mice administered a low-dose of TCDD (5, 50, or 500 ng/kg bw day^{-1}) without subchronic toxicity for 18 days, and determined the hepatic concentrations of TCDD using gas chromatography–mass spectrometry (GC/MS). We also compared the changes in gene expression between wild-type and *Ahr*-null mice to determine whether alterations in TCDD-induced gene expression was mediated by AHR. DNA microarray analysis and quantitative RT-PCR-based mRNA analysis revealed that low-dose TCDD modulated the expression of numerous genes apart from the xenobiotic response genes and those involved in circadian rhythm, cholesterol biosynthesis, fatty acid synthesis, and glucose metabolism.

Materials and methods

Materials. C57BL/6N mice were purchased from CLEA Japan Inc. (Tokyo, Japan). *Ahr*-null mice were described earlier (Fernandez-Salguero et al., 1995). 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

TCDD treatment. C57BL/6N wild-type and *Ahr*-null male mice, aged 6–7 weeks, were orally administered by gavage at the dose of 5, 50, or 500 ng TCDD/kg bw in corn oil, once a day for 18 days. Animals in the control groups were administered the same volume of corn oil without TCDD. The animals were housed in individual cages, each with a wire-mesh floor, at 23±2 °C under a 12:12 h light:dark cycle (lights switched on at 8:00 AM) in plastic chambers installed with HEPA filters. Throughout the experimental term, the animals had free access to AIN93G standard diets (Reeves et al., 1993) and desalted water. On the 19th day, the experimental animals were killed and livers were removed, quick frozen in liquid N_2 and stored at −80 °C until analyses.

The experimental plan of the present study was approved by the Animal Research–Animal Care Committee of the Graduate School of Agricultural Science, Tohoku University. The experiments were performed under the guidelines framed by this committee in accordance with the Japanese governmental legislation (1980). The same committee supervised the care and use of the mice in this study.

Gas chromatography–mass spectrometry (GC/MS) analysis. Mouse liver samples were spiked with [^{13}C] 2,3,7,8-TCDD as an internal standard and digested in 2.0 M potassium hydroxide solution. The digested material was extracted with *n*-hexane, and the extract was washed with concentrated sulfuric acid. The *n*-hexane layer was concentrated and sequentially subjected to silica gel, alumina, and active carbon-impregnated silica gel column chromatography. The GC/MS analysis was performed in the selected ion mode with a JMS 700D (JEOL, Akishima, Japan) coupled to an HP 6890 gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) with a DB-5MS column (Agilent Technologies, Palo Alto, CA, USA).

RNA preparation and DNA microarray experiments. Total RNA was isolated from the livers of experimental animals with the phenol/guanidine-isothiocyanate-based reagent, Isogen (Nippon Gene, Toyama, Japan), as detailed in the instruction manual. In each group, equivalent amounts of RNA from individual animals were pooled and RNA prepared, amplified and labeled with Cy3-dCTP or Cy5-dCTP (Perkin Elmer, Boston, MA, USA) by use of the Agilent Low Input Linear Amplification kit, according to the instruction manual (Agilent Technologies, Santa Clara, CA, USA). Following labeling and purification, Cy3- and Cy5-labeled cRNA were quantified and combined for hybridization. The Cy5-labeled cRNA of each of the experimental and control groups and the Cy3-labeled cRNA of the control group were competitively hybridized to a 44K Whole Mouse Genome Microarray slide (Agilent Technologies). After washing, the microarrays were imaged using an Agilent DNA Microarray Scanner, and the images were extracted with Agilent Feature Extraction version A7.5.1. The signal ratio (Cy5/Cy3) of each probe in the experimental groups was normalized based on the Cy5/Cy3 signal ratio obtained from competitive hybridization using RNA from the control group.

Quantitative RT-PCR experiments. The total RNA from the liver was used as a template for cDNA synthesis using Superscript III reverse transcriptase (Invitrogen, Carlsbad, CA, USA). An aliquot of synthesized cDNA was used as the template for quantitative RT-PCR using an Applied Biosystems 7300