LACK OF THYROXINE AND RETINOID METABOLIC RESPONSE TO 2,3,7,8-TETRACHLORODIBENZO-*P*-DIOXIN IN ARYLHYDROCARBON RECEPTOR -NULL MICE BUT NOT IN TRANSTHYRETIN-NULL MICE

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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 potent isomer among the large dioxin family of compounds. In addition to a wide variety of toxicities of TCDD, such as reproductive, immunological, neurobehavioral toxicities, teratogenicity and carcinogenicity, the alteration of thyroid hormone metabolism and function by TCDD is a major concern not only for laboratory animals but also for human populations because thyroid hormone is required for brain development, neuronal maintenance as well as metabolic function during fetal and early neonatal period.

The most consistent effect of TCDD exposure is a marked decrease in serum levels of thyroxin (T4) in mammals. We previously reported that a single oral dose of TCDD to rats caused a significant decrease in serum levels of T4 with concomitant increase in thyroid stimulating hormone (TSH) levels (1). Induction of cytochrome P4501A1 (CYP1A1) and UDP-glucuronosyltransferase (UGT1) genes,genes, has been shown in many studies as characteristic response to TCDD. Induction of UGT1 is considered to play a key role in reduced circulating T4 levels in TCDD-exposed animals. UGT1, induced through an AhR-mediated mechanism (2), catalyzes T4-glucuronide formation and is likely to contribute enhanced biliary excretion of T4 glucuronide.

Another factor which is thought to be responsible for reducing circulating thyroxine concentrations particularly in rodents has been suggested to be transthyretin (TTR). Transthyretin, which has high affinity for the thyroid hormones T3 and T4 in blood, is widely accepted to function as a major transport protein of T4 in rodents. Episkopou et al (3) demonstrated that TTR-null mice showed severe reduction in T4 concentrations although the plasma level of T3 was 65 % of that of wild-type mice. In addition, it has been suggested that TCDD exposure resulted in disruption in retinoid metabolism which was characterized by depletion of the hepatic storage of retinoids. An additional function of TTR is postulated to be a carrier protein and to prevent filtration of retinol from renal glomeruli by forming macromolecular complex with retinol binding protein.

It is established that most of the toxicity features that TCDD evoke in laboratory animals and human is mediated through aryl hydrocarbon receptor (AhR) (4-6). However, whether a disruption of thyroid hormone and retinoid homeostasis by TCDD is mediated by AhR is not known. Here we investigated the mechanisms involved in the disturbance of thyroxine and retinoids homeostasis in response to TCDD exposure by using AhR-null and TTR-null mice.

ENDOCRINE DISRUPTORS

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 potent isomer among the large dioxin family of compounds. In addition to a wide variety of toxicities of TCDD, such as reproductive, immunological, neurobehavioral toxicities, teratogenicity and carcinogenicity, the alteration of thyroid hormone metabolism and function by TCDD is a major concern not only for laboratory animals but also for human populations because thyroid hormone is required for brain development, neuronal maintenance as well as metabolic function during fetal and early neonatal period

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Materials and Methods

Animals and TCDD treatment:

AhR heterozygous (AhR+/-) pregnant mice were dosed with 10 µg TCDD/kg bw by gavage on gestational day (GD)12, and sera and tissues were collected from male and female offspring (AhR+/- or AhR-/-) on postnatal days (PND) 21. TTR-null (TTR-/-) and wild-type (TTR+/+) mice (13-weeks-old) were dosed with 10 or 20 µg TCDD/kg by gavage, and sera and tissues were collected 7 days after dosing.

Thyroid hormone analysis:

Serum levels of T4 and triiodothyronine (T3) were determined by radioimmunoassay (RIA), and TSH was determined by enzyme immunoassay (EIA).

RNA Extraction and RT-PCR:

Total hepatic RNA was extracted by Isogen (Nippon Gene, Tokyo, Japan). Expression of CYP1A1, CYP1A2, AhR, UGT1A6 and bactin was determined by reverse transcription and polymerase chain reaction (RT-PCR) using PCR primers for amplification as previously described (1). 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).

Analysis of retinoids by HPLC:

Retinoids from liver homogenates were extracted with n-hexane and analyzed by HPLC (Waters 2690), monitoring absorbance at 340 nm (excitation) and 460 nm (emission) by fluorescence detector (Waters 47). Retinol, retinyl palmitate, and other retinyl esters were quantified based on calibration curves using retinol or retinyl palmitate as standards.

Statistical Analysis:

Values were expressed as mean ± SEM for individual groups of animals. Difference in means among experimental groups was analyzed by one-way analysis of variance followed by Fisher's least significant difference test.

Results and Discussion

TCDD exposure drastically decreased serum levels of total T4 and free T4 levels in AhR +/- mice but not in AhR-null mice. A similar tendency was observed for gene expressions of UGT1A6, AhR, CYP1A1, and CYP1A2, and they were significantly induced by TCDD in AhR +/- mice, but not in AhR-null mice.

We found that TCDD administration decreased significantly hepatic levels of retinyl palmitate in AhR +/- mice, but not in the liver of AhR- null mice, either. Total T4 levels in the serum of TTR- null mice were almost half of the level of vehicle-treated wild type (TTR+/+) mice. TCDD exposure increased the induction of UGT1A6, AhR, CYP1A1 and CYP1A2 mRNA levels in the liver of in both TTR+/+ and TTR- null mice, which can be explained by the AhR-mediated mechanism. In accordance with these biochemical changes, TCDD administration resulted in a significant decrease in total T4 concentrations both in TTR+/+ mice and TTR-null mice.. Exposure to TCDD decreased retinyl palmitate content in the liver, in different from the presence or absence of TTR, almost half the level of the vehicle-treated control mice.

Taken together, the present study strongly suggests that not only changes in thyroxinee metabolism but also impairment of retinoid metabolism in response to TCDD was mediated entirely via the Ah receptor, and that TTR was minimally responsible for the reduction of serum T4 levels and hepatic retinoids induced by TCDD.

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