Alterations in Thyroid Function Following Gestational and Lactational Exposure to Dioxin

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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. TCDD induces a variety of toxic responses, including immunotoxicity, teratogenicity, and carcinogenicity.

In industrialized countries, contamination of breast milk and food including meat and dairy products as well as fish with polychlorinated biphenyls (PCBs) and dioxins has become matter of public concern. The survey has been conducted to evaluate effect of dioxins and PCBs on thyroid hormone status of pregnant women and their infants. They concluded that elevated levels of dioxins and PCBs in human milk can cause lower plasma T4 and higher plasma TSH levels in the mother and infants. Thyroid hormone is believed to play an important role in brain development during the early postnatal period. Therefore, whether or not gestational exposure to TCDD affects thyroid hormone metabolism in pups including the human newborn have gained a considerable attention.

A reduction in serum levels of T4 has been shown to occur both in maternally exposed-pups and in adult experimental animals following TCDD treatment. Unfortunately, most of studies reported previously have been investigated with relatively higher doses of TCDD or in subchronic feeding studies in adult or in weanling animals. Considering that thyroid hormone levels seem likely to change in response to other metabolic alterations elicited by TCDD, metabolic and toxic effects of TCDD on thyroid might be thus better understand if dose of TCDD administered just sufficient to affect thyroid functionally and morphologically was given. In a previous study, the alteration in thyroid hormone concentration has been revealed to occur in rats given single oral dose of TCDD at 6.25 μ g/kg.

It is still unknown whether gestational and lactational exposure to TCDD produces a thyroid disorder even in pups. At present, we investigated the effects of perinatal exposure to low

dose levels of 200 ng to 800 ng TCDD/kg body weight by single oral application on thyroid function of rat pups. We also conducted the study to clarify a major route of exposure of TCDD responsible for the disruption of thyroid hormone metabolism in neonatal Holtzman rats. Pregnant Holtzman rats were administered a single oral dose of 1000 ng/kg TCDD at gestation days (GD) 15 and pups were then corss-fosterd on postnatal day (PND) 1.

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

Additional toxic manifestations by TCDD are to disturb vitamin A metabolism. Growing attention has been focused on primarily on the mechanism of disturbance of vitamin A kinetics by TCDD in rodents. In general, exposure to TCDD has been reported to reduce hepatic vitamin A concentrations with a concomitant increase in renal and plasma levels of vitamin A. This effect on vitamin A homeostasis is likely to occur in all species investigated including in rats, guinea pigs, mice, and hamsters. Evidence has been presented that the AhR appears to be involved in the process of disturbed vitamin A homeostasis. AhR-null mice were demonstrated a three-fold increase in hepatic levels of retinyl palmitate with a tree-fold accumulation in retinoic acid and retinol compared to the AhR+/+ mice. TTR has been shown to be carrier protein of thyroid hormone and vitamin A in rodents. A possible involvement of the TTR as well as the AhR with vitamin A homeostasis in liver in response to TCDD exposure was also assessed.

Materials and Method

Effects of gestational and lactational exposure to TCDD on newborn rat thyroid

To investigate whether or not maternal exposure to TCDD affects thyroid function of pups, pregnant Holtzman rats (5 per group) were given a single oral dose of 200 or 800 ng TCDD/kg on gestational day (GD) 15, or equivalent volume of corn oil as the control. The pups were sacrificed on PND 21 and 49. Tissue and blood specimens were collected from 5 infant rats (one per litter) of each group on PND 21 and 49 and used for thyroid hormone, immunohistochemical, cytochromeP4501A1 (CYP1A1) and UGT-1 mRNA, and TCDD analysis

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 β-actin was determined by reverse transcription and polymerase chain reaction (RT-PCR) using PCR primers for amplification. 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).

Identification of principal source of TCDD in neonatal rats by cross-fostering studies

To identify major route for exposure of TCDD responsible for toxic manifestations in pup thyroid, pregnant Holtzman rats were given an oral dose of 1000 ng TCDD/kg bw on GD15. Pregnant rats were allowed to deliver, and litters were randomly culled to 8, 4 males and 4 females if possible on PND1. Half of the treated litters and that of the control litters were cross-fostered on PND1, which resulted in the following groups: C/C (control), T/T (perinatal exposure), T/C (prenatal exposure only), and C/T (postnatal exposure only). Pups were sacrificed on PND21 and PND49. Sera and tissues were collected and then subjected to biochemical and immunohistochemical examinations as well as to determination of TCDD concentrations.

Effects of TCDD on thyroid hormone and vitamin A homeostasis in the AhR- or TTR-null mice

To assess a possible involvement of the AhR and TTR with thyroid hormone and vitamin A homeostasis in TCDD-exposed animal, AhR heterozygous (AhR+/-) pregnant mice were dosed with 10 μg TCDD/kg bw by gavage on GD12, and sera and tissues were collected from male and female offspring (AhR+/- or AhR-/-) on 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.

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 474). Retinol, retinyl palmitate, and other retinyl esters were quantified based on calibration curves using retinol or retinyl palmitate as standards.

Results and Discussion

Effects of gestational and lactational exposure of TCDD on newborn rat thyroid

The TCDD analysis showed that almost the same amounts of TCDD were accumulated in the liver and adipose tissue of pups on PND 21, containing 2000 pg/g tissue in liver of an 800 ng TCDD/kg dose group. However, on PND 49, the amount of hepatic TCDD was dramatically decreased to approximately 2% of that of 21-day-old pup. While gene expression of CYP1A1 was markedly induced in the liver of TCDD-exposed rats on both PND 21 and 49, the level of UGT1 gene by TCDD was significantly induced on PND 21, but decreased

to control levels by PND 49, in consistent with a marked reduction of hepatic TCDD content. Serum T4 levels in both male and female pups were decreased significantly by exposure to TCDD on PND 21 in the 200 and 800 μg/kg dose groups, but, interestingly, restoration or even significant increase in T4 levels were observed on PND 49 in both dose groups. A significant increase in circulating T3 was also found in the pups exposed at 800 ng TCDD /kg. A dose of 800 ng TCDD/kg resulted in greater than a 2-fold increase in serum TSH levels in male pups on PND 21, and this increased levels continued until PND 49 even when circulating T4 levels reached more than those of the control.

The stimulatory effects of TCDD on T4 and TSH biosynthesis were confirmed by immunocytochemical examination. We showed that TCDD treatment resulted in an increase not only in intensity of immunostaining of thyroid hormones, but also in number of TSH-positive cells in target organs. In histological examinations, the gestational and lactational exposure at 800 ng TCDD/kg resulted in hyperplastic lesion of follicular cells in pup thyroid glands on PND 49. A quantification of hyperplastic changes in the pup thyroid by measuring the ratio of parenchymal area to follicular area revealed a significant increase in the ratio in response to TCDD. Proliferating cell nuclear antigen (PCNA) immunocytochemistry also supported this proliferative lesion of follicular cells by TCDD exposure.

It has been well-established that circulating T4 and T3 are regulated by TSH at the levels of the hypothalamus-pituitary-thyroid axis under physiological conditions. It seems likely that a decreased level of circulating T4 observed on PND 21 was due to enhanced excretion of T4-glucuronide by TCDD-induced hepatic UGT-1 as shown in the present study. There is no explanation for the unexpected increase in both T4 and TSH levels on PND 49. Sustained excretion of TSH resulted in hyperplastic lesion in thyroid as evidenced by morphometric evaluation and PCNA immunohistochemistry. It seems thus reasonable to postulate that this abnormality of thyroid hormone homeostasis is attributable to disorder of a feedback control system at step(s) on the hypothalamus-pituitary-thyroid axis.

Based on the experimental evidence from the present study, we would conclude that perinatal exposure to even low doses of TCDD could disturb thyroid hormone homeostasis including a sustained excessive secretion of TSH, leading to an irreversible hyperplasia of follicular cell in pup thyroid.

Identification of principal source of TCDD in neonatal rats by cross-fostering studies

We found that serum total T4 levels of male and female pups were significantly decreased in the C/T and T/T group but not in the T/C group compared to the C/C group on PND21 (n=4-6). In addition, mRNA levels of UGT1A6, UGT1A7 and cytochrome P450 (CYP) 1A1 in liver were highly elevated in the C/T and T/T group, but not in the T/C group compared to the C/C group. An induction of UGT1A6 and CYP1A1 in the liver of the C/T and T/T groups but only a trace in the T/C group was visualized immunohistochemically, supporting

the molecular biological data on PND21.

The present results led us to conclude that TCDD-induced disruption of thyroid hormone homeostasis observed in pups were mainly due to lactational exposure.

Effects of TCDD on thyroid hormone and vitamin A homeostasis in the AhR- or TTR-null mice

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. Total T4 levels in the serum of TTR-null mice were almost half 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 both in 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 in both TTR+/+ mice and TTR-null mice.

Exposure to TCDD decreased retinyl palmitate content in 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 thyroxine 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.