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Gestational exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin alters retinoid homeostasis in maternal and perinatal tissues of the Holtzman rat

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), one of the most widely studied environmental contaminants, causes a variety of adverse health effects including teratogenesis and altered development which may be related to disruptions in retinoid homeostasis. The purpose of this study was to determine the effect that gestational administration of TCDD has on retinoid homeostasis in both pregnant Holtzman rats and developing fetuses and neonates. A single oral dose of TCDD (0, 1.5, 3, or 6 μg/kg) was administered to pregnant rats on gestation day 10, with fetuses analyzed on gestation days 17 and 20, and neonates analyzed on post natal day 7. Exposure to TCDD generally produced decreases in the concentrations of retinyl esters, such as retinyl palmitate, and retinol in maternal and perinatal liver and lung, while increasing levels in the maternal kidney. Additionally, perinatal hepatic retinol binding protein 1-dependent retinyl ester hydrolysis was also decrease by TCDD. Sensitivity of the developing perinates to TCDD appeared to have an age-related component demonstrated by an increased rate of mortality and significant alterations to body weight and length on post natal day 7 relative to that observed at gestation day 20. A unique observation made in this study was a significant decrease in lung weight observed in the perinates exposed to TCDD. Taken together, these data demonstrate that TCDD significantly alters retinoid homeostasis in tissues of the developing fetus and neonate, suggesting that their unique sensitivity to TCDD may at least be in part the result of altered retinoid homeostasis.
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Keywords: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; Developmental toxicity; Altered lung development; Retinoid homeostasis; Retinol, retinyl esters; Retinyl palmitate; Cytochrome *P*450; Liver; Kidney

Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most widely studied and prototypical congener of the family of chemicals known as halogenated aromatic hydrocarbons. TCDD and other dioxin-like compounds are persistent environmental containments capable of causing a wide range of toxic manifestations that include a wasting syndrome, chloracne, immunosuppression, thymic atrophy, altered development, teratogenesis and tumor promotion (Birnbaum and Tuomisto, 2000; Mandal, 2005).

It is generally believed that the toxic manifestations exerted by TCDD and related compounds occur through the activation of the aryl hydrocarbon receptor (AhR). Upon ligand binding, AhR translocates into the nucleus and binds its dimerization partner AhR Nuclear Transporter (ARNT) (Hoffman et al., 1991; Reyes et al., 1992; Probst et al., 1993). The activated AhR/ARNT complex then binds dioxin–response elements (DREs), located in the 5′ region upstream of responsive target genes, and acts as a transcription factor resulting in a variety of differential changes in gene expression (Martinez et al., 2002; Vezina et al., 2004; Hanlon et al., 2005; Ovando et al., 2006; Tijet et al., 2006). Subsequent alterations in AhR-mediated gene expression are in turn associated with the observed toxicological responses since AhR knockout mice show a more limited toxic response to TCDD exposure (Fernandez-Salguero et al., 1996).

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Another well characterized signaling pathway leading to alterations in gene expression is the retinoic acid pathway (Balmer and Blomhoff, 2002). Through a series of reactions, dietary vitamin A is first hydrolyzed to retinol and either oxidatively metabolized to biologically active retinoic acid (RA) or esterified with long-chain fatty acids to form retinyl esters, primarily retinyl palmitate, and stored predominantly in the liver (Wang et al., 2006). As an activated metabolite, RA initiates signaling by binding to retinoic acid receptors (RAR). RARs can heterodimerize with retinoid X receptors (RXRs) and bind consensus sequences known as retinoic acid response elements (RARE) in the upstream promoter region of RA responsive genes, leading to alterations in gene expression. With different subfamilies of RARs and RXRs (α, β, γ) and several isoforms of each, a highly regulated system of gene expression is maintained (Chambon, 1996; Hoegberg et al., 2005). An additional level of control is established by the intracellular carrier proteins of retinoids, cellular retinol binding protein 1 and 2 (Rbp1 and Rbp2) and cellular retinoic acid-binding protein 1 and 2 (Crabp1 and Crabp2), which affects the cellular availability of RA (Noy, 2000).

Previous research suggests that some of the toxic manifestations of TCDD also may be a result of the disruption of retinoid homeostasis. First, some of the clinical pathologies associated with TCDD intoxication resemble both hypovitaminosis A (Thunberg et al., 1979; Thunberg and Ahlborg, 1980) and hypervitaminosis A (Nilsson and Hakansson, 2002). Second, AhR knockout mice have signs of altered retinoid homeostasis, indicated by increased levels of hepatic retinoids (Andreola et al., 1997). Third, altered retinoid homeostasis has been observed in studies with dioxin-like polychlorinated biphenyls, but not with non-dioxin-like polychlorinated biphenyls (Azais et al., 1987; Chen et al., 1992; Chu et al., 1994, 1995, 1996a,b; Morse and Brouwer, 1995; Lecavalier et al., 1997). Finally, genetic studies suggest cross talk between the AhR signaling pathway and the RA signaling pathway (Weston et al., 1995; Murphy et al., 2004; Toyoshiba et al., 2004).

The developing organism is particularly sensitive to both TCDD and RA. Previous studies in rats and in mice have found TCDD to be a potent embryo/fetal toxin, developmental toxin and teratogenic agent (Peterson et al., 1993; Birnbaum, 1995; ten Tusscher and Koppe, 2004; Kransler et al., 2007). Although RA is required for normal development, a teratogenic response is observed when concentrations are elevated. In laboratory animals, prenatal exposure to excess retinoids results in craniofacial malformations, such as cleft palate (Abbott and Birnbaum, 1989; Birnbaum et al., 1989), an effect also observed in humans after exposure to the pharmaceutical isotretinoin (Lammer et al., 1985).

The purpose of this study was to quantitatively determine the effect of gestational administration of TCDD on retinoid homeostasis in both pregnant Holtzman rats and developing fetuses and neonates, which are uniquely sensitive to TCDD, to further define the toxic response observed in developing organisms. Concentrations of various retinoids were determined in maternal and perinatal liver, lung and kidney that were collected on gestation day (GD) 17, GD20 or post natal day (PND) 7 from pregnant dams that were administered TCDD (1.5, 3 or 6 μg/kg) on GD10. Additionally, several parameters of perinatal development were assessed. Together, these data show that TCDD alters retinoid homeostasis in these tissues, that the toxic responses observed in the perinates are age-dependent and to the best of our knowledge, identifies the developing lung as a target organ for the toxic effects of TCDD.

Materials and methods

Chemicals. Retinol and retinyl palmitate were purchased from the Eastman Kodak Co. (Rochester, NY). 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (purity >99%) was a gift from the Dow Chemical Co. (Midland, MI) and dissolved in 1,4-dioxane. Concentrations of the TCDD dosing solutions were verified using GC-ECD (data not shown). HPLC grade acetone, hexane, tetrahydrofuran and 2-propanol were purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ). All other chemicals and reagents were obtained from commercial sources and were of the highest grade available.

Animals. Timed pregnant Holtzman rats were purchased from Harlan Sprague-Dawley Inc. (Indianapolis, IN). All animals were housed individually and allowed access to food and water *ad libitum*. Rat chow (Prolab RMH 2000 rodent chow) supplied 15,684 IU/kg vitamin A as retinyl palmitate. Animal cages were maintained under controlled temperature (25 °C) and light conditions (12/12 h light–dark). All animal protocols were reviewed and approved by the Institutional Use and Animal Care Committee at the University at Buffalo, The State University of New York.

Experimental design. Pregnant rats, 22-day gestation period, received a single oral dose of TCDD (1.5, 3 or 6 μg/kg) in a corn oil vehicle (1 ml/kg) via oral gavage or vehicle alone (control) on gestation day (GD) 10. The doses of TCDD were chosen to insure that the low dose would not be associated with an increase in perinatal lethality or changes in neonatal body weight and were based on a previous study (Kransler et al., 2007). Gestation day 10 corresponds to Carnegie Stage 9 of development, the approximate start of organogenesis (Hill, 2007). Animals were euthanized on GD17, GD20, or post natal day (PND) 7. Animals were anesthetized with sodium pentobarbital (100 mg/kg, i.p.) and euthanized via decapitation. All animals were monitored daily for signs of overt toxicity resultant from TCDD exposure.

Tissue collection and measurements. Maternal toxicity was evaluated by examining changes in organ and body weight during the study. The gravid uterus was examined after dissection with the number and uterine position of implantation sites, viable fetuses, resorptions and late fetal deaths recorded. Maternal net body weight gain was determined as the absolute gain in weight of the dam between GD10 and the time of collection minus the weight of the uterine contents. Viable fetuses were measured for body weight, crown-to-rump length, liver and lung weight. Fetuses were considered viable if after being removed from the uterus, palpation resulted in spontaneous movement. Non-viable fetuses were classified into one of two categories, embryo resorption or late fetal death (LFD). An embryo resorption was recorded when a non-distinct mass was visible at an implantation site. A fetus that failed to meet the criteria of being viable and in the initial stages of resorption was classified as a LFD. In all cases, the litter was considered the experimental unit for analysis.

Tissue extraction and retinoid analysis. Tissue samples were homogenized in buffer (250 mM sucrose, 10 mM HEPES, pH 7.4) 1:10 (w/v) for 5 to 10 s using a polytron homogenizer. An aliquot of homogenate (2–4 ml for fetal tissue or 1 ml serum) was then alkalinized with two volumes of 0.025 N KOH (in 95% ethanol) followed by extraction with 0.2% tetrahydrofuran (THF) in hexane (1:5 v/v homogenate/THF-hexane). Extractions were performed twice, combining 3 ml of the organic phase from each of the two extractions resulting in 6 ml total. Extracts were evaporated under N_2 at room temperature and resuspended in 200–250 μl of 2-propanol and were analyzed within 48 h. All procedures were carried out in subdued lighting conditions.

Extracts were analyzed by HPLC using a 3-cm Zorbax ODS reverse-phase column (Agilent Technologies, Palo Alto, CA). The mobile phase consisted of a gradient of 30% H_2O/70% 2-propanol to 100% 2-propanol over 16 min at a flow

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