Subchronic Effects of 2,3,3',4,4',5-Hexachlorobiphenyl or 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on Thyroid Hormone and Retinoid Metabolism: Possible Role of Cytochrome P450 and UDP-Glucuronyltransferases.

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Abstract

Female Sprague-Dawley rats were fed on diets containing 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) in various concentrations for three months. Both CYP1A1 and UGT1A1 inductions were observed at 0.2 µg/kg TCDD and 1.2 mg/kg PCB 156. UGT1A1 and CYP1A1 activities correlated well for TCDD or PCB 156 administration. Moreover, the relationship between UGT1A1 and CYP1A1 was identical for TCDD or PCB 156 treatment. Marked hepatic retinoid reductions were observed at 0.2 µg/kg TCDD and 6 mg/kg PCB 156. Both plasma thyroxine reductions and T4-UDP-GT inductions were observed at 0.4 µg/kg TCDD and 6 mg/kg PCB 156. A good correlation was found between T4-UDP-GT activities and plasma thyroxine concentrations after TCDD or PCB 156 administration. So, cytochrome P450 and UDP-glucuronyltransferases may be involved in the thyroid hormone and retinoid metabolism.

Introduction

Polyhalogenated compounds such as polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs) and biphenyls (PCBs) are persistent chemicals, which are widely distributed in the environment. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin and related isostereomers result in common toxic responses, like thymic atrophy, impairment of immune responses, hepatic retinoid loss and reduction in plasma thyroid hormone concentrations. Most of these effects are thought to be mediated by the Ah-receptor[1].

Recently, we have reported on dramatic decreases in hepatic retinoids and plasma thyroid hormone concentrations, following exposure of rats to 2,2',4,4',5,5'-hexachlorobiphenyl and TCDD in a 13-week feeding study[2].

In this study we investigated the effects of subchronic dosage of TCDD or 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) on thyroid hormone and retinoid metabolism, including effects on cytochrome P450 and UDP-GT enzyme activities.

Methods

Chemicals: 2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156) was synthesized by Cadogan coupling of 3,4-dichloroaniline and 1,2,3,4-tetrachlorobenzene[3], and purified by silica column chromatography. The reaction product was isolated in 25% yield, >98% pure as determined by HRGC-LRMS analysis. TCDD originated from Dow Chemical (Midland, Mi USA).

Animal treatment: Female Sprague-Dawley (Iva: S/V 50 (SD)) rats, 8 animals per group, starting weight about 150 grams, were fed on experimental diets for 13 weeks. The diets, pulverized feed (Nafag 890), contained 0, 0.2, 0.4, 0.7, 5, or 20 µg TCDD/kg diet, 1.2, 6, or 12 mg PCB 156/kg diet. Supplementation of the diets was performed according to Pluess *et al*[4].

Thyroid hormone measurements: Blood samples were analysed for plasma total (TT4) and free (FT4) thyroxine levels by Chemoluminescence immuno assays by using standard Amerlite kits (Amersham, U.K.).

Retinoid measurements: Liver retinoids were analysed according to Brouwer *et al*[5].

Enzyme induction: 7-Ethoxyresorufin-O-deethylation (EROD) was determined by using the method of Burke et al[6]. T4-UDP-GT activities were assayed as described by Beetstra *et al*[7]. UGT1A1 activity was measured as 1-naphtol-UDP-GT and was based on a method described by Bock *et al.*[8], and modified according to Beetstra *et al*[7].

Statistics: Data were analysed for differences to control by ANOVA (LSD test). Dose response was tested by linear regression with Student's T-test. Analysis of co-variance was used to test differences between slopes.

Results

Table 1 and 2 present thyroid hormone concentrations, hepatic retinoid levels, microsomal CYP1A1 enzyme activities, by using ethoxyresorufin-O-deethylation as a marker, and microsomal T4-UDP-GT and UGT1A1 activities. Hepatic retinoids were depleted by 0.2 µg/kg of TCDD to 26% and 56% of control for hepatic retinylpalmitate and retinol, respectively. Both CYP1A1 and UGT1A1 activities were induced by 0.2 µg/kg TCDD and 1.2 mg/kg PCB 156. A good correlation was found between CYP1A1 and UGT1A1 activities for both TCDD and PCB 156 ($r=0.91$, $p<0.001$ and 0.94, $p<0.001$, respectively). The slopes of both curves were the same.

Reductions in plasma total thyroxine (TT4) levels were found at 6 mg/kg PCB 156 and 0.7 µg/kg TCDD. At the same dose levels, T4-UDP-GT activities were induced up to 1.9 and 4.5 times control levels for TCDD and PCB 156, respectively. The correlation coefficients of the plasma TT4 levels and T4-UDP-GT activities for TCDD and for PCB 156 were 0.59 ($p<0.001$) and 0.69 ($p<0.001$), respectively.

Discussion

The decrease in plasma thyroid hormone levels clearly correlated with an elevation in microsomal T4-UDP-GT activity. This agrees with Barter and Klaassen that reduced plasma concentrations of TT4 or FT4 and induced hepatic microsomal T4-UDP-GT activities correlated well[9].

Not only CYP1A1 and UGT1A1 inductions, but also hepatic retinoid

Table 1. Thyroid hormone concentrations, T4-UDPGT-, 1-naphtol-UDPGT and EROD activities, and hepatic retinoid levels, after 13 weeks on diets containing TCDD (mean ± SE, n=8).

Dose TCDD (µg/kg)	TT4 (nmol/L)	FT4 (pmol/L)	T4-UDPGT (pmol/ mg.min)	Hepatic retinylpal-mitate (mg/g liver)	Hepatic retinol (mg/g liver)	1-Naphtol-UDPGT (nmol/mg/ min)[1]	EROD (nmol/ min.mg)
0	40.9 ± 6.7	23.4 ± 3.0	0.33 ± 0.07	472 ± 96	14.9 ± 3.1	20.2 ± 1.8	0.19 ± 0.05
0.2	41.1 ± 5.3	24.5 ± 5.6	0.60 ± 0.15	94 ± 24*	8.4 ± 1.2*	38.7 ± 4.1*	2.16 ± 0.48*
0.4	41.1 ± 6.4	22.4 ± 2.9	0.64 ± 0.16*	107 ± 27*	8.2 ± 0.8*	N.D.	4.10 ± 1.14*
0.7	32.3 ± 7.5*	19.3 ± 9.2	0.87 ± 0.32*	74 ± 14*	5.1 ± 0.3*	60.7 ± 1.9*	5.02 ± 0.86*
5	33.6 ± 6.1*	15.2 ± 3.9*	2.08 ± 0.47*	22 ± 8*	2.2 ± 0.3*	90.4 ± 5.6*	9.95 ± 1.05*
20	25.5 ± 7.7*	10.3 ± 4.9*	2.59 ± 0.31*	3 ± 1*	0.6 ± 0.2*	59.2 ± 17.1*	9.26 ± 1.21*

* Significant to control (LSD test, p<0.025)
[1] n=3
N.D.: not measured

Table 2. Thyroid hormone concentrations, T4-UDPGT-, 1-naphtol-UDPGT and EROD activities, and hepatic retinoid levels, after 13 weeks on diets containing PCB 156 (mean ± SE, n=8).

Dose PCB 156 (mg/kg)	TT4 (nmol/L)	FT4 (pmol/L)	T4-UDPGT (pmol/ mg.min)	Hepatic retinylpal-mitate (mg/g liver)	Hepatic retinol (mg/g liver)	1-Naphtol-UDPGT (nmol/mg/ min)[1]	EROD (nmol/ min.mg)
0	40.9 ± 6.7	23.4 ± 3.0	0.33 ± 0.07	472 ± 96	14.9 ± 3.1	20.2 ± 1.8	0.19 ± 0.05
1.2	35.6 ± 6.2	17.2 ± 3.8*	0.48 ± 0.26	383 ± 20	16.2 ± 1.0	51.8 ± 11.4*	2.46 ± 0.93*
6	25.8 ± 4.1*	14.5 ± 3.0*	1.48 ± 0.43*	219 ± 29*	9.8 ± 0.6*	103 ± 9*	9.02 ± 1.01*
12	20.6 ± 4.1*	11.4 ± 1.0*	2.35 ± 1.03*	50 ± 12*	3.8 ± 0.5*	97.5 ± 25*	9.84 ± 1.24*

* Significant to control (LSD test, p<0.025)
[1] n=3

reductions have already been observed at 0.2 µg/kg TCDD and 1.2 mg/kg PCB 156. Hepatic retinoid losses may be associated with an Ah-receptor mediated mechanism, since more toxic CYP1A inducers showed more profound effects on the hepatic retinoid content[10]. Leo and Lieber have found that retinol was actively oxidized to polar metabolites, including 4-hydroxyretinol, in a system requiring oxygen and NADPH[11]. Moreover, Roberts *et al.*[12] have reported involvement of several P450 isoenzymes in the catabolism of retinoids. They have found that purified rabbit cytochrome P450 isoenzymes could hydrolase retinoic acid, retinol, and retinal, mainly at the 4-position. TCDD has been reported to induce the glucuronidation of retinoic acid in the rat[13]. So, the measured loss in retinoid stores may be a consequence of the induction of certain cytochrome P450 isoenzymes, which may presumably be co-induced with several UDP-GTs.

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