Apparent Antagonistic Effects between 2,3,3',4,4',5-Hexachlorobiphenyl and 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin on CYP1A1 and CYP1A2 activities: Possible Role of Metabolism and Target Tissue Concentration.

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Abstract

Female Sprague-Dawley rats were fed on diets containing 0.2 µg/kg 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and/or 1.2, 6 or 12 mg/kg 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) for three months. CYP1A1 and CYP1A2 activities, and the *in vitro* metabolism of 3,3',4,4'-tetrachlorobiphenyl (TCB) were markedly induced by PCB 156 or TCDD. Co-administration of 1.2 mg/kg PCB 156 and 0.2 µg/kg TCDD did not further increase CYP1A1 and CYP1A2 activities, and the *in vitro* metabolism of TCB, when compared with 1.2 mg/kg PCB 156 or 0.2 µg/kg TCDD alone. TCDD liver retention was dose related reduced by PCB 156 to 57% of the levels in the 0.2 µg/kg TCDD group, whereas PCB liver retention was not influenced by the presence of TCDD. Assuming that TCB can be used as a model compound for studying TCDD metabolism, a possible contribution of increasing metabolism can not be excluded in the reduced liver retention of TCDD at 6 and 12 ppm PCB 156. In addition, the apparent antagonistic effects on enzyme induction at the 1.2 ppm PCB 156 dose level, may also be a consequence of decreased hepatic TCDD retention.

Introduction

Polyhalogenated aromatic compounds such as polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), and dibenzofurans (PCDFs) are highly lipophilic and resist to biodegradation. This has led to bioaccumulation in food chains and worldwide detectable amounts of PCBs, PCDDs and PCDFs as trace contaminants in the environment[1], including human adipose tissue and milk[2].

Risk assessment of polyhalogenated compounds is based on additive toxicity. However, antagonistic as well as synergistic effects have been reported between

PCBs and PCDDs[3-6]. A possible role of toxicokinetic factors may contribute to the nonadditive toxicological and biochemical effects observed. In fact, modulation of liver retention of TCDD by 2,2',4,4',5,5'-hexachlorobiphenyl has been reported in a subchronic feeding study[7].

We investigated possible interactive effects between TCDD and 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156) on CYP1A1, CYP1A2 activities, and the *in vitro* metabolism of 3,3',4,4'-tetrachlorobiphenyl as a model compound. At the same time we analysed the hepatic retention of the administered compounds.

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[8], and purified by silica column chromatography. The reaction product was isolated in 25% yield, >98% pure as determined by HRGC-LRMS analysis. A small amount of [^{3}H]TCDD (obtained from Givaudan, Switzerland) was added to TCDD, originating 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, 1.2, 6, or 12 mg PCB 156/kg diet, 0.2 µg TCDD/kg diet, or combinations of both. Supplementation of the diets was performed according to Pluess *et al*[9].

Enzyme induction: Microsomal CYP1A1 activity was determined as 7-ethoxyresorufin-O-deethylation (EROD) by using the method of Burke *et al*[10]. Microsomal CYP1A2 activity was measured as the 4-hydroxylation of acetanilide (4 OH-AA) according to Liu *et al*[11]. Protein levels were analysed according to Bradford[12].

In vitro metabolism of 3,3',4,4'-tetrachlorobiphenyl: Microsomal activity was determined based on the method of Morse *et al*[13]. In short, the assay system contained 20 µM 3,3',4,4'-tetrachlorobiphenyl (TCB), 0.1 M Tris-HCl buffer (pH 7.5), 0.5 mM NADPH, and 0.4 mg microsomal protein per mL in a final volume of 1 mL, and was followed for 10 min. Reaction was stopped by addition of 1 mL ice-cold methanol. Metabolites were extracted with hexane (including 2,2',4,4',5,5'-hexachlorobiphenyl as an internal standard) and were triethylated. Prior to analysis on GC-ECD, samples were purified on florisil. The response factor of 3,3',4,4'-tetrachloro-2-biphenylol (2-OH) was used for calculating the amount of 3,3',4,4'-tetrachloro-6-biphenylol (6-OH) in the samples, assuming the same response on GC-ECD. Standards of 2-OH and 3,3',4',5-tetrachloro-4-biphenylol (4-OH) were kindly supplied by Dr. E. Klasson-Wehler.

Residue analyses: Liver PCB 156 levels were analysed by GC-ECD after a clean up procedure described by De Jongh *et al.*[14], using only the first column described. Residues of TCDD in liver were determined as described earlier[7].

Statistics: Data were analysed for differences to control by ANOVA (least significant difference, LSD test).

Results and discussion

Table 1 presents the effects of PCB 156 on CYP1A1 activity, measured as EROD, CYP1A2 activity, measured as the 4-hydroxylation of acetanilide, and the in vitro formation of metabolites of TCB. At the 1.2 mg/kg PCB 156 dose level, CYP1A1, CYP1A2 activities and the *in vitro* TCB metabolism were markedly increased. Nearly maximum induction for the CYP1A1 and CYP1A2 assays were measured at the 12 mg/kg PCB 156 dose level. At this dose level, the *in vitro* formation of metabolites of TCB was about 90% of the maximum, as determined by a dose response curve of TCDD (not shown). Co-administration of 1.2 mg/kg PCB 156 with TCDD did not significantly differ in CYP1A1 and CYP1A2 activities, and the *in vitro* TCB metabolism compared with 1.2 mg/kg PCB alone. However, 0.2 µg/kg TCDD induced these activities up to levels as for 1.2 mg/kg PCB 156 only.

Liver retention of the administered compounds showed that TCDD liver levels

Table 1. CYP1A1 (EROD), CYP1A2 (4 OH-AA) activities, and metabolism of 3,3',4,4'-tetrachlorobiphenyl (4-OH and 6-OH) in liver microsomes of rats fed diets containing PCB 156 and/or TCDD for 13 weeks (mean ± SE, n=8).

Dose PCB (mg/kg diet)	PCB in liver (% of dose)	Dose TCDD (µg/kg diet)	TCDD in liver (% of dose)	EROD (nmol/mg.min)	4 OH-AA (µg/mg.min)	4-OH (ng/mg.min)[1]	6-OH (ng/mg.min)[1]
0	0	0	0	0.12 ± 0.03	0.75 ± 0.06	0	0
1.2	0.62 ± 0.18	0	0	1.67 ± 0.29	1.65 ± 0.28*	1.9 ± 0.5*	2.3 ± 0.6
6	0.73 ± 0.16	0	0	5.67 ± 0.92*	2.65 ± 0.26*	4.1 ± 0.3*	5.9 ± 0.6*
12	0.81 ± 0.32	0	0	8.61 ± 0.93*	3.64 ± 0.44*	6.1 ± 1.4*	7.0 ± 0.7*
0	0	0.2	3.48 ± 0.76	2.01 ± 0.17	1.70 ± 0.16	1.8 ± 0.2	2.3 ± 0.5
1.2	0.86 ± 0.21	0.2	2.66 ± 0.72	2.54 ± 0.18	1.56 ± 0.09	1.8 ± 0.2	2.8 ± 0.01
6	1.03 ± 0.38	0.2	1.87 ± 0.45	5.77 ± 1.13#	2.86 ± 0.38#	5.0 ± 0.4#	5.8 ± 1.1#
12	0.79 ± 0.25	0.2	1.50 ± 0.32	9.18 ± 0.79#	3.88 ± 0.33#	6.0 ± 1.1#	7.0 ± 2.3#

* Significant to control (LSD test, $p<0.025$)
\# Significant to 0.2 µg/kg TCDD (LSD test, $p<0.025$)
[1] n=3

were dose related reduced by PCB 156 to 57% of the levels in the 0.2 µg/kg TCDD group. In contrast, the liver retention of PCB 156 itself was not influenced by the presence of TCDD.

In conclusion, the PCB dose dependent decrease in hepatic TCDD levels can not be excluded to be a consequence of induced metabolism, assuming TCB is a good *in vitro* model compound. In addition, the apparent antagonistic effects on CYP1A1 and CYP1A2 induction at the 1.2 ppm PCB 156 dose level may also be a direct consequence of the decreased hepatic TCDD levels.

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