CYP1A2: The Inducible Binding Protein for TCDD Sequestration

Janet J. Diliberto[1], Deborah Burgin[2], and Linda S. Birnbaum[1]
[1]ETD, National Health Effects and Environmental Research Laboratory, United States Environmental Protection Agency, Research Triangle Park, NC 27711, USA
[2]University of North Carolina-CH, Curriculum in Toxicology, Chapel Hill, NC 27599, USA

Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD, dioxin), most potent of the polyhalogenated aromatic hydrocarbons, has been studied in a variety of genetically normal species. Transgenic mice lacking a cytochrome P450 1A2 gene were used to study the influence of the 1A2 gene on the hepatic sequestration and distribution of TCDD, 4-PeCDF (2,3,4,7,8-pentachlorodibenzofuran; dioxin-like compound), and PCB 153 (2,2',4,4',5,5'-HxCB; non-dioxin-like of PCB). The knock-out mice were compared to their age-matched lineage strains of C57BL/6N (1A2 +/+; AhR$^{b/b}$) and 129/Sv (1A2 +/+; AhR$^{d/d}$) for each compound.

Introduction

Cytochrome P450 1A2 (CYP1A2) is highly expressed constitutively in the liver and is involved in the metabolism of many toxicological important compounds (such as, food-derived heterocyclic amines, aflatoxin B_1, and acetaminophen)[1,2]. CYP1A2 (both mRNA and constitutive expression) is induced after exposure to aromatic hydrocarbons such as TCDD and is regulated by the Ah (Aryl hydrocarbon) receptor[3]. However in the null mutant Ahr -/- transgenic mouse, CYP1A2 mRNA was not induced by TCDD and the constitutive expression of CYP1A2 was decreased 90% by TCDD[4]. Many carcinogenic arylamines are known to be substrates for the human CYP1A2 enzyme[5,6]; as are endogenous substrates, such as estrogen and uroporphyrin.

The development of a null mutant CYP1A2 mouse by means of gene targeting, designated as CYP1A2(-/-), has been developed[7]. The importance of this dioxin-inducible gene on the pharmacokinetics and toxicity of dioxin has been suggested. Use of the knock-out mice will provide a valuable tool for defining the role of CYP1A2 in the pharmacokinetics of TCDD and related compounds. Moreover, because mouse and human CYP1A2 have similar cDNA derived amino acid sequence[8,9,10], this knock-out mouse may be an important model to understand the role of this protein in humans.

CYP1A2 has been hypothesized as the hepatic binding protein that sequesters TCDD and related compounds in the liver. By removing this hepatic binding protein, the pharmacokinetic behavior in liver may be similar to other non-sequestering tissues and the liver-to-fat (L/F) concentrations ratios may be less than one. Therefore, the objective of this study was to look at the role of CYP1A2 in knock-out transgenic mice lacking a cytochrome P4501A2 gene (1A2-/-) on distribution and hepatic sequestration of TCDD, 4-PeCDF (dioxin-like compound) and PCB 153 (nondioxin-like di-ortho substituted PCB). Both TCDD and 4-PeCDF are specific CYP1A iso-enzyme inducers; PCB 153 is an inducer of CYP2B which acts through a different mechanism of action than TCDD and 4-PeCDF. Also because the true pedigree and degree of

influence of the parental lineage on the genetic make-up and behavior of these mutant mice were not known, comparisons of the mutant mice to the age-matched parental lineage strains C57BL/6N (1A2+/+; AhR$^{b/b}$) and 129/Sv (1A2+/+; AhR$^{d/d}$) were evaluated.

Experimental Methods

Chemicals. TCDD was obtained from Radian Corporation (Austin, TX) with stated purity >98% as determined by gas chromatography/mass spectrometry. [1,6-^{3}H]TCDD was synthesized by Chemsyn Science Laboratories (Lenexa, KS) with a specific activity of 34.7 Ci/mmol and purity of ≥98%. Purity was verified by reverse-phase high-pressure (HPLC) (System Gold, Beckman Instruments, Inc., Fullerton, CA) using a C18 μBondapak stainless-steel column with a Guard-PAK precolumn insert (Waters, Milford, MA) and an isocratic solvent system of 85% methanol/15% water. Bioassay of rat biliary excretion of TCDD was used to verify the purity which was found to be 99%. 4-PeCDF was purchased from Ultra Scientific (North Kingston, RI) with a reported purity >98%. [^{14}C]4-PeCDF was obtained from Chemsyn Science Laboratories (Lenexa, KS) with purity >98%. PCB 153 was purchased from Ultra Scientific (North Kingston, RI) with a reported purity >98%. [^{14}C]PCB 153 was purchased from Sigma Chemical Co. (St. Louis, MO) with a specific activity of 12.6 Ci/mole and purity >98%.

Animals. Male mutant mice were bred to the F2 generation from mice generously donated by Dr. Frank P. Gonzalez at the NCI/NIH laboratories (Bethesda, MD). Parental lineage strains C57BL/6N and 129/Sv were obtained from Charles River Breeding Laboratories (Raleigh, NC) and Taconic Farms (Germantown, PA), respectively. All animals were 19 weeks old at the time of the study and were housed individually in Nalgene (Nalgene; Rochester, NY) metabolism cages. They were allowed 1 week to acclimate in metabolism cages before dosing. Throughout the study, animals were maintained on a 12-hr light/dark cycle at ambient temperature of 22±1°C and relative humidity of 55±5% and provided with dustless precision pellet feed (BioServe, Frenchtown, NJ) and tap water *ad libitum*.

Treatment. Mice (5/group) were given a single oral dose of 0, 25 μg [^{3}H]TCDD/kg body wt, 300 μg [^{14}C]-4PeCDF /kg body wt, or 35.8 mg [^{14}C]-PCB 153/kg body wt in a corn oil vehicle. The dosing volume was 10 ml/kg body wt.

Tissues. At 4 days after dosing, mice were euthanized by CO_2 asphyxiation followed by exanguination via cardiac puncture. Tissues were removed, weight, and quantitated for radioactivity.

Sample analysis. Radioactivity in tissues was determined by combustion (Packard 306B Biological Oxidizer, Downers Grove, IL) followed by liquid scintillation spectrometry (Beckman Scintillation Counter, Beckman Instruments, Fullerton, CA).

Data analysis. All data are presented as mean ± standard deviation.

Results and Discussion

Table 1 presents a comparison of the liver-to-fat (L/F) concentration ratios in the 1A2 knock-out mice and the two parental lineage strains (C57BL6N and 129/Sv) after treatment with TCDD (25 μg/kg), 4PeCDF (300 μg/kg), or PCB 153 (35.8 mg/kg). The animals were acutely dosed by oral gavage and killed four days after exposure. The doses for the various compounds were chosen for comparison to other studies conducted by this laboratory. The dose for TCDD was high enough to cause maximal CYP1A induction even in non-responsive strains.

The present study clearly demonstrated that CYP1A2 was the binding protein for dioxin in the liver as shown by the L/F concentrations ratios in Table 1. The L/F concentration ratio for TCDD and related compounds is one of the most sensitive indicators for hepatic

sequestration[11]. L/F concentration ratios represent the relationship between levels of radioactivity in liver and adipose tissue. Ratios less than one demonstrates a greater concentration in adipose tissue than liver. Whereas, ratios greater than one demonstrates a greater concentration in liver than adipose tissue.

No sequestration of TCDD was demonstrated in the liver of the knock-out mice. In contrast, hepatic sequestration of TCDD was seen in mice (C57BL/6N and 129/Sv) with the intact CYP1A2 gene (1A2+/+). By removing this hepatic binding protein, liver behaved as other highly perfused tissues with the tissue content being determined by simple partitioning. Because less TCDD was in the liver, more dioxin was available to other tissues, particularly the fat. The L/F concentration ratio was less than 0.2 in the animals with a knock-out gene for 1A2 (1A2-/-) as compared to 3.6 in the animals (C57BL/6N and 129/Sv) with the intact 1A2 gene (Table 1). Also as shown by the animals dosed with 4PeCDF, a chemical which has been suggested to bind more tightly to 1A2 than TCDD, removing the hepatic binding protein changed the pharmacokinetics of this compound. Similar to TCDD in the knock-out mice, more of the 4PeCDF-derived radioactivity was found in the fat with liver behaving like other tissues. The L/F concentration ratios were 0.3 compared to 17 to 19 for the knock-out and non-knock-out (C57BL/6N and 129/Sv) mice, respectively (Table 1). PCB 153, a non-1A2 binding PCB that does not sequester in the liver, demonstrated no differences in the L/F concentrations ratios in the knock-out and non-knock-out (C57BL/6N and 129/Sv) mice (Table 1).

Summary

This study provides direct confirmation of the hypothesis that CYP1A2 is the hepatic binding protein responsible for the sequestration of TCDD and related compounds in the liver.

This abstract does not necessarily reflect USEPA policy.

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Table 1. Liver/Fat Concentration Ratios[a]

Mouse Strain	1A2 Genotype	Compound	Dose	Liver/Fat Concentration Ratios
Knock-out	1A2 -/-	TCDD	25 µg/kg	0.17 ± 0.03
C57BL6N	1A2 +/+	TCDD	25 µg/kg	3.55 ± 0.41
129/Sv	1A2 +/+	TCDD	25 µg/kg	3.63 ± 0.22
Knock-out	1A2 -/-	4PeCDF	300 µg/kg	0.34 ± 0.08
C57BL6N	1A2 +/+	4PeCDF	300 µg/kg	18.68 ± 2.96
129/Sv	1A2 +/+	4PeCDF	300 µg/kg	17.31 ± 2.99
Knock-out	1A2 -/-	PCB 153	35.8 mg/kg	0.10 ± 0.03
C57BL6N	1A2 +/+	PCB 153	35.8 mg/kg	0.07 ± 0.02
129/Sv	1A2 +/+	PCB 153	35.8 mg/kg	0.06 ± 0.01

[a] Mean ± SD; n=5.