ROLE OF CYP1A2 ON TOXICOKINETIC BEHAVIOR OF PHAHS IN KNOCKOUT (CYP1A2-/-) VERSUS PARENTAL (CYP1A2+/+) STRAINS OF MICE

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Introduction

Polyhalogenated aromatic hydrocarbons (PHAHs), widely-dispersed environmental contaminants, are highly lipophilic and resistant to degradation resulting in their persistence in the environment and bioaccumulation in the food chain. Included in this class of compounds are dibenzo-*p*-dioxin (PHDD), dibenzofuran (PHDF), and biphenyl (PCB) isomers sharing a characteristic pattern of lateral substitution and relatively planar conformation. Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD) is the prototype and most potent member of the PHAHs that is known to produce a spectrum of toxic effects in animals via a common receptor-mediated mechanism (1). The PHAHs are often described as dioxin-like or nondioxin-like because of their hepatic enzyme induction and other responses (2,3). Among the dioxin-like compounds, toxic potency is derived not only from the ability of a specific congener to bind the cytosolic aryl hydrocarbon (Ah) receptor in target tissues, but also from its toxicokinetic behavior (2,4,5).

In acute and subchronic animal studies, tissue distribution demonstrates a dose-dependent specific hepatic localization for TCDD and related compounds (6-8) which suggests an inducible hepatic binding protein (9-13). Recently, a CYP1A2 knockout mouse has been developed with the null mutant CYP1A2 gene (14). Studies in our laboratory using CYP1A2 knockout (KO) mice have demonstrated that CYP1A2 is the inducible hepatic binding protein responsible for the hepatic sequestration of TCDD and dioxin-like compounds at inducing doses (15). We then tested the hypothesis that removal of hepatic CYP1A2 alters the toxicokinetic behavior of TCDD and dioxin-like compounds.

The objective of the present study was to investigate the role of CYP1A2 on disposition of TCDD, 2,3,4,7,8-pentachlorodibenzofuran (PeCDF), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) in CYP1A2 KO mice and their parental lineage strains with the intact CYP1A2 gene. Both TCDD and PeCDF are specific CYP1A isoenzyme inducers, and PeCDF is a dioxin-like PHAH. PCB 153 is a nondioxin-like di-ortho substituted PCB and is a non-CYP1A2 binding PCB that does not sequester in the liver (16). It induces cytochrome P450 2B (CYP2B) and acts through a different mechanism of action than TCDD and PeCDF. This PCB resists metabolism in many animals and is the most abundant congener detected in blood and adipose tissue in humans exposed to commercial PCB mixtures (17).

Materials and Methods

Chemicals: TCDD was purchased from Radian Corporation (Austin, TX; purity ≥98%). [1,6-^{3}H]TCDD (radiopurity ≥99%; specific activity 33 Ci/mmole at the time of use) was purchased from Chemsyn Science Laboratories (Lenexa, KS).

Animals and treatment: KO mice were bred from KO mice generously donated by Drs. Frank P. Gonzalez and P. Fernandex-Salguero at NCI/NIH laboratories (Bethesda, MD). C57BL/6N (CYP1A2 +/+; $Ah^{b/b}$) and 129/Sv (CYP1A2 +/+; $Ah^{d/d}$) mice were obtained from Charles River Breeding Lab. (Raleigh, NC) and Taconic Farms (Germantown, PA).

During the study, mice (male; 19 wks old; 5/group) were housed individually in Nalgene metabolism cages (Nalgene; Rochester, NY) with daily and separate collection of feces and urine, provided with food (dustless precision pellet feed; BioServe; Frenchtown, NJ) and tap water ad libitum, and maintained on a 12-hr light/dark cycle at 22±1°C and 55±5% relative humidity. The animals were given a single oral dose of 0 (corn oil), 25 µg [^{3}H]-TCDD/kg, 300 µg [^{14}C]-4PeCDF/kg, or 35.8 mg [^{14}C]-PCB 153/kg in a corn oil vehicle at dosing volume of 10 ml/kg. Four days after dosing, mice were euthanized by CO_2 asphyxiation followed by exanguination.

At necropsy, tissues were removed, weighed, and quantitated for radioactivity by combustion (Packard 306B Biological Oxidizer; Downers Grove, IL), followed by liquid scintillation spectrometry (LSS; Beckman Scintillation Counter, Beckman Instruments; Fullerton, CA). Daily fecal samples were air-dried, weighed, and analyzed for radioactivity by combustion and LSS. Daily urine samples were analyzed for radioactivity by aliquoting samples directly into scintillant for counting by LSS. For determination of CYP1A1 and CYP1A2 activities, S-9 fractions of liver and lung were prepared on same day of necropsy and stored at -70° C until analysis. Microsomal fractions were prepared on day of CYP1A activity analyses.

Sample analysis: Radioactivity in tissues was determined by combustion followed by LSS. Form of radioactivity localized in hepatic and adipose tissue has been demonstrated to be predominantly (if not all) unmetabolized TCDD. Daily fecal samples were air-dried, weighed, and analyzed for radioactivity by combustion and LSS. Daily urine samples were analyzed for radioactivity by aliquoting samples directly into scintillant for counting by LSS.

Cytochrome P450 activity: Ethoxyresorufin *O*-deethylase (EROD), marker for CYP1A1, was measured in hepatic and lung microsomes.

Statistics: All data are presented as mean ± SD. Intergroup comparisons were performed by a one-way analysis of variance (ANOVA) followed by Protected Fisher's Least Significant Difference test. Differences between the parental lineage strains and the knockout mice were considered statistically significant when $p < 0.05$.

Results and Discussion

In both TCDD- and PeCDF-treated mice, comparison of KO mice to parental strains clearly demonstrates that the absence of CYP1A2 affected disposition of both compounds. Mice with CYP1A2 (+/+) gene had 10x > TCDD and 15x > PeCDF in total liver than KO mice. In contrast, KO mice had 4x > TCDD and 6x > PeCDF than CYP1A2 (+/+) mice in adipose tissue. Also, total doses of TCDD- and PeCDF-derived radioactivity in most other measured tissues were 2-6x > in KO mice than CYP1A2 (+/+) mice. Because TCDD and PeCDF were not sequestered in the liver of KO mice, more of these compounds were available for extrahepatic distribution. Unlike TCDD and PeCDF, absence of CYP1A2 did not affect the disposition of PCB 153--in the three groups of genetically different mice, tissue dispositions were similar. This nondioxin-like PCB was not sequestered in liver but was mainly found in adipose tissue.

Significant differences in urinary excretion were demonstrated in TCDD- and PeCDF-treated KO vs normal mice but not in PCB 153-treated mice. Surprisingly, PeCDF-treated KO mice excreted 10x > PeCDF-derived radioactivity than CYP1A2 (+/+) mice. Fecal eliminations of TCDD and PeCDF were very similar in the three groups of different mice. In PCB 153-treated mice, the C57BL/6N strain had significant greater fecal elimination than the KO and 129/SV strains.

At the high inducing dose for both TCDD and PeCDF, similar induction of hepatic EROD enzymatic activity was seen in KO and C57BL/6N mice; but in 129/Sv mice, only ~ half induction was seen--indicative of the 129/Sv $Ah^{d/d}$ genotype. In lung, EROD activity was induced in the 3 groups of mice after TCDD and PeCDF.

In summary, the present study demonstrated the importance of CYP1A2 in the disposition of TCDD and dioxin-like compounds. Whereas, in nondioxin-like compounds, it does not play a role on the toxicokinetic behavior. The increased availability of unbound TCDD and PeCDF to extrahepatic tissues seen in KO mice has important implications for extrahepatic toxicity for dioxin and dioxin-like compounds. Because of no CYP1A2, the KO mice have a low hepatic affinity for dioxin. Likewise, the general human population at low non-inducing environmental levels to dioxin will have a low hepatic affinity for dioxin and relatively more available to extrahepatic tissues including immune and reproductive systems--potential targets for adverse health effects and toxicity.

(This abstract does not necessarily reflect EPA policy.)

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