CYP1A2 Knockout Mice: Decreased Hepatic Microsomal Localization of TCDD

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Abstract

In a previous study[1], we demonstrated that the subcellular distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) differed between hepatic and non-hepatic tissues and demonstrated that the liver specific microsomal localization of TCDD in female B6C3F1 mice and Sprague-Dawley rats may be due to the interaction of TCDD with CYP1A2. In this study, we compared the subcellular distribution of TCDD in the liver, lungs and kidneys of mice deficient in the CYP1A2 gene (-/-) to the subcellular distribution in a normal CYP1A2 mouse strain (B6C3F1)[1]. Male CYP1A2 (-/-) mice received a single oral dose of 25 μg TCDD/kg body weight and subcellular fractions of the liver, lungs and kidneys were prepared by differential centrifugation 4 days after exposure. In comparison to the B6C3F1 mouse strain, the CYP1A2 (-/-) mouse had a lower liver/adipose tissue (L/F) ratio of the TCDD concentration. In addition, the CYP1A2 (-/-) mouse strain had a three-fold increase in the TCDD concentration found in extra-hepatic tissues (lungs, kidneys) as compared to the B6C3F1 mouse strain. Analysis of the subcellular fractions revealed that TCDD was equally distributed between the hepatic P9 (mitochondrial, lysosomal, and nuclear) and S9 (cytosolic and microsomal) fractions in both strains. In comparison to the hepatic P100 (microsomal) localization of TCDD present in the B6C3F1 mouse strain, a decreased concentration of TCDD was associated with the hepatic P100 fraction in the CYP1A2 (-/-) mouse strain. The subcellular distribution of TCDD in extra-hepatic tissues was similar in both mouse strains. This study suggests that the subcellular distribution of TCDD in B6C3F1 mice differs from that in mice lacking a functional CYP1A2 gene and supports the role of CYP1A2 as the microsomal binding protein involved in TCDD sequestration in the liver.

Introduction

Tissue distribution studies for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and related halogenated aromatic hydrocarbons (HAHs) suggest a dose-dependent and specific hepatic localization of these chemicals[2-4]. This dose-dependent hepatic sequestration of TCDD and related HAHs suggests that an inducible hepatic binding protein exists[3,5-8]. Pretreatment with isosafrole, a selective CYP1A2 inhibitor, results in a 3.5-fold decrease in hepatic TCDD concentration, suggesting that CYP1A2 is involved in hepatic localization of TCDD[3]. A high affinity association of TCDD and other congeners with immunoprecipated CYP1A2 exists[6,8]. This data implies that hepatic sequestration of TCDD and related HAHs may be due to an interaction with CYP1A2. Recently, a CYP1A2 (-/-) mouse was developed by disrupting the first coding exon of the CYP1A2 gene (exon 2) rendering the CYP1A2 gene non-functional[9]. In addition, recent studies from our laboratory suggest that hepatic sequestration of TCDD does not occur in the CYP1A2(-/-) mouse strain[10]. However, subcellular distribution of TCDD was not

examined in CYP1A2 (-/-) mice. Therefore, this study investigated the subcellular distribution of TCDD in the liver, lungs and kidneys of acutely exposed CYP1A2 knockout mice as compared to the subcellular distribution in mice possessing a functional CYP1A2 gene[1].

Experimental Methods

Chemicals 2,3,7,8-Tetrachloro[1,6-^{3}H]dibenzo-*p*-dioxin ([^{3}H]TCDD) and unlabeled TCDD were obtained from Chemsyn Science Laboratory (Lenexa, KS) and Radian Corporation (Austin, TX), respectively. Chemical purity (>98%) was determined as described[1].

Treatment of animals and tissue isolation Nineteen-week old male CYP1A2 (-/-) mice (20-25 g) were bred to the F2 generation from CYP1A2 (-/-) mice generously donated by Dr. Frank Gonzalez at the NCI/NIH Laboratories (Bethesda, MD)[9]. Eight-week-old female B6C3F1 mice (20-25 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC). Mice were maintained at the NHEERL, USEPA for 1 week prior to dosing. Male CYP1A2 (-/-) mice, offspring from the above mating, were administered a single dose of 25 µg TCDD/kg body weight (bw) in corn oil at a dosing volume of 10 ml/kg by gavage. Four days after dosing, mice were terminated by CO_2 asphyxiation and the liver, lungs, kidneys and a sample of adipose tissue (50 mg) were excised. B6C3F1 mice were administered a single dose of 10 µg TCDD/kg bw in corn oil at a dosing volume of 10 ml/kg by gavage and tissues were isolated as described[1].

Preparation of S9, P9, S100 and P100 fractions Liver, lungs and kidneys were resuspended in 250 mM sucrose, 1 mM dithiothreitol, 0.5 mM EDTA, 25 mM KCl, 10 mM HEPES and 10% glycerol, pH 7.4 and homogenized with 20 strokes using a Teflon pestle/drill apparatus. 100-200 µl fractions of the liver, lung and kidney homogenates were removed. The remainder of the homogenate was centrifuged at 9000*g* (4°C) for 30 min. The supernatant (S9) and pellet (P9) were separated and 100-200 µl aliquots from each tissue S9 and P9 fraction were removed. The remainder of the S9 fraction was centrifuged at 100,000*g* for 1 hr at 4°C. The supernatant (S100) was separated from the pellet (P100) and 100-200 µl fractions of the S100 and P100 fractions were removed.

Oxidation and quantitation of TCDD Adipose tissue (50 mg), and homogenates, S9, P9, S100 and P100 fractions of the liver, lungs and kidneys, were oxidized in triplicate using a Packard 307 Sample Oxidizer followed by counting in a Beckman Model LS6000 LL liquid scintillation spectrometer. For calculation of percentage total dose, fat content was 7%[1]. The data was expressed as a percentage of TCDD present in the subcellular fractions compared to TCDD levels observed in the homogenate (set at 100%) ± standard deviations[1].

Statistical analysis The statistical intergroup comparisons were determined using a one-way analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD). The levels of probability of statistical significance are $p<0.05$. All data are represented as the mean ± standard deviation.

Results and Discussion

Tissue distribution of TCDD in B6C3F1 and CYP1A2 (-/-) mice

Figure 1 illustrates a comparison of the tissue distribution of TCDD in the B6C3F1 and CYP1A2 (-/-) mouse strains. A significantly ($p<0.05$) higher concentration of TCDD was found in the liver of the B6C3F1 mice as compared to the CYP1A2 (-/-) mouse strain. The B6C3F1 mouse strain had approximately a 12-fold higher concentration of TCDD in the liver as compared to the CYP1A2 (-/-) mouse strain. In contrast, a significantly ($p< 0.05$) elevated concentration of TCDD was found in the adipose tissue of the CYP1A2 (-/-) mouse strain as compared to the B6C3F1 mice. Liver/adipose tissue (L/F) ratios of TCDD concentrations showed a decreased accumulation of TCDD in the liver as compared to the adipose tissue in the CYP1A2 (-/-) mouse strain as compared to the B6C3F1 mouse strain. The L/F ratio of TCDD concentrations was 2.5 in the B6C3F1 mouse strain demonstrated a hepatic localization of TCDD as compared to the adipose tissue. In contrast, the L/F ratio of TCDD concentrations was 0.1 in the CYP1A2 (-/-) mouse strain. The L/F ratio of TCDD concentration is one of the most sensitive indicators of hepatic

sequestration[1]). Figure 1 shows the reduced hepatic localization of TCDD in the CYP1A2 (-/-) mouse as compared to a mouse strain (B6C3F1) possessing a functional CYP1A2 gene. In the extra-hepatic tissues, the lungs/kidneys concentration of TCDD was three-fold greater ($p<0.05$) in the CYP1A2 (-/-) mouse as compared to the B6C3F1 mouse strain, indicating an elevated concentration of TCDD available to extra-hepatic tissues of mice with a deficient CYP1A2 gene. Thus, altered tissue localization of TCDD in the CYP1A2 (-/-) mouse strain after an acute exposure to 25 μg TCDD/kg compared to the B6C3F1 mouse strain further supports the hypothesis that CYP1A2 is the binding protein responsible for hepatic sequestration of TCDD.

Subcellular distribution of TCDD in B6C3F1 and CYP1A2 (-/-) mice

Figure 2 illustrates the subcellular distribution of TCDD in the P9 (nuclear, lysosomal and mitochondrial) subcellular fraction prepared from the liver, lungs and kidneys of B6C3F1 and CYP1A2 (-/-) mice acutely-exposed to TCDD. In both mouse strains, approximately 70% of the total dose of TCDD was concentrated in the P9 of both the kidneys and lungs along with even distribution of TCDD in the hepatic P9 and S9 fractions. The high concentration of TCDD found within the hepatic, pulmonary and renal P9 fractions of both the B6C3F1 and CYP1A2 (-/-) mouse strains may be due to the interaction of TCDD with proteins in the nuclear fraction. TCDD is known to exert its effects by binding to a protein, the aryl hydrocarbon (Ah) receptor (AhR), which acts in the nucleus as a ligand-activated transcriptional enhancer for genes including CYP1A1 and CYP1A2[11]).

Figure 2 also illustrates the subcellular distribution of TCDD in the P100 (microsomal) and S100 (cytosolic) subcellular fractions from the lungs and kidneys of B6C3F1 and CYP1A2 (-/-) mice acutely-exposed to TCDD. Differential centrifugation of the renal S9 fraction demonstrated that TCDD was evenly distributed within the P100 and S100 fractions of both the B6C3F1 and CYP1A2 (-/-) mouse strains. In addition, differential centrifugation of the S9 fraction from the lungs demonstrated that TCDD predominantly localized within the S100 subcellular fraction of both mouse strains. These data suggest the existence of a similar mechanism for subcellular distribution of TCDD within extra-hepatic tissues in both the CYP1A2 (-/-) mouse strain and a mouse strain (B6C3F1) expressing a functional CYP1A2 gene. The mechanism of the differences in subcellular localization of TCDD within the renal and pulmonary S100 and P100 subcellular fractions of both mouse strains may be related to differences in the tissue AhR concentration in the S100 (cytosolic) fraction. For example, analysis of AhR mRNA from C57BL/6J and DBA/2J mice revealed that the highest concentration of the AhR were found in lung, liver, thymus, brain and placenta of both strains[12]). A low concentration of AhR mRNA were identified within the spleen, kidney and muscle.

Figure 2 also illustrates the subcellular distribution of TCDD in the P100 (microsomal) and S100 (cytosolic) subcellular fractions from the liver of B6C3F1 and CYP1A2 (-/-) mice acutely-exposed to TCDD. In both the B6C3F1 and CYP1A2 (-/-) mouse strains, differential centrifugation of the S9 fraction showed an elevated concentration of TCDD associated with the hepatic P100 fraction. However, examination of the hepatic P100/S100 ratios of TCDD in the B6C3F1 mouse strain indicated a 12-fold increase in the concentration of TCDD in the P100 fraction as compared to the S100 fraction. In contrast, the P100/S100 ratios of TCDD in the CYP1A2 (-/-) mouse strain was slightly elevated in the P100 fraction as compared to the S100 fraction (P100/S100 ratio = 1.5). The results of the present study are consistent with the proposed mechanism that the hepatic microsomal sequestration of TCDD and related congeners may be related to the interaction of these compounds with the hepatic inducible binding protein, CYP1A2. The slightly elevated P100/S100 ratio of TCDD concentration observed in the CYP1A2 (-/-) mouse strain may be due to the non-specific association of TCDD with other proteins/factors present within the microsomal fraction.

Summary

This is the first study to suggest that the subcellular distribution of TCDD in B6C3F1 mice differs from the subcellular distribution of TCDD in mice lacking a functional CYP1A2 gene and support the hypothesis that CYP1A2 is responsible for TCDD sequestration in the liver.

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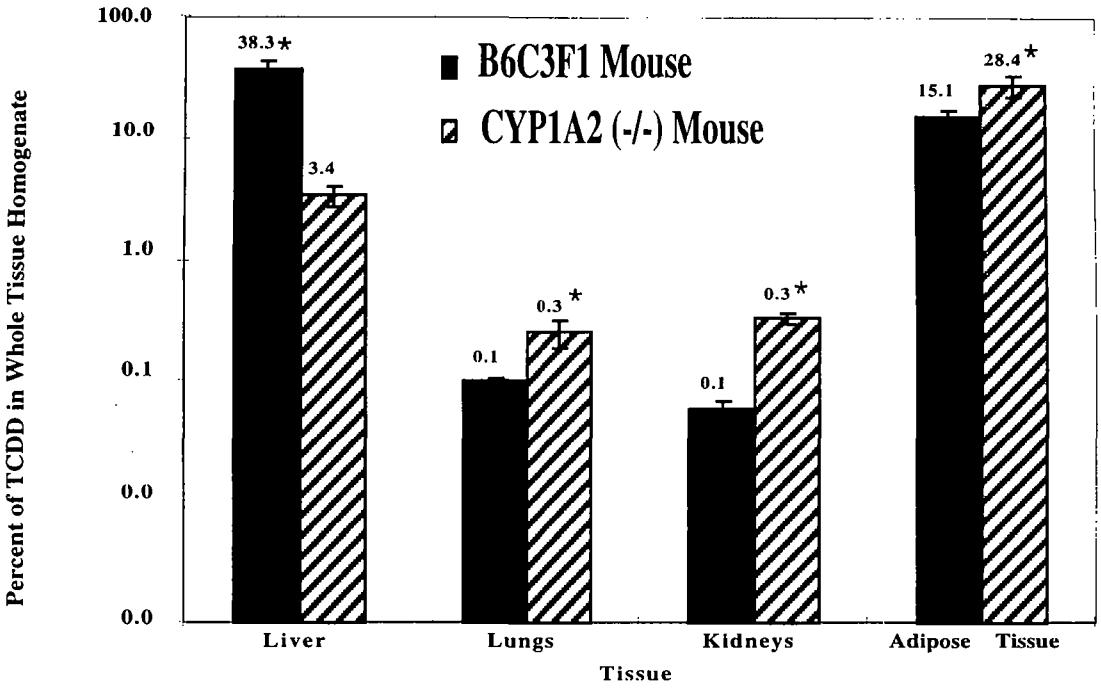

Figure 1. Comparison of Tissue Distribution of TCDD in B6C3F1 and CYP1A2 (-/-) mice. B6C3F1 mice received a single oral dose of 10 μg TCDD/kg and were sacrificed as described[1] CYP1A2 (-/-) mice received a single oral dose of 25 μg TCDD/kg and were sacrificed as described in the Experimental Methods section. Whole liver, lungs, kidneys and a sample of adipose tissue were excised and the concentration of TCDD in each tissue was determined by sample oxidation as described[1]. Data are presented as the mean ± standard deviation (n=5). * Statistically higher ($p<0.05$) than the corresponding tissue when comparing the B6C3F1 mouse strain versus the CYP1A2 (-/-) mouse strain.

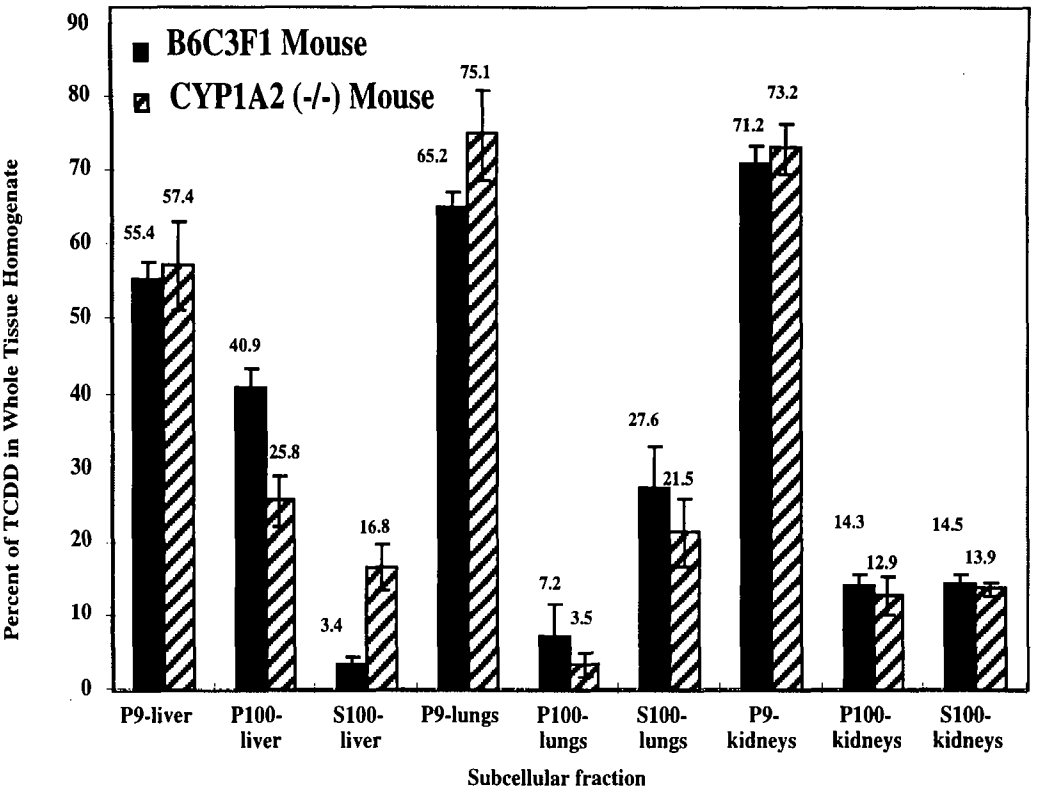

Figure 2. The Distribution of TCDD in Subcellular Fractions of Liver, Lungs and Kidneys of B6C3F1 and CYP1A2 (-/-) mice. B6C3F1 mice received a single oral dose of 10 μg TCDD/kg and were sacrificed as described[1]). CYP1A2 (-/-) mice received a single oral dose of 25 μg TCDD/kg and were sacrificed as described in the Experimental Methods section. The P9, S100 and P100 subcellular fractions were prepared and the concentration of TCDD in each fraction was determined by sample oxidation as described[1]). The subcellular fraction data are expressed as pecentages of the radioactivity present in each fraction as compared to the total radioactivity obtained in the intial tissue homogenates. P9=nuclear, mitochondrial and lysosomal fractions, S100=cytosolic fraction and P100=microsomal fraction. Data are presented as the mean ± standard deviation (n=5).