The Role of CYP1A2 in Localization of TCDD in Subcellular Fractions of Rat and Mouse Tissues

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1. Abstract

This study was designed to examine the role of CYP1A2 in the subcellular distribution of TCDD in the liver and kidneys of mice and rats. Subcellular fractions of the liver and kidneys from TCDD-treated female mice and rats were prepared and oxidized to determine [^{3}H]TCDD levels. The results show an accumulation of TCDD in the rat liver nuclear fraction (P9) after an acute dose. Almost all of the [^{3}H]TCDD present in the cytosolic plus microsomal (S9) fraction localized in the hepatic microsomes (P100). The results also show an accumulation of [^{3}H]TCDD in the rat kidney P9 fraction (which is not dose-related). However, [^{3}H]TCDD was evenly distributed between the kidney P100 and cytosol (S100) fractions. Sub-chronic exposure of mice to [^{3}H]TCDD results in a dose-dependent distribution of [^{3}H]TCDD within the liver, but not in the kidney. At the low dose, [^{3}H]TCDD concentrations are localized to the P9 fraction of mouse liver. However, with increasing dose, an even distribution of [^{3}H]TCDD in the S9 and P9 fractions was observed. In contrast, within the kidney, [^{3}H]TCDD is found predominately in the mouse P9 fraction. A dose-dependent increase in deposition of [^{3}H]TCDD within the microsomes is observed in mouse liver, but not in the kidney. Increased [^{3}H]TCDD in rat hepatic microsomes was associated with increased levels of CYP1A2. The data suggest that the deposition of [^{3}H]TCDD is more concentrated in the liver microsomes than the renal microsomes, which may be caused by sequestering of TCDD by liver CYP1A2.

2. Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic member of the halogenated aromatic hydrocarbon (HAH) family, which includes polychlorinated dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), biphenyls (PCBs) and naphthalenes (PCNs)[1]. These environmental compounds, present in complex mixtures, elicit a diverse spectrum of sex-, strain-, age-, and species-specific toxic and biological responses[2]. The best characterized effect of TCDD in animals and humans is the induction of CYP1A1 and CYP1A2, which is aryl hydrocarbon (Ah) receptor (AhR)-mediated and involves transcriptional activation of the gene[1]. Immunolocalization studies have indicated an apparent linear relationship between dose and TCDD liver concentrations over a dose range which produces CYP1A1 and CYP1A2 induction[3]. This dose-dependent hepatic accumulation of TCDD is believed to occur via the interaction of TCDD with CYP1A2[4,5]. The concentration and affinity of the induced microsomal binding protein in the liver appears to be responsible for the preferential TCDD localization in the liver as compared to fat in C57BL/6J and DBA/2J mice[5]. However, the induction of P450 isozymes by TCDD is tissue-specific[6-8]. Induced CYP1A1 levels are found in TCDD-treated rat liver, kidneys and lungs[6]; as well as in TCDD-treated rabbit lungs and kidneys[7]. TCDD-induced CYP1A2 has been identified in hepatic tissues[6,7] and constitutive levels of CYP1A2 are detected by immunofluorescent staining in rabbit kidneys and lungs[8]. Due to the species- and tissues-variability in CYP1A2 levels, we chose to

investigate the role of CYP1A2 in the dose-dependent localization of TCDD in rat and mouse liver and kidney subcellular fractions after acute and sub-chronic exposure.

3. Materials and Methods

Chemicals ^{3}H-TCDD was obtained from Radian Corporation (Austin, TX) and was purified by reverse-phase-high-pressure liquid chromatography to ≥ 99% (radiochemical purity, Sp. Ac. 34.7 Ci/mmole)

Treatment of animals Eight-week-old female Sprague-Dawley rats (150-200 g) and female B6C3F1 mice (20-25 g) were obtained from Charles River Breeding Laboratories (Raleigh, NC) and maintained at the Health Effects Research Laboratory, USEPA. Rats received a single oral dose of 0.1, 1.0, or 10.0 µg [^{3}H]TCDD/kg in 5 ml/kg corn oil. Mice were treated orally with 1.5 or 150 ng [^{3}H]TCDD/kg/day in 10 ml/kg corn oil 5 days/week for 17 weeks.

Tissue isolation Three days after final dosing, rats and mice were terminated by CO_2 asphyxiation and the liver and kidneys were excised.

Preparation of S9, P9, S100 and P100 fractions Liver and kidneys (peeled) 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 and kidney homogenates were oxidized. The remainder of the homogenate was centrifuged at 9000*g* (4°C) for 30 min. The supernatant (S9) and pellet (P9) was removed and 100-200 µl fractions were oxidized. The S9 fraction was centrifuged at 100,000*g* for 1 hr at 4°C. The supernatant (S100, cytosol) was separated from the pellet (P100, microsomes) and 100-200 µl fractions of the S100 and P100 fractions were oxidized.

Oxidation and quantitation of subcellular fractionsHomogenates, S9, P9, S100 and P100 fractions, of the liver and kidney were oxidized using a Packard 307 Sample Oxidizer followed by counting in a Beckman Model LS6000 LL liquid scintillation spectrometer and the data was expressed as a percentage of [^{3}H]TCDD present in the subcellular fractions compared to [^{3}H]TCDD levels observed in the homogenate (set at 100%) ± standard deviations.

Ethoxyresorufin-*O*-deethylase (EROD) assay: EROD levels, a marker for CYP1A1 activity, were determined as described[9].

Determination of protein concentration: Protein measurements were measured as described[10].

Western blot analysis of CYP1A2: Rat microsomal proteins (10-50 µg) were resolved on a 8% tris-glycine mini gel (Novex, San Diego, CA.) and transferred onto a 0.2 mm PVDF membrane (Novex, San Diego, CA.) using a Trans-Blot SD Semi Dry Transfer Cell apparatus (Biorad Laboratories Inc., Hercules, CA.). After blocking, the membrane was probed with a 1:1000 dilution of a rabbit polyclonal antibody against rat CYP1A1 and CYP1A2 (Human Biological, Inc. Phoenix, AZ) and coated with a secondary Goat Anti-Rabbit IgG (H+L)-(human adsorbed) alkaline phosphatase conjugate (Gibco BRL, Gaithersberg, MD). CYP1A2 was visualized by an alkaline phosphatase reaction and quantified by densitometry with an Olympus Cue 2 system. CYP1A2 levels in liver and kidney are expressed as a percentage of CYP1A2 levels observed in the samples compared to CYP1A2 levels found in liver microsomes from TCDD-treated rats ± standard deviations.

4. Results/Discussion

The results in Table 1 illustrate the distribution of TCDD in the S9, P9, S100 and P100 fractions of rat liver and kidneys. The data shows an accumulation of TCDD in the liver P9 (nuclear) fraction with subsequent decreases in S9 (cytosol and microsomal) fractions. The data also shows that a higher concentration of [^{3}H]TCDD is found in the kidney P9 fraction compared to the S9 fraction (not dose-related). This data coincides with the molecular mechanism of action of the AhR as a nuclear ligand-activated transcription factor[1]. However, tissue-differences in [^{3}H]TCDD concentrations in liver and kidney P9 and S9 fractions are apparent and may reflect subcellular tissue differences in TCDD uptake/localization and/or AhR levels[1,2].

Previous studies have suggested that TCDD is sequestered in the liver by binding to CYP1A2[4,5]. Table 1 illustrates that within the S9 fraction, TCDD is mainly localized within the hepatic microsomal fraction (P100). This liver microsomal localization may be related to elevated levels of CYP1A2 (Table 2) which sequesters TCDD. Elevated levels of TCDD result in the induction of CYP1A1 and its associated enzymatic activity (Table 2). A near maximal localization of [^{3}H]TCDD (i.e.

92.5%=(P100/(S100+P100) *100)) in the hepatic microsomes was observed at the lowest dose tested; which also produced near maximal levels of EROD induction and CYP1A2 levels (Table 2). In contrast, within the kidney S100 and P100 fractions, a more even localization of [^{3}H]TCDD is observed (Table1). The lower concentration of TCDD observed in the kidney P100 fraction compared to the liver P100 fraction may reflect lower levels of CYP1A2 found in the kidney (Table 2). Moreover, EROD induction reflects the differential dosimetry between the kidney and liver (Table 2). However, this data does indicate the presence of CYP1A2 in a rat extrahepatic tissue.

The results in Table 3 depict the distribution of TCDD between S9 and P9 fractions in the mouse liver and kidneys. This data suggests that sub-chronic exposure of mice to TCDD results in nuclear accumulation of TCDD in liver and kidneys and a dose-dependent localization of TCDD in the hepatic microsomes. This data suggests that mouse liver microsomes sequester TCDD. There is no apparent accumulation of [^{3}H]TCDD within the renal microsomes (Table 2). For example, at the low dose, [^{3}H]TCDD is evenly localized within the kidney S100 and P100 fractions. At the high dose, a higher [^{3}H]TCDD concentration is found within the kidney S100 fraction. The absence of localization of TCDD within the renal microsomes suggests that there is no or little sequestering mechanism (CYP1A2) present in the mouse renal microsomes.

5. Conclusions

The data appears to demonstrate a sequestering of TCDD in the hepatic microsomes of mice and rats, which is not observed in the kidneys. The results are consistent with the hypothesis that inducible CYP1A2 sequesters TCDD in the liver resulting in increased induction of CYP1A1 and its associated enzymatic activity (EROD). The results also suggest a difference in tissue TCDD localization in animals with acute versus subchronic dosing. Future studies involve delineating the role of CYP1A2 on tissue localization of TCDD within CYP1A2 knockout mice.

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6. References

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Table 1. Subcellular distribution of [^{3}H]TCDD in rat liver and kidneys. Rats were treated and subcellular fractions of liver and kidneys were prepared as described. [^{3}H]TCDD levels were determined by fraction oxidation and the data are expressed as a percentage of [^{3}H]TCDD found in the subcellular fractions compared to [^{3}H]TCDD levels (*ng TCDD/g) observed in the homogenate (set at 100%) (#= ((P100/(P100+S100))*100)).

Sub. Fr.	Tissue	0.1 µg TCDD/kg	1 µg TCDD/kg	10 µg TCDD/kg
Hom.	liver	100 (*1.0)	100 (*11.7)	100 (*106.6)
P9	liver	53.8 ± 8.4	71.0 ± 10.6	82.1 ± 7.9
S9	liver	42.3 ± 1.6	36.6 ± 5.6	31.5 ± 1.9
S100	liver	4.1 ± 1.4	2.1 ± 0.5	1.8 ± 0.6
P100	liver	50.0 ± 4.6 (#92.5)	39.6 ± 3.9 (#95.0)	28.2 ± 3.3 (#94.0)
Hom.	kidneys	100 (*0.02)	100 (*0.09)	100 (*0.7)
P9	kidneys	62.6 ± 6.8	51.1 ± 4.8	50.2 ± 11.3
S9	kidneys	22.1 ± 8.5	33.4 ± 12.0	27.5 ± 10.5
S100	kidneys	6.2 ± 3.0	10.9 ± 5.4	13.7 ± 10.2
P100	kidneys	15.4 ± 5.0	14.7 ± 7.7	17.0 ± 5.1

Table 2. TCDD-induction of EROD activity and CYP1A2 levels in rat liver and kidney microsomes. Rats were treated as described and EROD and CYP1A2 levels were measured according to the Materials and Methods section. EROD is expressed as pmoles/min/mg protein. CYP1A2 levels are expressed as a percentage of O.D. units/µg protein in the sample compared to O.D. units/µg protein observed in a TCDD-treated rat liver microsomal sample (set at 100%).

µg TCDD/kg	Tissue	EROD Activity	CYP1A2 Levels
0.0	liver	811.6 ± 98.2	8.8 ± 2.3
0.1	liver	6302.1 ± 1563.3	165.2 ± 26.9
1.0	liver	11223.1 ± 2732.7	228.3 ± 40.9
10.0	liver	13792.0 ± 1965.3	217.6 ± 80.9
0.0	kidneys	142.9 ± 52.4	1.8 ± 0.8
0.1	kidneys	532.5 ± 63.1	2.0 ± 1.0
1.0	kidneys	3263.4 ± 1126.8	2.5 ± 1.2
10.0	kidneys	6166.9 ± 778.4	2.5 ± 1.0

Table 3. Subcellular distribution of [^{3}H]TCDD in mouse liver and kidneys. Mice were treated and subcellular fractions from liver and kidneys were prepared according to the Materials and Methods section. [^{3}H]TCDD levels were determined by oxidation and the data are expressed as a percentage of [^{3}H]TCDD found in the subcellular fractions compared to [^{3}H]TCDD levels (*ng TCDD/g) observed in the homogenate (set at 100%).

Subcellular Fraction	Tissue	1.5 ng TCDD/kg/day	150 ng TCDD/kg/day
Homogenate	liver	100 (*0.09)	100 (*21.5)
P9	liver	96.3 ± 0.3	47.2 ± 1.6
S9	liver	3.7 ± 0.3	52.8 ± 1.6
S100	liver	42.2 ± 6.8	13.8 ± 2.4
P100	liver	57.8 ± 6.8	86.2 ± 2.4
Homogenate	kidneys	100 (*0.01)	100 (*0.27)
P9	kidneys	70.9 ± 19.0	72.1 ± 8.2
S9	kidneys	29.1 ± 19.0	27.9 ± 8.2
S100	kidneys	52.3 ± 5.2	69.8 ± 3.5
P100	kidneys	47.7 ± 5.2	30.2 ± 3.5