In Vivo Regulation of the Hepatic Cytosolic Ah Receptor (AhR) Protein by TCDD

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1. INTRODUCTION

Several studies have investigated the regulation of the aryl hydrocarbon (Ah) receptor (AhR) upon treatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) (Sloop and Lucier, 1987; Prokipcak and Okey, 1991; Abbott *et al.*, 1994b; Abbott and Probst, 1995; Pollenz, 1996). A time-dependent decrease in cytosolic [^{3}H]TCDD:AhR binding in female Sprague-Dawley rats was observed 30-60 min post treatment, followed by an increase from 1-9 days (Sloop and Lucier, 1987). A time-dependent loss of immunoreactive AhR protein expression by TCDD has been reported using a number of rodent cultured cell lines (Pollenz, 1996). These results suggest that AhR expression may be altered in response to treatment with TCDD. Although regulation of the AhR by TCDD has been demonstrated under certain conditions, no investigation has characterized the time-course relationships between TCDD tissue dosimetry, CYP1A1- and CYP1A2-dependent enzyme induction and AhR protein expression. Therefore, the time-dependent effects of TCDD on tissue localization, enzyme-induction, and AhR protein expression in the liver of female Sprague-Dawley rats treated with 10 μg [^{3}H]TCDD/kg for 0.5, 1, 3 or 8 hours, or 1, 7, 14 or 35 days were examined. In addition, we also investigated the dose-dependent effect of TCDD on hepatic cytosolic AhR protein expression in female Sprague-Dawley rats 3 days after exposure to a single oral dose of 0.0 (corn oil vehicle), 0.01, 0.1, 0.3, 1.0, 10.0 or 30.0 μg [^{3}H]TCDD/kg.

2. MATERIALS AND METHODS

Chemicals. 2,3,7,8-Tetrachloro[1,6-^{3}H]dibenzo-*p*-dioxin (^{3}H-TCDD) was purchased from Chemsyn Science Laboratory (Lenexa, KS) and radiochemical purity (≥99%) was verified as described (Diliberto *et al.*, 1995). TCDD was obtained from Radian Corp. (Austin, TX) with a ≥98% stated chemical purity.

Animals. Eight week old female Sprague-Dawley rats (200-225 g) were purchased from Charles River Laboratories (Raleigh, NC) and acclimated for one week. Rats were housed at NHEERL of the USEPA (Research Triangle Park, NC) and followed a diurnal cycle of 12 hours of light and dark at ambient temperature (22 ± 1°C) and relative humidity (55 ± 5%). Five (5) animals per dose group were placed in polycarbonate cages with free access to Purina 5001 Rodent Chow (Ralston Purina Co., St Louis, MO) and water.

Treatment/Tissues. Rats (5 per time point) were administered a single oral dose of either a corn oil solution containing 10 μg (31 μmol, 5 μCi) [^{3}H]TCDD/kg or corn oil vehicle alone at 5 ml/kg. At 0.5, 1, 3 or 8 hours, or 1, 7, 14 or 35 days after dosing, rats were euthanized by CO_2 asphyxiation and liver and adipose tissues (perirenal fat) were removed. In a separate experiment, rats were administered a single oral dose of 0.0 (corn oil), 0.01, 0.1, 0.3, 1.0, 10.0 or 30.0 μg [^{3}H]TCDD/kg at 5 ml/kg and terminated 3 days post-dosing (n=4 rats/treatment group). Liver and adipose tissues were also removed.

TCDD determination. TCDD tissue concentrations were determined by combustion in a Packard 307 Sample Oxidizer (Packard, Downers Grove, IL) and analyzed by liquid scintillation spectrometry. One hundred mg of the liver and 50 mg of adipose tissue were combusted in triplicate.

Enzyme Assays. Ethoxyresorufin *O*-deethylase (EROD) and methoxyresorufin *O*-demethylase (MROD) activities, markers for CYP1A1 (Pohl and Fouts, 1980) and CYP1A2 (Chaloupka *et al.*, 1995) respectively, were quantitated spectrofluorimetrically (Pohl and Fouts, 1980) as modified by Diliberto *et al.*, 1995.

Western analysis of AhR. Rat hepatic cytosol was prepared (Bandiera *et al.*, 1982) and protein concentrations were determined (Bradford, 1976). Cytosolic proteins (50 µg) were resolved on a 10% acrylamide resolving gel and a 4% acrylamide stacking gel as described (Laemmli, 1970) and transferred onto a 0.2 µm nitrocellulose membrane at 200 mA for 30 min (Towbin *et al.*, 1979). Membranes were blocked in Tris-buffered-saline pH 7.5 with 0.05 % Tween (TBST), containing 5 % commercial non-fat milk for 1 hour at 22°C and probed with a 1:1000 dilution of rabbit polyclonal antibody against AhR overnight at 4°C (Pollenz *et al.*, 1994) followed by coating with a 1:5000 dilution of a secondary goat anti rabbit IgG (H+L)-(human adsorbed) alkaline phosphatase conjugate (Gibco BRL, Gaithersburg, MD). The AhR was visualized by an alkaline phosphatase reaction at pH 9.8 for 15 min and quantified with a Masterscan interpretive densitometer using Scanalytics software (Billerica, MA). The hepatic cytosolic AhR protein concentration is expressed as optical denisity units/µg protein. The molecular weights of the AhR immunostained protein was determined in comparison to molecular weight standards (Novex, San Diego, CA).

Data/statistical analysis. For calculation of percentage total dose, fat content was defined as 7% (ILSI, 1994). All data are represented as the mean ± standard deviation. The statistical intergroup comparisons were determined using a one-way analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

Figures 1a & 1b show the tissue dosimetry data for the liver and adipose tissue as expressed as a percentage administered dose/gram tissue. Both tissues show an initial accumulation of radioactivity that was similar to tissue localization studies of male Fischer 344 rats acutely-exposed to 2,3,7,8-tetrachlorodibenzofuran (TCDF) (Birnbaum *et al.*, 1980) or 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) (Buckley-Kedderis *et al.*, 1991). In the liver, the concentration of TCDD reached a maximum at approximately 24 hours post-treatment (Figure 1a). In the adipose tissue, the concentration of TCDD reached a maximum approximately 7 days after dosing (Figure 1b) which was similar to studies by Birnbaum and coworkers (1980). This data supports previous tissue disposition studies showing that TCDD and related compounds are cleared more rapidly from the liver than the adipose tissue of rats (Birnbaum *et al.*, 1980; Abraham *et al.*, 1988; Buckley-Kedderis *et al.*, 1991).

Hepatic EROD and MROD activities are presented in Figures 2a & 2b. Control animals exhibited similar EROD and MROD activities at all time-points examined (Figures 2a & 2b). After a 0.5, 1 or 3 hours exposure, EROD & MROD activity in TCDD-treated animals was similar to controls animals. TCDD-induced EROD activity increased for 8 hours, was maximum at 7 days and then decreased. TCDD-induced MROD activity was also increased for 8 hours, but did not reach a maximum until 14 days post-treatment, followed by a slight decrease at 35 days. The time-dependent effect of TCDD on hepatic enzyme-activity was similar to previous CYP1A1 and CYP1A2-associated enzymatic studies in TCDD-treated rats and mice (Abraham *et al.*, 1988; Diliberto *et al.*, 1995).

Figure 3a shows the *in vivo* time-dependent expression of the AhR protein in control- and TCDD-treated female Sprague-Dawley rats as determined by western blot analysis. The hepatic cytosolic AhR protein exhibited a molecular weight of 105 kDa (data not shown) as previously reported (Poland and Glover, 1987). At all time-points and doses examined, preliminary western blot analysis showed that the hepatic cytosolic AhR protein expression was highly variable (Figures 3a & 3b) TCDD-treatment resulted in an increase in animal to animal variablity of the hepatic cytosolic AhR expression (Figure 3a & 3b), which has been previously observed by

analysis of AhR message by quantitative RT-PCR (B. Abbott, USEPA, Personal Communication). In control animals the hepatic cytosolic AhR protein expression was relatively constant. After a 0.5, 1 or 3 hours exposure, cytosolic AhR protein expression in TCDD-treated animals was similar to controls animals. A non significant decrease in cytosolic AhR protein expression was observed 8 hours post dosing compared to controls. This non significant decrease in hepatic cytosolic AhR protein expression in TCDD-treated animals compared to control was also observed 7, 14 and 35 days after exposure. A more pronounced time-dependent decrease in hepatic AhR protein expression by TCDD was observed in total cell lysates and nuclear/cytosolic fractions of TCDD-treated Hepa 1c1c7 cultured cells (Pollenz, 1996). The *in vivo* dose-dependent expression of the hepatic cytosolic AhR protein expression in female Sprague-Dawley rats treated with TCDD was also determined by western blot analysis (Figure 3b). The data suggest a dose-dependent increase in the *in vivo* hepatic cytosolic AhR protein expression by TCDD. However, this trend was not significant. These data are similar to previous rat studies that showed a time-dependent increase in ligand-bound hepatic AhR complexes after TCDD-exposure (Sloop and Lucier, 1987). In contrast, a recent *in situ* study reported a dose-dependent decrease in immunoreactive total hepatic AhR expression in TCDD-treated Hepa 1c1c7 cultured cells (Pollenz, 1996). The reason for this difference in TCDD-induced modulation (upregulation/downregulation) of the hepatic AhR in cultured cells and *in vivo* by is unknown, but may related to a cell type- and/or tissue-specific effects (Sloop and Lucier, 1987; Prokipcak and Okey, 1991; Abbott *et al.*, 1994a; Abbott *et al.*, 1994b; Pollenz, 1996).

4. CONCLUSIONS:

The results demonstrate a time-dependency of TCDD on hepatic and adipose tissue localization and CYP1A1/CYP1A2-associated enzymatic activities. However, there is little effect of TCDD exposure on the cytosolic AhR concentration in liver.

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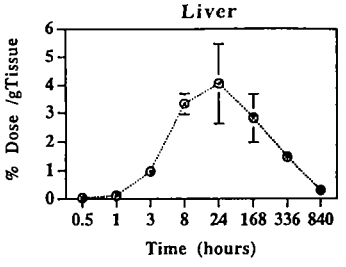

Figure 1a. Time-dependent tissue localization of TCDD in the liver of female Sprague-Dawley rats treated orally with 10 µg TCDD/kg. Each data point represents the mean values (±SD) obtained from 5 animals.

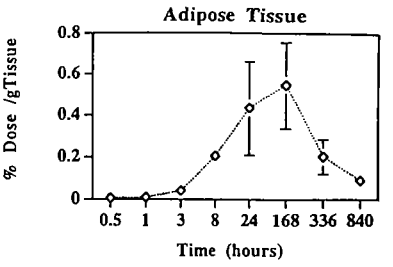

Figure 1b. Time-dependent tissue localization of TCDD in adipose tissue of female Sprague-Dawley rats treated orally with 10 µg TCDD/kg. Each data point represents the mean values (±SD) obtained from 5 animals.

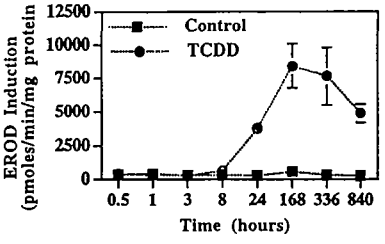

Figure 2a. Time-dependent EROD induction in the liver of control- and TCDD-treated female Sprague-Dawley rats. Each data point represents the mean values (±SD) obtained from 5 animals.

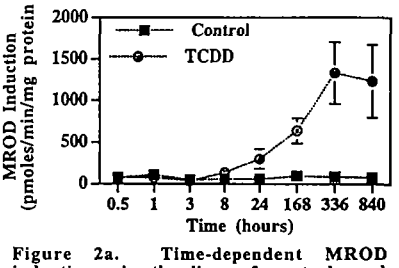

Figure 2a. Time-dependent MROD induction in the liver of control- and TCDD-treated female Sprague-Dawley rats. Each data point represents the mean values (±SD) obtained from 5 animals.

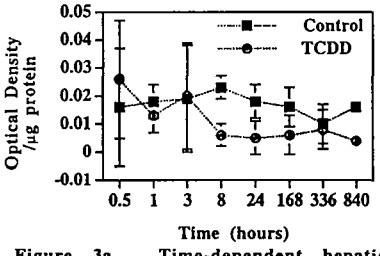

Figure 3a. Time-dependent hepatic cytosolic AhR protein expression in control- and TCDD- treated female Sprague-Dawley rats. Each data point represents the mean values (±SD) obtained from 5 animals.

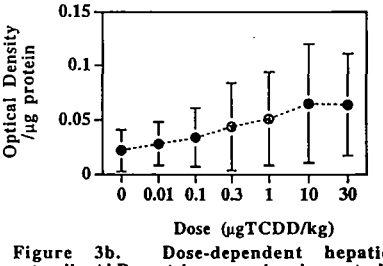

Figure 3b. Dose-dependent hepatic cytosolic AhR protein expression in control- and TCDD- treated female Sprague-Dawley rats. Each data point represents the mean values (±SD) obtained from 4 animals.