Porphyrin Profile Alterations From Subchronic 2,3,7,8-Tetrachlorodibenzo-p-Dioxin Exposure In Non-Traditional Laboratory Species.

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1. Introduction

Porphyrins are precursors in the biosynthesis of heme, a component in hemoproteins, cytochromes and chlorophylls. As a result of their biochemical importance, porphyrins are found in virtually all eukaryotes. The precursors to heme are four linked, cyclized pyrole rings with either 8 (URO), 7 (HEPTA), 6 (HEXA), 5 (PENTA), 4 (COPRO) or 2 (PROTO) carboxyl groups attached. A series of decarboxylation steps transform highly carboxylated porphyrins to the dicarboxylated porphyrin which becomes heme. Organisms typically overproduce the carboxylated porphyrins in predicable patterns which can be quantified in a wide range of matrixes including liver, kidney, blood, urine and feces [1]. Contaminant induced changes in these patterns have been widely reported [2]. Alterations of carboxylated porphyrin concentrations due to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure have been previously described in the literature and typical results include the accumulation and excretion of elevated levels of uroporphyrin (8- or URO) and heptacarboxylporphyrin (7- or HEPTA) [3]. The potential to alter baseline porphyrin profiles with environmental contaminants and the ease with which porphyrin concentrations can be quatified, has lead to the suggestion that porphyrin profiles can be used as a wildlife biomarker [4].

The mechanism of TCDD-induced porphyria, while not entirely elucidated, is thought to include several components. The direct inhibition of heme synthesis enzymes, and the induction of delta-aminolevulinic acid synthesis have been suggested. These have not been shown, however, to be the exclusive basis of TCDD-produced porphyrias [5]. The induction of CYP1A2, via the Ah receptor protein, is also suspect in the onset of the TCDD porphyria [6]. Oxidation of uroporphyrinogen III to uroporphyrin III, which cannot be utilized as a uroporphyrinogen decarboxylase (UROD) substrate, via a CYP1A2 dependent mechanism is also suspect [1], [7]. It is also possible that the P450 enzyme activates heme intermediates, which can then bind to UROD and inactivate the enzyme, causing an 8- and 7- carboxylporphyrin overproduction.

Few laboratory studies have been conducted utilizing environmentally significant TCDD dosing regimes or wildlife species. The present experiments study two distinctly different species responses to TCCD exposure. In one experiment, chickens were used as a surrogate model for wild turkeys inhabiting a TCDD contaminated site in Times Beach, Missouri. A high dose group simulated the pre-remediated contamination scenario, where

an average of 200 ppb TCDD soil concentrations were observed while a low dose group simulated the post remediation exposure potential with 1 ppb soil concentrations. Although contaminated areas averaged 200 ppb3 much of the site was not contaminated. The contamination at this site was limited to roads and roadsides; therefore, the overall site (500+ acres) TCDD level before remediation began was approximately 40ppb4.In the second study, a mammalian wildlife species, the deer mouse (*Peromyscus maniculatus*), was dosed with TCDD. Liver tissue from each species was assessed for changes in porphyrin profiles.

2. Materials and Methods

Animal Handling and Sample Collection

Reproductively mature white leghorn chickens (*Gallus gallus*) were obtained from ISE of America, Newbery, SC. The animals acclimated in laboratory conditions for a period of four weeks before beginning treatments. Four female chickens of the same dose group were housed per cage. Animals were maintained on a 16 hour light / 8 hour dark cycle and provided with food and water ad libitum. Chickens were dosed intraperitoneally twice per week for six weeks with TCDD in olive oil. Low dose animals received 5.6 ng/kg/day and high dose animals received 1.17 ug/kg/day. Chickens were euthanized at the end of weeks six by CO_2 narcosis, and necropsied. Livers were stored at -80 ° C until analysis.

Male deer mice (Peromyscus maniculatus), 7-8 weeks old, were allowed to acclimate for a period of one week. Mice were housed in nalgene cages and provided a 12 hour light / 12 hour dark cycle. Food and water were provided ad libitum. Six mice of the same dose group were kept per cage. The mice were dosed intraperitoneally with TCDD in corn oil at a volume of 10 ul/gram. Low, medium and high dose organisms received 0.030, 0.30 and 3.0 mg/kg/day, respectively. Control mice received corn oil vehicle only. Dosing was carried out every other day for 12 days. On day 12, mice were euthanized by CO_2 narcosis and necropsied. Livers were stored at -80 ° C until analysis.

Tissue Porphyrin Analyses

Reagents and materials were the same as those previously reported [3]. Tissue porphyrins were extracted using a modification [3] of the extraction procedure of Kennedy and James (1993) [8]. Briefly, a portion of liver (0.2 gram) was homogenized with 6 ml of acetonitrile/1 N HCl (1:1) using a Brinkmann polytron homogenizer (Littau, Switzerland). The mixture was centrifuged at 3,000 g for 15 minutes, and the resulting supernatant was saved. The pellet was rehomogenized with another 6 ml of acetonitrile/1 N HCl (1:1) and centrifuged as before. The combined supernatants were diluted with water to achieve a final volume of 70 ml. The porphyrins were isolated on the SepPak C-18 columns, eluted with acetonitrile (10 ml), brought to complete dryness with nitrogen, and reconstituted with 1 N HCl (0.5 ml) and filtered (0.45 um).

Porphyrin analyses were performed by reverse phase HPLC [9), 1)]. The 8-, 7-, 6-, 5-, 4- and 2- carboxylporphyrins were separated on a Waters HPLC system equipped with two pumps (model 501, Waters, Milford, MA, USA), autosampler (WISP 710B, Waters), computer integration program (Baseline 820, Waters), and a fluorescence detector (RF-535, Schimadzu, Kyoto, Japan) with excitation/emission wavelengths of 390/615 nm.

Individual porphyrin peaks from 100 ul injections were separated at a flow rate of 1.5 ml/min with a methanol and phosphate buffer (0.5 M, pH 3.5) gradient program [3].

Individual porphyrin peak areas were integrated by the Baseline program (Waters). Individual porphyrin levels were calculated from standard curve values and expressed as pmole porphyrin/gram tissue. Total porphyrin levels were calculated as the sum of the six individual porphyrins. Differences between dose levels were compared using analysis of variance and the Tukey-Kramer procedure. The level of significance was chosen as $p<0.05$.

3. Results

All six measured individual hepatic carboxylporphyrin concentrations were significantly ($p<0.05$) elevated in chickens administered 1.17 ug TCDD/kg/day (Figure 1). The lower dosing regime (5.6 x 10-6 mg/kg/day) resulted in significant increases of 7-, 5-, 4- and 2-carboxylporphyrin.

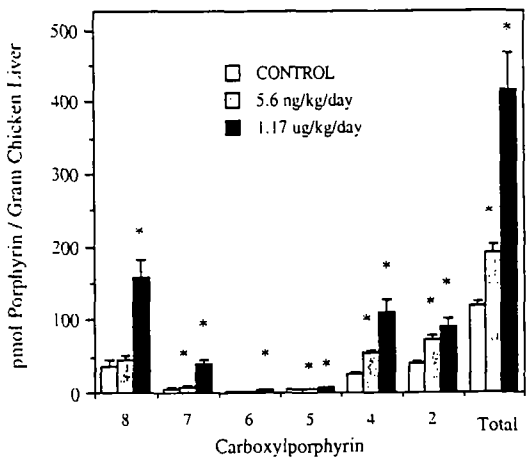

Figure 1. Hepatic porphyrin concentration from TCDD-dosed chickens. Values are means ± standard error. * indicates significance ($p<0.05$).

A significant increase was observed in the total porphyrin concentration of both the low and high TCDD doses, with total porphyrin concentrations 1.6-fold and 3.5-fold over controls, respectively. The 8-, 7-, 4- and 2-carboxylporphyrins contributed the most substantially to the detected total increase.

Significant increases were observed in deer mice URO, HEPTA and COPRO carboxylporphyrins at the highest (3.0 mg/kg/day) dose (Figure 2), resulting in total liver porphyrin elevations 1.6-fold over controls. The 8-, 7- and 4-carboxylporphyrins represented the majority of the total porphyrin increase; 2-carboxylporphyrin, however, was uneffected. Lower dosing levels failed to produce an observable porphyrin effect.

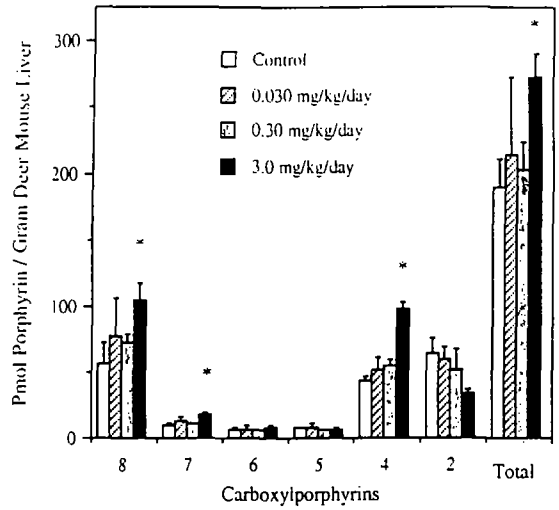

Figure 2. Hepatic porphyrin concentrations from TCDD-dosed mice. Values are mean ± standard error. * indicates significance ($p<0.05$).

4. Conclusions

The TCDD dosing regimes resulted most significantly in increases of URO, HEPTA and COPRO porphyrin levels in both species. Modest alterations, yet not significant ($p<0.05$), in deer mouse porphyrin concentrations at lower dose levels may have been masked by relatively higher levels of baseline variation. This increase in variation is predictable in outbred wildlife species, and may be compensated for by using a greater sample size.

These data indicate that differences in baseline porphyrin profiles exist between the chicken and the deer mouse. While the majority of the baseline total porphyrin concentration consists of 8-, 4- and 2- in both species, the deer mice has approximately a 1.6-fold higher concentration than the chicken. These differences may be the result of distinct enzyme concentrations, activities or transport rates.

Deer mouse hepatic porphyrins were significantly different only at the highest dose levels, while the chicken hepatic porphyrins were demonstrably different in both the low and high dose group. These findings are relatively low compared to the results of van Birgelen et al. [3]. This apparent discrepancy might be best attributed to the longer dose period of 13 weeks, pointing out that both duration and quantity of dosing is critical in the TCDD-induced porphyria. In the present study, while the chicken and the mouse doses were in a similar range, the chickens were dosed for a period 3.5-fold longer than the mice. This indicates that lower doses require longer periods to manifest an observable porphyric response. This point is critical for biomarker implementation, as one must be able to

approximate the time an organism is in contact with contaminants. for accurate interpretation of results.

The results of this study indicate that total porphyrin concentrations are an effective measure for assessing TCDD exposure in dosed animals. One may loose sensitivity, however, as variability in porphyrins with high baseline concentrations. such as 4-carboxylporphyrin. may obscure significant alterations in porphyrins with lower baseline concentrations. Further measurement of total porphyrin concentrations. only eliminates one's ability to differentiate between exposures to distinct classes of environmental contaminants [2].

These data suggest that porphyrin profiles are dependent on duration and level of TCDD exposure in the animal species tested and that porphyrin profiles can serve as a biomarker of contaminant exposure for diverse species.

5. References Cited

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