DOSE-DEPENDENT EFFECTS OF TCDD ON PEROXISOMAL ENZYMES AND CYP4A

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I. Introduction

Polyhalogenated aromatic hydrocarbons (PHAHs) may accumulate in the food chain and are extremely toxic to certain species. Hence, PHAHs have become the focus of intense public debate and controversy because of their presence in the environment and the associated potential health risks. Many PHAHs and the prototypic 2,3,7,8-tetrachloro-dibenzo-*p*-dioxin (TCDD) produce a spectrum of toxic changes thought to be receptor-mediated. A second class of environmental contaminants, composed of some pesticides and plasticizers, are known as peroxisome proliferators. TCDD and peroxisome proliferators elicit similar effects such as hepatomegaly, hepatic hyperplasia and thymic atrophy in rodents [1], and are potent promoters in two-stage hepatocarcinogenesis [2, 3]. PHAHs, which are good ligands for the aryl hydrocarbon (*Ah*) receptor, induce cytochrome P450(CYP)1A enzymes while the CYP4A subfamily is also inducible by peroxisome proliferators. The *Ah* receptor and the peroxisome proliferator activated receptor (PPAR) system act by similar mechanisms [4].

Preliminary data had given us some indication that the genes associated with peroxisomes eg. fatty acyl-CoA oxidase (FAO), total peroxisomal ß-oxidation and catalase, may be down-regulated by PHAHs. The enzyme FAO is the first and rate-limiting step in the peroxisomal β-oxidation pathway. The following studies therefore were designed to study gene regulation and toxicity. Using TCDD, the expression of peroxisomal enzymes and CYP4A was studied in rat liver at very low doses of TCDD. The following specific questions were to be answered: does TCDD down-regulate the expression of the peroxisomal and CYP4A enzymes in rat liver and is this reflected at the protein and mRNA levels?

2. Materials and Methods

A first study was carried out to determine the effects of 1 µg and 10 µg/kg TCDD on the peroxisomal and CYP4A enzymes. Nine immature male Sprague Dawley rats were purchased from Harlan Sprague Dawley, Indianapolis, Indiana. TCDD was dissolved in stripped corn oil and administered to the rats via a single ip injection at doses of 1 µg and 10 µg/kg body weight with corn oil as control (n=3 for each group). The rats were killed 7 days later by carbon dioxide asphyxiation. The livers were weighed, and whole liver homogenates and microsomal fractions were prepared by differential centrifugation.

For the second study, thirty-six immature male Sprague Dawley rats (75-100g) were purchased as above and administered a single ip injection of TCDD at doses of 0.1, 1, 5, 10 and 40 µg/kg body weight (n=6). The rats were killed 7 days later as described above. Liver pieces were removed, immediately stored in liquid nitrogen and later at -80°C, for mRNA analysis. The remaining livers were weighed and whole liver homogenates and microsomal fractions were prepared by differential centrifugation as described above.

In whole liver homogenates, enzyme activities associated with peroxisome proliferation (FAO, total peroxisomal ß-oxidation and catalase) were determined. Lauric acid hydroxylase activity, an indicator of CYP4A activity, was determined in the microsomes. Using the polyclonal antibody CYP4A (kindly provided by Dr. R. Okita), which recognized CYP4A1, 4A2 and 4A3, the western blot analysis was done on the microsomes to determine the effect of TCDD on the levels of CYP4A proteins. To determine the effect of TCDD on CYP4A1, CYP4A2 and CYP4A3 mRNA levels, slot blot analyses were done using oligonucleotides (20-base pair oligomer) specific for CYP4A1, 4A2 and 4A3 [5]. They were made by the Department of Biochemistry, University of Kentucky, and had the following sequences:

CYP4A1	5'-TAT-GGG-AAG-GGT-GCT-GGC-TT-3'
CYP4A2	5'-GCT-GGG-AAG-GTG-TCT-GGA-GT-3'
CYP4A3	5'-ACT-GGG-ATG-GAG-TCT-GGA-GG-3'

3. Results and Discussion

While peroxisome proliferators greatly increase FAO activity (up to 20-fold), TCDD caused a significant stepwise decrease in its activity with increasing dose (Fig. 1). However, the data showing peroxisomal ß-oxidation and catalase were not as clear cut but showed similar trends (data not shown). Lauric acid hydroxylase activity was significantly decreased at all doses of TCDD (Fig. 2).

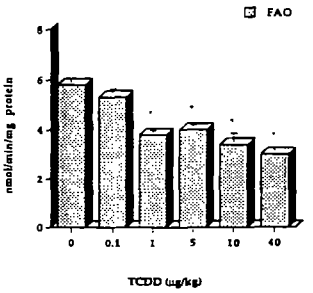

Fig. 1. Dose-dependent effect of TCDD (0, 0.1, 1, 5, 10, 40 µg/kg) on FAO activity in male Sprague Dawley rats. Results are expressed as mean ± SEM with n=6 (*significantly different from control, p=0.05).

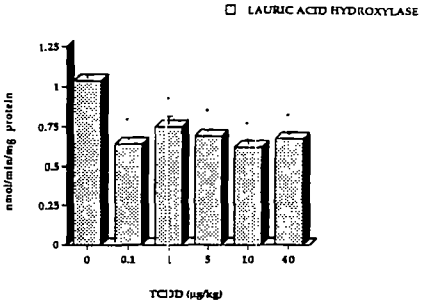

Fig. 2. Dose-dependent effect of TCDD (0, 0.1, 5, 10, 40 µg/kg) on lauric acid hydroxylase activity. Results are expressed as mean ± SEM with n=6 (*significantly different from control, p=0.05).

Western blot analyses were performed to determine if the decrease observed in lauric acid hydroxylase activity was reflected at the protein level. The levels of CYP4A protein were unchanged from control values at all doses of TCDD (data not shown), which was in contrast with the enzyme activity data (Fig. 2). Similarly, the mRNA levels for CYP4A1, 4A2 and 4A3 were unchanged at all doses of TCDD (data not shown).

The activities of the enzymes FAO and catalase (which are located within or attached to the membrane of the peroxisome) as well as the measurement of the flux through the peroxisomal β-oxidation pathway are all highly specific peroxisomal activities. In male rats, FAO and total peroxisomal β-oxidation increase 10-20-fold when potent peroxisome proliferators are administered, whereas catalase only increases about 2-fold. Lauric acid hydroxylase activity, a specific indicator of CYP4A activity, is also highly induced (10-30-fold) by peroxisome proliferators. In this study, TCDD did not increase any of these activities. Instead, it significantly lowered FAO and CYP4A enzyme activities. These data are consistent with the first study, which also showed a decrease in both the FAO and CYP4A enzymes in livers of rats treated with 1 and 10 μg TCDD/kg body weight, one week after treatment (data not shown). Similarly, Robertson and co-workers showed that the two most acutely toxic halogenated biphenyls, 3,3',4,4'-tetrabromobiphenyl and 3,3',4,4',5-pentachlorobiphenyl, significantly diminished the liver content of CYP4A isozymes and the activities of the peroxisomal enzymes studied (unpublished data). However, the western blot analysis of CYP4A protein levels in the present study indicated that total CYP4A protein was unchanged at all doses of TCDD.

The induction of CYP4A isozymes following treatment with peroxisome proliferators is accompanied by peroxisome proliferation in the rodent, and it has recently been suggested that the CYP4A and peroxisomal enzymes are coordinately regulated. Hence, this could explain the accompanying decrease seen in CYP4A activity in this study. In contrast, an increase in CYP4A1 content was found in the livers of neonates which had been exposed to PCBs via lactational transfer, while FAO and peroxisomal β-oxidation activities were diminished [6].

It is also possible that TCDD could be exerting its effects on the peroxisomal and CYP4A enzymes via the *Ah* receptor. These enzymes could be a part of the *Ah* gene battery which are down-regulated by TCDD via negative response elements [7]. There may be consensus sequences present in the peroxisome proliferator receptor elements (PPREs) found in the 5' flanking regions of the FAO or CYP4A genes that the TCDD-receptor complex recognizes and binds, hence suppressing peroxisomal and CYP4A enzyme activities. However, in this study, TCDD had no effect on CYP4A1, 4A2 and 4A3 mRNA levels at the various doses, which indicated that regulation of CYP4A was not at the transcriptional level but at the post-translational level.

Another possible explanation for the down-regulation of CYP4A enzyme activity by TCCD observed in this study could be due to TCDD's ability to influence the expression of various transcription factors, for example chicken ovalbumin upstream transcription factors (COUP-TFs). COUP-TFs are orphan members of the steroid hormone receptor family [8]. By competing for their binding sites and heterodimerization with the retinoic X receptor (RXR), COUP-TFs repress the hormonal induction of vitamin D_3, thyroid and retinoic acid genes. Human COUP-TFs were also bound to the bifunctional (now trifunctional) enzyme

PPREs *in vitro* and repressed PPAR-mediated peroxisome proliferation *in vivo* [8]. It is possible TCDD could be activating COUP-TFs to bind to the PPREs of FAO and bifunctional enzymes, thus decreasing the activities of the peroxisomal and CYP4A enzymes.

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4. References

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