COMPARISON OF HEPATIC GENE EXPRESSION PROFILES IN FEMALE RATS EXPOSED SUBCHRONICALLY TO TCDD, PECDF, PCB126 AND PCB153 USING DNA MICROARRAY

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Introduction

2,3,7,8 Tetrachlorodibenzo-*p*-dioxin (TCDD, Dioxin) is a persistent environmental contaminant and a human carcinogen (IARC, 1997).[1] The toxicity of TCDD is partially mediated by binding to and activation of the cytosolic aromatic hydrocarbon receptor (AhR). Upon activation, the AhR translocates into the nucleus and serves as an enhancer for gene expression. AhR–mediated gene activation is a critical element in the etiology of TCDD toxicity and may play a role in the genesis of reproductive and developmental toxicity, immunotoxicity, endocrine disruption, anorexia and "wasting," porphyria, hepatotoxicity, and cancer. Hepatocellular carcinoma is a prominent response to chronic TCDD exposure in rats and emerges most often in females. Although TCDD is considered the most potent ligand for the AhR, several compounds exhibit affinity for this receptor and many are capable of inciting biological responses similar to dioxin. Considering that environmental exposure to TCDD often occurs in combination with other dioxin-like compounds, including polychlorinated dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and biphenyls (PCBs), it is necessary to accommodate for their presence when performing dioxin risk assessments. Accordingly, a classification scheme that assigns relative potency factors to dioxin-like chemicals was established for estimating aggregate exposure to dioxin-like chemicals. In this system, individual chemicals are assigned Toxic Equivalency Factors (TEF) based on their potency compared to TCDD (Van den Berg *et al*., 1998).[2] In an attempt to delineate whether the cancer risks of dioxin-like compounds reflect their TEFs, the National Toxicology program initiated a 2 year exposure study with female Sprague-Dawley rats treated with equivalent doses (based on TEFs) of three dioxin like compounds (TCDD; 3,3',4,4',5-PeCB, PCB126; 2,3,4,7,8-PeCDF) and one non-dioxin like compound (2,2',4,4',5,5'-HxCB, PCB153). The purpose of the current study was to investigate changes in gene expression profiles in the liver of rats treated subchronically with these compounds.

Materials and Methods

In the National Toxicology Program's study on cancer and TEFs for dioxin and dioxin-like chemicals, , female Sprague Dawley Rats were administered *toxicologically equivalent* doses of TCDD (3, 10, 22, 46, 100 ng/kg/day), PeCDF (6, 20, 44, 92, 200 ng/kg/day), PCB126 (10, 30, 100, 175, 300, 550, 1000 ng/kg/day), PCB153 (10, 100, 300, 1000 ug/kg/day), or corn oil (vehicle control) by gavage for 2 years. Subgroups of additional rats were also sacrificed after 14, 31, or 53 weeks for mechanistic studies. Target organs from these rats were then removed, flash frozen in liquid nitrogen and stored at –70ºC.

The present study utilized liver tissue from rats treated for 14 weeks with the highest dose of each of these compounds. Frozen hepatic tissue was disrupted by homogenization with a rotor stator homogenizer and total RNA was isolated using the Qiagen RNA Isolation Mini-Kit. There were a total of six animals in each dose group. For each group, RNA from two rats was randomly paired and pooled, thus reducing the sample size to $n = 3$ while preserving biological variability. Pooled total RNA was further purified using the Qiagen Poly(A)RNA Isolation Kit. The Agilent Bioanalyzer 2100 was utilized to assess RNA integrity. This study utilized high quality RNA that displayed two distinct, sharp peaks and a 28S/18S ribosomal RNA ratio greater than 1. Poly(A) RNA was converted into labeled cRNA by the Roswell Park Cancer Institute Microarray Core Facility. cRNA quality was evaluated by comparing 3'/5' signal ratios of housekeeping genes using Affymetrix GenChip Test3 arrays. High quality cRNA (3'/5' signal ratio greater than 1) was then hybridized to Affymetrix RGU34A GeneChips and chips were scanned to create two-dimensional images containing probe intensity data.

Cell Intensity Files (.CEL) files were generated with Affymetrix Microarray Suite software and probe level data was background subtracted, normalized, and converted to gene expression measures using the MAS 5.0 algorithm included in the Bioconductor implementation for R (www.bioconductor.org). Gene expression data from the three GeneChips in each dose group was combined and changes in gene expression were calculated as the average Log_2-fold change versus control. The Gene expression profiles of TCDD, PeCDF, PCB126, and PCB153 were analyzed by Principal Component Analysis (PCA)[3] to identify relationships between compounds. Pavlidis Template Matching (PTM)[4] was utilized to identify genes specifically regulated by dioxin-like compounds but not PCB153. GenMapp[5] (www.GenMapp.org) was employed to identify biochemical processes perturbed by exposure to each agent. For selected genes, the quantitative expression estimates obtained by microarray analysis were validated by real-time RT-PCR.

Results and Discussion

A fundamental advantage of the microarray approach towards toxicological research is the robust nature of data that can be acquired from this technology. The Affymetrix RGU34A GeneChip utilized in these studies simultaneously assesses the expression of approximately 7,000 annotated genes and 1,000 Expressed Sequence Tag (EST) clusters, the latter of which represent unclassified transcribed genes. Accordingly, these studies were capable of evaluating global changes in hepatic gene expression and determining signature effects of TCDD, PeCDF, PCB126, and PCB153 on gene expression in rat liver. Principal Component Analysis (PCA) of TCDD, PeCDF, PCB126, and PCB153 samples revealed a unique relationship between toxicants. The global gene expression profile of PeCDF and PCB126 were very similar and were closely related to that of TCDD. The global expression profile of the non-coplanar PCB153, however, was unique from the dioxin-like compounds. Considering that PCB153 demonstrates little or no affinity for the AhR, the different expression profiles of these compounds underscores the significance of AhR activation in propelling phenotypic changes associated with the biological and toxicological effects of dioxin-like compounds in hepatic tissues.

The dioxin response gene battery represents genes that are directly activated by the AhR and include Cytochromes P450 (CYP) 1A1, 1B1, and 1A2 as well as several phase II drug metabolizing enzymes. While the biological activity of these proteins has been useful towards inferring a biological role for the AhR, identification of additional AhR gene targets may facilitate

a more complete understanding of AhR function. In order to discover new AhR gene targets, Pavlidis Template Matching (PTM) was utilized to identify genes that were specifically induced by the AhR ligands TCDD, PeCDF, and PCB126, but not by PCB153. This approach recovered many genes previously classified as being dioxin responsive, thus validating the efficacy of this approach while further verifying the integrity of sample RNA. PTM also revealed the increased expression of several novel genes, including C-CAM4, Cox8h, and protocadherin 2, as well as several ESTs, which have not previously been characterized with regard to activation by the AhR.

A variety of biological and toxicological responses have been associated with exposure to dioxin or dioxin like toxicants, but the molecular mechanisms of many of these responses remains unclear. Hepatic gene expression data from TCDD, PeCDF, PCB126, and PCB153 treated animals was analyzed by GenMapp to investigate toxicant-related transformation of biochemical processes that may relate the wide range of dioxin-like responses. Toxicant exposure was associated with deregulation of genes involved in mitochondrial long chain-fatty acid beta-oxidation, fatty acid homeostasis, and regulation of cell growth. These effects may be related to dioxin-induced liver steatosis or hepatotoxicity, fat redistribution, and deregulated cell growth.

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