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Progression of micronutrient alteration and hepatotoxicity following acute PCB126 exposure

W.D. Klaren[a,b], G.S. Gadupudi[a,b], B. Wels[c], D.L. Simmons[c], A.K. Olivier[d,1], L.W. Robertson[a,b,*]

[a] Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, Iowa, USA
[b] Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, Iowa, USA
[c] State Hygienic Laboratory, University of Iowa, Ankeny, Iowa, USA
[d] Department of Pathology, University of Iowa, Iowa City, Iowa, USA

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ABSTRACT

Polychlorinated Biphenyls (PCBs) are industrial chemicals that have become a persistent threat to human health due to ongoing exposure. A subset of PCBs, known as dioxin-like PCBs, pose a special threat given their potent hepatic effects. Micronutrients, especially Cu, Zn and Se, homeostatic dysfunction is commonly seen after exposure to dioxin-like PCBs. This study investigates whether micronutrient alteration is the byproduct of the ongoing hepatotoxicity, marked by lipid accumulation, or a concurrent, yet independent event of hepatic damage. A time course study was carried out using male Sprague–Dawley rats with treatments of PCB126, the prototypical dioxin-like PCB, resulting in 6 different time points. Animals were fed a purified diet, based on AIN-93G, for three weeks to ensure micronutrient equilibration. A single IP injection of either tocopherol-stripped soy oil vehicle (5 mL/kg) or 5 μmol/kg PCB126 dose in vehicle was given at various time points resulting in exposures of 9 h, 18 h, 36 h, 3 days, 6 days, and 12 days. Mild hepatic vacuolar change was seen as early as 36 h with drastic changes at the later time points, 6 and 12 days. Micronutrient alterations, specifically Cu, Zn, and Se, were not seen until after day 3 and only observed in the liver. No alterations were seen in the duodenum, suggesting that absorption and excretion may not be involved. Micronutrient alterations occur with ROS formation, lipid accumulation, and hepatomegaly. To probe the mechanistic underpinnings, alteration of gene expression of several copper chaperones was investigated; only metallothionein appeared elevated. These data suggest that the disruption in micronutrient status is a result of the hepatic injury elicited by PCB126 and is mediated in part by metallothionein.

1. Introduction

PCBs continue to be an enduring threat with an almost constant exposure whether through air or food (Ampleman et al., 2015; Hu et al., 2010). Given the recent elevation of PCBs to a group I human carcinogen by International Agency for Research on Cancer (IARC) and current research tying them to neurologic and metabolic disorders, a better understanding of their toxicity is needed (Baker et al., 2013; Grandjean and Landrigan, 2006; Lauby-Secretan et al., 2013). A particularly persistent and toxic PCB conger, 3,3′,4,4′,5-pentachlorobiphenyl (PCB126), exerts adverse effects on many different systems from hepatic to immunological; including the homeostasis of micronutrients (Lai et al., 2010). PCB126, along with other dioxin-like PCBs, imparts its toxicity through activation of the aryl-hydrocarbon receptor (AhR) which in turn alters the expression of important xenobiotic metabolizing enzymes (CYPs) and also antioxidant proteins (Abel and Haarmann-Stemmann, 2010). In addition, recent evidence suggests that supplementing the diet with certain micronutrients can actually mitigate some of the toxicity of PCB126, a finding that may propose a therapy for PCB exposure (Lai et al., 2012, 2013, 2011). So clearly, micronutrient status has some basis in PCB toxicity, but the extent and mechanism behind its role are unknown.

Micronutrient status is a crucial aspect of many different biological systems that is often overlooked in a diverse range of fields. It contributes to the redox status of the cell by modulating antioxidant enzymes or acting as prooxidants, e.g. copper's fenton

* Corresponding author at: Department of Occupational and Environmental Health, The University of Iowa, College of Public Health, 100 Oakdale Campus #219 IREH, Iowa City, Iowa 52242-5000, USA. Fax: +1 319 335 4290.
E-mail address: larry-robertson@uiowa.edu (L.W. Robertson).
[1] Present Address: Department of Pathobiology and Population Medicine, Mississippi State University, Starkville, Mississippi.

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properties (Brewer 2008; Gadupudi and Chung, 2011). In addition, gene expression can be altered by the lack of zinc in zinc-finger motifs in transcription factors (Cho et al., 2007). Various pathologies have been associated with changes in micronutrient status, including liver disease, Alzheimer's disease, and glucose and insulin homeostasis (Adlard and Bush, 2006; Fung et al., 2015; Mohammad et al., 2012). Whether the modifications to micronutrient homeostasis play an active role in disease progression or are just a symptom of it, need to be clarified. In any context, micronutrient status is fundamental for the normal function of the cell and the body as a whole.

Dioxin-like compounds, in particular dioxin-like PCBs, cause disruptions in serum, hepatic, and renal micronutrients consisting of changes in tissue levels of copper, zinc, selenium and iron (Elsenhans et al., 1991; Wahba et al., 1988; Wahba et al., 1990). Yet a clear explanation of what is causing this disruption and how this contributes to the overall toxicity is still lacking. The current study aims to elucidate the progression of micronutrient alteration following an acute exposure to PCB126. With this timeline of metals disturbance, better insights into the mechanism behind this phenomenon can be obtained. Hepatocellular degeneration was observed as early as 36 h following exposure to PCB126, whereas substantial micronutrient change, in particular copper, was not observed until day 3. This suggests that micronutrient alteration is mediated by PCB126 and is likely the result of hepatic injury.

2. Methods and materials

2.1. Chemicals

Unless otherwise noted, all chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). PCB126 was synthesized by an improved Suzuki-coupling method of 3,4-dichlorophenyl boronic acid and 3,4,5-trichlorobenzene employing a palladium-catalyzed cross coupling reaction (Luthe et al., 2006). Crude product was purified on an aluminum oxide column and flash silica gel column chromatography followed by recrystallization from methanol. The final product purity was >99.8%, determined by GC/MS and the structure was confirmed by ^{13}C NMR. Analytical standards, 2,4,6-trichlorobiphenyl (PCB30), 2,3,4′,5,6-pentachlorobiphenyl (PCB117), and 2,2′3,4,4′,5,6,6′-octachlorobiphenyl (PCB204), were purchased from AccuStandard (New Haven, CT). Caution: PCBs and their metabolites should be handled as hazardous compounds in accordance with NIH guidelines.

2.2. Animals

Experimental design and procedures were performed with the approval of the Institutional Animal Care and Use Committee of the University of Iowa. 4–5 week old male Sprague–Dawley rats were acquired from Harlan Sprague–Dawley (Indianapolis, IN). This age of animal was selected based on (1) their sensitivity/induction potential and (2) recent evidence of PCB-contaminated schools and the risk that poses to children (Grimm et al., 2015; Kato and Takanaka, 1968). The 75–100 g animals were housed one per cage in wire cages in a controlled environment maintained at 22 °C with a 12 h light-dark cycle. Animals were fed ad libitum a refined diet, AIN-93G, with a 10% fat content provided by tocopherol stripped soy oil purchased from Harlan Teklad (Madison WI), throughout the study. Since diets can vary widely in micronutrient content (Chen et al., 1990), rats were fed this refined diet for three weeks to allow for equilibration. Animals were then administered a single IP injection of vehicle (5 mL/kg body wt. of tocopherol stripped soy oil; Harlan Tekland, Madison, WI) or vehicle with 5 μmol/kg (1.63 mg/kg) dose of PCB126 at time points that result in exposures of 9 h, 18 h, 36 h, 3 days, 6 days and 12 days (Reeves and DeMars, 2006). To conserve the number of animals used in the study, three animals were used in each treatment group and only the 12 day vehicle control was used. The rats were then euthanized by carbon dioxide asphyxiation followed by cervical dislocation. Blood was collected via cardiac puncture and other organs were excised, weighed and processed for analysis.

2.3. Histology

Tissue sections from each animal were fixed in 10% neutral buffered formalin. Fixed tissues were routinely processed, embedded in paraffin and sectioned at 4 μm. Sections were further processed and stained with hematoxylin and eosin (H and E) followed by an analysis by a pathologist.

2.4. PCB126 and lipid analysis

2.4.1. Extraction of tissues

Liver samples weighing between 0.5 g and 1 g were homogenized with diatomaceous earth and separated into two portions, one for PCB126 analysis and the other for total extractable lipids. The portion for PCB126 analysis was spiked with a recovery standard, PCB117, then extracted with hexane: acetone (1:1 v/v) using Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) as described previously (Rignall et al., 2013). Cells containing only diatomaceous earth and florisil as well as the ongoing precision and recovery (OPR) standard (blanks spiked with all analytes) were extracted along with the hepatic samples. After extraction, the samples were spiked with internal standards, PCB30 and PCB204, then concentrated followed by a sulfur clean-up and a final treatment of sulfuric acid as described earlier (Rignall et al., 2013). The portion for total extractable lipids was extracted with chloroform:methanol (2:1 v/v) using Accelerated Solvent Extractor (Dionex, Sunnyvale, CA) as described (Bunaciu et al., 2007). Samples were concentrated and evaporated to dryness in pre-weighed vials. Vials were kept in a desiccator and weighed three times over the course of several days, to constant weight.

2.4.2. GC analysis

An Agilent 6890N gas chromatograph equipped with a SPB-1 column (poly(dimethyl siloxane), 60 m, 0.25 mm ID, 0.25 μm film thickness; Supelco, St. Louis, MO) and ^{63}Ni μ-electron capture detector was used for PCB126 quantification. 280 °C and 300 °C were used as the injector and detector temperatures, respectively. The temperature program was as follows: 80 °C for 1 min, 15 °C/min increase to 260 °C, 1 °C/min increase to 274 °C, 15 °C/min increase to 300 °C. The level of PCB126 was calculated using PCB204 as a volume corrector and normalized to tissue weight.

2.4.3. Quality assurance/quality control

The recoveries for PCB117 were 91.5 ± 8.3%. PCB126 concentrations were corrected by the recoveries of surrogate standards. OPR standard, which was run in parallel with hepatic samples, recovery was 92.1%

2.5. Micronutrient analysis

Roughly 0.5 g of liver and PBS flushed duodenum were used to determine copper, zinc, iron and selenium content using a previously described method (Lai et al., 2010). Briefly, samples were acid (HNO_3) digested by microwave-assisted closed vessel digestion prior to instrument measurement. Metal concentrations were quantitatively determined with an elemental mass spectrometer by Inductively Coupled Plasma-Mass Spectrometry