Toxicology Reports 3 (2016) 21–28

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

Metallothionein's role in PCB126 induced hepatotoxicity and hepatic micronutrient disruption

W.D Klaren [a,b], S. Flor [b], K.N. Gibson-Corley [c], G. Ludewig [a,b], L.W. Robertson [a,b,*]

[a] Interdisciplinary Graduate Program in Human Toxicology, University of Iowa, Iowa City, IA, United States
[b] Department of Occupational and Environmental Health, College of Public Health, University of Iowa, Iowa City, IA, United States
[c] Department of Pathology, University of Iowa, Iowa City, IA, United States

ARTICLE INFO

Article history:
Received 25 November 2015
Accepted 2 December 2015

Chemical compound studied in this article:
3,3′,4,4′,5-pentachlorobiphenyl PCB126
(PubChem CID63090)

Keywords:
Metallothionein
Micronutrients
Metals
PCB
AhR
Hepatotoxicity

ABSTRACT

Polychlorinated biphenyls (PCBs), industrial chemicals and persistent environmental pollutants, are found in rural and urban settings. Rodent studies have shown that exposure to PCB126, a dioxin-like PCB, causes a significant disruption of hepatic micronutrient homeostasis and an increase in metallothionein (MT), an antioxidant protein and metal carrier. A MT knockout mouse strain was used to assess metallothionein's role in micronutrient disruption and overall hepatotoxicity. Twenty four 129S male mice (12 wild type (WT) and 12 MT knockout (MTKO)) were placed on a purified diet (AIN-93G) for 3 weeks to achieve hepatic metal equilibrium. Mice were then given a single IP injection of either vehicle or 150 μmol/kg PCB126 in vehicle. The animals were sacrificed 2 weeks later and organs processed for analysis. Liver histology, hepatic lipids, gene expression, micronutrient and ROS status were investigated. Liver weights, liver lipids, ROS, and hepatocyte vacuolation were increased with PCB126 exposure along with AhR responsive genes. The MTKO animals had more severe histological changes in the liver and elevated liver lipids than their wild type counterparts. Hepatic and renal metals levels (Cu, Zn, Se and Mn) were mostly reduced by PCB126 treatment. Renal micronutrients were more affected by PCB126 treatment in the MTKO animals. This research suggests that MT may not be the sole/primary cause of the metal disruption caused by PCB126 exposure in mice, but may provide protection against overall hepatotoxicity.

© 2015 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Polychlorinated biphenyls (PCBs) are persistent environmental and industrial chemicals that continue to pose a threat to human health because of their toxicity and recurrent exposure [2]. The recent elevation by IARC of these chemicals to group I carcinogens exemplifies this threat [17]. Of the 209 congeners, the dioxin-like PCBs, in particular PCB126 (3,3′,4,4′,5-pentachlorobiphenyl), affect multiple targets through activation of the aryl-hydrocarbon receptor (AhR) [1]. This activation drives the induction of a multiplicity of genes including xenobiotic metabolizing enzymes (e.g., cytochrome P450s (CYPs)) as well as antioxidant proteins, like paraoxonases and metallothionein [15,33]. In addition, studies have shown that PCB126 can alter the micronutrient status of the liver causing hepatic copper to increase whereas hepatic zinc, selenium and manganese decrease [13]. The extent to which micronutirent alterations exacerbate the ongoing liver damage is not fully understood as is the mechanism by which these micronutrients are being altered.

Metallothionein is an important protein family that has several roles alongside metal transport and reactive oxygen scavenging [31]. The metallothionein family consists of 4 isoforms in mammals. Two main metallothioneins are ubiquitously expressed, MTI and MTII, with especially high levels seen in the liver and kidney [38]. They consist of a 6 kDa cytosolic protein with a large percentage of cysteine residues (≈30%) which mainly chelates intracellular copper and zinc, but can also bind other metals [28]. The high thiol content results in its antioxidant property and allows it to interact with several metal ions at a time, in particular 7 zinc atoms or 12 copper atoms [4,28]. Given the molar equivalence, a small change in its expression can result in a very marked change in the levels of the metals bound to metallothionein. Metallothionein expression is altered by many different inducers, including cytokines, hormones, specifically glucocorticoids, and some metals [19,26]. Sato and

* Corresponding author at: Department of Occupational and Environmental Health, The University of Iowa, College of Public Health, 100 Oakdale Campus #219 IREH, Iowa City, IW 52242-5000, United States. Fax: +1 319 335 4290.
E-mail address: larry-robertson@uiowa.edu (L.W. Robertson).

http://dx.doi.org/10.1016/j.toxrep.2015.12.001
2214-7500/© 2015 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

co-workers have shown that activation of the AhR induced changes in metallothionein expression through interaction with the glucocorticoid receptor which corroborates work showing PCB126 can alter metallothionein expression [12,32]. Aside from metal binding, metallothionein has been shown to mitigate the toxicity of some chemicals, including carbon tetrachloride and cadmium, and is believed to facilitate zinc's abrogative properties in alcohol induced liver damage [7,11,39]. Overall, metallothionein is a versatile protein that positively contributes to different aspects of cellular and organ health and whose properties may be involved in the dynamics of PCB126 mediated liver damage.

The liver injury characteristic of PCB126 exposure is believed, in part, to be the result of reactive oxygen species (ROS) generated by idle CYPs, among other mechanisms [36]. Given the ROS scavenging aspects of metallothionein and its metal binding ability, metallothionein could be central to the hepatic toxicity of PCB126 in the context of micronutrient alterations and ROS. The hypothesis of this study is that loss of metallothionein will result in increased hepatotoxicity with PCB126 exposure with alterations in micronutrient homeostasis. The role of metallothionein in micronutrient alteration and hepatic injury caused by PCB126 is addressed using a metallothionein knockout mouse line.

2. Materials and methods

2.1. Chemicals

Unless stated otherwise, all chemicals were obtained from Sigma–Aldrich Chemical Company (St. Louis, MO). The synthesis of PCB126 followed the Suzuki coupling of 3,4-dichlorophenyl boronic acid and 3,4,5-trichlorobenzene using a palladium catalyzed cross coupling reaction [24]. The product was purified using an aluminum oxide column with flash silica gel column chromatography, finally being recrystallized with methanol. GC/MS determined the final product purity to be >99.8% and ^{13}C NMR was used for confirmation of structure. Caution: PCBs and their metabolites should be handled as hazardous compounds in accordance with NIH guidelines.

2.2. Animals

All animal procedures and experimental designs were performed with the approval of the Institutional Animal Care and Use Committee of the University of Iowa. Twelve male wild type [129S1/SvlmJ] mice (WT) and twelve male double knockout (MTI and MTII) MT−/− mice [129S7/SvEvBrd-Mt1-Mt2] (MTKO), 8–10 weeks of age, were purchased from Jackson Laboratories (Sacramento, CA). The mice were housed three per cage and assigned a treatment to effectively age match the different groups. The age of the mice was chosen given recent evidence of adolescent PCB exposure and the sensitivity/induction potential of that age of animal [9,10]. Access was provided to water and a pelletized AIN-93G diet (Harlan Teklad; Madison, WI) ad libitum throughout the study. The animals were acclimatized for three weeks to reach hepatic micronutrient equilibrium [29]. Mice were then given a single i.p. injection (5 ml/kg) of either vehicle (tocopherol stripped soy oil) or a dose of 150 μmol/kg PCB126 in vehicle. This dose and route of administration was chosen based on previous studies with similar AhR nonresponsive mouse strains that showed comparable hepatic effects to earlier rat studies [30]. The resulting four groups of six animals each (WT—vehicle; WT—PCB126; MTKO—vehicle; MTKO—PCB126) were monitored for two weeks following the injections, then mice were euthanized with carbon dioxide followed by cervical dislocation; blood, liver and other organs were removed and processed for further analysis. The genotype of the animals was confirmed from a small piece of liver with the help of Transnetyx (Cordova, TN).

2.3. Histology

A small piece of liver was fixed in 10% neutral buffered formalin. Formalin fixed tissue was routinely processed and embedded in paraffin followed by sectioning at 4 μm. Sections were then stained with hematoxylin and eosin (H&E) and examined by a board-certified veterinary pathologist (KN G-C).

2.4. Micronutrient analysis

0.5 g of liver and kidney were weighed and placed into acid washed 15 ml polyethylene tubes. Acid digestion was conducted with a 4:1 ratio of nitric acid and 30% hydrogen peroxide followed by heat block treatment at >110 °C for 2 h. Once the samples were thoroughly digested, a Thermo X-series II inductively coupled plasma–mass spectrometer (ICP-MS) with collision cell and Cetac autosampler was used to determine micronutrient status. Analysis was performed at the Iowa Trace Element Analysis Laboratory at the University of Iowa with the assistance of Dr. David Peate.

2.5. Lipid extraction

0.25–0.75 g pieces of liver were homogenized with diatomaceous earth with a mortar and pestle and extracted using Accelerated Solvent Extraction (Dionex, Sunnyvale, CA), as described previously, utilizing chloroform:methanol (2:1 v/v) [5]. Extracted samples were initially concentrated and placed in preweighed vials followed by evaporating and heating to dryness. Dried samples were placed in a desiccator and weighed several times until constant weight was achieved, usually taking several days. Weights were expressed relative to weight of the liver piece used.

2.6. Gene expression

The gene expression of several proteins was assessed using a two-step RT-PCR method utilizing an Eppendorf Realplex Mastercycler. First mRNA was isolated from a small piece of liver using a Qiagen (Hilden, Germany) RNeasy Mini kit, as per the manufacturer's instructions. Total mRNA concentration and purity was confirmed by measuring absorbance at 260 nm and 280 nm. A High Capacity cDNA Reverse Transcription Kit from Applied Biosystems (Waltham, MA) was used to reverse transcribe the mRNA to cDNA. 10 ng cDNA was used along with 300 nM primers and a SYBR green PCR master mix (Applied Biosystem) for the real time RT-PCR. The reaction conditions for all primer sets were optimized and two technical replicates were used. Primer sequences were obtained from references and are given in Supplementary Table 1 [18,20]. All primers were purchased from Integrated DNA Technologies (IDT) (Coralville, IA). GAPDH was used as a reference gene and the wild type vehicle group functioned as the biological control.

2.7. ROS determination

An OxiSelect InVitro ROS/RNS Assay Kit from Cell Biolabs (San Diego, CA) was used to determine the level of ROS in the liver. Briefly, liver samples were homogenized with PBS and diluted to a uniform concentration. Samples were added to both catalyst and a fluorescein based dye in a 96 well plate. The plate was incubated at room temperature for 45 min. The fluorescence of the dichlorofluorescein dye (DCF) was determined at 480 nm excitation and 530 nm emission. Samples were analyzed in triplicate. A DCF stan-

Download English Version:

https://daneshyari.com/en/article/2572103

Download Persian Version:

https://daneshyari.com/article/2572103

Daneshyari.com