Toxicology 380 (2017) 11–22

Contents lists available at ScienceDirect

Toxicology

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

A compromised liver alters polychlorinated biphenyl-mediated toxicity

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ARTICLE INFO

Article history:
Received 24 August 2016
Received in revised form 10 January 2017
Accepted 1 February 2017
Available online 2 February 2017

Keywords:
PCB126
Aroclor1260
MCD
Liver
Steatohepatitis
Toxicity

ABSTRACT

Exposure to environmental toxicants namely polychlorinated biphenyls (PCBs) is correlated with multiple health disorders including liver and cardiovascular diseases. The liver is important for both xenobiotic and endobiotic metabolism. However, the responses of an injured liver to subsequent environmental insults has not been investigated. The current study aims to evaluate the role of a compromised liver in PCB-induced toxicity and define the implications on overall body homeostasis. Male C57Bl/6 mice were fed either an amino acid control diet (CD) or a methionine-choline deficient diet (MCD) during the 12-week study. Mice were subsequently exposed to either PCB126 (4.9 mg/kg) or the PCB mixture, Arcolor1260 (20 mg/kg) and analyzed for inflammatory, calorimetry and metabolic parameters. Consistent with the literature, MCD diet-fed mice demonstrated steatosis, indicative of a compromised liver. Mice fed the MCD-diet and subsequently exposed to PCB126 showed observable wasting syndrome leading to mortality. PCB126 and Aroclor1260 exposure worsened hepatic fibrosis exhibited by the MCD groups. Interestingly, PCB126 but not Aroclor1260 induced steatosis and inflammation in CD-fed mice. Mice with liver injury and subsequently exposed to PCBs also manifested metabolic disturbances due to alterations in hepatic gene expression. Furthermore, PCB exposure in MCD-fed mice led to extra-hepatic toxicity such as upregulated circulating inflammatory biomarkers, implicating endothelial cell dysfunction. Taken together, these results indicate that environmental pollution can exacerbate toxicity caused by diet-induced liver injury which may be partially due to dysfunctional energy homeostasis. This is relevant to PCB-exposed human cohorts who suffer from alcohol or diet-induced fatty liver diseases.

1. Introduction

Polychlorinated biphenyls (PCBs) are synthetic organochlorine compounds that were manufactured over three decades ago and used as dielectric fluids in electrical equipment. The global production of PCBs is estimated at 1.5 million tons with the United States being the single largest producer. PCB production in the United States was banned by the United States Congress in 1979 and worldwide in 2001 by the Stockholm Convention on Persistent Organic Pollutants (Xu et al., 2013). Despite being banned for over 30 years, the chemical and thermodynamic stability of PCBs allowed them to resist degradation and hence they still persist in the ecosystem. Exposure to persistent organic pollutants such as PCBs is positively correlated with increased risk of developing multiple diseased outcomes such as liver disease, hypertension, diabetes and vascular diseases (Cave et al., 2010; Perkins et al., 2016; Taylor et al., 2013). Based on their chemical structure, PCBs

Abbreviations: AhR, aryl hydrocarbon receptor; ANF, natriuretic peptide type A; AUC, area under the curve; BP, blood pressure; CAE, chloroacetate esterase; CAR, constitutive androstane receptor; CD, control diet; Cd36, cluster of differentiation 36; Cpt1a, carnitine palmitoyl transferase 1a; Cyp, cytochrome P450; EE, energy expenditure; Fas, fatty acid synthase; Fmo3, flavin monooxygenase 3; Fgf21, fibroblast growth factor 21; G6Pase, glucose-6-phosphatase; H&E, hematoxylin–eosin; Hmox-1, heme oxygenase 1; HOMA- β, homeostasis model assessment of β -cell function; HOMA-IR, homeostasis model assessment of insulin resistance; ICAM-1, intracellular adhesion molecule 1; IL-6, interleukin-6; LW/BW, liver weight to body weight; MCD, methionine-choline deficient diet; Mcp-1, macrophage chemoattractant protein-1; NAFLD, non-alcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; Nqo1, (NAD(P)H dehydrogenase quinone 1; PCBs, polychlorinated biphenyls; PCR, polymerase chain reaction; Pepck-1, phosphoenol-pyruvate carboxy kinase; Ppar, peroxisome proliferator-activated receptor; PXR, pregnane-xenobiotic receptor; QUICKI, quantitative insulin sensitivity check index; RER, respiration exchange rate; SEM, standard error mean; Timp-1, tissue inhibitor of metalloproteinase 1; Tnfα, tumor necrosis factor alpha; PAI-1, plasminogen activator inhibitor 1.

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http://dx.doi.org/10.1016/j.tox.2017.02.001
0300-483X/

can be classified as either coplanar or non-coplanar. The coplanar PCBs are mostly the lower-chlorinated congeners that have less than one-*ortho* chlorine substitution in their phenyl ring while the non-coplanar ones have more than one-*ortho* substitutions and usually comprise of the higher chlorinated congeners. Furthermore, based on the structure of the specific congener, PCBs have also been proposed to bind and/or activate different receptors in the body, eventually leading to different pathological outcomes (Wahlang et al., 2014a). Animal studies have shown that coplanar PCB exposure is mostly associated with vascular cell dysfunction and inflammation (Petriello et al., 2014) while exposure to higher chlorinated, non-coplanar PCBs is associated with obesity and fatty liver disease (Wahlang et al., 2013, 2014b). Humans are exposed to multiple PCB congeners especially the heavily-chlorinated ones that are more resistant to degradation and thus, tend to persist in the ecosystem. Therefore, in the context of the human exposure paradigm, the presence of both classes of PCBs in the body may result in more complex health outcomes by affecting different organ systems.

The liver, being the primary site for xenobiotic detoxification, is the principal target organ for toxic effects induced by environmental pollutants including PCBs. However, the liver possesses additional functions such as maintaining energy homeostasis in the body attributed to its role in endobiotic metabolism (Rui, 2014). Liver injury and liver diseases such as non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) have been linked to other health complications such as obesity, diabetes, insulin resistance and the metabolic syndrome (Firneisz, 2014). Moreover, liver disease has been considered to be a risk factor for other disorders such as cardiovascular diseases (Bhatia et al., 2012; Magida and Leinwand 2014; Naschitz et al., 2000). In fact, NAFLD occurs worldwide and it is the most common form of liver disease in industrialized countries. In the United States, NAFLD accounts for 75% of chronic liver diseases and affects all ages (Hassan et al., 2014). Moreover, the liver is the site for PCB metabolism and any injury to the liver may compromise the ability of the body to metabolize and excrete these compounds, which could result in more deleterious effects exerted by the parent compound. Therefore, it is important to evaluate the effects of chemicals such a PCBs on a compromised liver and to determine if this would disrupt normal energy metabolism. This will allow us to better understand how crucial and relevant is the liver's role in mitigating the toxic effects of environmental pollutants. Furthermore, it will also identify interactions between the heart and extra-hepatic organ systems that may act cumulatively in exacerbating PCB toxicity and other health complications such as obesity and the metabolic syndrome.

In the current study, we aim to investigate the effects of PCB exposure in the presence of a compromised liver and evaluated hepatic and peripheral (extra-hepatic) toxicity endpoints. In order to test our hypothesis, mice were fed a methionine-choline deficient (MCD) diet to induce hepatic fibrosis and injury (Liu et al., 2013). Mice were then exposed to PCBs, either as a single congener (PCB126) or a mixture of congeners, using the commercial PCB mixture, Aroclor1260. The results obtained from the study demonstrated that the liver is indeed crucial for maintenance of energy homeostasis in the body and that exposure of a compromised liver to different PCB congeners can consequently lead to severe toxicological outcomes.

2. Materials and methods

2.1. Animals, diets and PCB exposure

The animal protocol was approved by the University of Kentucky Institutional Animal Care and Use Committee. Eight week-old wild type male C57Bl/6 mice were purchased form Taconic (Hudson, NY, USA). Mice were divided into 6 study groups (n = 10) based on either diet type, PCB126 exposure or Aroclor1260 exposure during this 12-week study utilizing a 2×3 design. Mice were housed in a temperature- and light controlled-room (12 h light; 12 h dark) with food and water *ad libitum*. For the first two weeks, all animals received the amino acid control diet (CD; TD.94149; Harlan Teklad, Madison, WI, USA). Diet components are described in Supplementary Table 1. The groups designed to receive the methionine-choline deficient (MCD) diet were fed with the MCD diet (TD.90262, Harlan Teklad) from week 3 onwards. On week 6–7 and week 8–9, the MCD-fed mice were restored back to CD- feeding because of the excess weight loss that was non-compliant with the IACUC protocol. PCB126 or Aroclor1260 (both purchased from AccuStandard, CT, USA) was administered in corn oil by oral gavage (*vs.* corn oil alone). PCB126 (2.45 mg/kg) and Aroclor1260 (10 mg/kg) were gavaged in two individualized doses on week 5 and week 7 to minimize acute toxicity, resulting in a cumulative dosage of 4.9 mg/kg for PCB126 and 20 mg/kg for Aroclor1260. The dosages selected were based on previous PCB studies (Newsome et al., 2014; Wahlang et al., 2016, 2014b) where PCB126 has been shown to induce inflammation in mouse models while Aroclor1260 resembled PCB levels found in a PCB-exposed human cohort. The different groups based on diet and exposure timelines are shown in Supplementary Fig. 1. After the second gavage was administered (week 7), some of the mice from the MCD + PCB126 group died (n = 3). The rest of the mice were extremely sick and lethargic, and their body weight was under the weight limit required by the IACUC protocol (>20% loss in body weight), hence they were subsequently euthanized at the end of week 8. Due to their extremely low bodyweight, only their livers were collected for hepatic analysis. A glucose tolerance test was performed at week 10. During weeks 11–12, 5 mice were randomly selected from each group and placed in metabolic chambers (PhenoMaster, TSE systems, Chesterfield, MO, USA) overnight to assess food/water consumption and physical activity. Animals were euthanized (ketamine/xylazine, 100/20 mg/kg body weight, i.p.) at the end of week 12. Prior to euthanasia, the animals were analyzed for lean and body fat composition using the EchoMRI (EchoMRI LLC, Houston, TX, USA). Ethylenediaminetetraacetic acid was added to collected blood samples, briefly mixed, and centrifuged at 2000*g* for 15 min at 4 °C to separate blood plasma. Plasma samples were frozen in liquid nitrogen and stored at −80 °C until processing. Tissues were harvested for mRNA or protein and stored at −80 °C prior to analysis. Thus, six different groups were evaluated; CD + vehicle, CD + PCB126, CD + Aroclor1260, MCD + vehicle, MCD + PCB126, MCD + Aroclor1260.

2.2. Ultrasound imaging and blood pressure measurements

On week 8, 2 mice were randomly chosen from each group and high-frequency ultrasound imaging was performed using the Vevo 2100 Imaging System (VisualSonics Inc., Toronto, Ontario, Canada) to determine the development/extent of liver steatosis and fibrosis (Fernandez-Dominguez et al., 2011). During imaging sessions, mice were kept under anesthesia using 1.5% isoflurane in oxygen and restrained on a heated stage. The mouse abdomen was depilated with commercial hair removal cream (Veet, Reckitt Benckifer, Granollers, Spain), and an ultrasound coupling gel was applied to the depilated skin. Images of the liver were acquired through the ventral body wall in transverse and sagittal orientations, employing a 40-MHz probe. Systolic and diastolic blood pressure measurements were measured using a non-invasive tail-cuff system (Coda 8; Kent Scientific Corporation, Torrington, CT, USA). The measurements were performed for four sequential days on week 11 of the study.