Cod: 1.1006

POLYCHLORINATED BIPHENYLS AFFECT THE HEPATIC-PERIPHERAL VASCULAR AXIS SUGGESTING A NOVEL MECHANISM FOR PERSISTENT ORGANIC POLLUTANTS

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Introduction

Exposure to persistent organic pollutants such as polychlorinated biphenyls (PCBs) is positively correlated to multiple non-communicable disease outcomes including liver disease, hypertension, diabetes and vascular diseases(1, 2). Liver diseases such as non-alcoholic fatty liver disease and non-alcoholic steatohepatitis have been risk factors for other health complications, namely peripheral vascular diseases and associated cardiovascular diseases (CVD)(3). However, information on the relationship between the liver and peripheral vasculature is scarce, and potential cross-talk between the two organ systems is understudied. Notably, the liver is the site for PCB metabolism and liver injury will compromise its ability to metabolize and excrete these compounds. Therefore, it is important to evaluate the effects of chemicals such a PCBs on an injured liver and determine if this would further disrupt metabolic processes. Moreover, the concept that an underlying disease can impact toxicant exposure, eventually resulting in CVD is novel. Elucidating the hepatic-heart axis is relevant to PCB-exposed human cohorts that may already have a compromised liver due to alcohol or diet-induced liver disease and this could subsequently impact the cardiovascular system.

In the current study, we aim to study the effects of PCB exposure in the presence of a compromised liver and look at cardiovascular and hepatic toxicity. In order to test our hypothesis, mice were fed a methionine-choline deficient (MCD) diet to induce hepatic fibrosis and 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 essential for maintaining energy homeostasis and that exposure to different PCB congeners consequently led to different pathological outcomes.

Materials and Methods

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 (Taconic, Hudson, NY) were divided into 6 study groups (n=10) during this 12-week study utilizing a 2x3 design. For the first two weeks, all animals received the amino acid control diet (CD; TD.94149; Harlan Teklad, Madison, WI). The groups designed to receive the MCD diet (TD.90262, Harlan Teklad) were fed from Week 3 onwards. PCB126 or Aroclor1260 (AccuStandard, CT) was administered in corn oil by oral gavage as previously described(4 5). High-frequency ultrasound imaging was performed using Vevo 2100 Imaging System to determine steatosis/fibrosis. Systolic blood pressure (BP) measurements were measured using a non-invasive tail-cuff system (Coda 8; Kent Scientific Corporation, Torrington, CT). Animals were euthanized (ketamine/ xylazine, i.p.) on Week 12. Prior to euthanasia, the animals were analyzed for lean and body fat composition using the EchoMRI.

Histological, cytokine and real-time PCR measurements. Liver sections were stained with hematoxylin-eosin (H&E) and examined by light microscopy. The Milliplex Map Mouse Adipokine Magnetic Bead Panel was utilized to measure plasma cytokines on the Luminex Xmap MAGPIX system. Polymerase chain reaction (PCR) was performed on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA) using the Taqman Fast Advanced Master Mix (Thermo Fisher Scientific Inc). Statistical analysis was performed using GraphPad Prism software.

Results and Discussion

The current study revealed that the MCD groups exhibited hepatic fibrosis (Fig. 1) and this was worsened with PCB exposure. Mice fed the MCD diet demonstrated decreased bodyweight and lower % fat composition, consistent with other MCD-liver fibrosis mouse models(6). Interestingly, during the study, PCB126 induced mortality in the MCD-fed mice and they were removed from the study at Week 8. Hence the MCD+PCB126 group was absent in some experimental analyses. Hepatic steatosis and inflammation was assessed histologically (Fig. 2). The MCD-fed mice developed steatosis by the end of the study. PCB126 induced steatosis in the CD-fed mice but this was absent in the Aroclor1260-exposed mice. The plasma and hepatic expression of multiple cytokines including plasminogen activator inhibitor (tPAI-1)

were upregulated in PCB126-exposed mice irrespective of the diet type, and in Aroclor1260-exposed mice on MCD-feeding (Fig. 3). Elevated PAI-1 is a risk factor for thrombosis and atherosclerosis since this serine protease inhibitor prevents breakdown of fibrin clots(7).
Liver function was evaluated by measuring hepatic expression of genes involved in hepatic metabolism. The MCD groups had lower Cpt1a mRNA levels while PCB126 reduced Pparα expression in the MCD group, indicating perturbations in hepatic lipid metabolism. Because we proposed that the combination of a compromised liver and PCB exposure could hamper cardiac function and induce injury on the related vasculature, systolic BP was measured in these animals (Fig. 4). The CD+PCB126 group appeared hypotensive (lower systolic BP) while MCD feeding increase BP, indicative of hypertension. Cardiac expression of genes that play a role in heart injury were evaluated including atrial natriuretic peptide (ANF, counters high blood pressure). ANF expression was increased with MCD+Aroclor1260 exposure consistent with the observed increase in BP. Furthermore, PCB126 and Aroclor1260 induced different hepatic receptor target genes, leading to different mechanisms of toxicity.
Taken together, our results indicate that PCBs either as a single congener ('dioxin-like' PCB126) or as a mixture (congeners comprising majorly of non-coplanar, 'non-dioxin-like' PCBs) can disrupt hepatic homeostasis and alter cardiac parameters. PCBs also activate different receptors, eventually leading to different disease outcomes(8). Therefore, in the context of the human exposome, the presence of different congeners in the body result in complex health outcomes by affecting different organ systems simultaneously.

Acknowledgements
This research was supported by grants from NIEHS/NIH (P42ES007380) and NIGMS/NIH (8 P20 GM103527-06). The authors would like to acknowledge Drs. Wendy Katz (COBRE Research Core); Jessica Moorleghen and Anju Balakrishnan (Saha Cardiovascular Research Center); Andrew Morris and Arnold Stromberg (Core B, UK Superfund Research Center).

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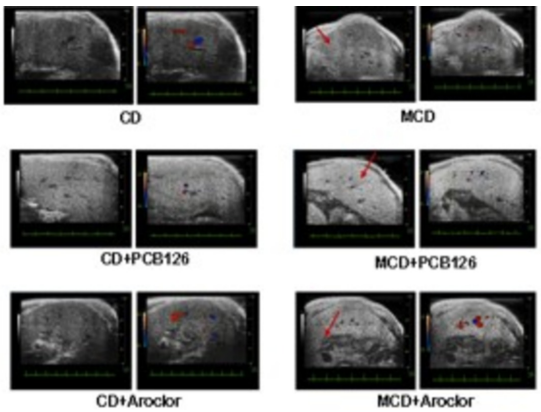

Fig. 1. A high-frequency, high-resolution ultrasound imaging system was used to detect the progression of liver damage (steatohepatitis) during the study period (Week 8). The MCD-fed mice appeared to have irregular liver surfaces and increased echogenicity, indicating fatty infiltration.

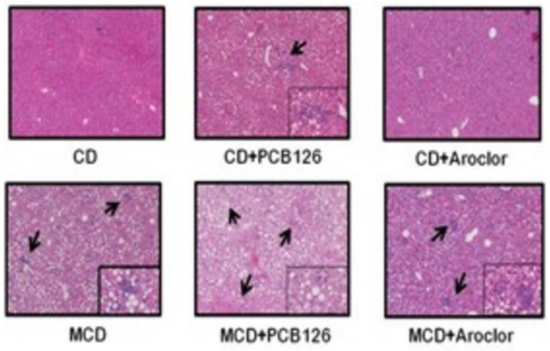

Fig. 2. H&E staining of hepatic sections established the occurrence of steatosis, and inflammatory foci in the MCD groups as well as in the PCB-126 exposed group on CD.

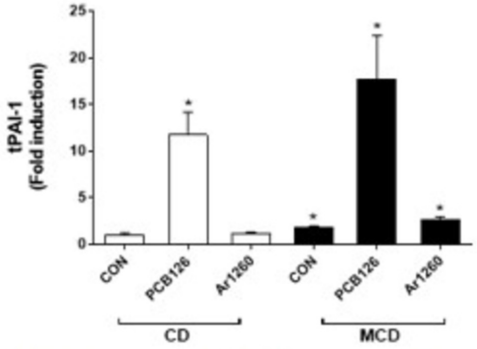

Fig. 3. The hepatic mRNA expression for the serine protease inhibitor tPAI-1 was measured using real time PCR. Values are represented as mean ± SEM, $p < 0.05$.

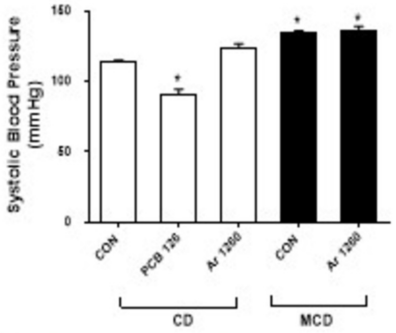

Fig. 4. The systolic blood pressure was measured using the tail-cuff method. Values are represented as mean ± SEM, $p < 0.05$.