Published in final edited form as:

Toxicology. 2012 December 16; 302(2-3): 212–220. doi:10.1016/j.tox.2012.08.001.

Lipopolysaccharide Potentiates Polychlorinated Biphenyl-induced Disruption of the Blood–Brain Barrier via TLR4/IRF-3 Signaling

Jeong June Choi^a, Yean Jung Choi^b, Lei Chen^c, Bei Zhang^{a,d}, Sung Yong Eum^a, Maria T Abreu^e, and Michal Toborek^a

^aDepartments of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, FL

^bDepartment of Neurosurgery, University of Kentucky Medical Center, Lexington, KY

^cDepartment of Neuroscience, Mount Sinai School of Medicine, New York, NY

^dGraduate Center for Nutritional Sciences, University of Kentucky Medical Center, Lexington, KY

^eDivision of Gastroenterology, Department of Medicine, University of Miami School of Medicine, Miami, FL

Abstract

Exposure to polychlorinated biphenyls (PCBs) is associated with numerous adverse health effects. Although the main route of exposure to PCBs is through the gastrointestinal tract, little is known about the contribution of the gut to the health effects of PCBs. We hypothesize that PCBs can disrupt intestinal integrity, causing lipopolysaccharide (LPS) translocation into the bloodstream and potentiation of the systemic toxicity of PCBs. C57BL/6 mice were exposed to individual PCB congeners by oral gavage, followed by the assessment of small intestine morphology and plasma levels of proinflammatory mediators. In addition, mice and human brain endothelial cells were exposed to PCB118 in the presence or absence of LPS to evaluate the contribution of LPS to PCB-induced toxicity at the blood–brain barrier (BBB) level. Oral administration of PCB153, PCB118, or PCB126 disrupted intestinal morphology and increased plasma levels of LPS and proinflammatory cytokines. Direct injection of LPS and PCB118 into the cerebral microvasculature resulted in synergistic disruption of BBB integrity and decreased expression of tight junction proteins in brain microvessels. *In vitro* experiments confirmed these effects and indicated that stimulation of the toll-like receptor 4 (TLR4) pathway can be responsible for these effects via activation of interferon regulatory factor-3 (IRF-3). These results indicate that LPS may be a contributing factor in PCB-induced dysfunction of the brain endothelium via stimulation of the TLR4/IRF-3 pathway.

© 2012 Elsevier Ireland Ltd. All rights reserved.

Corresponding Author: Michal Toborek, MD, PhD; Department of Biochemistry and Molecular Biology, University of Miami School of Medicine, Gautier Bldg., Room 517, 1011 NW 15th Street Miami, FL 33136, Phone# 305-243-0230, mtoborek@med.miami.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

blood–brain barrier; inflammation; interferon regulatory factor-3; polychlorinated biphenyls; tight junctions; Toll-like receptor 4

1. INTRODUCTION

Polychlorinated biphenyls (PCBs) are persistent organic pollutants associated with numerous adverse health effects in humans. Based on their chemical structure and affinity to specific cellular receptors, they are classified as dioxin-like (e.g., PCB126 used in the present study), non-dioxin-like (e.g., PCB153), or mono-*ortho* PCB congeners (e.g., PCB118), which partially preserve both dioxin-like and non-dioxin-like biological activity. Due to their lipophilic properties and resistance to biodegradation, PCBs are bioconcentrated at each level of the food chain. As a result of this process, dietary intake is the primary route of PCB exposure in humans (Fitzgerald et al. 2007). To mimic such an experimental exposure, several recent studies evaluated the toxic effects of individual PCB congeners or PCB mixtures when administered to mice by oral gavage (Lee et al. 2012). Treatment of mice with Aroclor 1254 (a commercial PCB mixture) disrupted spermatogenesis, as evidenced by decreased sperm count, testicular dysgenesis, and lowered estradiol levels (Cai et al. 2011). Oral treatment with selected PCB congeners also affected growth, adrenal development, and cortisol production in a fetal sheep model (Zimmer et al. 2011). Developmental oral exposure to PCB52 or PCB180 induced alterations of the brainstem auditory evoked potentials (Lilienthal et al. 2011). The results from our laboratory indicated that oral exposure to individual PCB congeners resulted in increased permeability across the blood–brain barrier (BBB) and intestinal barrier and facilitated formation of brain metastases (Choi et al. 2010; Seelbach et al. 2010). However, the mechanisms by which oral PCB exposure compromised the BBB in these studies are unknown. In particular, it is unclear whether PCBs act independently or in concert with gut factors, like lipopolysaccharide (LPS), that translocate into systemic circulation as the result of disrupted intestinal barrier function.

The BBB regulates the entry of nutrients and trafficking of cells into the CNS. It is composed of specialized cerebral microvascular endothelium, which interacts with astrocytes, neurons, and pericytes (Weiss et al. 2009). Cerebral microvascular endothelial cells, by expressing tight junction proteins which seal the neighboring cells, are responsible for the barrier properties of the BBB. Occludin is a transmembrane protein and the first described tight junction protein (Feldman et al. 2005). Zonula occludens-1 and -2 (ZO-1 and -2) interact directly with the COOH-terminal domain of occludin, bridging between the tight junctions and the actin cytoskeleton (Itoh et al. 1999). ZO-2 was also suggested to act as a transcriptional regulator in the nucleus (Betanzos et al. 2004). Decreased expression or redistribution of tight junction proteins in the brain endothelium results in the disruption of the BBB, contributes to neurotoxicity and neuroinflammation, and may facilitate cell trafficking into the brain (Weiss et al. 2009).

The intestinal lumen provides a specific environment for bacteria, which interact with other pathogens and substances. Since Gram-negative bacteria are a normal constituent of the gastrointestinal tract, disruption of the intestinal barrier function may stimulate systemic exposure to bacteria or pathogen-associated microbial products, such as LPS. Indeed, bacteria can translocate from the leaky gut to the portal and systemic circulation. Clinically, these events are frequently observed during disruption of intestinal barrier function in inflammatory bowel disease or liver cirrhosis (Gardiner et al. 1995). In addition, enteric

Escherichia coli K1 is known to translocate from the gut and elicit systemic infections, resulting in meningitis (Teng et al. 2005).

The aim of the present study was to evaluate the influence of the disruption of the intestinal barrier function on the systemic toxicity of PCBs. We demonstrate that lipopolysaccharide (LPS), which translocates from the gut into the bloodstream as the result of alterations of intestinal barrier function, contributes to the cerebrovascular toxicity of PCBs. In a series of *in vivo* and *in vitro* experiments, we demonstrate that co-exposure to PCBs and LPS results in disruption of the brain endothelium via a signaling pathway that involves the toll-like receptor 4 (TLR4) and interferon regulatory factor-3 (IRF-3) pathway.

2. MATERIALS AND METHODS

2.1. Animals and treatment

All animal protocols employed in this study were approved by the Institutional Committee on Animal Care and abide by the NIH guidelines. Male C57BL/6 mice (12–14 weeks old, Charles River Laboratories, Wilmington, MA) were housed under 12:12 h light/dark conditions with access to food and water *ad libitum*. Mice were administered by oral gavage with PCB126, PCB153, or PCB118 (99.8% pure, AccuStandard, New Haven, CT), which belong to three distinct classes of PCBs. To evaluate structure-function relationship, all PCBs were used in the equimolar dose of 150 $\mu\text{mol/kg}$ body weight, which corresponds to 416 ng/kg PCB153 or 460 ng/kg PCB126 and PCB118. These doses of PCBs administered orally to mice result in a 5 μM PCB plasma level (Choi et al. 2010), i.e., a concentration that is widely used *in vitro* (Eum et al. 2008) and corresponds to plasma levels of PCBs in acutely exposed population (Jensen 1989; Wassermann et al. 1979). PCBs were dissolved in vitamin-stripped safflower oil and administered in a 0.1-ml volume using a 3''-long, 18-G curved gavage needle with a 2¼-mm ball diameter (Popper and Sons, New Hyde Park, NY) for 24 h. Control animals received an equal volume of vehicle.

For cerebrovascular administration, PCB118 (67 $\mu\text{mol/kg}$; i.e., 206 pg/kg body weight, dissolved in DMSO) and/or LPS (67 ng/kg) were injected into the internal carotid artery (ICA) as described previously (Chen et al. 2009). The selectivity of substance delivery via ICA injection was standardized by injection of carbon black or FITC-labeled albumin, followed by visualization of the stained brain area. The amounts of the injected PCB118 and LPS were estimated to result in plasma levels of 2 μM PCB118 and 2 ng/ml LPS, assuming 100% bioavailability, a blood volume of 6% body weight, and that plasma represents 45% of blood volume. In these experiments, PCB118 was dissolved in DMSO and the total injection volume was 200 μl . The LPS and control groups were injected with the same amount of DMSO. Mice were sacrificed 24 h post PCB118 and/or LPS administration.

2.2. Intestinal morphology and LPS and cytokine determination

Histopathology was performed on 1–3-cm gastroduodenal segments, which were fixed with 10% buffered formaldehyde, paraffin-embedded, and processed into 10- μm -thick sections, which were stained with hematoxylin-eosin (HE).

To assess plasma levels of LPS and inflammatory cytokines, mice were anesthetized with isoflurane, and blood samples were collected by heart puncture in heparin-containing microtubes (Sarstedt, Germany). Plasma was separated by centrifugation at $900 \times g$ for 20 min. The Limulus amoebocyte lysate pyrochrome chromogenic test kit was used to determine plasma LPS levels, as described by the manufacturer (Charles River, Charleston, SC). Plasma cytokine levels were measured using the Milliplex Mouse Cytokine/Chemokine Kit (Millipore Corp., Billerica, MA) according to the manufacturer's protocol.

2.3. Immunofluorescence and BBB functional permeability assay

Isolation of brain microvessels and immunofluorescence detection of tight junction integrity in isolated microvessels was performed as described earlier (Seelbach et al. 2010). Briefly, after removal of the choroid plexus, meninges, cerebellum, and brainstem, brains were homogenized in buffer containing 103 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 15 mM HEPES, 25 mM NaHCO₃, 10 mM glucose, 1 mM Na pyruvate, and 10 g/L dextran. Following homogenization, the samples were mixed with 26% dextran, centrifuged at $5,800 \times g$ for 20 min, and filtered through a 70- μ m mesh filter. Following isolation, microvessels were spread onto glass slides, heat-immobilized at 95°C for 10 min, and fixed with 4% formaldehyde in PBS for 10 min. Microvessels were permeabilized with 0.1% Triton X-100 for 30 min and incubated with occludin or ZO-2-specific antibody overnight at 4°C. Occludin and ZO-2 were visualized with a Texas Red- and FITC-conjugated secondary antibody, respectively. Slides were mounted with ProLong Gold Antifade reagent containing 4',6-diamidino-2-phenylindole (DAPI, Invitrogen, Carlsbad, CA) to stain the nuclei.

BBB permeability was assessed as previously described (Chen et al. 2009). Briefly, treated animals were injected i.p. with 200 μ l of sodium fluorescein (10%), which was allowed to circulate for 15 min. Blood collection via cardiac puncture was followed by transcardial perfusion with 0.9% saline and brain harvesting. Brain homogenates were prepared in PBS (1:10 w/v), precipitated in 15% trichloroacetic acid (1:1 v/v), and centrifuged at $1,000 \times g$ for 10 min. The supernatant pH was adjusted by adding 125 μ l of 5 M NaOH. Brain fluorescence intensity was determined at 485 nm excitation and 530 nm emission and normalized to plasma fluorescence intensity.

2.4. Cell cultures, transendothelial permeability, and cell migration assays

Human brain endothelial cell line (hCMEC/D3 cells) was recently developed by transfection of primary cells with human telomerase reverse transcriptase (hTERT) and SV40 large T antigen. The cells were cultured in EBM-2 medium (Lonza, Walkerville, MD) supplemented with growth factors and 0.5% fetal bovine serum (FBS) as previously described (Eum et al. 2009). Twelve hours prior to treatment, the medium was changed, and cells were incubated with serum-free EBM medium. In a typical experiment, cells were exposed to PCB118 at 2 μ M and/or LPS at 2 ng/ml concentrations, which were based on extensive preliminary experiments to determine the subtoxic levels of these agents. Control cells were treated with the same volume of vehicle (DMSO, 0.01%). To inhibit TLR4 signaling, 10 μ M of CLI-095 (InvivoGen, San Diego, CA) was pretreated for 1 h before PCB118 and/or LPS treatments.

Endothelial integrity was assessed as described earlier (Eum et al. 2004). Briefly, hCMEC/D3 cells were seeded on collagen type I-coated Transwell tissue culture filters (12-mm diameter, 0.4- μ m pore size, Corning) at the density of 1×10^5 cells per filter insert. Confluent cultures (typically, 3 days after seeding) were exposed to PCB118 and/or LPS for 24 h. The treatment factors were added to both the lower and the upper compartments of the Transwell system. Following treatment, the cultures were rinsed with Krebs-Ringer glucose (KRG) solution and the transendothelial transfer of 20-kDa FITC-dextran (FD-20, 0.5 mg/ml) was assessed by microplate reader at 485 nm excitation and 530 nm emission.

In order to perform transendothelial migration assay (Ramirez et al. 2008), hCMEC/D3 cells were seeded on collagen type I-coated Transwell filters with 3- μ m pore size (12-mm diameter) at 1×10^5 cells/filter insert. The cultures were maintained for 3 days, at which time the cells reached confluency. THP-1 cells were then labeled with calcein-AM (5 μ M, Invitrogen) for 30 min, suspended in 100 μ l of the serum-free EBM medium, and added onto hCMEC/D3 monolayers at 5×10^5 cells/filter insert. CCL-2 (50 ng/ml) was added to

the lower chamber to enhance cell migration. After 2 h incubation, THP-1 cells that adhered to the upper side of the filter were gently scraped off using a cotton swab. The migrating cells and the cells on the lower side of the filter were lysed by adding 150 μ l of 4% Triton-X100 to 400 μ l of cell culture media in the lower chamber and incubating the filter with the solution for 5 min. The fluorescence intensity was measured at 485 nm excitation and 530 nm emission.

2.5. Gene silencing

hCMEC/D3 cells at 70–80% confluency were transfected with 75 nM control or IRF-3-specific siRNA (Applied Biosystems, Carlsbad, CA) using GeneSilencer (Genlantis, San Diego, CA). The cells were incubated with transfection mixtures for 24 h, allowed to recover in complete medium for 48 h, transferred to new cell culture dishes, and then maintained in complete culture medium for an additional two days.

2.6. Western blotting

Whole cell lysates were prepared in RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 1% NP-40, 0.25% sodium deoxycholate, 150 mM NaCl, and 1 mM EDTA). Nuclear extracts were isolated using NE-PER® Nuclear and Cytoplasmic Extraction kit (Thermo Scientific) according to the manufacturer's protocol.

Brain microvessels were isolated as in section 2.3 and lysed with 6 M urea lysis buffer (6 M urea, 0.1% Triton X-100, 10 mM Tris, 5 mM MgCl₂, 5 mM EGTA, 150 mM NaCl). Immunoblotting was performed as described earlier (Eum et al. 2009) on lysates from individual animals, and proteins of interest were semi-quantitated with ImageJ software (version 1.45s, <http://rsb.info.nih.gov/ij/>).

2.7. Statistical analysis

Comparisons between treatments were assessed by one-way or two-way ANOVA, followed by Tukey's pairwise multiple comparison procedure. Data are presented as means $\pm$ SEM. A statistical probability of p0.05 was considered significant.

3. RESULTS

3.1. Oral administration of individual PCB congeners disrupts the morphology of the small intestine and elevates plasma LPS and cytokine levels *in vivo*

In the first series of experiments, we investigated whether oral administration of individual PCB congeners can affect the gut epithelial structure. Following a 24-h exposure to individual PCB congeners (PCB153, PCB118, or PCB126, 150 μ mol/kg), histopathology was performed on 10- μ m duodenal longitudinal sections using 6 mice per group. Representative data from these experiments are illustrated in Figure 1A. Villi of the control animals revealed typical highly organized morphology with evenly aligned columnar epithelial cells (enterocytes). Treatment with all PCB congeners induced prominent morphological changes in the small intestine, with PCB126 displaying the most pronounced alterations. Administration of PCB126 highly distorted villi and resulted in thinning and disruption of the inner circular muscle layer combined with necrosis and significant loss of the villus epithelium. Treatment with PCB153 caused disruption of the muscle layer and induced infiltration of the small intestine with inflammatory cells. Administration of PCB118 also caused structural damage to the villi and loss of epithelial cells.

We next determined the plasma levels of inflammatory cytokines in mice exposed to individual PCB congeners by oral gavage. As shown in Table 1, the most pronounced induction of proinflammatory mediators was observed following PCB118 exposure, which

significantly increased plasma levels of IL-6, TNF- α , and CCL-2. Exposure to PCB153 increased levels of IL-6; however, treatment with PCB126 did not affect plasma levels of proinflammatory cytokines at the time point when the analyses were performed. Based on these results and our previous findings showing that PCB118 is the most effective PCB congener in facilitating brain metastasis formation (Seelbach et al. 2010), the remaining experiments were performed only with PCB118.

To determine whether PCB118 administration results in pathogen translocation from the gut into the bloodstream, we measured plasma LPS levels as the marker of bacterial translocation. As shown in Figure 1B, at 24 h post oral administration of PCB118, plasma LPS levels had increased more than two fold compared with control mice. Treatment with PCB126 or PCB153 also increased plasma LPS levels (data not shown).

3.2. LPS and PCB118 cross-potentiate disruption of BBB integrity *in vivo*

We hypothesized that increased plasma levels of LPS contribute to the systemic vascular effects of PCB118, including disruption of the BBB. To address this possibility, we altered the route of PCB118 administration and directly injected PCB118 and/or LPS into the cerebral microvasculature. The doses of PCB118 and LPS (54 μ mol/kg and 54 ng/kg, respectively) were calculated to achieve similar plasma concentrations as those determined after oral administration of PCBs at 150 μ mol/kg.

Both immunoblotting (Figure 2A) and immunofluorescence results (Figure 2B) indicated that administration of PCB118 or LPS affect the levels of occludin and ZO-2 in brain microvessels. However, combined exposure to PCB118 and LPS generally potentiated the effects induced by individual treatment factors. Importantly, co-treatment with PCB118 and LPS resulted in markedly increased BBB permeability, while exposure to PCB118 or LPS alone did not alter the integrity of the cerebral microvasculature (Figure 2C).

3.3. Co-exposure to PCB118 and LPS disrupts the barrier integrity of hCMEC/D3 cells

Next, we investigated the effects of treatment with subtoxic levels of PCB118 and/or LPS (2 μ M and 2 ng/ml, respectively) on hCMEC/D3 cell integrity using *in vitro* model systems. Confluent cultures were exposed to PCB118 and/or LPS for 24 h. A combined exposure significantly decreased expression of both occludin and ZO-2 compared with exposure to individual treatment factors or control (Figures 3A and 3B).

In the next series of experiments, we evaluated permeability across the monolayers created by hCMEC/D3 cells. FITC-labeled dextran of molecular weight 20 kDa (FD-20) was used as a marker of endothelial integrity. Treatment with PCB118 or LPS alone did not affect endothelial permeability; however, combined exposure to these treatment factors resulted in a dramatic increase in permeability across hCMEC/D3 monolayers (Figure 3C).

Because disruption of the BBB may stimulate transendothelial cellular migration (Choi et al. 2003; Eum et al. 2004), we also evaluated the effects of PCB118 and/or LPS co-treatment on leukocyte passage through the monolayers of hCMEC/D3 cells. While treatment with PCB118 or LPS alone at subtoxic levels did not affect leukocyte transmigration, the combined treatment significantly increased cell migration across the endothelial monolayers (Figure 3D).

3.4. Co-treatment with PCB118 and LPS activates TLR4 signaling in hCMEC/D3 cells

The biological effects of LPS are mediated by activation of TLR4, which branches into the MyD88-dependent and MyD88-independent pathways. Treatment of hCMEC/D3 cells with LPS and PCB118 did not activate the MyD88-dependent pathway as determined by lack of

changes in IRAK-1 phosphorylation, a signaling molecule involved in this pathway (supplementary Figure 1). In fact, the band corresponding to phosphorylated IRAK-1 was hardly detectable, which also prevented reliable data quantification. Therefore, we focused on MyD88-independent signaling, which leads to phosphorylation of cytoplasmic IRF-3, which then translocates into the nucleus and acts as a transcription factor by modulating gene expression (Yoneyama et al. 2002).

Cultured hCMEC/D3 cells were treated with PCB118 plus LPS for up to 8 h, and the levels of phosphorylated IRF-3 were determined in total cell lysates. Treatment with PCB118 or LPS alone did not affect phosphorylated IRF-3 (Supplementary Figure 2A and 2B). Importantly, PCB118 and LPS co-treatment increased phosphorylation of IRF-3 in a time-dependent manner, with the maximum effect at 30 min to 2 h (Figure 4A). The effect of co-treatment on IRF-3 phosphorylation was statistically significant compared with treatment using PCB118 or LPS alone (Figure 4B). In addition, co-treatment with PCB118 plus LPS potentiated translocation of phosphorylated IRF-3 into the nucleus compared with the effects of individual treatment factors alone (Figure 4C).

3.5. PCB118 and LPS-induced disruption of endothelial integrity is mediated by TLR4 via IRF-3 signaling

In the last series of experiments, we assessed the role of TLR4 and IRF-3 signaling in the disruption of endothelial integrity by PCB118 and LPS. The activity of TLR4 was inhibited with CLI-095, followed by exposure to PCB118 and/or LPS for 24 h and determination of occludin expression. Consistent with the results in Figure 3A, occludin levels were greatly reduced by co-treatment with PCB118 and LPS. Importantly, pre-treatment with CLI-095 inhibited these effects (Figure 5A), suggesting that TLR4 may be involved in PCB118 and LPS-induced alteration of occludin expression.

Because pharmacological inhibition of TLR4 may lack full specificity, we silenced expression of IRF-3 with a specific siRNA (Figure 5B), which was followed by treatment with PCB118 and/or LPS. Silencing of IRF-3 markedly protected against PCB118 plus LPS-mediated reduction in occludin levels (Figure 5C). Moreover, PCB118 plus LPS-induced hyperpermeability was attenuated by IRF-3 silencing (Figure 5D).

4. DISCUSSION

The primary human exposure to PCBs is via the oral route by contaminated food or water. Therefore, the intestine is the initial, yet largely neglected, organ exposed to PCB toxicity. We previously reported that PCBs are toxic to intestinal epithelial cells and that oral exposure to PCBs results in increased permeability through the intestinal epithelium (Choi et al. 2010). We also demonstrated that PCBs administered orally can affect cerebral microvessels, leading to induction of inflammatory responses and transcapillary migration of tumor cells (Seelbach et al. 2010).

The results of the present study confirm that PCBs are highly toxic to the small intestine, which is in line with our earlier study suggesting that PCBs can induce alterations of gut integrity via activation of NADPH oxidase, stimulation of oxidative stress, and disruption of tight junction proteins (Choi et al. 2010). In the present study, administration of specific PCB congeners resulted in disruption of villi morphology, loss of the epithelium, and thinning and disruption of the inner circular muscle layer. Importantly, these morphological changes were associated with increased plasma cytokine and LPS levels. Although treatment with all PCB congeners resulted in morphological alterations of the small intestine, the mechanisms underlying these effects were likely different and related to the specific mode of action of individual congeners. PCB126 is a dioxin-like coplanar PCB that binds to the

aryl hydrocarbon receptor (AhR) and induces toxic effects via activation of this receptor (Zhang et al. 2012). Importantly, AhR may regulate responses mediated by LPS, as AhR-deficient mice are more sensitive to LPS-induced lethal shock than wild-type mice and LPS-induced production of IL-6, TNF- α , and IL-12p40 is increased in AhR-deficient macrophages (Kimura et al. 2009). On the other hand, there are also contrary reports that an AhR ligand, TCDD, can synergistically increase LPS-induced IL-1 β and IL-6 and enhance LPS-induced toxicity in mice (Pestka and Zhou 2006; Taylor et al. 1992). Therefore, it was unexpected that oral administration of PCB126 did not result in increased plasma levels of pro-inflammatory cytokines in the present study. One of the modes of action induced by AhR is cell death via apoptotic pathways (Camacho et al. 2005; Ito et al. 2004). Thus, PCB126 may be less pro-inflammatory than other PCB congeners used in the present study. Non-dioxin-like PCB congeners such as PCB153 possess a non-coplanar biphenyl ring and do not interact with AhR (Kopeck et al. 2010). Instead, constitutively active androstane receptor (CAR), epidermal growth factor receptor (EGFR), and pregnane X receptor (PXR) have been suggested as mediating the toxic effects of non-coplanar PCB congeners (Al-Salman and Plant 2012; Eum et al. 2009). Finally, PCB118, a mono-*ortho* PCB congener, has a multi-potent mode of action that partially preserves both dioxin-like and non-dioxin-like biological activity (Hornbuckle and Robertson 2010).

Our observations that oral administration of PCBs induces alterations of gut morphology and LPS translocation are consistent with the role of the intestine in the development of systemic inflammatory conditions associated with a leaky gut (Groschwitz and Hogan 2009). Nevertheless, these results do not answer the important question of whether PCB-induced plasma LPS levels are sufficient to induce dysfunction of the BBB or whether other factors released from the leaky gut are required to contribute to cerebrovascular toxicity. To address this question, we changed the route of exposure and administered LPS alone and/or PCB118 directly into the cerebrovascular circulation to produce the same plasma levels as those determined after oral administration of PCBs. Interestingly, direct administration of such a dose of PCB118 did not affect the integrity of the BBB and induced only relatively small changes in tight junction protein expression. Statistically significant disruption of the BBB was observed only when administration of subtoxic doses of PCB118 was accompanied by LPS injection. Clinically, these data may be important because PCBs are known to induce neurotoxicity, and disruption of the BBB may contribute to the development of brain neurotoxicity (Carpenter 2006; Weiss et al. 2009; Winneke et al. 2002). The effects of LPS as stimulators of BBB dysfunction are consistent with literature reports demonstrating the role of LPS in the opening of the BBB via disruption of tight junctions (Singh and Jiang 2004; Zhou et al. 2009). We further confirmed the contribution of LPS to the vascular toxicity of PCBs in a series of *in vitro* experiments in which brain endothelial cells were exposed to subtoxic levels of PCB118 and LPS.

To explore the potential mechanisms of LPS-mediated influence on PCB118 cerebrovascular toxicity, we focused on the role of TLR4, a specific receptor for LPS which is widely expressed in various tissues, including the brain endothelium and endothelial cell line (hCMEC/D3) used in the present study (Nagyoszi et al. 2010; Singh and Jiang 2004). While activation of TLR4 was suggested to be involved in LPS-induced BBB disruption (Zhou et al. 2009), the functional role of this receptor in regulation of BBB integrity is not fully understood. We observed that pharmacological inhibition of TLR4 by CLI-095 attenuated a decrease in occludin expression induced by co-treatment with PCB118 and LPS. These observations are consistent with literature reports indicating that TLR4 may be involved in regulation of tight junction protein expression. For example, alterations of ZO-1 expression induced by LPS were prevented by TLR4 siRNA in cholangiocyte monolayers (Sheth et al. 2007), and TLR4 knockout mice were protected against intestinal barrier breakdown after thermal injury (Peterson et al. 2010).

Activated TLR4 branches into two relatively well-established pathways involving MyD88-dependent and MyD88-independent signaling. In the MyD88-dependent pathway, IL-1 receptor-associated kinases (IRAKs) are phosphorylated by recruiting MyD88, which transduces signals to TRAF6, MAPKs, and NF- κ B. In preliminary experiments, we evaluated whether this pathway is activated in our model; however, phosphorylation of IRAK-1 was not affected in response to PCB118 and LPS compared with control cultures. In contrast, exposure to PCB118 and LPS stimulated phosphorylation of IRF-3, a signal molecule in the MyD88-independent pathway. Importantly, our results demonstrate that IRF-3 is involved in PCB118 and LPS-induced downregulation of occludin and endothelial cell barrier dysfunction. These data are novel in that there are no literature reports linking IRF-3 to increased endothelial permeability and alterations of tight junction protein expression.

IRF-3 is a transcription factor that binds specifically to a consensus DNA binding motif and regulates gene transcription. In search of the mechanisms of IRF-3-mediated alterations of tight junction protein expression, we evaluated the promoter regions of occludin and ZO-2 for putative IRF-3 binding sites using the Transcription Element Search System (TESS) (<http://www.cbil.upenn.edu/cgi-bin/tess/tess>). Since no IRF-3 binding sites were found in the sequences 2 kb upstream of genes encoding these tight junction proteins, it is highly unlikely that IRF-3 directly controls expression of ZO-2 or occludin by binding to their promoter sequences. However, IRF-3 can form a holocomplex with cAMP responding element binding protein (CBP)/p300 (Yoneyama et al. 1998) and thus regulate expression of occludin and ZO-2 via binding to the CRE sites in their promoter regions. This hypothesis is supported by the observations that binding to the CRE sequence in the ZO-1 promoter suppresses expression of this tight junction protein (Chen et al. 2008).

IRF-3 may also affect the integrity of brain endothelial cells by a variety of indirect mechanisms. For example, IRF-3 transactivates expression of proinflammatory cytokines and chemokines, including IL-6, MIP-1 α , and RANTES (Sweeney et al. 2010), which are known to contribute to the disruption of BBB integrity (Terao et al. 2008; Toborek et al. 2003). Activated IRF-3 can also stimulate expression of matrix metalloproteinases (MMPs) (Sweeney et al. 2010), which were shown to be involved in proteolytic degradation of tight junction proteins (Huang et al. 2011).

While LPS is an established ligand for TLR4, the mechanisms by which exposure to PCB118 contributes to activation of this receptor are less understood. TLR4 is localized in caveolae and lipid rafts, which also control its activation (Wong et al. 2009). Interestingly, PCBs can accumulate in caveolae in endothelial cells (Lim et al. 2007) and exert their biological effects via signaling mechanisms associated with these membrane microdomains. For example, PCB-induced upregulation of cell adhesion molecules is inhibited in mice lacking caveolae (Han et al. 2010) or by disruption of lipid rafts (Eum et al. 2009). Thus, it is possible that PCB-mediated alterations of caveolae and lipid rafts participate in modulation of TLR4 and thus influence IRF-3 signaling.

5. CONCLUSION

The results of the present study provide the first evidence that LPS may be a co-factor in PCB-induced BBB disruption. Oral administration of PCB118 disrupted the intestinal barrier function and induced LPS translocation into the bloodstream. Importantly, co-exposure to PCB118 and LPS potentiated alterations of tight junction protein expression and disruption of endothelial barrier functions via the TLR4/IRF-3 pathway. Overall, these results underline the role of a leaky gut and endotoxin translocation in the systemic health effects induced by PCBs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by the grants from the National Institutes of Health: CA133257, P42 ES 07380, MH63022, MH072567, and DA027569.

Abbreviations

BBB	blood–brain–barrier
IRF-3	interferon regulatory factor-3
LPS	lipopolysaccharide
PCB	polychlorinated biphenyl
TLR4	toll-like receptor-4
ZO	zonula occludens

References

- Al-Salman F, Plant N. Non-coplanar polychlorinated biphenyls (PCBs) are direct agonists for the human pregnane-X receptor and constitutive androstane receptor, and activate target gene expression in a tissue-specific manner. *Toxicol Appl Pharmacol.* 2012;1016/j.taap.2012.05.016
- Betanzos A, Huerta M, Lopez-Bayghen E, Azuara E, Amerena J, Gonzalez-Mariscal L. The tight junction protein ZO-2 associates with Jun, Fos and C/EBP transcription factors in epithelial cells. *Exp Cell Res.* 2004; 292:51–66. [PubMed: 14720506]
- Cai J, Wang C, Wu T, Moreno JM, Zhong Y, Huang X, Chen Y, Zuo Z. Disruption of spermatogenesis and differential regulation of testicular estrogen receptor expression in mice after polychlorinated biphenyl exposure. *Toxicology.* 2011; 287:21–28. [PubMed: 21664404]
- Camacho IA, Singh N, Hegde VL, Nagarkatti M, Nagarkatti PS. Treatment of mice with 2,3,7,8-tetrachlorodibenzo-p-dioxin leads to aryl hydrocarbon receptor-dependent nuclear translocation of NF-kappaB and expression of Fas ligand in thymic stromal cells and consequent apoptosis in T cells. *J Immunol.* 2005; 175:90–103. [PubMed: 15972635]
- Carpenter DO. Polychlorinated biphenyls (PCBs): routes of exposure and effects on human health. *Rev Environ Health.* 2006; 21:1–23. [PubMed: 16700427]
- Chen J, Xiao L, Rao JN, Zou T, Liu L, Bellavance E, Gorospe M, Wang JY. JunD represses transcription and translation of the tight junction protein zona occludens-1 modulating intestinal epithelial barrier function. *Mol Biol Cell.* 2008; 19:3701–3712. [PubMed: 18562690]
- Chen L, Swartz KR, Toborek M. Vessel microport technique for applications in cerebrovascular research. *J Neurosci Res.* 2009; 87:1718–1727. [PubMed: 19115415]
- Choi W, Eum SY, Lee YW, Hennig B, Robertson LW, Toborek M. PCB 104-induced proinflammatory reactions in human vascular endothelial cells: relationship to cancer metastasis and atherogenesis. *Toxicol Sci.* 2003; 75:47–56. [PubMed: 12805654]
- Choi YJ, Seelbach MJ, Pu H, Eum SY, Chen L, Zhang B, Hennig B, Toborek M. Polychlorinated biphenyls disrupt intestinal integrity via NADPH oxidase-induced alterations of tight junction protein expression. *Environ Health Perspect.* 2010; 118:976–981. [PubMed: 20299304]
- Eum SY, Andras I, Hennig B, Toborek M. NADPH oxidase and lipid raft-associated redox signaling are required for PCB153-induced upregulation of cell adhesion molecules in human brain endothelial cells. *Toxicol Appl Pharmacol.* 2009; 240:299–305. [PubMed: 19632255]
- Eum SY, Andras IE, Couraud PO, Hennig B, Toborek M. Pcb's and tight junction expression. *Environ Toxicol Pharmacol.* 2008; 25:234–240. [PubMed: 18438464]

- Eum SY, Lee YW, Hennig B, Toborek M. VEGF regulates PCB 104-mediated stimulation of permeability and transmigration of breast cancer cells in human microvascular endothelial cells. *Exp Cell Res*. 2004; 296:231–244. [PubMed: 15149853]
- Feldman GJ, Mullin JM, Ryan MP. Occludin: structure, function and regulation. *Adv Drug Deliv Rev*. 2005; 57:883–917. [PubMed: 15820558]
- Fitzgerald EF, Belanger EE, Gomez MI, Hwang SA, Jansing RL, Hicks HE. Environmental exposures to polychlorinated biphenyls (PCBs) among older residents of upper Hudson River communities. *Environ Res*. 2007; 104:352–360. [PubMed: 17382313]
- Gardiner KR, Halliday MI, Barclay GR, Milne L, Brown D, Stephens S, Maxwell RJ, Rowlands BJ. Significance of systemic endotoxaemia in inflammatory bowel disease. *Gut*. 1995; 36:897–901. [PubMed: 7615280]
- Groschwitz KR, Hogan SP. Intestinal barrier function: molecular regulation and disease pathogenesis. *J Allergy Clin Immunol*. 2009; 124:3–20. [PubMed: 19560575]
- Han SG, Eum SY, Toborek M, Smart E, Hennig B. Polychlorinated biphenyl-induced VCAM-1 expression is attenuated in aortic endothelial cells isolated from caveolin-1 deficient mice. *Toxicol Appl Pharmacol*. 2010; 246:74–82. [PubMed: 20406653]
- Hornbuckle K, Robertson L. Polychlorinated biphenyls (PCBs): sources, exposures, toxicities. *Environ Sci Technol*. 2010; 44:2749–2751. [PubMed: 20384370]
- Huang W, Andras IE, Rha GB, Hennig B, Toborek M. PPARalpha and PPARgamma protect against HIV-1-induced MMP-9 overexpression via caveolae-associated ERK and Akt signaling. *FASEB J*. 2011; 25:3979–3988. [PubMed: 21840940]
- Ito T, Tsukumo S, Suzuki N, Motohashi H, Yamamoto M, Fujii-Kuriyama Y, Mimura J, Lin TM, Peterson RE, Tohyama C, Nohara K. A constitutively active arylhydrocarbon receptor induces growth inhibition of jurkat T cells through changes in the expression of genes related to apoptosis and cell cycle arrest. *J Biol Chem*. 2004; 279:25204–25210. [PubMed: 15069065]
- Itoh M, Furuse M, Morita K, Kubota K, Saitou M, Tsukita S. Direct binding of three tight junction-associated MAGUKs, ZO-1, ZO-2, and ZO-3, with the COOH termini of claudins. *J Cell Biol*. 1999; 147:1351–1363. [PubMed: 10601346]
- Jensen, AA. Background levels in humans. In: Kimbrough, RD.; Jensen, AA., editors. *Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products*. Elsevier Science Publishers; New York: 1989. p. 345–364.
- Kimura A, Naka T, Nakahama T, Chinen I, Masuda K, Nohara K, Fujii-Kuriyama Y, Kishimoto T. Aryl hydrocarbon receptor in combination with Stat1 regulates LPS-induced inflammatory responses. *J Exp Med*. 2009; 206:2027–2035. [PubMed: 19703987]
- Kopec AK, Burgoon LD, Ibrahim-Aibo D, Mets BD, Tashiro C, Potter D, Sharratt B, Harkema JR, Zacharewski TR. PCB153-elicited hepatic responses in the immature, ovariectomized C57BL/6 mice: comparative toxicogenomic effects of dioxin and non-dioxin-like ligands. *Toxicol Appl Pharmacol*. 2010; 243:359–371. [PubMed: 20005886]
- Lee DW, Notter SA, Thiruchelvam M, Dever DP, Fitzpatrick R, Kostyniak PJ, Cory-Slechta DA, Opanashuk LA. Subchronic polychlorinated biphenyl (aroclor 1254) exposure produces oxidative damage and neuronal death of ventral midbrain dopaminergic systems. *Toxicol Sci*. 2012; 125:496–508. [PubMed: 22094459]
- Lilienthal H, Heikkinen P, Andersson PL, van der Ven LT, Viluksela M. Auditory effects of developmental exposure to purity-controlled polychlorinated biphenyls (PCB52 and PCB180) in rats. *Toxicol Sci*. 2011; 122:100–111. [PubMed: 21464466]
- Lim EJ, Smart EJ, Toborek M, Hennig B. The role of caveolin-1 in PCB77-induced eNOS phosphorylation in human-derived endothelial cells. *Am J Physiol Heart Circ Physiol*. 2007; 293:H3340–3347. [PubMed: 17933968]
- Nagyoszi P, Wilhelm I, Farkas AE, Fazakas C, Dung NT, Hasko J, Krizbai IA. Expression and regulation of toll-like receptors in cerebral endothelial cells. *Neurochem Int*. 2010; 57:556–564. [PubMed: 20637248]
- Pestka J, Zhou HR. Toll-like receptor priming sensitizes macrophages to proinflammatory cytokine gene induction by deoxynivalenol and other toxicants. *Toxicol Sci*. 2006; 92:445–455. [PubMed: 16687389]

- Peterson CY, Costantini TW, Loomis WH, Putnam JG, Wolf P, Bansal V, Eliceiri BP, Baird A, Coimbra R. Toll-like receptor-4 mediates intestinal barrier breakdown after thermal injury. *Surg Infect (Larchmt)*. 2010; 11:137–144. [PubMed: 20374005]
- Ramirez SH, Heilman D, Morsey B, Potula R, Haorah J, Persidsky Y. Activation of peroxisome proliferator-activated receptor gamma (PPARgamma) suppresses Rho GTPases in human brain microvascular endothelial cells and inhibits adhesion and transendothelial migration of HIV-1 infected monocytes. *J Immunol*. 2008; 180:1854–1865. [PubMed: 18209083]
- Seelbach M, Chen L, Powell A, Choi YJ, Zhang B, Hennig B, Toborek M. Polychlorinated biphenyls disrupt blood-brain barrier integrity and promote brain metastasis formation. *Environ Health Perspect*. 2010; 118:479–484. [PubMed: 20064788]
- Sheth P, Delos Santos N, Seth A, LaRusso NF, Rao RK. Lipopolysaccharide disrupts tight junctions in cholangiocyte monolayers by a c-Src-, TLR4-, and LBP-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol*. 2007; 293:G308–318. [PubMed: 17446308]
- Singh AK, Jiang Y. How does peripheral lipopolysaccharide induce gene expression in the brain of rats? *Toxicology*. 2004; 201:197–207. [PubMed: 15297033]
- Sweeney SE, Kimbler TB, Firestein GS. Synovocyte innate immune responses: II. Pivotal role of IFN regulatory factor 3. *J Immunol*. 2010; 184:7162–7168. [PubMed: 20483755]
- Taylor MJ, Lucier GW, Mahler JF, Thompson M, Lockhart AC, Clark GC. Inhibition of acute TCDD toxicity by treatment with anti-tumor necrosis factor antibody or dexamethasone. *Toxicol Appl Pharmacol*. 1992; 117:126–132. [PubMed: 1440607]
- Teng CH, Cai M, Shin S, Xie Y, Kim KJ, Khan NA, Di Cello F, Kim KS. Escherichia coli K1 RS218 interacts with human brain microvascular endothelial cells via type 1 fimbria bacteria in the fimbriated state. *Infect Immun*. 2005; 73:2923–2931. [PubMed: 15845498]
- Terao S, Yilmaz G, Stokes KY, Russell J, Ishikawa M, Kawase T, Granger DN. Blood cell-derived RANTES mediates cerebral microvascular dysfunction, inflammation, and tissue injury after focal ischemia-reperfusion. *Stroke*. 2008; 39:2560–2570. [PubMed: 18635850]
- Toborek M, Lee YW, Pu H, Malecki A, Flora G, Garrido R, Hennig B, Bauer HC, Nath A. HIV-Tat protein induces oxidative and inflammatory pathways in brain endothelium. *J Neurochem*. 2003; 84:169–179. [PubMed: 12485413]
- Wassermann M, Wassermann D, Cucos S, Miller HJ. World PCBs map: storage and effects in man and his biologic environment in the 1970s. *Ann N Y Acad Sci*. 1979; 320:69–124. [PubMed: 110205]
- Weiss N, Miller F, Cazaubon S, Couraud PO. The blood-brain barrier in brain homeostasis and neurological diseases. *Biochim Biophys Acta*. 2009; 1788:842–857. [PubMed: 19061857]
- Winneke G, Walkowiak J, Lilienthal H. PCB-induced neurodevelopmental toxicity in human infants and its potential mediation by endocrine dysfunction. *Toxicology*. 2002; 181–182:161–165.
- Wong SW, Kwon MJ, Choi AM, Kim HP, Nakahira K, Hwang DH. Fatty acids modulate Toll-like receptor 4 activation through regulation of receptor dimerization and recruitment into lipid rafts in a reactive oxygen species-dependent manner. *J Biol Chem*. 2009; 284:27384–27392. [PubMed: 19648648]
- Yoneyama M, Suhara W, Fujita T. Control of IRF-3 activation by phosphorylation. *J Interferon Cytokine Res*. 2002; 22:73–76. [PubMed: 11846977]
- Yoneyama M, Suhara W, Fukuhara Y, Fukuda M, Nishida E, Fujita T. Direct triggering of the type I interferon system by virus infection: activation of a transcription factor complex containing IRF-3 and CBP/p300. *EMBO J*. 1998; 17:1087–1095. [PubMed: 9463386]
- Zhang W, Sargis RM, Volden PA, Carmean CM, Sun XJ, Brady MJ. PCB 126 and Other Dioxin-Like PCBs Specifically Suppress Hepatic PEPCK Expression via the Aryl Hydrocarbon Receptor. *PLoS One*. 2012; 7:e37103. [PubMed: 22615911]
- Zhou H, Andonegui G, Wong CH, Kubes P. Role of endothelial TLR4 for neutrophil recruitment into central nervous system microvessels in systemic inflammation. *J Immunol*. 2009; 183:5244–5250. [PubMed: 19786543]
- Zimmer KE, Kraugerud M, Aleksandersen M, Gutleb AC, Ostby GC, Dahl E, Berg V, Skaare JU, Olsaker I, Ropstad E. Fetal adrenal development: Comparing effects of combined exposures to PCB 118 and PCB 153 in a sheep model. *Environ Toxicol*. 2011; 1002/tox.20703

Figure 1. Oral administration of PCB congeners disrupts intestinal morphology

Mice were administered by oral gavage with individual PCB congeners (PCB153, PCB118, or PCB126, 150 μ mol/kg) dissolved in vitamin-stripped safflower oil or with vehicle (control group). Analyses were performed at 24 h post PCB administration. (A) Paraffin-embedded cross-sections of mouse duodenum were stained with hematoxylin and eosin. Arrowheads indicate the inner circular muscle layer surrounding the epithelium (white), the tunica propria (yellow) and villi (grey). (B) Plasma levels of LPS 24 h post oral gavage of PCB118 (150 μ mol/kg). The results are means $\pm$ SEM, n=6. *p<0.05 vs. vehicle group.

C

Figure 2. LPS and PCB118 cross-potentiate disruption of the BBB *in vivo*

PCB118 and/or LPS were injected into the cerebral microcirculation via the internal carotid artery and allowed to circulate for 24 h. Expression of occludin and ZO-2 was analyzed by (A) immunoblotting and (B) immunofluorescence in brain microvessels. In (B), red staining indicates occludin; green, ZO-2; and blue, nuclei stained with DAPI. The white arrows indicate distortions of occludin or ZO-2 integrity (C) Brain permeability was measured using sodium fluorescein as a marker. The results are expressed as the ratio of brain to plasma fluorescence level. The blots or images are representative data from 4–6 independent experiments and the bar graphs present the quantitative data from these studies. The results are means $\pm$ SEM. * $p < 0.05$ or ** $p < 0.01$ vs. control.

Figure 3. Co-exposure to PCB118 and LPS disrupts the barrier integrity of cultured hCMEC/D3 cells

Confluent cultures were exposed to PCB118 and/or LPS for 24 h, followed by immunoblotting for occludin (A) and ZO-2 (B). Levels of occludin and ZO-2 were normalized to β -actin and plotted as the percentage of control. (C) The barrier function of endothelial monolayers was measured by the transendothelial permeability of 20-kDa FITC-dextran. (D) Transendothelial migration was assessed with THP-1 cells stained with calcein AM. The results are means $\pm$ SEM, $n=6$. * $p<0.05$, *** $p<0.001$ vs. vehicle.

Figure 4. Co-treatment with PCB118 and LPS activates IRF-3 in hCMEC/D3 cells

(A) Cultures were exposed to PCB118 plus LPS for the indicated time, and phosphorylation of IRF-3 was determined by immunoblotting. The levels of phosphorylated IRF-3 (p-IRF-3) were normalized to total IRF-3 expression. (B) Cells were exposed to PCB118 and/or LPS for 30 min and phosphorylation of IRF-3 was assessed as in (A). (C) Cells were exposed to PCB118 and/or LPS for 2 h. Phosphorylated IRF-3 was assessed by immunoblotting of nuclear extracts and normalized to nucleoporin p62 expression. Representative images from three independent experiments are shown, and the bar graphs present the quantitative data from these studies. The results are means $\pm$ SEM. * $p < 0.05$ vs. control.

Figure 5. PCB118 and LPS-induced disruption of endothelial integrity is mediated by TLR4/IRF-3 signaling

(A) Confluent hCMEC/D3 cultures were exposed to PCB118 and/or LPS for 24 h. Selective cultures were pre-treated with TLR4 inhibitor CLI-095 (10 μ M) or vehicle (DMSO, 0.01%) for 1 h. Occludin levels were measured by immunoblotting and normalized to β -actin levels. (B) Cells were transfected with IRF-3 siRNA and immunoblotting against IRF-3 was performed to determine the efficiency of the silencing procedure. (C) Following IRF-3 silencing, cells were exposed to PCB118 and/or LPS for 24 h and occludin levels were measured as in (A). Images in A–C are representative results from four independent experiments and the bar graphs summarize the quantitative results from these studies. (D) Following IRF-3 silencing, cells were exposed to PCB118 and/or LPS for 24 h and the integrity of the endothelial monolayers was assessed as in Figure 3C. The results are means $\pm$ SEM, n=4. *p<0.05, **p<0.01, ***p<0.001 vs. vehicle. #p<0.05. ###p<0.001 vs. scramble (control) siRNA or CLI-095.

Table 1

Plasma levels of proinflammatory cytokines following oral administration of individual PCB congeners (150 $\mu\text{mol/kg}$). The results are means $\pm$ SEM, n=3.

Cytokine	Vehicle	PCB118	PCB126	PCB153
IL-6 (pg/ml)	20.1 $\pm$ 3.7	55.8 $\pm$ 7.9 *	23.2 $\pm$ 7.3	55.1 $\pm$ 16.5 *
TNF-α (pg/ml)	0.9 $\pm$ 0.2	2.3 $\pm$ 0.6 *	2.2 $\pm$ 0.7	1.8 $\pm$ 0.4
CCL-2 (pg/ml)	25.2 $\pm$ 4.7	33.8 $\pm$ 10.7 *	20.2 $\pm$ 7.1	21.5 $\pm$ 7.3

* Statistically significant at $p < 0.05$ compared with vehicle-treated mice. Plasma levels of IL-1 β , IL-2, IL-4, IL-7, IL-10, IL-12, IL-14, GM-CSF, and IFN- γ were unchanged.