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Up-regulation of Endothelial Monocyte Chemoattractant Protein-1 by Coplanar PCB77 is Caveolin-1-dependent

Zuzana Majkova[a], **Eric Smart**[b], **Michal Toborek**[c], and **Bernhard Hennig**[a,d,*]

a *Graduate Center for Toxicology, University of Kentucky, Lexington, KY 40536-0200, USA*

b *Department of Pediatrics, University of Kentucky, Lexington, KY 40536-0200, USA*

c *Department of Neurosurgery, University of Kentucky, Lexington, KY 40536-0200, USA*

d *Department of Animal and Food Sciences, University of Kentucky, Lexington, KY 40536-0200, USA*

Abstract

Atherosclerosis, the primary cause of heart disease and stroke is initiated in the vascular endothelium, and risk factors for its development include environmental exposure to persistent organic pollutants. Caveolae are membrane microdomains involved in regulation of many signaling pathways, and in particular in endothelial cells. We tested the hypothesis that intact caveolae are required for coplanar PCB77-induced up-regulation of monocyte chemoattractant protein-1 (MCP-1), an endothelium-derived chemokine that attracts monocytes into sub-endothelial space in early stages of the atherosclerosis development. Atherosclerosis-prone LDL-R$^{-/-}$ mice (control) or caveolin-1$^{-/-}$/-LDL-R$^{-/-}$ mice were treated with PCB77. PCB77 induced aortic mRNA expression and plasma protein levels of MCP-1 in control, but not caveolin-1$^{-/-}$/LDL-R$^{-/-}$ mice. To study the mechanism of this effect, primary endothelial cells were used. PCB77 increased MCP-1 levels in endothelial cells in a time- and concentration-dependent manner. This effect was abolished by caveolin-1 silencing using siRNA. Also, MCP-1 up-regulation by PCB77 was prevented by inhibiting p38 and c-Jun N-terminal kinase (JNK), but not ERK1/2, suggesting regulatory functions via p38 and JNK MAPK pathways. Finally, pretreatment of endothelial cells with the aryl hydrocarbon receptor (AhR) inhibitor α-naphthoflavone (α-NF) partially blocked MCP-1 up-regulation. Thus, our data demonstrate that coplanar PCB77 can induce MCP-1 expression by endothelial cells and that this effect is mediated by AhR, as well as p 38 and JNK MAPK pathways. Intact caveolae are required for these processes both *in vivo* and *in vitro*. This further supports a key role for caveolae in vascular inflammation induced by persistent organic pollutants.

Keywords

Endothelial cells; 3,3′,4,4′-tetrachlorobiphenyl (PCB77); caveolin-1; monocyte chemoattractant protein (MCP-1)

Corresponding author: Bernhard Hennig, Molecular and Cell Nutrition Laboratory, College of Agriculture, University of Kentucky, Room 591, Wethington Health Sciences Building, 900 South Limestone, Lexington, KY 40536-0200, USA, Phone: +1 859 323-4933 ext. 81387, Fax: +1 859 257 1811, E-mail: E-mail: bhennig@email.uky.edu.

Conflict of interest statement

The authors have no conflict of interest.

Introduction

Endothelial activation is one of the earliest events in the development of atherosclerosis (Ross, 1999). Exposure to circulating persistent organic pollutants, such as polychlorinated biphenyls (PCBs), can facilitate this process (Hennig *et al*., 2002). Coplanar PCBs, for example 3,3′, 4,4′-tetrachlorobiphenyl (PCB77), bind to the aryl hydrocarbon receptor (AhR) in endothelial cells, causing up-regulation of cytochrome P450 1A1 (CYP1A1) (Toborek *et al*., 1995). Subsequent uncoupling of CYP1A1 by PCB77 leads to overproduction of reactive oxygen species (ROS) (Schlezinger *et al*., 2006), activation of oxidative stress-sensitive signaling pathways, and up-regulation of inflammatory mediators (Slim *et al*., 1999).

Monocyte chemoattractant protein-1 (MCP-1) is an endothelium-derived chemokine that plays an essential role in the recruitment of leukocytes to the site of injury during inflammation. The recruitment of monocytes into the artery wall, followed by their differentiation into macrophages and foam cells, is also one of the earliest events in the pathology of atherosclerosis (Packard and Libby, 2008). It has been reported that AhR ligands, for example polycyclic aromatic hydrocarbon benzo[a]pyrene (B[a]P) (Knaapen *et al*., 2007) or the strongest known AhR ligand, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Vogel *et al*., 2007), have the ability to induce MCP-1 production. Understanding mechanisms of MCP-1 up-regulation by coplanar PCBs would help to dissect mechanisms responsible for the increased incidence of atherosclerosis observed after PCB77 treatment *in vivo* (Arsenescu *et al*., 2008), as well as epidemiological evidence implicating PCBs in increased cardiovascular risk in exposed populations (Gustavsson and Hogstedt, 1997; Goncharov *et al*., 2008).

Many endothelial functions, including signal transduction, seem to be regulated through caveolae (Frank *et al*., 2003; Mineo and Shaul, 2006), which are 50–100 nm membrane microdomains enriched in cholesterol and sphingolipids, as well as its major structural protein caveolin-1 (Sargiacomo *et al*., 1993). Interestingly, a significant reduction in the size of atherosclerotic lesions has been observed in ApoE−/− mice deficient in caveolin-1 (Frank *et al*., 2004). Recent evidence from our laboratory implicate caveolae as a regulatory platform involved in endothelial activation by environmental contaminants, namely B[a]P (Oesterling *et al*., 2008) and coplanar PCBs (Lim *et al*., 2007; Lim *et al*., 2008). Caveolin-1 was required for eNOS activation by PCB77 (Lim *et al*., 2007), and AhR binding to caveolin-1 seems to play a role in up-regulation of downstream AhR targets including CYP1A1 (Lim *et al*., 2008).

Thus, the current study was designed to test the hypothesis that functional caveolae are required for MCP-1 up-regulation by coplanar PCB77 in endothelial cells. Our data provide clear evidence that PCB77 increases MCP-1 expression through AhR signaling and that caveolin-1 is a possible biomarker of inflammatory events mediated through AhR.

Materials and Methods

Materials and chemicals

The inhibitors α-naphthoflavone, N-acetyl-L-cysteine, SB203580 and PD98059, as well as sterile, endotoxin-tested, dimethyl sulfoxide (DMSO), were purchased from Sigma-Aldrich (St. Louis, MO). PCB77 was a generous gift from Dr. Larry W. Robertson, University of Iowa, Iowa City, IA. 3,3′,4,4′,5-pentachlorobiphenyl (PCB126) and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were purchased from AccuStandard, Inc. (New Haven, CT).

Cell culture

Primary porcine endothelial cells were isolated from pulmonary arteries as described previously (Hennig *et al*., 1984). Cells were cultured in M199 media (Invitrogen, Carlsbad,

CA) supplemented with 10% FBS (Hyclone Laboratories, Logan, UT). Prior to treatments, cells were grown until confluent and then synchronized by maintaining them in 1% FBS for 16 h. All vehicle controls and treated cultures contained the same amount of DMSO (0.1% v/v). This concentration of DMSO did not have any effect on MCP-1 expression.

Animals

All animals were housed in the Association for Assessment and Accreditation of Laboratory Animal Care-certified animal facilities at the University of Kentucky. For our experiments mice were used with low density lipoprotein receptor (LDL-R)-deficient background. These mice are a preferred model for atherosclerosis studies because they mimic human lipoprotein levels and atherosclerosis development (Daugherty, 2002). We also have demonstrated previously that PCB77 increases aortic adhesion molecule expression in LDL-R-deficient mice (Hennig et al., 2005). LDL-R deficient (LDL-$R^{-/-}$) mice on a C57BL/6 background and caveolin-1 (Cav-$1^{-/-}$) deficient mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Cav-1 deficient mice were generated in the Sv129 strain and backcrossed onto a C57BL/6 background. Mice were bred at the University of Kentucky to generate LDL-R/caveolin-1 double null mice (LDL-$R^{-/-}$ Cav-$1^{-/-}$ mice). At 8 weeks of age, mice were placed on a standardized diet containing 20% calories from fat (Dyets Inc., Bethlehem, PA). After 2 weeks, mice were injected intraperitoneally with PCB77 (170 μmol/kg body weight) or vehicle (olive oil) and then 6 days later they were injected again. This dose of PCB77 was previously sufficient to induce aortic adhesion molecule expression *in vivo* (Hennig *et al*., 2002; Hennig *et al*., 2005), as well as the development of atherosclerotic lesions over the course of 6 weeks (Arsenescu *et al*., 2008). 24 h after the last treatment, mouse tissues were harvested.

Caveolin-1 small-interfering RNA (siRNA) and transfection

Caveolin-1 protein levels in endothelial cells were silenced using small inhibitory (si)RNA technique as described previously (Lim *et al*., 2007). Briefly, the cells were transfected with a mixture of two siRNAs targeted against caveolin-1 (40 nM each) or control siRNA (80 nM), synthetized by Dharmacon (Lafayette, CO) according to previously published sequences (Repetto *et al*., 2005), and by using GeneSilencer transfection reagent (Genlantis, San Diego, CA) in OptiMEM media (Invitrogen). Subsequently, cells were treated with vehicle (DMSO) or PCB77.

Real-time PCR

Total RNA was extracted from the cells using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA) according to the manufacturer's protocol. Mouse aortas were cleaned of periadventitial tissue and stored in RNAlater RNA stabilizing reagent (Qiagen, Valencia, CA) at −80°C. Total mRNA was purified using the RNeasy Fibrous Tissue Mini Kit (Qiagen) according to the manufacturer's instructions. Reverse transcription was performed using the AMV reverse transcription system (Promega, Madison, WI). The levels of mRNAs expression were then assessed by real-time PCR using 7300 Real Time PCR System (Applied Biosystems, Foster City, CA) and SYBR Green (in endothelial cells) or TaqMan (in tissues) master mix (Applied Biosystems). MCP-1 or interleukin-6 (IL-6) mRNA levels were divided by β-actin (internal control). β-Actin and MCP-1 primer sequences for SYBR Green chemistry were designed using the Primer Express Software 3.0 for real-time PCR (Applied Biosystems) and synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). β-actin sequences: sense, 5′-TCATCACCATCGGCAACG-3′; antisense, 5′-TTCCTGATGTCCACGTCG-3′; MCP-1 sequences:5′-CGGCTGATGAGCTACAGAAGAGT-3′; antisense, 5′-GCTTGGGTTCTGCACAGATCT-3′. For TaqMan reactions, TaqMan gene expression assays (Applied biosystems) were used.

MCP-1 and IL-6 protein level

Cell culture media were harvested on ice and centrifuged at 2000 × g for 10 min at 4°C. The supernatants were collected and MCP-1 protein levels were measured using Quantikine ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. Mouse blood was collected in tubes containing EDTA, centrifuged at 1500 g for 20 min at 4°C, and plasma was removed and stored at −80°C. Plasma levels of MCP-1 and IL-6 were measured using Mouse Adipokine LINCOplex kit (Millipore, St. Charles, MO) according to the manufacturer's instructions. Luminex 100 (Luminex Corporation, Austin, TX) and Multiplex Data Analysis Software 1.0 (Upstate USA, Inc., Chicago, IL) were utilized for signal detection and data analysis, respectively.

Cell viability

Cell viability was evaluated using the commercially available MTS test (Sigma-Aldrich). Cells were plated into 96-well culture plates and treated with vehicle (DMSO) or increasing concentrations of PCB77 for 24 h. The MTS test was performed according to the manufacturer's instructions. Absorbance at 490 nm was measured using a SpectraMax M2 (Molecular Devices, Sunnyvale, CA).

Statistical analysis

Values are reported as means ± SE of at least three independent groups. Comparisons between two treatments were made by t-test; comparisons among three or more groups by one-way or two-way analysis of variance (ANOVA) followed by post-hoc Fisher's LSD test using SigmaStat 2.0 software (Systat Software, Point Richmond, CA). Statistical probability of $p < 0.05$ was considered significant.

Results

PCB77, as well as other AhR agonists, increase MCP-1 mRNA and protein levels in endothelial cells

MCP-1 is a chemokine involved in recruitment of monocytes from blood stream to sub-endothelial space in early stages of atherosclerosis development. In order to test whether coplanar PCB77 can increase the expression of MCP-1 in endothelial cells, cells were treated with vehicle control or PCB77 (5 μM) for various time intervals ranging from 2–24 hours. Significant increases in both mRNA expression (measured by RT PCR) (Fig. 1A) and protein levels released into culture media (measured by ELISA) (Fig. 1B) were observed only after 24 hours. In order to find out the lowest concentration of PCB77 to achieve MCP-1 up-regulation, increasing concentrations of PCB77 ranging from 1–10 μM were used. A significant increase in MCP-1 mRNA a protein expression was observed at both 5 and 10 μM concentrations of PCB77 (Figs. 2A and 2B). Using the MTS test, cell viability was not affected at any of these concentrations. Since 5 μM was the lowest effective concentration of PCB77, this dose was used in the subsequent inhibitor studies. This concentration also was used in our previous work on endothelial dysfunction (Slim *et al.*, 1999), and is similar to PCB levels reported in acutely exposed populations (3.4 μM, or 1 ppm). PCB77 is an example of a coplanar PCB with dioxin-like activity; thus, we also tested other AhR ligands, such as PCB126 (3,3′,4,4′,5-pentachlorobiphenyl) and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin), on induction of MCP-1 expression. Both coplanar PCB126 (Fig. 3A) and TCDD (Fig. 3B) significantly induced MCP-1 mRNA levels after 24 h. Toxicant concentrations used in these experiments were normalized relative to their toxic equivalency factor (TEF). In contrast to the AhR ligands, non-coplanar PCB153 (2,2′,4,4′,5,5′-hexachlorobiphenyl) did not affect MCP-1 expression (data not shown).

MCP-1 up-regulation is regulated through the aryl hydrocarbon receptor (AhR)

Many endothelial responses to coplanar PCBs are mediated through their binding to AhR (Hennig *et al*., 2002). In order to test the role of AhR in MCP-1 up-regulation by PCB77, the AhR antagonist α-naphthoflavone (α-NF) was used. Cells were pre-treated with α-NF (0.01 μM), followed by vehicle (DMSO) or PCB77 treatment. MCP-1 mRNA expression was significantly increased by PCB77, but this was prevented by α-NF pre-treatment (Fig. 4A).

MCP-1 up-regulation is oxidative stress-dependent

PCB77 is known to cause AhR-mediated up-regulation and subsequent uncoupling of cytochrome P450 (CYP) 1A1 (Schlezinger *et al*., 2006). Oxidative stress can cause MCP-1 up-regulation in vascular endothelium (Woo Lee *et al*., 2001). Here, the glutathione precursor and antioxidant N-acetyl cysteine (NAC) was used to increase antioxidant potential of endothelial cells. Cells were pre-treated with 100 μM NAC for 1 h followed by vehicle (DMSO) or PCB77 (5 μM) treatment. PCB77-induced MCP-1 mRNA levels were blocked by NAC pre-treatment (Fig. 4B).

MCP-1 up-regulation is mediated by p38 and JNK MAPKs

Mitogen activated protein kinases (MAPKs) play a role in cellular responses to environmental stress, including endothelial activation. SB203580 (SB), a selective p38 kinase inhibitor, was used to examine the role of p38 kinase in MCP-1 expression. Pre-treatment for 1 h with 5 μM SB blocked PCB77-induced MCP-1 mRNA expression (Fig. 5A). Similarly, SP600125, a selective inhibitor of c-Jun K-terminal kinase (JNK) pretreatment (1 h, 20 μM) prevented PCB77-induced MCP-1 mRNA expression (Fig. 5B). In contrast, the ERK1/2 inhibitor, PD98029, at concentrations ranging from 1–20 μM had no effect on PCB77-induced over-expression of MCP-1 (data not shown).

Caveolin-1 silencing prevents MCP-1 up-regulation by PCB77in vitro and in vivo

To investigate the role of caveolae in MCP-1 induction by PCB77, caveolin-1 was silenced using the siRNA technique. Cavelin-1 is the major structural protein of caveolae that is required for caveolae formation in endothelial cells (Drab *et al*., 2001). PCB77 induction of MCP-1 mRNA was abolished in the cells lacking caveolin-1, suggesting that intact caveolae are required for this response (Fig. 6A). To confirm these findings *in vivo*, atherosclerosis-prone LDL-$R^{-/-}$ mice were compared to LDL-$R^{-/-}$ Cav-$1^{-/-}$ mice. PCB77 treatment increased both aortic mRNA expression levels of MCP-1 in LDL-$R^{-/-}$ mice, as measured by real time PCR, and plasma protein levels of MCP-1 protein, assessed by LincoPLEX kit. However, no MCP-1 induction was detected in LDL-$R^{-/-}$ Cav-$1^{-/-}$ mice (Figs. 6B and 6C). Taken together, caveolin-1 is required for MCP-1 up-regulation by PCB77 both *in vitro* and *in vivo*. In addition to MCP-1, interleukin-6 (IL-6) also is an important mediator of acute phase response and risk factor for cardiovascular disease (Yudkin *et al*., 2000; Song and Schindler, 2004). Upregulation of aortic IL-6 mRNA (Fig. 7A) and plasma protein (Fig. 7B) levels by PCB77 followed a similar pattern as observed with MCP-1. These data suggest that caveolin-1 is a common regulator of PCB77-induced vascular inflammatory response.

Discussion

Persistent organic pollutants (Ha *et al*., 2007) and in particular PCBs (Gustavsson and Hogstedt, 1997), were associated with an increased risk of cardiovascular disease. In this study, we have documented for the first time that coplanar PCBs, such as PCB77, can increase transcription and secretion of MCP-1 by endothelial cells. This is likely an important step in vascular inflammatory events involved in the promotion of atherosclerosis development by coplanar

PCBs (Arsenescu *et al.*, 2008). For example, MCP-1-mediated recruitment of monocytes is a critical event in atherosclerotic lesion formation (Boring *et al.*, 1998; Gu *et al.*, 1998).

There is evidence that the aryl hydrocarbon receptor (AhR) may play a critical regulatory role in the induction of proatherogenic inflammatory markers. For example, MCP-1 was previously reported to be induced in various tissues by other environmental toxicants, such benzo[a]pyrene (B[a]P) (Knaapen *et al.*, 2007), or 2,3,7,8-tetrachlorodibenzo-para-dioxin (TCDD) (Vogel *et al.*, 2007). AhR is a nuclear receptor that, after a ligand binding, translocates into the nucleus and initiates transcription through dioxin responsive elements (DREs) in regulatory regions of AhR-responsive genes. The majority of toxic effects of coplanar PCBs are initiated by their binding to AhR (Safe *et al.*, 1985). Our data demonstrate that both coplanar PCB77 and PCB126, as well as TCDD, induce MCP-1 mRNA expression levels, suggesting AhR to be a common mediator of this pathway. In the present study, we used the AhR antagonist α-naphthoflavone (α-NF) (Merchant *et al.*, 1993) to test the hypothesis that MCP-1 up-regulation by coplanar PCB77 is AhR-dependent, and we were able to abolish MCP-1 up-regulation by α-NF pre-treatment. We have previously demonstrated that coplanar PCBs trigger AhR-mediated up-regulation of cytochrome P450 (CYP1A1) levels and activity in endothelial cells (Ramadass *et al.*, 2003). PCB-induced up-regulation of CYP1A1 can cause overproduction of reactive oxygen species and activation of oxidative stress-sensitive transcription factors such as nuclear factor-κB (NF-κB) (Hennig *et al.*, 2002), a possible prerequisite for MCP-1 induction. Indeed, pre-treatment with the glutathione precursor and anti-oxidant N-acetyl cysteine (NAC) prevented MCP-1 induction, thus supporting a role of oxidative stress in this process.

Mitogen-activated protein kinases (MAPKs) mediate cellular responses to various environmental stimuli and are important signaling components upstream of transcription factors that regulate an inflammatory response. There are three best characterized MAPKs sub-families, extracellular signal-regulated kinases (ERK), c-Jun N-terminal kinases (JNK), and p38 kinases (Johnson and Lapadat, 2002), and all of them can be regulated by oxidative stress (McCubrey *et al.*, 2006).

JNK and p38 in particular seem to be important mediators of pro-atherogenic events (Hoefen and Berk, 2002; Zakkar *et al.*, 2008) (Zakkar *et al.*, 2008), and p38 activation can lead to endothelial MCP-1 up-regulation (Sung *et al.*, 2001; Liu *et al.*, 2008). Our data demonstrated that MCP-1 up-regulation by PCB77 could be diminished by both p38 and JNK inhibition.

We have evidence that caveolae may provide a critical signaling platform in the regulation of PCB-induced endothelial cell dysfunction and that caveolin-1 can be a biomarker of PCB toxicity (Lim 2008). Caveolae are membrane domains enriched in cholesterol and sphingolipids, and the major structural proteins caveolins (Thomas and Smart, 2008). Caveolae are highly abundant in endothelial cells (Frank *et al.*, 2003) where they are proposed to serve as platforms to compartmentalize and selectively modulate cell signaling events. Caveolin-1, a 22 kDa protein (Rothberg *et al.*, 1992) is required for caveolae formation in endothelial cells (Drab *et al.*, 2001), and mice which lack caveolin-1 demonstrate increased severity of atherosclerosis (Frank *et al.*, 2004). In the current study we used caveolin-1 siRNA to test the hypothesis that functional caveolae facilitate MCP-1 up-regulation by PCB77. Indeed, we found that in the absence of caveolin-1, MCP-1 up-regulation was diminished. These results were confirmed *in vivo* following PCB77 treatment of mice lacking the caveolin-1 gene. LDL-R deficient mice were used as a background control in this study as a model of atherosclerosis because their elevated LDL fraction resembles the lipoprotein profile of hypercholesterolemic humans (Daugherty, 2002). We found that PCB77 increased aortic mRNA expression of both MCP-1 and IL-6 in control LDL-$R^{-/-}$ mice, which was accompanied by increased plasma MCP-1 and IL-6 protein levels. In contrast, caveolin-$1^{-/-}$ LDL-$R^{-/-}$ mice were resistant to

PCB77-induced MCP-1 and IL-6 up-regulation, suggesting the physiological importance of caveolae in PCB-induced inflammation and atherosclerosis.

Our recent study showed that AhR binds caveolin-1 in endothelial cells and that deletion of the caveolin-1 gene partially decreased CYP1A1 induction by coplanar PCBs (Lim *et al.*, 2008). We also demonstrated that the production of reactive oxygen species induced by PCB77 was diminished in the absence of caveolin-1 (Lim 2008). Since AhR activation and oxidative stress were required for MCP-1 up-regulation, our data suggest that PCB77-mediated AhR binding and activation and subsequent up-regulation of MCB-1 requires functional caveolae. In a related study we also found that caveolin-1 was required for ICAM-1 up-regulation by B [a]P in endothelial cells and that this effect was also mediated by p38 activation (Oesterling *et al.*, 2008). In addition, caveolin-1 was demonstrated to bind p38 in endothelial cells and to facilitate its phosphorylation and activation of downstream targets (Siddiqui *et al.*, 2007). These data all suggest that regulation of inflammatory pathways through caveolae might be a mechanism shared by environmental toxicants that are AhR agonists.

In conclusion, we have demonstrated that coplanar PCB77 can up-regulate endothelial levels of MCP-1, a critical regulator of early stages of atherosclerosis. This process is regulated by caveolin-1 and caveolae-associated signaling pathways, including induction of AhR and reactive oxygen species, as well as p38 and JNK MAP kinases. It appears that functional caveolae are important for endothelial cell dysfunction, and caveolin-1 may emerge as a critical biomarker of cardiovascular toxicity by environmental contaminants.

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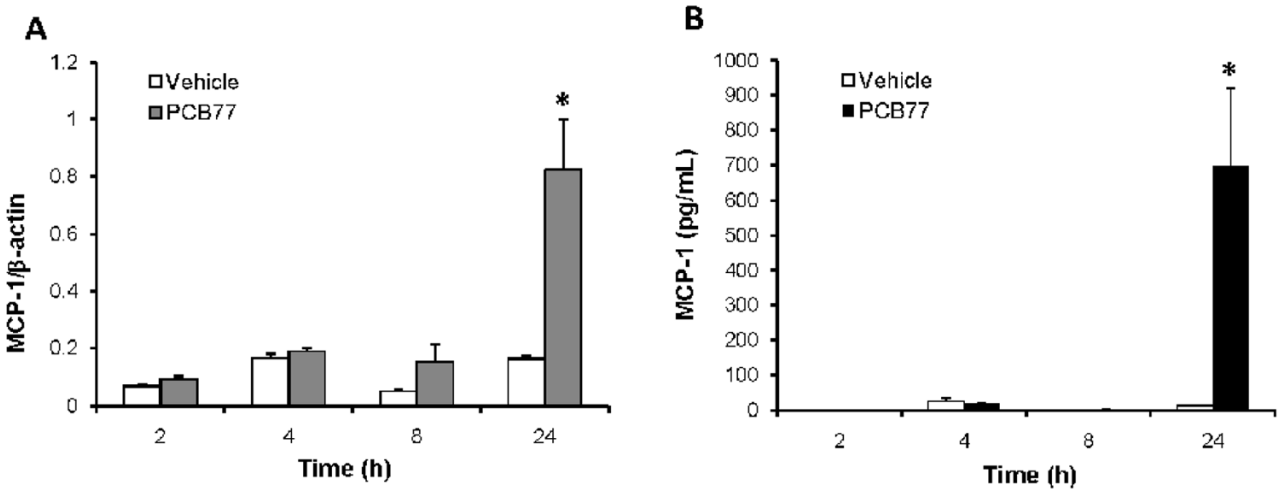

Figure 1. PCB77 up-regulates MCP-1 in a time-dependent manner

Cells were treated with vehicle (DMSO) or PCB77 (5 μM) for indicated time periods. MCP-1 mRNA expression levels (A) were measure using Real-time PCR, and MCP-1 protein levels in culture media (B) were measured using a human MCP-1 OptiEIA ELISA Kit. Results represent mean ± SEM of 4 experiments. *Significantly different compared to vehicle control ($p<0.05$).

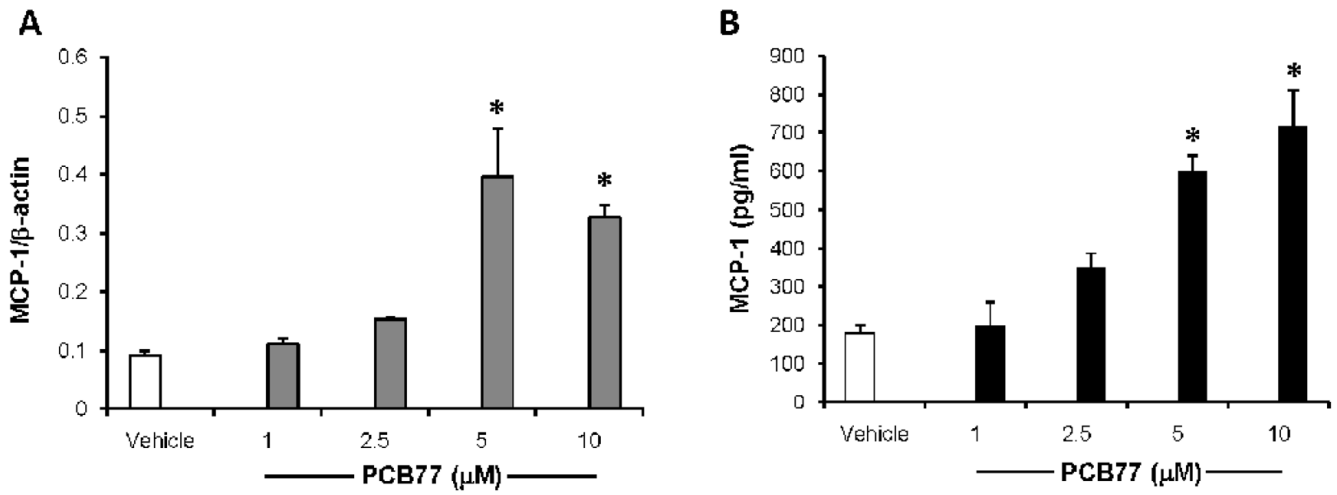

Figure 2. PCB77 up-regulates MCP-1 in a concentration-dependent manner

Cells were treated with vehicle (DMSO) or increasing concentrations of PCB77 for 24 h. MCP-1 mRNA expression levels (A) were measure using Real-time PCR, and MCP-1 protein levels in culture media (B) were measured using the MCP-1 OptiEIA ELISA Kit. Results represent mean ± SEM of 4 experiments.

*Significantly different compared to vehicle control ($p<0.05$).

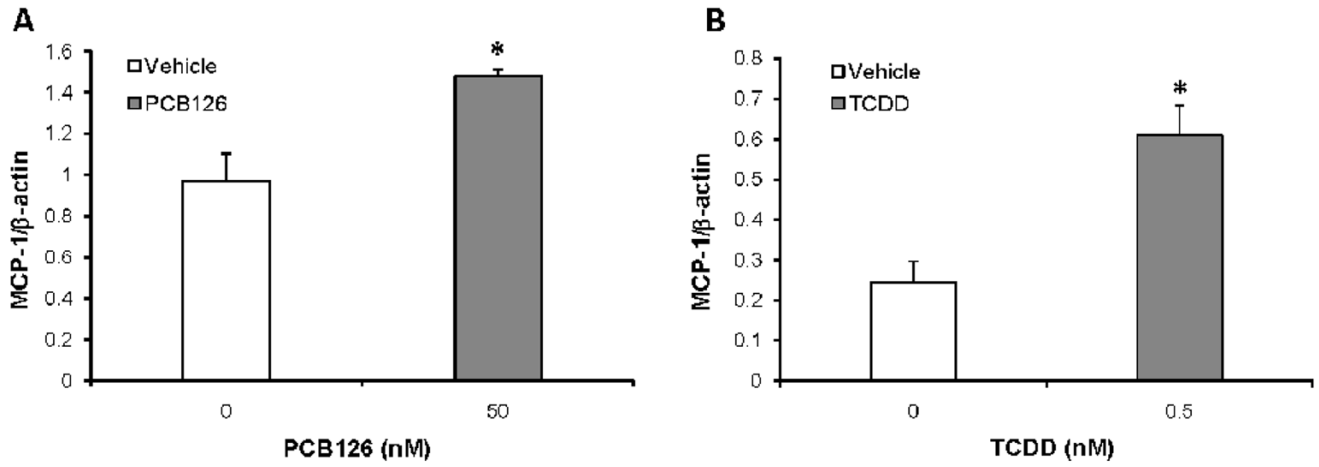

Figure 3. PCB126 and TCDD (2,3,7,8-tetrachlorodibenzo-p-dioxin) induce MCP-1 mRNA expression

Cells were treated with vehicle (DMSO) or PCB126 (50 nM) (A), or TCDD (0.5 nM) (B) for 24 h. MCP-1 mRNA expression levels were measured using Real-time PCR. Results represent mean ± SEM of 3 experiments. *Significantly different compared to vehicle control ($p<0.05$).

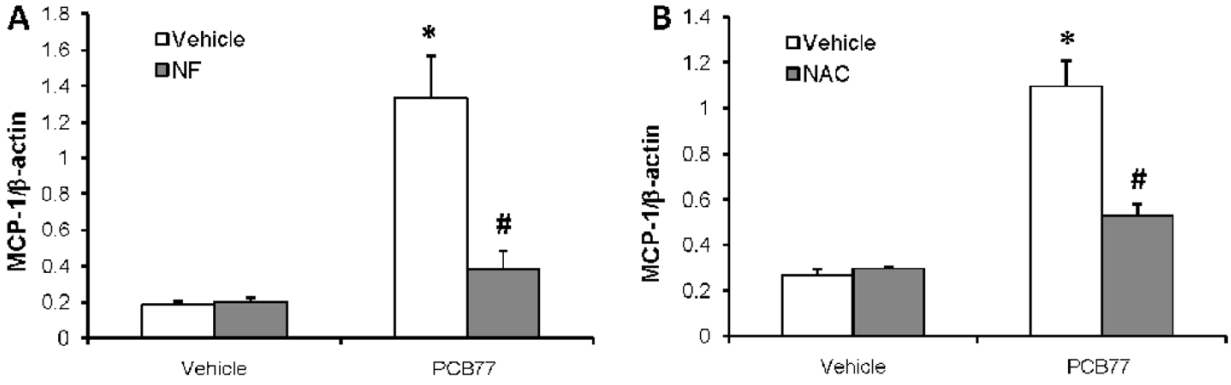

Figure 4. Endothelial MCP-1 up-regulation by PCB77 is AhR- and reactive oxygen species-dependent

Cells were pre-treated with the AhR antagonist α-naphthoflavone (NF, 0.01 μM) (A) or the glutathione precursor N-acetyl cysteine (NAC, 0.1 mM) (B) for 1 h followed by vehicle (DMSO) or PCB77 (5 μM) treatment for 24 h. MCP-1 mRNA expression levels were measured using Real-time PCR. Results represent mean ± SEM of 4 experiments. *Significantly different compared to vehicle control ($p<0.05$). #Significantly different compared to PCB77-treated control without NF (A) or without NAC (B) ($p<0.05$).

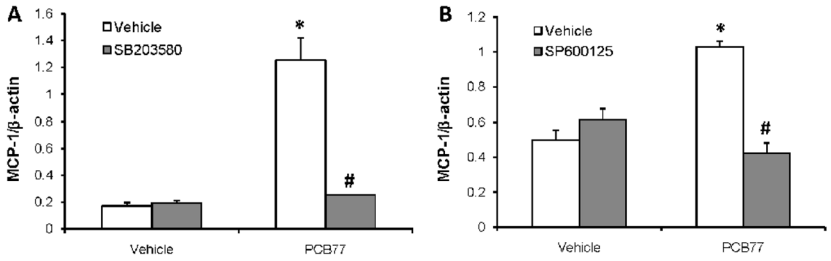

Figure 5. MCP-1 up-regulation by PCB77 is regulated by p38 and JNK

Cells were pre-treated with p38 (SB203580, 5 μM) (A) or JNK (SP600125, 20 μM) (B) inhibitors, respectively, for 30 min followed by vehicle (DMSO) or PCB77 (5 μM) treatment for 24 h. MCP-1 mRNA expression levels were measured using Real-time PCR. Results represent mean ± SEM of 4 experiments. *Significantly different compared to vehicle control ($p<0.05$). #Significantly different compared to PCB77-treated control without inhibitors ($p<0.05$).

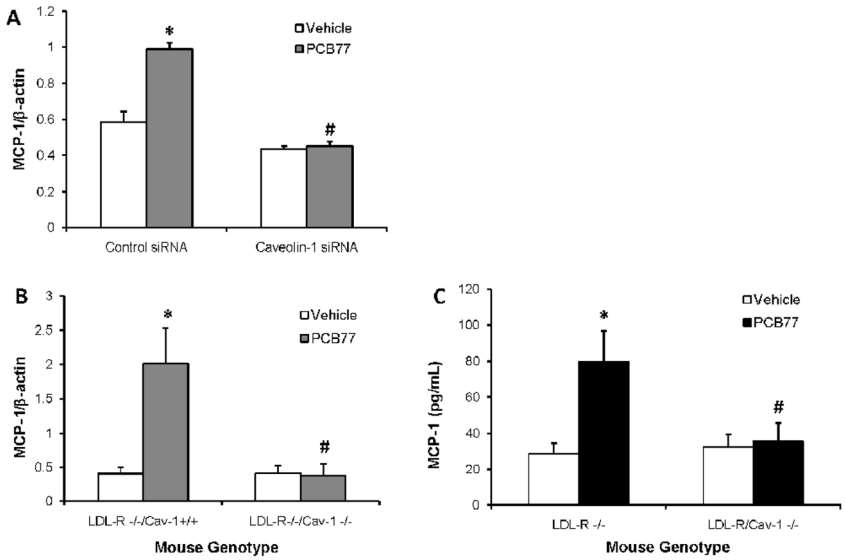

Figure 6. Caveolin-1 deficiency prevents MCP-1 up-regulation by PCB77 in endothelial cells and mouse vasculature

Cells were transfected with control or caveolin-1 siRNAs for 48 hours and treated with vehicle (DMSO) or PCB77 (5 μM) for 24 h. MCP-1 mRNA expression levels were measured using Real-time PCR with SYBR Green chemistry (A). LDL-$R^{-/-}$/Cav-$l^{+/+}$ (control) and LDL-$R^{-/-}$/Cav-$1^{-/-}$ (caveolin-1 deficient) mice were treated with vehicle control (olive oil) or PCB77 (170 μmole/kg). Aortic mRNA was isolated and MCP-1 mRNA expression levels were measured using Real-time PCR with TaqMan chemistry (B). Plasma samples were analyzed for MCP-1 levels using mouse adipokine LINCOplex kit (C). Results represent the mean ± SEM of 6 experiments/animals. *Significantly different compared to vehicle control ($p<0.05$). #Significantly different compared to PCB77-treated control, i.e., siRNA cell or LDL-$R^{-/-}$/Cav-$l^{+/+}$ mice ($p<0.05$).

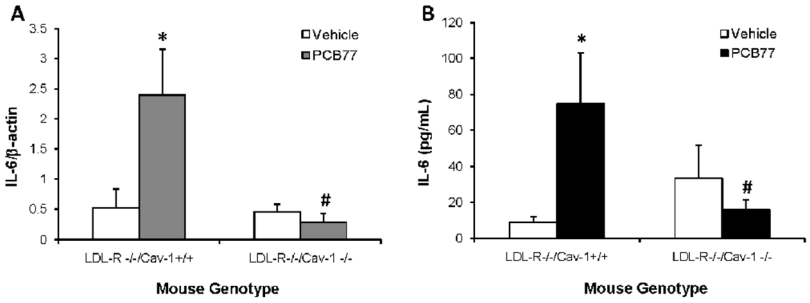

Figure 7. Caveolin-1 deficiency prevents IL-6 up-regulation by PCB77 in mouse vasculature

LDL-$R^{-/-}$/Cav-$1^{+/+}$ (control) and LDL-$R^{-/-}$/Cav-$l^{-/-}$ (caveolin-1 deficient) mice were treated with vehicle control (olive oil) or PCB77 (170 μmole/kg). Aortic mRNA was isolated and IL-6 mRNA expression levels were measured using Real-time PCR with TaqMan chemistry (A). Plasma samples were analyzed for IL-6 levels using mouse adipokine LINCOplex kit (B). Results represent the mean ± SEM of 6 animals. *Significantly different compared to vehicle control ($p<0.05$). #Significantly different compared to PCB77-treated LDL-$R^{-/-}$/Cav-$l^{+/+}$ mice ($p<0.05$).