

Caveolin-1 mediates up-regulation of pro-inflammatory cytokines by 3,4,3',4'-tetrachlorobiphenyl (PCB77)

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ABSTRACT

Polychlorinated biphenyls (PCBs) are persistent organic pollutants that are ubiquitous in the environment. They can contribute to the development of atherosclerosis by endothelial activation, a critical first step in the development of the atherosclerotic plaque. PCBs in plasma are carried mainly by albumin and lipoprotein particles that interact with endothelial cells through their receptors in caveolae. Our recent data suggest that caveolae, membrane microdomains that regulate multiple signaling pathways in particular in endothelial cells, play a role in coplanar PCB77 toxicity. Endothelium-derived monocyte chemoattractant protein-1 (MCP-1) is a key activator of monocyte adhesion to activated endothelium, whereas interleukin-6 (IL-6) is an inflammatory mediator associated with increased risk of cardiovascular disease. The hypothesis that lack of caveolin-1, the major structural protein of caveolae can prevent up-regulation of these cytokines in endothelial cells was tested. PCB77 up-regulated both MCP-1 and IL-6 expression in primary porcine endothelial cells, however MCP-1 up-regulation was more pronounced. Subsequently, treatment with PCB77 induced gene expression of MCP-1 in primary mouse aortic endothelial cells and this was prevented in cells isolated from caveolin-1 *-/-* mice. The hypothesis that caveolae are mediators of PCB77 cardiovascular toxicity was also tested *in vivo*. PCB77 induced gene expression of inflammatory MCP-1 and IL-6 in the aortic tissue of control, but not caveolin-1 *-/-* mice. As a result of this up-regulation, plasma levels of both MCP-1 and IL-6 were up-regulated by PCB77 in control, but not caveolin-1 *-/-* mice. In conclusion, these data show that induction of pro-inflammatory cytokines IL-6 and MCP-1 by coplanar PCBs in vasculature is regulated through caveolae and can be prevented in absence of caveolin-1. This suggests that caveolin-1 might be an important target for preventing PCB toxicity in exposed human populations.

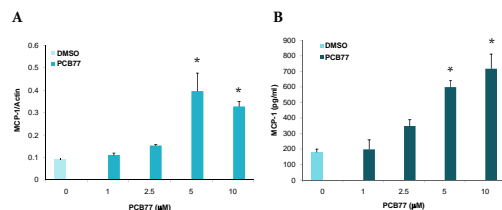
INTRODUCTION

Exposure of vascular endothelium to persistent organic pollutants, such as PCBs, leads to activation of pro-inflammatory signaling pathways (Hennig et al., 2002). This causes increased adhesion and extravasation of circulation monocytes and initiation of an early atherosclerotic lesion. Endothelial cells are particularly rich in membrane microdomains called caveolae and their major structural protein, caveolin-1. Caveolae have a specific lipid composition and our recent studies have shown that PCBs after *in vitro* exposure accumulate mainly in caveolae. Caveolae also play a role in transduction and regulation of many pro-inflammatory signaling pathways. Caveolin-1 knockout mice have been shown to be protected against atherosclerosis (Frank et al., 2004). MCP-1 and IL-6 are inflammatory mediators produced by vascular endothelium and, as shown in this study, can be up-regulated by coplanar PCB77. Whereas IL-6 is an important predictor of human atherosclerosis, MCP-1 is a key regulator of monocyte recruitment produced by endothelial cells and MCP-1-deficient models are protected from atherosclerosis. As many of the pathways involved in MCP-1 and IL-6 up-regulation are associated with caveolae, the hypothesis that caveolae are involved in the up-regulation of MCP-1 and IL-6 by PCB77 was tested in this study.

IN VITRO

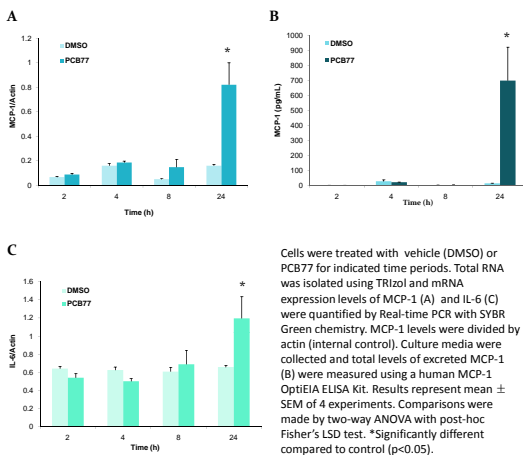
Primary porcine endothelial cells (PEC) were isolated from porcine pulmonary arteries as described previously (Toborek et al., 2002) and cultured in M199 medium enriched with 10% fetal bovine serum (FBS). Endothelial cells were grown until confluent, and synchronized by being maintained overnight in M199 containing 1% FBS, before treatment with either vehicle (DMSO) or PCB77.

PCB77 Up-regulates MCP-1 Levels in Concentration-dependent Manner in Endothelial Cells



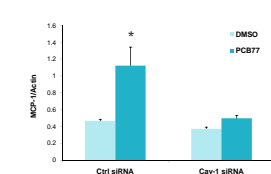
Cells were treated with vehicle (DMSO) or increasing concentrations of PCB77 for 24 h. Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA) and mRNA expression levels (A) were quantified by Real-time PCR with SYBR Green chemistry. MCP-1 levels were divided by actin (internal control). Culture media were collected and total levels of excreted MCP-1 (B) were measured using a human MCP-1 OptiEIA ELISA Kit (BD Pharmingen, Los Angeles, CA). Results represent mean \pm SEM of 4 experiments. Comparisons were made by one-way ANOVA with post-hoc Fisher's LSD test. *Significantly different compared to control (p<0.05).

PCB77 Up-regulates MCP-1 and IL-6 in Time-dependent Manner in Endothelial Cells



Cells were treated with vehicle (DMSO) or PCB77 for indicated time periods. Total RNA was isolated using TRIzol and mRNA expression levels of MCP-1 (A) and IL-6 (C) were quantified by Real-time PCR with SYBR Green chemistry. MCP-1 levels were divided by actin (internal control). Culture media were collected and total levels of excreted MCP-1 (B) were measured using a human MCP-1 OptiEIA ELISA Kit. Results represent mean \pm SEM of 4 experiments. Comparisons were made by two-way ANOVA with post-hoc Fisher's LSD test. *Significantly different compared to control (p<0.05).

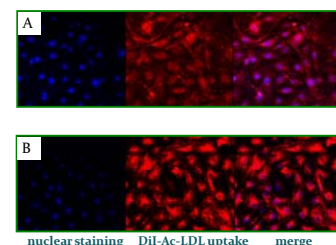
Caveolin-1 Silencing Prevents MCP-1 Up-regulation by PCB77 in PEC



Caveolin-1 was silenced using siRNA as described previously (Lim et al., 2007). Cells were treated with vehicle (DMSO) or PCB77. Total RNA was isolated using TRIzol and mRNA expression levels were quantified by Real-time PCR with SYBR Green chemistry. MCP-1 levels were divided by actin (internal control). Results represent mean \pm SEM of 6 experiments. Comparisons were made by two-way ANOVA with post-hoc Fisher's LSD test. *Significantly different compared to control (p<0.05).

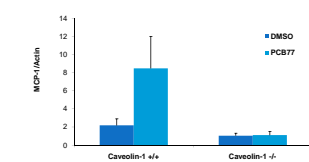
Primary mouse aortic endothelial cells (MAEC) were isolated and cultured as described previously (Srinivasan et al., 2004). The cells were grown in DMEM medium enriched with 20% FBS until confluent, then synchronized by being maintained overnight in DMEM containing 1% FBS, and treated with either vehicle (DMSO) or PCB77.

MAEC are Positive for DiI-Ac-LDL Uptake



MAECs (A) and PECs (B) stained for DiI acetylated low-density lipoprotein (DiI-Ac-LDL, Texas Red fluorescence) uptake after 4 h, followed by nuclear staining (DAPI blue fluorescence) using a laser scanning confocal microscope.

PCB77-induced MCP-1 mRNA Up-regulation Is Reduced in MAEC from Caveolin-1 *-/-* Mice

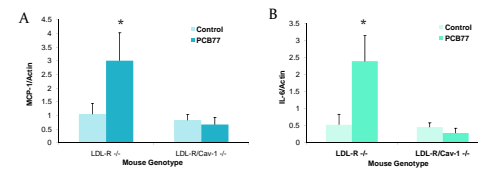


MAEC were treated with vehicle (DMSO) or PCB77. Total RNA was isolated using TRIzol and mRNA expression levels were quantified by Real-time PCR with SYBR Green chemistry. MCP-1 levels were divided by actin (internal control). Results represent mean \pm SEM of 4 experiments.

IN VIVO

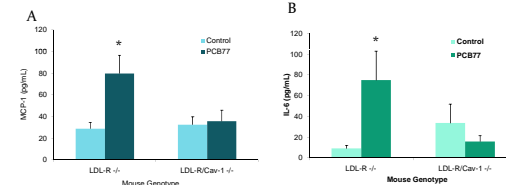
Low density lipoprotein receptor null (LDL-R^{-/-}) and caveolin-1 null (Cav-1^{-/-}) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were bred at the University of Kentucky to generate LDL-R^{-/-}/caveolin-1 double null mice (LDL-R^{-/-}/Cav-1^{-/-} mice). At 8 weeks of age, mice from each genotype were placed on a standardized diet containing 20% calories from fat (Dyets Inc., Bethleham, PA). After 2 weeks, mice were injected intraperitoneally with PCB77 or vehicle (olive oil) and then 6 days later they were injected again. 24 h after the last treatment, the tissue samples were collected for further analysis.

PCB77 Up-regulates Aortic mRNA Levels of Pro-inflammatory Cytokines *In Vivo* in Caveolin-1-dependent Manner



Aortas were cleaned of peri-adventitial tissue and stored in RNalater (Qiagen, Valencia CA). Total RNA was isolated using the RNeasy kit (Qiagen). mRNA expression levels were measured using Real-time PCR with TaqMan chemistry. IL-6 (A) and MCP-1 (B) levels were divided by actin (internal control). Results represent mean \pm SEM of 6 animals. Comparisons were made by two-way ANOVA with post-hoc Fisher's LSD test. *A significant difference compared with control (vehicle) mice (p<0.05).

PCB77 Up-regulates Plasma Protein Levels of Pro-inflammatory Cytokines *In Vivo* in Caveolin-1-dependent Manner



Plasma samples were analyzed for MCP-1 (A) and IL-6 (B) levels using mouse adipokine LINCplex kit (Linco Research, St. Charles, MO) according to the manufacturer's instructions. Results represent mean \pm SEM of 6 animals. Comparisons were made by two-way ANOVA with post-hoc Fisher's LSD test. *A significant difference compared with control (vehicle) mice (p<0.05).

CONCLUSIONS

- PCB77 up-regulates endothelial expression of MCP-1 and IL-6 in caveolin-1-dependent manner *in vitro*
- PCB77 up-regulates aortic mRNA expression and plasma levels of MCP-1 and IL-6 in caveolin-1-dependent manner *in vivo*

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