

# Caveolae mediate pro-inflammatory properties of coplanar polychlorinated biphenyls

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## ABSTRACT

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent organic environmental pollutants that have been implicated in development of cardiovascular pathologies, such as atherosclerosis. Caveolae are invaginated membrane microdomains with distinct lipid and protein composition that regulate multiple signaling pathways involved in atherosclerosis, in particular in endothelial cells (ECs). We hypothesized that caveolae play an important role in endothelial activation by PCBs. Primary endothelial cells exposed to coplanar PCBs (PCB77 and PCB126), but not to non-coplanar PCB153, had increased expression of caveolin-1, a major structural component of caveolae. This caveolin-1 up-regulation resulted in increased membrane caveolae formation demonstrated by transmission electron microscopy. PCBs in plasma associate mainly with albumin and lipoproteins. The receptors for these carriers are located in caveolae, therefore we can expect the caveolae compartment to mediate the interaction between PCBs and their molecular targets. After treatment, PCB77 accumulated mainly in the caveolae-rich fraction, as determined by gas chromatograph-mass spectrometry. The hypothesis that caveolae are required for coplanar PCB toxicity was tested in caveolin-1-deficient mice. In liver of caveolin-1<sup>-/-</sup> mice, a significant decrease in PCB77-induced CYP1A1 and CYP1B1 expression was observed compared to control mice. Furthermore, PCB77 induced expression of the pro-inflammatory cytokines interleukin-6 and monocyte chemoattractant protein-1 in the aortas, but this up-regulation was diminished in caveolin-1<sup>-/-</sup> mice, further supporting the critical role of caveolae in the PCB toxicity. These and other findings showing that lack of caveolae prevents coplanar PCB toxicity might be important for nutritional recommendations in PCB-exposed populations.

## RESULTS

### Hypothesis 1: Caveolae mediate cellular PCB77 uptake

Endothelial cells (ECs) were isolated from porcine pulmonary arteries 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 accumulates in caveolae fraction of endothelial cells

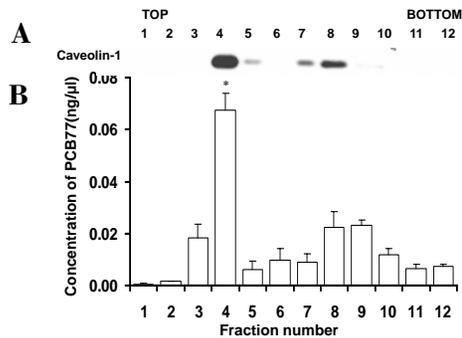


Fig. 1. Treatment of cell lysates with mild detergent and subsequent sucrose gradient centrifugation yielded 12 fractions, with fraction 4 (caveolae fraction) highly enriched in caveolin-1 protein, measured by Western blot (A). PCB77 concentrations in each fraction were quantified by GC-MS (B). The means  $\pm$  SEM of PCB77 concentrations for 3 different experiments are shown as ng/ $\mu$ l per fraction. Comparisons were made by one-way ANOVA with post-hoc Fisher's LSD test. \* A significant difference compared with all other cell fractions ( $p < 0.05$ ).

### Hypothesis 2: Coplanar PCBs increase caveolae formation in endothelial cells

#### Coplanar PCBs induce caveolin-1 expression

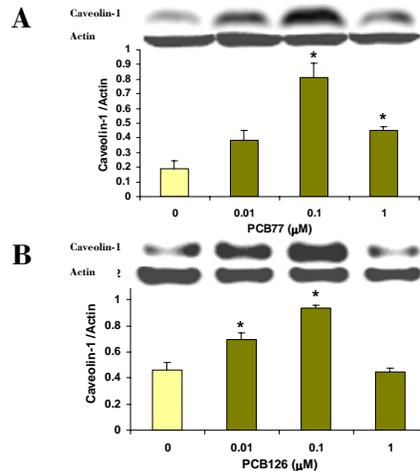


Fig. 2. Endothelial cells were incubated for 8 h with vehicle (DMSO) or increasing concentrations of PCB77 (A) or PCB126 (B). Total cell proteins were resolved using SDS-PAGE, and caveolin-1 and actin protein levels were detected by Western blot. Densitometry results represent mean  $\pm$  SEM of three independent experiments. Comparisons were made by one-way ANOVA with post-hoc Fisher's LSD test. \*Significantly difference compared with control cultures ( $p < 0.05$ ).

### PCB77 induces caveolae formation in endothelial cells

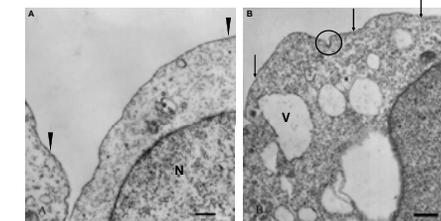


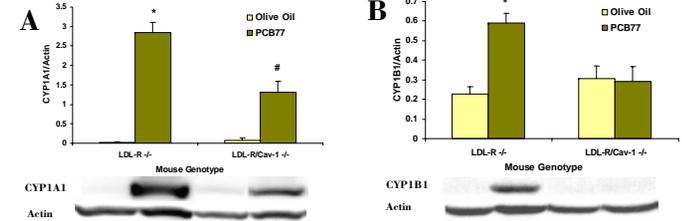
Fig. 3. Endothelial cells were treated for 6 h with vehicle control (A) or PCB77 (2.5  $\mu$ M) (B), and cells were fixed for TEM analysis. Subsequently, plasma membranes of numerous cells were scanned for caveolae (defined as uniform 50 to 100 nm flask-shaped membrane invaginations). DMSO (vehicle) treatment did not impart cytological changes or cause an accumulation of endocytic vesicles (A). After PCB exposure (B), cells exhibited an increase in caveolae formation (arrows). Some showed aggregation and coalescing and inward migration (circle). Cell interior also displayed vacuoles of varying sizes. Scale bar = 1  $\mu$ m; arrowheads = endocytic vesicles; arrows = caveolae; circle = caveolae opening; V = vacuole, N = nucleus.

### Hypothesis 3: Caveolin-1 regulates pro-inflammatory properties of PCB77

Low density lipoprotein receptor null (LDL-R<sup>-/-</sup>) and caveolin-1 null (Cav-1<sup>-/-</sup>) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Mice were bred at the University of Kentucky to generate LDL-R<sup>-/-</sup>/Cav-1<sup>-/-</sup> mice. At 8 weeks of age, mice from each genotype 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  $\mu$ mol/kg body weight) or vehicle (olive oil) and then 6 days later they were injected again. 24 h after the last treatment, liver and aorta tissue samples were obtained and frozen in liquid nitrogen for further analysis.

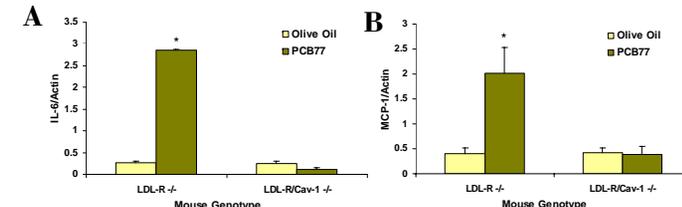
### Up-regulation of cytochrome P450s (CYPs) by PCB77 in mouse liver is reduced in LDL-R<sup>-/-</sup> mice lacking the caveolin-1 gene

Fig. 4. Liver proteins were extracted and levels of CYP1A1 (A) and CYP1B1 (B) were assessed using Western blot and divided by the values for actin (loading control). Densitometry results represent mean  $\pm$  SEM of 6 animals. Comparisons were made by one-way ANOVA with post-hoc Fisher's LSD test. \* A significant difference compared with control (vehicle) mice ( $p < 0.05$ ). # A significant difference compared with PCB77-treated LDL-R<sup>-/-</sup> mice ( $p < 0.05$ ).



### Up-regulation of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) mRNA in mouse aorta by PCB77 is reduced in LDL-R<sup>-/-</sup> mice lacking the caveolin-1 gene.

Fig. 5. Aortic tissue was collected in RNAlater and total RNA was isolated using the RNeasy kit (Qiagen, Valencia CA). 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 one-way ANOVA with post-hoc Fisher's LSD test. \* A significant difference compared with control (vehicle) mice ( $p < 0.05$ ).



## CONCLUSIONS

- Caveolae serve as a platform for interaction with PCB77 and its uptake in vascular endothelial cells.
- Coplanar PCBs, such as PCB77, can increase caveolin-1 levels and caveolae formation in endothelial cells.
- Lack of caveolin-1 gene (and caveolae) *in vivo* results in a decreased response to PCB77 toxicity, i.e., both up-regulation of liver metabolizing enzymes and cytokine production in the vasculature were reduced.

\*Certain diet-derived compounds can down-regulate caveolae; thus, findings demonstrating that lack of caveolae prevents coplanar PCB toxicity might be important for nutritional recommendations in PCB-exposed populations.

## ACKNOWLEDGEMENTS

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