

Endothelial cell caveolae are involved in activation of aryl hydrocarbon receptor pathway by coplanar polychlorinated biphenyls



Majkova, Zuzana¹; Lim, Eun Jin²; Smart, Eric³; Tseng, Michael T.⁴; Toborek, Michal⁵; Hennig, Bernhard²

¹Graduate Center for Toxicology, ²Molecular and Cell Nutrition Laboratory, College of Agriculture, Departments of ³Pediatrics and ⁵Neurosurgery, University of Kentucky, Lexington, KY 40536, USA, ⁴Department of Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY 40202, USA

ABSTRACT

Polychlorinated biphenyls (PCBs) are ubiquitous and persistent environmental pollutants. Epidemiological and experimental data have suggested that PCBs play a significant role in development of cardiovascular pathologies, such as atherosclerosis. Caveolae are membrane microdomains that mediate endocytosis and regulate multiple signaling pathways involved in atherosclerosis, in particular in endothelial cells. We hypothesized that caveolae play an important role in endothelial activation by coplanar PCBs. Primary endothelial cells exposed to PCB77 had increased expression of caveolin-1, a major structural component of caveolae. This caveolin-1 up-regulation resulted in enrichment of caveolin-1 in the caveolae fraction collected by sucrose gradient isolation, as well as increased membrane caveolae formation demonstrated by transmission electron microscopy. Next we evaluated whether functional caveolae are required for PCB77 toxicity mediated by activation of the aryl hydrocarbon receptor (AhR) and up-regulation of downstream genes such as cytochrome P450 1A1 (CYP1A1). AhR was found to co-immunoprecipitate with caveolin-1, suggesting that this scaffolding protein plays a role in regulation of activation of AhR and associated genes. Compared to control cells, PCB77 failed to induce CYP1A1 expression in endothelial cells after caveolin-1 was silenced by siRNA. Similarly, in liver of caveolin-1 null mice a significant decrease in PCB77-induced CYP1A1 expression was observed compared to control mice. In conclusion, these data demonstrate that coplanar PCB77 induces caveolae formation and that the presence of functional caveolae is required for activation of the AhR pathway in endothelial cells.

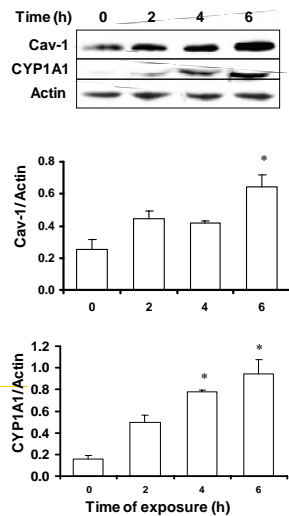
RESULTS

PCB77 induces caveolin-1 and CYP1A1 protein expression in endothelial cells.

Cell culture:

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, before treatment with either vehicle (DMSO) or PCB77.

Fig. 1. Cells were incubated with PCB77 (2.5 μM) or control (vehicle) for the times indicated. Total cell proteins were resolved using SDS-PAGE, and caveolin-1 (Cav-1), CYP1A1 and actin protein levels were detected by Western blot. Densitometry results represent mean ± SEM of three independent experiments. * A significant difference compared with control (p<0.05).



PCB77 induces caveolae formation in endothelial cells.

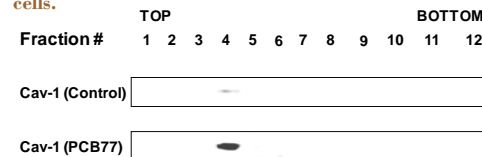


Fig. 2. Cells were treated with control (vehicle) or PCB77. Cell fractions were obtained by sucrose gradient centrifugation and analyzed for caveolin-1 (Cav-1) levels by Western blot. Fraction 4 represents the caveolae-rich fraction as described previously (Song et al., J Biol Chem. 1996 Apr 19;271(16):9690-7).

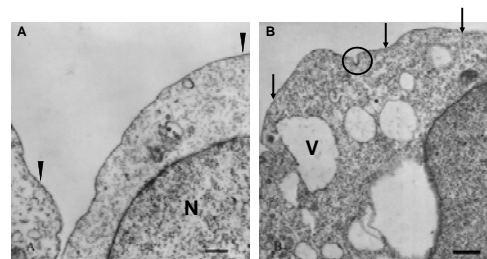


Fig. 3. Endothelial cells were treated with control (vehicle) (A) or PCB77 (B), and cells were fixed for analysis by transmission electron microscopy. Subsequently, plasma membranes of numerous cells were scanned for caveolae (defined as uniform 50 to 100 nm flask-shaped membrane invaginations). DMSO (vehicle) treatment did not impact 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 of caveolae. Cell interior also displayed vacuoles of varying sizes. Scale bar = 1 μm; arrowheads = endocytic vesicles; arrows = caveolae; circle = caveolae opening; V = vacuole; N = nucleus.

CONCLUSIONS

- Coplanar PCBs, such as PCB77, can increase caveolin-1 levels and caveolae formation in endothelial cells.
- Caveolin-1 interaction with AhR is increased after PCB77 treatment.
- Lack of caveolin-1 gene (and caveolae) *in vitro* and *in vivo* results in a decreased induction of AhR-mediated responses, such as induction of cytochrome P450 1A1 protein levels.

PCB77 induces AhR binding to caveolin-1 in endothelial cells.

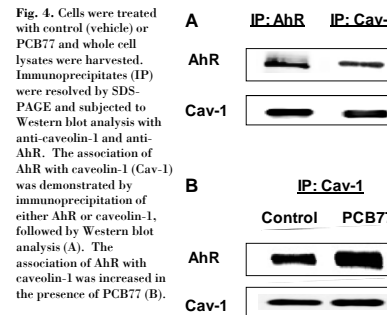


Fig. 4. Cells were treated with control (vehicle) or PCB77 and whole cell lysates were harvested. Immunoprecipitates (IP) were resolved by SDS-PAGE and subjected to Western blot analysis with anti-caveolin-1 and anti-AhR. The association of AhR with caveolin-1 (Cav-1) was demonstrated by immunoprecipitation of either AhR or caveolin-1, followed by Western blot analysis (A). The association of AhR with caveolin-1 was increased in the presence of PCB77 (B).

Caveolin-1 silencing decreases CYP1A1 induction by PCB77.

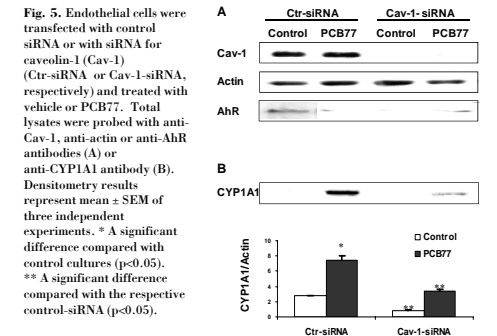


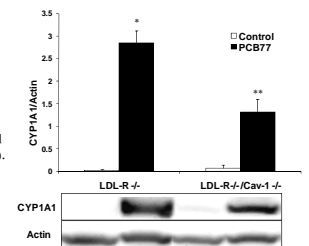
Fig. 5. Endothelial cells were transfected with control siRNA or with siRNA for caveolin-1 (Cav-1) (Ctr-siRNA or Cav-1-siRNA, respectively) and treated with vehicle or PCB77. Total lysates were probed with anti-Cav-1, anti-actin or anti-AhR antibodies (A) or anti-CYP1A1 antibody (B). Densitometry results represent mean ± SEM of three independent experiments. * A significant difference compared with control cultures (p<0.05). ** A significant difference compared with the respective control-siRNA (p<0.05).

CYP1A1 induction is reduced in LDL-R^{-/-} mice lacking the caveolin-1 gene.

Animals:

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). Mice were injected intraperitoneally with PCB77 (170 μmol/kg body weight) or vehicle (olive oil). Liver tissue samples were obtained and frozen in liquid nitrogen for further analysis.

Fig. 6. LDL-R^{-/-} mice and LDL-R^{-/-}/Cav-1^{-/-} mice were injected with olive oil (vehicle control) or PCB77 and tissue samples were collected. Liver protein levels of CYP1A1 were assessed using Western blot and divided by the values for actin (loading control). * A significant difference compared with control (vehicle) mice (p<0.05). ** A significant difference compared with PCB77-treated the LDL-R^{-/-} mice (p<0.05).



ACKNOWLEDGEMENTS

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