Caveolae mediate pro-inflammatory properties of coplanar polychlorinated biphenyls

Zuzana Májková1, Eun Jin Lim2, Shiften Xu3, Leonidas Bachas3, Eric Smart4, Michael T. Tseng5, Michal Toborek6, Bernhard Henning1,2

1Graduate Center for Toxicology, 2Molecular and Cell Nutrition Laboratory, College of Agriculture, Departments of 3Chemistry, 4Pediatrics and 6Neurosurgery, University of Kentucky, Lexington, KY 40536, USA, 5Department of Anatomical Sciences & Neurobiology, University of Louisville, Louisville, KY 40202, USA

ABSTRACT

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., Bethlehem, PA). After 2 weeks, mice were injected intraperitoneally with PCB77 (150 µg/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.

Hypothesis 2: Coplanar PCBs increase caveolin formation in endothelial cells

Coplanar PCBs induce caveolin-1 expression

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

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., Bethlehem, PA). After 2 weeks, mice were injected intraperitoneally with PCB77 (150 µg/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.

Fig. 4. Liver proteins were extracted and levels of CYP1A1 (A) and Cav1 (B) were assayed using Western blot and divided by the values for actin (loading control). Densitometry results represent mean ± 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-/- 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-/- mice lacking the caveolin-1 gene

Fig. 5. Aortic tissue was collected in RNAlater and total RNA was isolated using the RNAzol kit (Innogen, 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). Densitometry results represent mean ± 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

This work was supported by grants from NEHS/NHI (P42ES007380) and the University of Kentucky Agriculture Experiment Station.