Exposure to PCB 126 triggers cellular defense through cross-talk of caveolae and Nrf2 signaling

Michael C. Petriello[1] , Sung Gu Han[2] & Bernhard Hennig[1,2]

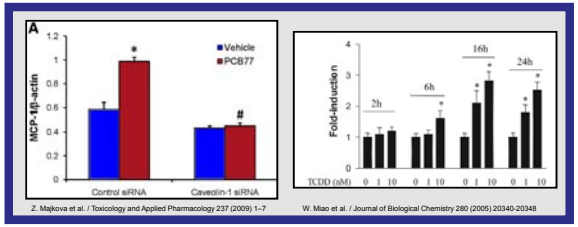
[1]Graduate Center for Toxicology, University of Kentucky, Lexington, KY

[2]Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY

Abstract

Environmental toxicants such as polychlorinated biphenyls have been implicated in the promotion of multiple inflammatory diseases including cardiovascular disease, but information regarding mechanisms of toxicity and cross-talk between relevant cell signaling pathways is lacking. We have reported that coplanar PCBs promote endothelial cell activation through the lipid microdomain caveolae, and the loss of caveolin-1 (Cav-1) ameliorates these detrimental effects. We have also shown that PCB 126 can activate the antioxidant transcription factor Nrf2 resulting in upregulation of antioxidant genes and downregulation of inflammatory markers. Normally, Nrf2 is sequestered in the cytoplasm and degraded through inhibitory action of Keap1, but upon activation via toxicants such as PCBs, can enter the nucleus and activate the transcription of a battery of protective genes. Our previous data suggests downregulation of Cav-1 and upregulation of Nrf2 protects against PCB-induced cellular dysfunction, but here we show for the first time an intimate example of cross-talk between these two cellular signaling pathways. To examine the cross-talk between Cav-1 and Nrf2 pathways in PCB-induced inflammation we silenced cav-1 in vascular cells. Importantly, Cav-1silenced cells treated with PCB126 resulted in increased levels of Nrf2-ARE binding determined by EMSA. Also, in cells treated with PCB 126, silencing of Cav-1 resulted in decreased protein levels of both inhibitory Keap1 and Fyn kinase, which both have been shown previously to be implicated in Nrf2 deactivation. Finally, Cav-1 silencing allowed for a more effective antioxidant response, as observed by higher levels of the antioxidant genes glutathione s-transferase (GST) and NADPH dehydrogenase quinone 1 (NQO1) in cells exposed to PCB 126. Ultimately, these data introduce novel cross-talk between Cav-1 and Nrf2 and implicates the ablation of Cav-1 as a protective mechanism of PCB-induced cellular dysfunction and inflammation.

Prior Research



Methods

Caveolin-1 silencing: Primary endothelial cells were transfected with control or Cav-1 siRNA at a final concentration of 100 nM using GeneSilencer transfection reagent (Genlantis, San Diego, CA) in OptiMEM serum free media. Cells were incubated with the transfection mixture for 4 h followed by adding FBS to achieve a final FBS concentration of 10%. Forty-eight hours after transfection, cells were equilibrated in 1% FBS containing media for 16 hours, then treated with 0.25 μ M PCB.
Western Blot Analysis: Whole cells were lysed in RIPA lysis buffer (Cell Signaling Technology) containing protease inhibitors(Thermo Scientific) and centrifuged at 12,000 g for10 min at 4 C. The supernatants were collected and the protein levels were determined by BCA assay (Pierce). Proteins were separated on 10% SDS-PAGE, transferred onto nitrocellulose membranes, blocked at room temperature with 5% non-fat milk in Tris-buffered saline containing 0.05% Tween 20 (TBST), and subsequently incubated overnight at 4 C with primary antibodies. Horseradish peroxidase-conjugated secondary antibodies were added. After TBST washes, the blots were visualized by using ECL detection reagents (Amersham Biosciences,) and exposure to film.
Nuclear Translocation The nuclear fraction of cellular lysate was isolated via the Assay:NE-PER Nuclear and Cytoplasmic Extraction Reagents kits (Thermo). Upon isolation the nuclear fraction underwent western blot analysis as above.
EMSA: Nuclear fraction was collected as above and EMSA was completed with Nrf2:ARE consensus binding sequences :AGATTCTGCTGAGTCACTGTGACTG; CAGTCACAGTGACTCAGCAGAATCT as described before
Real Time PCR Cells were grown in 6-well plates, and total RNA was extracted from the cells using TRIzol reagent (Life Technologies). Reverse transcription was performed using the AMV reverse transcription system (Promega). The levels of mRNAs expression were then assessed by real-time PCR using 7300 Real-Time PCR System(Applied Biosystems,) and SYBR Green master mix with appropriate forward and reverse primers

Figures

Figure 1. Silencing of Caveolin-1 promotes increased baseline and toxicant-induced Nrf2-ARE binding as determined by EMSA .

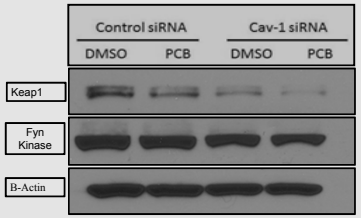


Figure 2. Silencing of Caveolin-1 promotes decreases in cellular Keap1 and Fyn Kinase protein levels; both of which are known to inhibit Nrf2 activation.

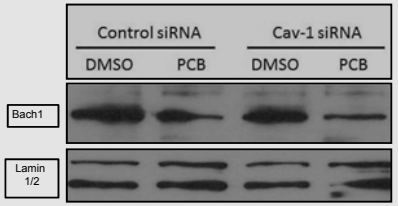


Figure 3. Silencing of Caveolin-1 also decreases nuclear localization of Bach1 protein levels during PCB 126-insult. Bach1 is known to export Nrf2 from the nucleus and inhibits Nrf2 activation.

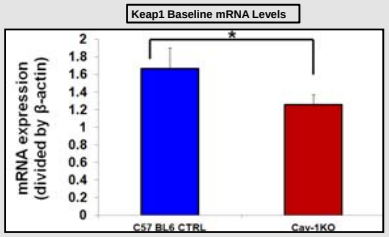


Figure 4. Caveolin-1 Knock Out mice exhibit lower baseline mRNA levels of inhibitory Keap1 when compared to age matched control mice of the same background. * denotes p-value =0.045

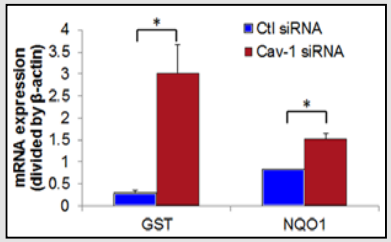


Figure 5. Silencing of Caveolin-1 ultimately increases mRNA levels of Nrf2 target genes Glutathione S-transferase (GST) and NAD(P)H dehydrogenase [quinone] 1 (NQO1). * denotes p-value <0.05.

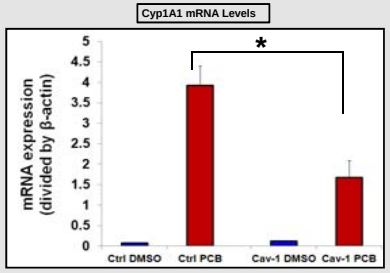


Figure 6. Silencing of Caveolin-1 decreases PCB-induced Cytochrome P450 1A1 upregulation. * denotes p-value <0.05

Results

- Silencing of Cav-1 resulted in multiple levels of Nrf2 cross-talk:
  1. Decreased whole cell levels of inhibitory Keap1
  2. Decreased whole cell levels of inhibitory Fyn Kinase
  3. Decreased nuclear localization of inhibitory Bach1
- ❖ Downregulation of multiple Nrf2 inhibitory proteins resulted in increased Nrf2:ARE binding and upregulation of Nrf2 target genes GST and NQO1
- ❖ A decrease in Cav-1 coupled with an increase in Nrf2 activation results in decreased Cyp1A1 expression and endothelial toxicity

Discussion

- Coplanar PCBs can cause endothelial cell dysfunction
  - Upregulation of Cyp1A1, ROS, & NFκB
- Caveolin-1 is integral to endothelial PCB Toxicity
  - May alter PCB transport or cellular signaling pathways
- The antioxidant response controller Nrf2 is activated in response to Dioxin-like compounds.
- We show here that cross-talk between caveolae and Nrf2 signaling pathways exists
- Decreasing Cav-1 increases Nrf2 activation which leads to a more efficient and effective antioxidant defense against PCB 126.

Possible Future Directions

- Determine which signaling pathway, caveolae or Nrf2, is most important in PCB-induced endothelial toxicity (or if cross-talk is critical)
- Utilize nutrition to modulate PCB vascular toxicity
  - ❖Effective bioactive food components will
    1. Downregulate Caveolin-1
    2. Increase Nrf2 activation
- Evidence that Omega-3 PUFAs and Flavonoids can protect via mechanisms involving caveolae and Nrf2
- Elucidate the mechanisms of protection
  - Investigate the Parent Vs. Metabolite controversy via HR Mass Spec

Acknowledgments

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