PPARα AGONISTS PROTECT AGAINST PCB-INDUCED VASCULAR ENDOTHELIAL CELL ACTIVATION

Xabier Arzuaga[1,2] Gudrun Reiterer[1,2], Zuzana Majkova[2], Michal Toborek[2,3], Bernhard Hennig[1,2].
College of Agriculture[1], Graduate Centers for Nutritional Sciences, and Toxicology[2], and Department of Surgery[3], University of Kentucky

Abstract:

Exposure to polychlorinated biphenyls (PCBs) can contribute to the pathology of atherosclerosis by activating inflammatory responses in vascular endothelial cells (ECs). Preventing the activation of such responses through pharmacologic or nutritional intervention could help alleviate the toxic effects caused by PCB exposure. Activation of peroxisome proliferator activated receptors (PPARs) by nutrients (e.g. omega-3-fatty acids) or synthetic agonists (e.g. fibrates and glitazones) has been shown to block pro-inflammatory responses both in-vivo and in-vitro. In this study we demonstrated that activation of PPARα though synthetic agonists was able to reduce PCB77-induced inflammation. Porcine primary vascular ECs were pre-incubated with the PPARα ligands fenofibrate or WY14643 (1-20 μM) followed by exposure to PCB77 (3.4μM). PPARα activation protected ECs against PCB77-induced mRNA and protein expression of the downstream proinflammatory genes: vascular cell adhesion molecule-1 (VCAM-1), cycloxygenase-2 (COX-2) and interleukin-6 (IL-6). Exposure to PPARα ligands decreased PCB77-induced expression of the aryl hydrocarbon receptor (AHR) responsive gene cytochrome P4501A1 (CYP1A1), and decreased AHR protein expression. We also investigated the possible interactions between PCBs and PPAR activity and expression. PCB77 reduced PPARα and PPARγ protein expression in a dose dependent manner. PCB77 also reduced PPAR transactivation activity as determined in MCF-7 cells stably transfected with a PPRE-driven luciferase reporter gene. These findings suggest that PPARα activation by synthetic agonists can significantly reduce PCB-induced EC activation by reducing AHR expression and function, and that the inflammation induced by PCBs is mediated, in part, by inhibition of anti-inflammatory PPARs. (Supported by grants from NIEHS, NIH (P42ES07380) and the University of Kentucky AES)

Key words: atherosclerosis, vascular endothelial cells, PPAR, PCB77, inflammation.

Background:

In vascular endothelial cells coplanar PCBs such as PCB77 increase cellular damage and activation of oxidative stress sensitive and pro-inflammatory transcription factors NF-κB and AP-1 with a subsequent expression inflammatory genes such as VCAM-1, COX-2 and IL-6. Most of the toxic effects caused by these compounds are regulated by the aryl hydrocarbon receptor (AHR) (Slim *et al.*, 1999; Ramadass *et al.*, 2003).

PPARα agonists have been shown to be protective against inflammation by down-regulating underlying pro-inflammatory signaling pathways. PPARs have been shown to negatively interfere with NF-κB, and AP-1 signaling pathways and prevent the expression of inflammatory genes such as adhesion molecules and cytokines. Indeed, clinical and experimental evidence suggests that PPARα activation decreases the incidence of cardiovascular diseases (Chinetti *et al.*, 2003).

Objectives:

The experiments described below were designed to answer the following questions:

- *Does PPARα inhibit co-planar PCB induction of the pro-inflammatory genes: VCAM-1, COX-2, and IL-6?*
- *What are the molecular mechanisms involved in PPARα dependent inhibition of PCB induced inflammation?*
- *Do co-planar PCBs inhibit expression and transcriptional activity of PPARα*

Methods:

Cell culture and experimental media

Endothelial cells (ECs) were isolated from porcine pulmonary arteries as described previously (Toborek *et al.*, 2002). MCF-7 cells stably transfected with a luciferase gene driven by PPRE sites were utilized for selected experiments. Cells were sub-cultured in medium-199 (endothelial cells) and DMEM (MCF-7 cells) containing fetal bovine serum (FBS, HyClone Laboratories, Logan, UT) using standard techniques. PCB77 and PPAR agonists (FF or WY) were added from a stock solution in DMSO. All treatment groups contained an equal amount of DMSO.

PPARα, PPARg, VCAM-1 and COX-2 protein expression studies

Total cellular protein was extracted as previously described (Slim *et al.*, 1999). Protein extracts were electrophoresed on SDS-polyacrilamide gels transferred to nitrocellulose membranes. PPARα, PPARγ, VCAM and COX-2 proteins were probed with commercial rabbit and goat antibodies.

CYP1A activity assay (EROD)

Cytochrome P450 1A (CYP1A) activity or ethoxyresorufin-o-deethylase (EROD) activity was measured as described previously (Ramadass *et al.*, 2003) using 7-ethpxyresorufin as a CYP1A substrate.

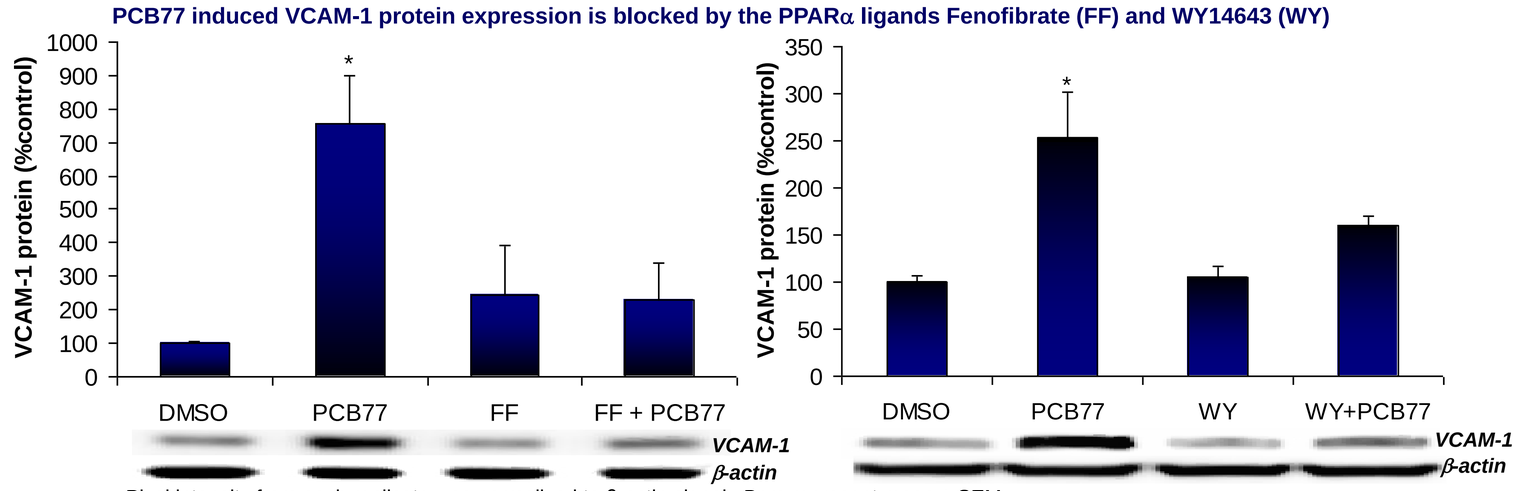
PPAR reporter gene studies

The human breast cancer epithelial cell line MCF-7 was stably transfected with a vector containing repeats of PPREs in the promoter region driving luciferase gene expression and a HSV-TK-driven renilla. Cells were plated and exposed to PCBs prior to cell lysis. Cell lysis and reporter gene assay was performed using a dual reporter assay kit. Values were expressed as a luciferase/renilla ratio.

Statistics

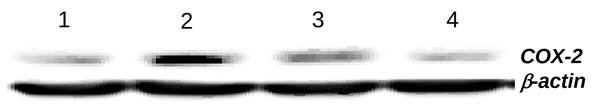
Data was analyzed using one or two way ANOVA, depending on the number of variables used in the experiments, followed by Tukey's test for post hoc comparisons.

PCB77 induced VCAM-1 protein expression is blocked by the PPARα ligands Fenofibrate (FF) and WY14643 (WY)



Pixel intensity from each replicate was normalized to β-actin signal. Bars represent mean ± SEM.

COX-2 protein induction by PCB77 is significantly reduced by the PPARα ligand Fenofibrate

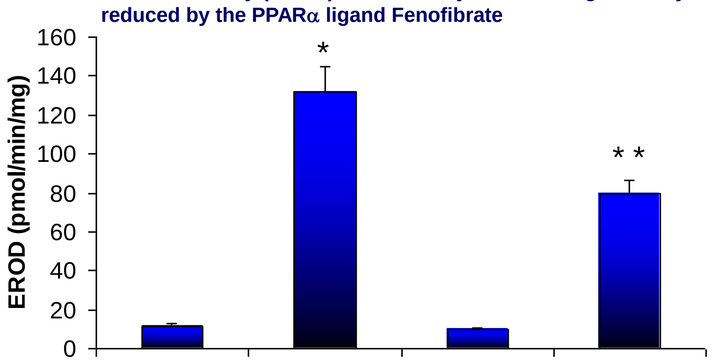


1-Vehicle (DMSO) **3**-PCB77 + FF (1μM)
2-PCB77 (3.4μM) **4**-PCB77 + FF (10μM)

PCB77 induced IL-6 mRNA expression is blocked by the PPARα ligand Fenofibrate

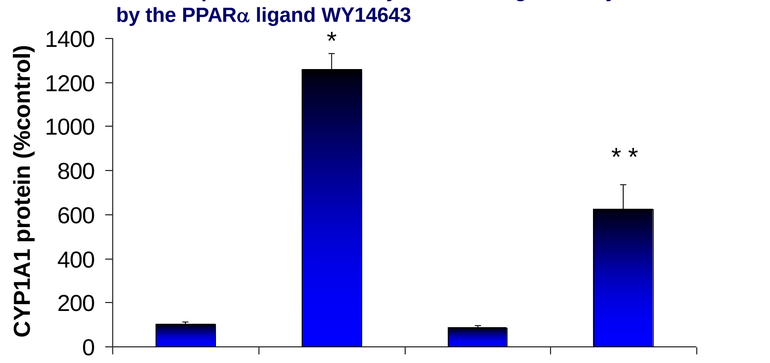
mRNA expression levels were determined by RT-PCR

CYP1A1 activity (EROD) induction by PCB77 is significantly reduced by the PPARα ligand Fenofibrate



Bars represent mean EROD activity (pmol/min/mg) SEM.

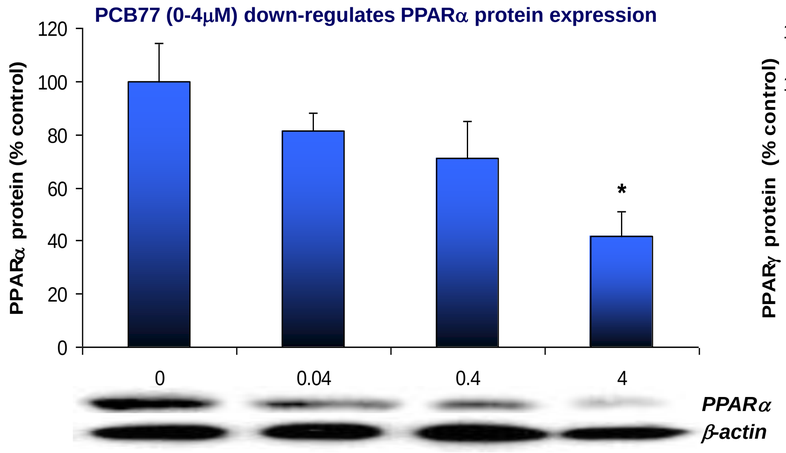
CYP1A1 protein induction by PCB77 is significantly reduced by the PPARα ligand WY14643



Pixel intensity from each replicate was normalized to β-actin signal. Bars represent % control mean ± SEM.

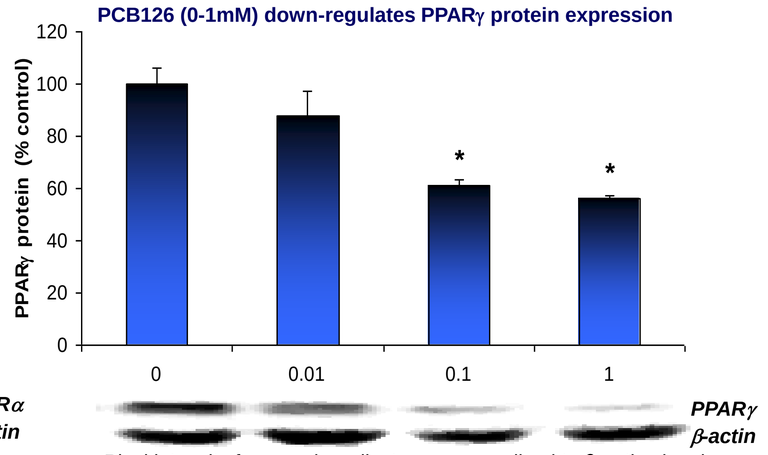
The PPARα ligand Fenofibrate reduces basal aryl hydrocarbon receptor (AHR) protein expression

PCB77 (0-4μM) down-regulates PPARα protein expression



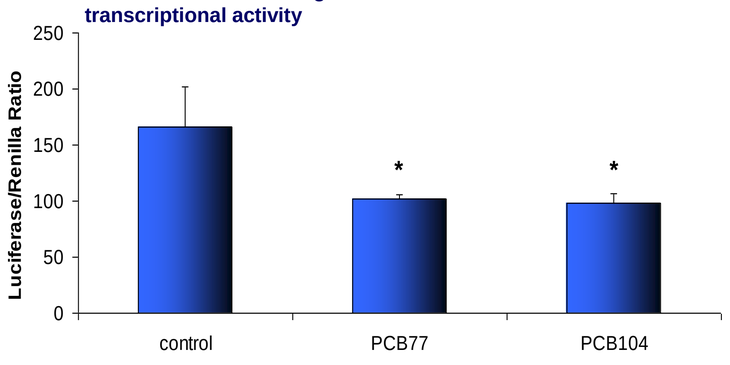
Pixel intensity from each replicate was normalized to β-actin signal. Bars % control mean ± SEM.

PCB126 (0-1mM) down-regulates PPARγ protein expression

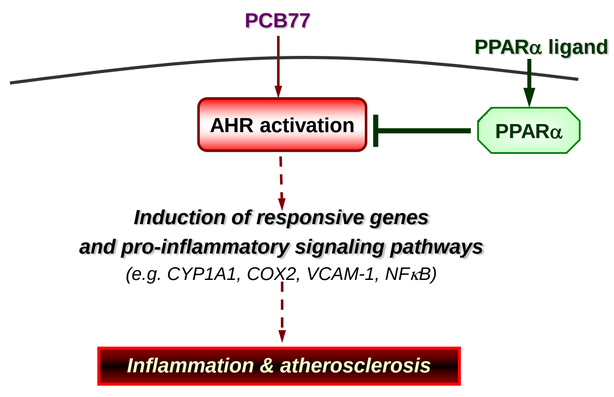


Pixel intensity from each replicate was normalized to β-actin signal. Bars % control mean ± SEM.

PCBs 77 & 104 down-regulate PPAR transcriptional activity



Possible mechanism for PPARα dependent inhibition of PCB77 induced inflammation



Conclusions:

In summary, our findings demonstrate that PPARα agonists can downregulate PCB77-induced expression of pro-inflammatory genes and might be promising candidates in the prevention of atherosclerosis in populations exposed to PCBs and other chlorinated AHR ligands. The effects of PPARα ligands on AHR and CYP1A1 expression suggest that PPARα activation protects endothelial cells against PCB77 induced inflammation, in part, through inhibition of the AHR pathway. Our results also suggest that the pro-inflammatory properties of the coplanar PCB77 could be mediated, in part, by inhibiting expression and activity of protective PPARs.

Selected references:

Chinetti, G., Fruchart, J. C., and Staels, B. (2003). *Int J Obes Relat Metab Disord* **27 Suppl 3**, S41-45.
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Slim, R., Toborek, M., Robertson, L. W., and Hennig, B. (1999). *Toxicol Sci* **52**, 232-239.
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