Proinflammatory Mechanisms Induced by PCBs: Implications in Vascular Diseases

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Introduction

Atherosclerotic lesions are thought to be initiated by vascular endothelial cell dysfunction. Because the endothelium is in immediate contact with the blood, endothelial cells are particularly susceptible to the effect of environmental contaminants and their downstream mediators present in the bloodstream. These risk factors induce certain cell signaling pathways leading to the activation of proinflammatory transcription factors such as nuclear factor-kB (NF-kB), activator protein-1 (AP-1) or signal transducer and activator or transcription-3 (STAT-3). These transcription factors control inflammatory genes in endothelial cells, including cyclooxygenase-2 (COX-2), interleukin-6 (IL-6) and vascular cell adhesion molecule-1 (VCAM-1).

Peroxisomeproliferator-activated receptors (PPARs) alpha and gamma agonists have been shown to be protective against inflammatory events by down-regulating proinflammatorysignaling pathways. PPARs have been shown to negatively interfere with NF-kB, AP-1, and STAT signaling pathways and can therefore prevent the expression of inflammatory genes such as cytokines and adhesion molecules. Proinflammatory compounds, such as PCBs could, at least in part, act by antagonizing PPARs.

There is also increasing evidence that caveolae play a critical role in atherosclerosis. Caveolae are a subset of lipid rafts characterized by the presence of specific caveolin proteins. Caveolae are highly expressed in endothelial cells and are thought to play a role in regulation of endothelial vesicular trafficking. Caveolae have been shown to be involved in uptake of lipids and possibly lipophilicxenobiotics such as PCBs. Besides their role in cellular uptake of lipophilic substances, caveolae contain an array of cell signaling molecules. In fact, numerous genes involved in endothelial cell dysfunction, inflammation and PCB toxicity are associated with caveolae.

Materials and Methods

PCBs: PCBs were synthesized in our laboratory or purchased from AccuStandard, Inc. (New Haven, CT).

Cell culture and experimental media: Endothelial cells were isolated from porcine pulmonary arteries or aortas and cultured using standard culture techniques. The experimental media were composed of M199 enriched with 5% FBS and the coplanar PCB 77 (3,3',4,4'-tetrachlorobiphenyl) or the non-coplanar PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl). In most experiments, PCBs were solubilized in DMSO and used at a concentration of 3.4 µM. This level was chosen because it reflects serum concentrations after exposure to PCBs. Optimal specific time points for gene expression used in the present experimental design were characterized and reported by us previously (1-3).

DNA binding activity: Following overnight treatment with PPARa agonist (fenofibrate, 10 mM) or PPARg agonist (thiazolidinedeione, 25 mM), porcine primary endothelial cells were treated with PCB 77 (6 h) or PCB 153 (1h). Nuclear proteins were incubated with P^{32} end labeledoligonucleotides specific for PPAR response element (PPRE), NF-kB, or STAT-3 and DNA binding was determined by gel shift assay.

Expression of inflammatory genes: Endothelial cells were exposed to PCB 77 and 153 for 4 h to measure COX-2 expression or 6 h for IL-6 expression. Total cellular RNA was extracted followed by RT-PCR. PCR products were separated on 2% agarose gel and stained with SYBR gold. In some experiments, cells were pretreated with PPAR agonists.

PPAR transcriptional activity: MCF-7 cells were stably transfected with a luciferase reporter gene driven by a triple repeat of PPRE. Cells were exposed to PCBs for 6 h. Luciferase readings were normalized by readings obtained from measuring the CMV-driven renilla.

Expression of genes associated with lipid rafts: Endothelial cells were exposed to PCB 77, and time-dependent changes in mRNA levels of caveolae-associated genes (caveolin-1, annexin-2 COX-2) were studied. Total cellular RNA was extracted followed by RT-PCR. PCR products were separated on 2% agarose gel and stained with SYBR gold.

Bovine serum albumin cotransporation: Endothelial Cells were treated with PCBs (e.g., PCB 153) or DMSO for 3 hours in media containing various plasma binding proteins. Total cellular RNA was extracted followed by RT-PCR using E-selectin as a model adhesion molecule leading to activation. PCR products were separated on 2% agarose gel and stained with SYBR gold.

Statistical analysis: The data were analyzed using SYSTAT 7.0. Comparisons between treatments were made by one-way ANOVA with post-hoc comparisons of the means.

Results and Discussion

Many environmental contaminants, and especially persistent organic pollutants, are risk factors for atherosclerosis because they may exacerbate an underlying disease by altering gene expression patterns (4). For example, exposure to dioxin or TCDD or the co-planar PCB 126 (3,3',4,4',5-pentachlorobiphenyl) for up to two years was associated with treatment-related increases in the incidence of degenerative cardiovascular lesions (5). There is evidence linking the aryl hydrocarbon receptor (AhR) with mechanisms associated with cardiovascular diseases (6) and that AhRligands may be atherogenic by disrupting the functions of endothelial cells in blood vessels.

The mechanisms by which environmental chemicals induce endothelial cell activation are not fully understood, and little is known about how PCB-mediated cell dysfunction can be prevented. Oxidative stress-induced transcription factors (e.g., NF-kB, AP-1), which regulate inflammatory cytokine and adhesion molecule production, play critical roles in the induction of inflammatory responses. The binding sites for these transcription factors are present in the promoter regions of a variety of inflammatory genes such as IL-6, VCAM-1 or COX-2, all of which are upregulated during PCB toxicity (7-9).

There is increasing evidence that caveolae play a critical role in the pathology of atherosclerosis and that the lack of the caveolin-1 gene may provide protection against the development of atherosclerosis (10). Caveolae are particularly abundant in endothelial cells, where they are believed to play a major role in the regulation of endothelial vesicular trafficking and are involved in the uptake of lipids, as well as related compounds/chemicals and possibly environmental pollutants. The role of caveolae in cellular PCB-trafficking is not known. In our experiments we found caveolin-1 to be upregulated in cultured endothelial cells after exposure to PCB 77 (Figure 1a), and both caveolin-1 and CD36 were upregulated in liver tissues derived from LDL-R-/- mice (11).

Besides their role in cellular uptake of lipophilic substances, caveolae contain an array of cell signaling molecules. In fact, numerous genes involved in endothelial cell dysfunction, inflammation and PCB toxicity are associated with caveolae. We found that both coplanar and non-coplanar PCBs increased expression of COX-2 (Figure 1b) andactivated STAT 3, both are present in caveolae. We hypothesize that caveolae are critical cell-surface plasma membrane invaginations, which facilitate the lipid and PCB-mediated cellular uptake and subsequent endothelial inflammatory response and cytotoxicity.

Peroxisomeproliferator activated receptors (PPARs) appear to possess potent anti-inflammatory signaling properties. Very little is known about the effect of environmental contaminants on PPAR signaling. There is evidence that the classical AhRligand 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) can suppress PPAR gamma expression (12). It has been suggested that AhR functions may antagonize PPAR functions (13). Our data suggest that PCBs can down-regulate PPAR activation. We also have data that show PPAR agonist-mediated protection against COX-2, IL-6 and expression induced by PCB 77 (Figure 2 a and b). We hypothesize that PCBs contribute to an endothelial inflammatory response in part by down-regulating PPAR signaling.

Many environmental contaminants exhibit human toxicity and disease via oxidative stress-sensitive signaling pathways. We found that both coplanar and non-coplanar PCBs can induce inflammatory pathways in endothelial cells. Although different pathways might be responsible for the PCB-mediated endothelial cell activation, both coplanar and non-coplanar PCBs appear to be pro-atherogenic. Coplanar PCBs such as PCB 77 are able to increase caveolin-1, annexin-2, and COX-2 mRNA expression possibly indicating internalization due to caveolae.

We also propose that specific PCBs can decrease PPAR activation and thus contribute to inflammatory responses within the vascular endothelium. We demonstrated that PCBs can inhibit PPAR DNA binding and transcriptional activity. Finally, pretreatment with PPAR alpha and gama agonists protected against PCB-mediated activation of endothelial cells, suggesting that PCBs contribute to an endothelial inflammatory response in part by down-regulating PPAR signaling.

Acknowledgements

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Figure legends

Figure 1: Time-dependent changes in mRNA levels of (a) caveolae and (b) COX-2 in endothelial cells exposed to 3.4 mM PCB 77 after 0, 1, 4, and 8 hours of treatment. Total cellular RNA was extracted followed by RT-PCR. PCR products were separated on a 2% agarose gel and stained with SYBR gold.

Figure 2: PPAR ligands block PCB 77 induced expression of COX-2 (a) and IL-6 (b) in porcine endothelial cells. Endothelial cells were pretreated with the PPAR agonistsfenofibrate (FF) troglitazone (TG) or thiazolidinedione (TZD) and exposed to PCB 77 for 4 h to measure COX-2 expression or 6 hours for IL-6 expression. Total cellular RNA was extracted followed by RT-PCR. PCR products were separated on 2% agarose gel and stained with SYBR gold. β-actin was used as a housekeeping gene.

Figure 1

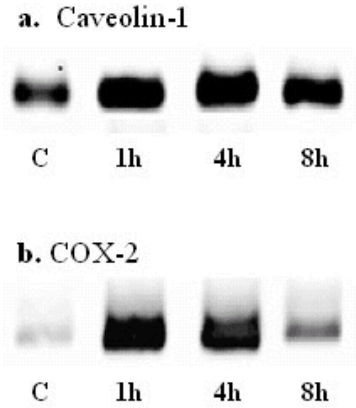

Figure 2