3,3'4,4'-tetrachlorobiphenyl disrupts PPAR signaling in vascular endothelial cells

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Introduction

From epidemiological studies, there is substantial evidence that cardiovascular diseases are linked to environmental pollution. 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 cytotoxic metabolites, inflammatory cytokines and environmental contaminants present in the bloodstream [1]. These risk factors induce cell signaling pathways, leading to the activation of pro-inflammatory transcription factors such as nuclear factor-kB (NF-kB) which control many inflammatory genes in endothelial cells.

We have previously reported that the coplanar 3,3'4,4'-tetrachlorobiphenyl (PCB 77) can induce oxidative stress and expression of the inflammatory genes, such as IL-6 and VCAM-1, in vascular endothelial cells [1, 2]. The expression of cytokines and adhesion molecules enhances the inflammation, and loss of the endothelial barrier function can lead to the formation of fatty streaks.

PPARa and g agonists have been shown to be protective against these events by down-regulating underlying pro-inflammatory signaling pathways. PPARs have been shown to negatively interfere with NF-κB, STAT and AP-1 signaling pathways [3] and can therefore prevent the expression of inflammatory genes such as adhesion molecules and cytokines. On the other hand, the PPAR signaling could also be impaired due to negative crosstalk by inflammatory pathways [4]. Pro-inflammatory compounds, such as PCBs could, at least in part, act by antagonizing PPARs. In fact, little is known about the effects of environmental contaminants, such as PCBs, on PPARa and g signaling.

In the current study, we hypothesize that PCBs can down-regulate the anti-inflammatory transcription factors PPARa and g and that PPAR agonists can prevent the inflammatory response induced by PCBs. This hypothesis was tested in the current study with the coplanar PCB 77.

Materials and Methods

Cell culture and experimental media

Endothelial cells were isolated from porcine pulmonary arteries as described previously [5]. MCF-7 cells stably transfected with a luciferase gene driven by a triple repeat of the PPAR response element (PPRE) were utilized for selected experiments. The experimental media contained 5% (v/v) FBS. PCB 77 (0.04 - 3.4 mmol/L) and PPAR agonists (10 - 25 mmol/L) were added from a stock solution in DMSO. PCB concentrations used are calculated values from plasma levels reported after acute exposure [6], or derived from concentration-response curves measuring CYP1A activity (ethoxyresorufin-o-deethylase or EROD) after cellular exposure to PCB77. For most experimental settings, cells were treated with PPAR agonists for 6-18 h, and with PCB77 for 1.5-18 h.

Electrophoretic mobility shift assay (EMSA)

Nuclear extracts containing active proteins were prepared from cells as previously described [1].

RT-PCR

Total RNA was extracted from endothelial cells by the use of TRI reagent. Gene expression was determined through reverse transcription-polymerase chain reaction (RT-PCR) as described earlier [7]. The following primers were employed in the PCRs; IL-6 forward: 5' AAT TCG GTA CAT CCT CGA CG 3', reverse: 5' GCG CAG AAT GAG ATG AGT TG 3', COX-2 forward: 5' GGA GAG ACA GCA TAA ACT GC 3', reverse: 5' GTG TGT TAA ACT CAG

CAG CA 3', b-actin forward: 5' ggg acc tga ccg act acc tc 3', reverse: 5' ggg cga tga tct tga tct tc3'.

CYP1A1/2 catalytic activity (ethoxyresorufin-o-deethylase, EROD)

Endothelial cell EROD activity was measured as previously described by [8].

PPAR reporter gene studies

The human breast cancer epithelial cell line MCF-7 was stably transfected with pGL3 vector (Promega, Madison, WI) containing a triple repeat of peroxisome proliferators response element (PPRE) in the promoter region driving luciferase gene expression and a HSV-TK-driven renilla. Transfected cells were selected using G418. Cells were exposed to PCB for 6 h prior to cell lysis. Reporter gene assay was performed using the dual reporter assay kit.

Statistical analysis

All experiments were confirmed 3 to 4 times. Data were quantified and analyzed using the Scion Image, Image Gauge and Sigma Stat software. Comparisons between treatments were made by one or two way ANOVA and Tukey or Least Square Difference (LSD) test for post hoc comparisons using SYSTAT 10 (Richmond, CA). Statistical probability of $p < 0.05$ was considered significant.

Results and Discussion

PPAR agonists downregulate the PCB 77-induced activity of the AhR pathway and NF-kB.

CYP1A activity (or EROD) was used as a marker for AhR activation and responsive gene induction. Incubation with 0.04 mM PCB 77 resulted in a significant (11.7 fold) EROD induction. Co-treatment with the PPARa agonist fenofibrate (FF, 100 mM) caused a significant decrease (54.5%) in PCB 77 induced EROD. Similar results were obtained in *in vivo* studies using rats [9] and suggest that PPARacan interfere with AhR function.

To determine if PPAR agonists interfere with PCB 77-induced NF-kB DNA binding activity, endothelial cells were treated with PCB 77 (3.4 mM) ± the PPARg agonist thiazolidinedione (TZD, 25mM), followed by EMSA. The DNA binding activity of NF-kB was significantly induced by exposure to PCB 77, and co-treatment with TZD protected against PCB 77-induced NF-kB DNA binding activity. These results suggest that PPAR agonists can interfere with pro-inflammatory signaling cascades activated by PCB 77.

PPAR agonists protect against PCB 77 - induced IL-6 and COX-2 gene expression

IL-6 mRNA expression was upregulated after a 6 h exposure to PCB 77 (3.4 mM). Maximal mRNA expression of COX-2 was measured following 4 h exposure to PCB 77. The expression of both PCB-induced genes could be significantly decreased by pre-treatment (18 h) of cells with the PPARa agonist FF (10 mM) or the PPARg agonists TZD or troglitazone (25 mmol/L). These results suggest that PPARa and g agonists are protective against PCB 77-mediated induction of pro-inflammatory genes.

PCB 77 decreases PPAR DNA binding and transcriptional activation

To determine the effect of PCB 77 on PPAR activity, endothelial cells were treated with 3.4 mM PCB 77 for 2 h, followed by EMSA analysis of PPAR-DNA binding. Treatment with PCB 77 led to a significant decrease in PPAR-DNA binding activity. In addition, PCB 77 significantly reduced PPAR-dependent reporter gene expression in MCF-7 cells stably transfected with luciferase driven by a triple repeat of the PPRE.

Previous studies by others have shown that the classical AhR ligands can suppress PPAR expression [10]. By measuring reporter gene activity we provide evidence that PCB 77 does not only decrease PPAR-DNA binding but also transcriptional activity. The mechanism of PCB-mediated inhibition of PPARs is not fully known. One possibility is that PCB 77 binds to PPARs, thus acting as competitive antagonists and preventing DNA binding and transcriptional activity, or that PCB 77 triggers pro-inflammatory pathways that negatively crosstalk with PPAR signaling pathways. Further studies are required to determine the molecular and biochemical mechanisms by which co-planar PCBs downregulate PPAR expression, activity and associated signaling pathways.

In summary, our results suggest that the pro-inflammatory properties of the coplanar PCB 77 could be mediated, in part by inhibiting the anti-inflammatory properties of PPARs. We also demonstrate that PPARa and g agonists can potently downregulate PCB-mediated toxicity and might therefore be promising candidates in the prevention of atherosclerosis in populations exposed to PCBs and other chlorinated AhR ligands.

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