Role of Caveolae in PCB-Induced Vascular Endothelial Cell Activation

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Abstract

PCBs are persistent environmental contaminants that can contribute to the pathology of atherosclerosis by activating inflammatory responses in vascular endothelial cells. We hypothesize that the plasma membrane microdomains called caveolae are critical in endothelial activation and toxicity mediated by PCBs. Caveolae are particularly abundant in endothelial cells and play a major role in endothelial trafficking and the regulation of signaling pathways associated with the pathology of vascular diseases. Our data suggest that PCB uptake into endothelial cells may occur via caveolae-related endocytosis. Mass spectrometry of subcellar membrane fractions revealed that high levels of PCB77 were localized to the caveolin-rich fractions. TEM confirmed increased invaginations of caveolae after PCB77 treatment. PCBs also can induce caveolae-linked signaling pathways during endothelial cell activation. We tested the hypothesis that PCBs can activate endothelial cells via eNOS signaling. PCB77 rapidly induced caveolin-1 and eNOS phosphorylation as well as activation of PI3K and Akt. Caveolin-1 silencing abolished the PCB-stimulated eNOS phosphorylation. PCB77 also increased nitrotyrosine levels, which may have implications in oxidative-stress mediated inflammatory mechanisms. Our data provide evidence that caveolae play a critical role in regulating vascular endothelial cell activation and toxicity induced by persistent environmental pollutants such as coplanar PCBs.

Introduction

From epidemiological studies, there is substantial evidence that cardiovascular diseases are linked to environmental pollution. For example, a recent study reported increased hospitalization rates for coronary heart disease in populations residing near areas contaminated with persistent organic pollutants (1). The lining of blood vessels is protected by the endothelium, and endothelial cells play an active role in physiological processes such as regulation of vessel tone, blood coagulation, and vascular permeability. Dysfunction of endothelial cells is a critical underlying cause of the initiation of cardiovascular diseases such as atherosclerosis. One functional change in atherosclerosis is the activation of the endothelium, which is manifested as an increase in the expression of specific cytokines and adhesion molecules. These cytokines and adhesion molecules are proposed to mediate the inflammatory aspects of the disease by regulating the vascular entry of leukocytes. We have demonstrated previously that coplanar as well as non-coplanar PCBs can cause endothelial cell dysfunction as determined by markers such as expression of cytokines and adhesion molecules (reviewed in 2). 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 (3). We and others have shown that

persistent organic pollutants (such as PCBs) can induce certain cell signaling pathways leading to the activation of proinflammatory transcription factors such as nuclear factor-κB (NF-κB), which control inflammatory genes in endothelial cells, including cyclooxygenase-2 (COX-2), interleukin-6 (IL-6) and vascular cell adhesion molecule-1 (VCAM-1) (reviewed in 3). However, mechanisms, and in particular intracellular signaling pathways, responsible for the regulation of PCB-mediated endothelial cell activation are not well understood.

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 (4). 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 lipophilic xenobiotics 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. Our data provide evidence that caveolae may play a critical role in regulating vascular endothelial cell activation and toxicity induced by persistent environmental pollutants such as coplanar PCBs. This may have implications in understanding novel mechanisms of PCB-mediated inflammatory diseases such as atherosclerosis.

Materials and Methods

Cell culture and experimental media:

Human-derived endothelial cells (EAhy926 cells) were maintained in medium DMEM, supplemented with 10% fetal calf serum (Hyclone, Logan, UT) and antibiotics. Subsequently, cells were treated for various time periods with 2.5 μM PCB77 (AccuStandard, New Haven, CT).

Detergent-free purification of caveolin-enriched membrane fractions:

Caveolin-enriched membrane fractions were prepared from endothelial cells as described earlier (5). Cells were washed with PBS and were lysed with ice-cold MES-buffered saline. A light-scattering band confined to the 5-35% sucrose interface contained caveolin.

Gene silencing by siRNA:

Caveolin-1 gene (Genbank Accession NO. NM001753) silencing was performed using a mix of two siRNAs directed against the following target sequences: 5'-AACATCTACAAGCCCAACAAC-3', 5'-AACCAGAAGGGACACACAGTT-3'. The siRNA to mRNA caveolin-1 was synthesized by Dharmacon. Control scrambled siRNA 5'-AAAGAGCGACUUUACACAC-3' (Dharmacon) also was used. Cells were seeded and transfected with siRNA using GeneSilencer (Genlantis).

Measurement of total NO (Nitrite-Plus-Nitrate):

Culture media were concentrated through micropore filtration. The filtrates were analyzed using a NO_2-/NO_3- assay kit-C II (Dojindo, Kumamoto, Japan).

Immunoprecipitation:

Nitrotyrosine was quantified as described earlier (6). Total lysates were incubated with 5 μg of monoclonal anti-nitro-tyrosine antibody, followed by precipitation with 30 μL of protein

A/G plus-agarose beads. Protein precipitates were separated by 7.5% SDS-PAGE and blotted onto a nitrocellulose membrane.
Statistical analysis: 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

We have shown previously that coplanar PCBs, such as PCB 77 can induce oxidative stress through the aryl hydrocarbon receptor (AhR)-cytochrome P450 1A1 (CYP1A1) pathway (7). Reactive oxygen species (ROS) can subsequently trigger oxidative stress sensitive pro-inflammatory pathways including the transcription factors NF-κB and AP-1, as well as downstream genes such as inflammatory cytokines (e.g., IL-6) and adhesion molecules (VCAM-1, ICAM-1, E-selectin) (8).

There is increasing evidence that membrane domains called 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 (4). A recent study suggests that the recruitment of leukocytes during inflammation occurs though translocation of endothelial intercellular adhesion molecule-1 (ICAM-1) to caveolae-rich membrane domains (9). Caveolae are particularly abundant in endothelial cells, where they are believed to play a major role in the regulation of endothelial vesicular trafficking as well as the uptake of lipids and related lipophilic compounds (10), possibly including lipoprotein- and albumin-associated persistent organic pollutants and bioactive food components such as flavonoids.

The mechanisms responsible for the uptake and cellular processing of PCBs are not well understood. We propose that PCB uptake into vascular endothelial cells may occur via caveolae-related endocytic processes. Caveolae are specialized membrane domains highly enriched in sphingolipids and cholesterol and particularly abundant in endothelial cells. There is evidence that caveolae play a major role in endothelial cell trafficking and in the regulation of signaling pathways associated with the pathology of vascular diseases. In this study, endothelial cells were treated with PCB77 and subjected to sucrose gradient fractionation in order to separate caveolae from other cellular components. Western blot analysis revealed that PCB77 significantly increases Cav-1, indicating increased formation of caveolae compared to control. Mass spectrometry of subcellular membrane fractions revealed that high levels of PCB77 were localized to the caveolin-rich fractions. Furthermore, transmission electron microscopy (TEM) confirmed that the invaginations of caveolae were observed along all membrane surfaces after PCB77 treatment at a higher frequency than compared to untreated cells. These data suggest that caveolae are critical cell-surface plasma membrane invaginations, which facilitate PCB-mediated cellular uptake and subsequent endothelial inflammatory response and cytotoxicity.

Besides their role in cellular uptake of lipophilic substances, we have demonstrated that numerous genes involved in endothelial cell dysfunction, inflammation and PCB toxicity are associated with caveolae. Our data suggest that PCB77 uptake into vascular endothelial cells occur via caveolae. We hypothesize that signaling pathways mediating release of caveolae from the plasma play a critical role in endothelial activation and toxicity mediated by PCBs. For example, endothelial nitric-oxide synthase (eNOS) is co-localized with caveolae and is a critical regulator of vascular homeostasis. Dysfunctional eNOS signaling

may be important in the early pathology of atherosclerosis, and the activation of eNOS can lead to excessive NO formation. We tested the hypothesis that PCBs can activate endothelial cells via eNOS signaling. Human endothelial cells (EA.hy926) were treated with PCB77 (2.5 μM) for 5 and 10 minutes to explore protein activation. Exposure to PCB77 rapidly induced caveolin-1 and eNOS phosphorylation as well as activation of PI3K and Akt. Caveolin-1 silencing abolished the PCB-stimulated eNOS phosphorylation. Furthermore, PI3K inhibitors (Wortmanin, LY294002) blocked eNOS and caveolin-1 phosphorylation. Cellular exposure to PCB77 also increased nitrotyrosine levels, which may have implications in oxidative-stress mediated inflammatory mechanisms induced by PCBs.

In summary, we provide evidence that caveolae may play a role in regulating cellular uptake and trafficking of environmental pollutants such as PCBs. Also, our data indicate that PCBs can activate of caveolae-associated signaling molecules. Our findings suggest that PCB77 induces eNOS phosphorylation in endothelial cells through a Src/PI3K/ Akt-dependent mechanism, events regulated by caveolin-1. Thus, our data provide evidence that caveolae may play a critical role in regulating vascular endothelial cell activation and toxicity induced by persistent environmental pollutants such as coplanar PCBs.

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