PCB-Mediated Endothelial Cell Dysfunction: Implications in Atherosclerosis

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Introduction

Factors implicated in the pathogenesis of atherosclerosis include chronic and cumulative metabolic alterations of the endothelium induced by certain lipids, prooxidants, inflammatory cytokines and environmental contaminants, such as polyhalogenated aromatic hydrocarbons. These risk factors may contribute to an overall cellular imbalance of the oxidative stress/antioxidant balance, thus leading to chronic activation or stimulation of the endothelium as well as to damage of vascular tissues. Maintenance of endothelial integrity is critical for the performance of normal barrier function in order to limit the entry of plasma components, such as cholesterol-rich lipoproteins into the vessel wall.

There is evidence that certain environmental contaminants, such as polychlorinated biphenyls (PCBs) or 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), can cause vascular endothelial cell dysfunction (1-3). PCBs (and especially non-planar PCBs) can activate circulating neutrophils, and thus further promote an oxidative burst and inflammatory reactions, which are all critical events in atherosclerotic lesion formation (4,5). Thus, exposure to PCBs and related polyhalogenated compounds may contribute to the pathology of cardiovascular disease (6). PCBs and related highly persistent, lipophilic and hazardous substances are wide-spread pollutants. In the blood, PCBs are transported in close association with lipoproteins and albumin. Numerous environmental contaminants and their metabolites may remain in the blood circulation for extended time periods. Endothelial cells are thus vulnerable to chemical insult, which can lead to severe endothelial cell dysfunction. Several studies suggest that a critical underlying mechanism of PCB-mediated endothelial cell activation and dysfunction is an increase in cellular oxidative stress (2,4,7). There is also evidence which suggests that the oxidative stress induced by specific environmental contaminants, i.e., polyhalogenated aromatic hydrocarbons like PCB 77 or TCDD, is due to the interaction of these compounds with the aryl hydrocarbon (Ah) receptor and activation of the cytochrome P450 1A subfamily (1,2,8). Induction of cytochrome P450 1A1 or 1A2 may lead to generation of reactive oxygen species (9) and thus exert cell injury. There is strong evidence that the vascular endothelium may be one of the major sites of PCB-mediated induction of cytochrome P450 1A1 (3,10), and that these enzymes play important roles in determining the metabolic fates of circulating protoxicants.

However, the mechanisms by which PCBs alter endothelial cell metabolism are not fully understood, and little is known about how PCB-mediated cell dysfunction can be prevented or blocked. We hypothesize that PCBs are atherogenic by causing endothelial cell dysfunction. We also propose that selected diet-derived lipids can potentiate and that antioxidant nutrients can downregulate disease-promoting and cytotoxic mechanisms of PCBs.

Materials and Methods

Endothelial cell culture: Endothelial cells were obtained from two sources, porcine pulmonary artery or aorta and human umbilical veins (HUVEC).

Experimental Design: PCBs levels in the range of up to 3.4 μM were chosen because they reflect serum concentrations after acute exposure to these environmental chemicals. Fatty acid concentrations used in our experimental settings ranged from 0 to 90 μM, assuming albumin concentrations of 30 to 60 μM in our culture media. Metabolic studies indicate that the molar ratio of free fatty acid to albumin is the main factor controlling free fatty acid availability to tissues. In humans, the vascular endothelium can be exposed to significant levels of free fatty acids derived from lipoprotein lipase-mediated hydrolysis of triglyceride-rich lipoproteins. Exposure times of endothelial cells to PCBs and/or fatty acids were up to 24 h. In some experiments, cells were first preenriched with antioxidant nutrients, such as vitamin E (25 μM), for 24 h prior to PCB treatment.

Oxidative stress: Cellular oxidative stress was determined using a cell imaging techniques based on reactive oxygen species-mediated conversion of 2',7'-dichlorofluorescin (DCF-H) into fluorescent 2',7'-dichlorofluorescein (DCF). Vitamin E was measured in cell and media samples by an isocratic non-aqueous reversed-phase HPLC method, which separates α-, β- and γ-tocopherol.

Endothelial barrier function in culture (albumin transfer studies): For the albumin transfer experiments, endothelial cells were plated on 13 mm diameter polycarbonate filters with 0.8 μm pores (Millipore Corp., Bedford, MA). Transendothelial flux of albumin was determined by measuring the change in absorbance at 630 nm following addition of bromcresol green (Sigma Chemical Company).

NF-κB activation studies (electrophoretic mobility shift assay): Nuclear extracts containing active proteins were prepared from cells and treated with ^{32}P-end-labeled oligonucleotide (Gibco/BRL, Gaithersburg, MD) probe containing the κB enhancer DNA element containing a tandem duplicate of a NF-κB binding site (-GGGGACTTTCC-).

Interleukin-6: Commercially available ELISA kits and a sensitive and reproducible *in vitro* bioassay using murine hybridoma cell line B9 were used for quantitating the IL-6 production.

Adhesion molecules: Activation of adhesion molecules in endothelial cells were estimated by immunofluorescence flow cytometry, i.e., by quantifying fluorescent-labeled antibodies against VCAM-1 and ICAM-1.

Statistical analysis: The data were analyzed using SAS (Statistical Analysis System). Comparisons between treatments were made by one-way ANOVA with post-hoc comparisons of the means made by Fischer's least significance difference method.

Results and Discussion

Polychlorinated biphenyls (PCBs), may be atherogenic by disrupting normal functions of the vascular endothelium. To investigate this hypothesis, we exposed porcine pulmonary artery-derived endothelial cells to 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3,4,4',5-pentachlorobiphenyl (PCB 114) or 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) for up to 24 hours. These PCBs were selected for their varying binding avidities with the aryl hydrocarbon (Ah) receptor and differences in their induction of cytochrome P450. PCB 77 and PCB 114 contributed markedly to cellular oxidative stress, as measured by 2,7-dichlorofluorescin (DCF) fluorescence and lipid hydroperoxides (1). Enhanced oxidative stress and $[Ca^{2+}]_i$ in PCB 77 and PCB 114 treated cells were accompanied by increased activity and content of cytochrome P450 1A and by a decrease in

the vitamin E content in the culture medium. These PCBs also disrupted endothelial barrier function by allowing an increase in albumin transfer across endothelial monolayers. In contrast to the effects of PCB 77 and PCB 114, cell exposure to PCB 153 had little or no effect on cellular oxidation, $[Ca^{2+}]_i$ or endothelial barrier function.

There is evidence that selected fatty acids, and especially n-6 (omega-6) unsaturated fatty acids, derived from the hydrolysis of triglyceride-rich lipoproteins, may be atherogenic by causing endothelial injury or dysfunction and subsequent endothelial barrier dysfunction (reviewed in 11). In support of this hypothesis, we have shown that saturated fatty acids in general had little effect on endothelial barrier function. On the other hand, unsaturated fatty acids, and mostly linoleic acid, can markedly disrupt endothelial barrier function, expressed as an increased transfer of both albumin and LDL across the endothelium. We hypothesize that selected dietary lipids may increase the atherogenicity of PCBs, by cross-amplifying mechanisms leading to dysfunction of the vascular endothelium. To investigate this hypothesis, we treated cultured endothelial cells with 90 μM linoleic acid (18:2n-6), followed by either one of two PCBs, PCB 77 or PCB 153. PCB 77 disrupted endothelial barrier function by allowing an increase in albumin transfer across endothelial monolayers. Prior cellular enrichment with linoleic acid before PCB treatment further diminished endothelial barrier function, as compared to cells treated only with the PCB. This phenomenon appeared to be mediated by increased oxidative stress, which was supported by enhanced 2,7-dichlorofluorescein fluorescence, as well as an observed decrease in vitamin E content in the culture media (4). Similar to the endothelial permeability data, pre-enrichment of cells with linoleic acid further increased the PCB-mediated induction of cytochrome P450 1A. In contrast to PCB 77, PCB 153 (or fatty acid plus PCB 153) had little or no effect on endothelial barrier function. Our results suggest that certain unsaturated fatty acids can potentiate PCB-mediated endothelial cell dysfunction and that oxidative stress and activation of the cytochrome P450 1A subfamily may be, in part, responsible for these metabolic events.

Our data also suggest that PCBs which are ligands for the Ah receptor (e.g., PCB 77) can activate the oxidative stress-sensitive transcription factor NF-κB. NF-κB plays a central role in regulating the cytokine network, and hence its activation may be a major consequence towards the pathogenesis of atherosclerosis. Many target genes in endothelial cells contain NF-κB or NF-κB-like binding sites in the promoter genes coding for inflammatory cytokines (e.g., tumor necrosis factor [TNF], interleukin-1 [IL-1], IL-6, IL-8) and adhesion molecules. Thus, activation of NF-κB by PCBs and selected lipids could result in increased expression of inflammatory cytokines and adhesion molecules, endothelial cell inflammation and increased monocyte infiltration and atherogenesis. We found that compared to control cultures, PCB treatment for 3 hours already activated NF-κB, with the highest activation at approximately 6 hours (2). Most interestingly, compared to a 6 hour treatment with fatty acid alone, exposure to linoleic acid for 3 hours followed by co-exposure to PCB 77 for an additional 3 hours (6 hour total exposure to linoleic acid) further increased activation of NF-κB. This suggests that unsaturated lipids can potentiate PCB-mediated endothelial cell activation. We also have evidence now that PCBs have inflammatory properties by promoting endothelial cell-mediated production of IL-6. Finally, we have preliminary data providing evidence that PCBs can lead to an inflammatory response and thus are atherogenic *in vivo*. To study the *in vivo* expression of adhesion molecules (VCAM-1 and ICAM-1), mice were injected intraperitoneally with PCB 77. Compared to the vehicle control, there was a positive staining for both VCAM-1 and ICAM-1 in the PCB-treated animals 24 hours after injection.

We also have tested the protective effects of antioxidants, such as vitamin E (α-tocopherol), on endothelial cell activation induced by PCB 77. Vitamin E completely blocked PCB 77-mediated endothelial barrier dysfunction. This protective effect by vitamin E was associated with a decrease in both oxidative stress, as measured by DCF fluorescence, as well as in NF-κB activation. Furthermore, vitamin E decreased PCB 77-mediated production of the inflammatory cytokine IL-6. Our findings suggest that exposure to specific environmental contaminants can trigger diseases of the vasculature, e.g., cardiovascular disease. In addition, high-fat diets may potentiate and diets high in antioxidant nutrients may protect against against PCB-mediated endothelial cell dysfunction.

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