PROINFLAMMATORY PROPERTIES OF COPLANAR PCBS

Bernhard Hennig[1,2], Purushothaman Meerarani[1], Michal Toborek[3], Alan Daugherty[4], Allen E. Silverstone[5] and Larry W. Robertson[2]

[1]Cell Nutrition Group, Department of Animal Sciences, [2]Graduate Center for Toxicology, [3]Department of Surgery, and [4]Department of Medicine, Gill Heart Institute; University of Kentucky, Lexington, KY 40546-0215; [5]Department of Microbiology and Immunology, SUNY Upstate Medical University, Syracuse, NY, USA.

Introduction

Dysfunction of vascular endothelial cells is a critical underlying cause of the initiation of cardiovascular diseases, such as atherosclerosis. In addition to endothelial barrier dysfunction, another functional change in atherosclerosis is the activation of the endothelium that is manifested as an increase in inflammatory aspects, such as the expression of specific cytokines and adhesion molecules. Certain environmental chemicals, such as PCBs TCDD, can cause vascular endothelial cell dysfunction (1-3), and a critical underlying mechanism of PCB-mediated endothelial cell activation and dysfunction is an increase in cellular oxidative stress. Specific environmental contaminants can induce oxidative stress via interaction of these compounds with the AhR and transcriptional activation of the cytochrome P450 1A subfamily. Induction of CYP1A1 or 1A2 may lead to oxidative stress as a result of excessive generation of reactive oxygen species, which can result in an imbalance in the cellular oxidative stress/antioxidant balance and thus cause cell injury. Recently, Schlezinger *et al.* (4) have demonstrated that PCB 77 can uncouple the catalytic cycle of cytochrome P4501A1, resulting in the formation of reactive oxygen species within the active site. There is strong evidence that the vascular endothelium may be one of the major sites of PCB-mediated induction of CYP1A1 (1,2), and that these enzymes play important roles in determining the metabolic fates of circulating toxic substances.

Transcriptional regulation of metabolic events leading to endothelial cell dysfunction and an inflammatory response induced by AhR agonists, such as coplanar PCBs, is not well understood. Our present data indicate that coplanar PCBs that function as AhR agonists (such as PCBs 77, 126 and 169), may be proinflammatory and atherogenic by activating NF-?B in vascular endothelial cells. We also provide *in vivo* evidence using an AhR-deficient mouse model that a functional AhR is critical for the proinflammatory events mediated by coplanar PCBs.

Materials and Methods

PCBs: PCBs were synthesized in our laboratory or purchased from Ultra Scientific (North Kingstown, RI).

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 PCBs, PCB 77 (3,3’,4,4’-tetrachlorobiphenyl), PCB 126 (3,3’,4,4’,5-pentachlorobiphenyl), or PCB 169 (3,3’,4,4’,5,5’-hexachlorobiphenyl) 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 previously (2,3,5).

Endothelial barrier function in culture (albumin transfer studies): For the albumin transfer experiments, endothelial cells were plated on 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).

Oxidative stress: Cellular oxidative stress was determined using cell imaging techniques based on reactive oxygen species-mediated conversion of 2',7'-dichlorofluorescin into fluorescent 2',7'-dichlorofluorescein (DCF).

NF-κB activation studies (EMSA): 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 possessing a tandem duplicate of a NF-κB binding site (-GGGGACTTTCC-).

RT-PCR: Total RNA was extracted by the use of TRI reagent and reverse-transcribed. The primer combinations used for VCAM-1 were: Sense: 5'-GTTTACCCGGTTGAAAAGTTGGAG-3'; Antisense: 5'-CACCGTGTCGCCTGTCTCT-3' and for CYP1A1: Sense: 5'-TGGAGAGGCAAGAGTAGTTGG-3'; Antisense: 5'-GGCACAACGGAGTAGCTCATA-3'.

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

Animal studies: The AhR-deficient mice used in this study have been characterized recently (6). The AhR+/+ and AhR-/- mice were derived from a heterozygous cross (+/-) from founder mice initially provided by P. Fernandez-Salguero and F. Gonzalez (National Cancer Institute, National Institutes of Health, Bethesda, MD). Mice were injected intraperitoneally with PCB 77 (170 μmoles/kg mouse) or the vehicle (stripped corn oil).

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.

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

There is evidence that exposure to PCBs can contribute to cardiovascular diseases such as atherosclerosis by promoting vascular endothelial cell dysfunction which predisposes the endothelium to inflammatory reactions (reviewed in 7). However, specific mechanisms by which coplanar PCBs cause endothelial cell activation and dysfunction and thus contribute to atherosclerosis are still unclear. We and others have demonstrated that certain environmental chemicals, such as PCBs, can cause vascular endothelial cell dysfunction (1-3). We also found that oxidative stress was induced only by the coplanar PCB 77 and not by the diortho-substituted PCB 153 (2,3). Presumably, this is due to the interaction of PCB 77 with the AhR and transcriptional activation of the CYP1A subfamily. We have evidence that, in addition to PCB 77, other coplanar PCBs, i.e., PCB 126 and PCB 169, also can induce oxidative stress in vascular endothelial cells. Our data also suggest that all three coplanar PCBs tested can induce cellular oxidative stress in a concentration-dependent manner, but that the concentration(s) needed for the apparent maximal induction of oxidative stress differs among these coplanar PCBs tested. Interestingly, PCB 126 exhibited maximal oxidative stress already at 0.5 μM, whereas the other two PCBs required 2.5 μM for maximal induction of cellular oxidative stress. These results may be explained by the fact that PCB 126 has comparatively the highest binding affinity for the AhR.

Some aspects of mechanisms by which environmental chemicals alter endothelial cell metabolism have been investigated. We have previously shown that PCB 77, a AhR ligand, but not PCB 153 (not a AhR ligand), significantly disrupted endothelial barrier function (2). PCB 77, but not PCB 153, also contributed markedly to cellular oxidative stress, which was accompanied by increased activity and

content of CYP1A and by a decrease in the vitamin E content in the culture medium. We also demonstrated that vitamin E and alpha-naphthoflavone (an AhR antagonist and inhibitor of CYP1A1) can markedly reduce PCB 77-mediated oxidative stress, activation of NF-κB and production of inflammatory cytokines (e.g., IL-6), as well as PCB-mediated endothelial barrier dysfunction (5). Furthermore, we studied the cellular glutathione redox status as a modulator of the endothelial defense against PCB toxicity (8), and we demonstrated that PCB 77 can induce a cellular stress response which is reflected by the activation of c-Jun N-terminal/stress-activated protein kinases (JNK/SAPK).

In our earlier studies we employed PCB 77 as a model AhR agonist. However, PCB 77 is also known to be a substrate for the induced CYP1A enzymes (2). A lingering question was whether the metabolic activation of PCB 77 also played a role in the associated oxidative stress events, as suggested by Dr. L. Birnbaum in Venice (Dioxin '99). Thus, our present study was extended beyond one coplanar PCB by including a series of coplanar PCBs, i.e., PCBs 77, 126, and 169. With increasing chlorination, the rate of metabolism would be greatly diminished. Should metabolic activation of PCBs markedly contribute to their biological effects, we could expect a marked decrease in proinflammatory properties of these coplanar PCBs relative to an increase in chlorination. However, this was not the case, and PCBs 126 and 169 were as efficacious as PCB 77 in disrupting endothelial barrier function, in causing oxidative stress and activation of NF-κB, in production of IL-6 and expression of the VCAM-1 gene in cultured cells.

Recent evidence suggests that the AhR agonist TCDD can activate NF-κB and AP-1, and it was proposed that CYP1A1-dependent and AhR complex-dependent oxidative signals are in part responsible for the observed activation of these transcription factors (9). We now also provide *in vivo* evidence using an AhR-deficient mouse model that a functional AhR is critical for the proinflammatory events mediated by coplanar PCBs and possible other AhR agonists. After a single administration of PCB 77, VCAM-1 expression was increased only in wild-type mice, while mice lacking the AhR gene showed no increased staining for VCAM-1.

In summary, our data suggest that coplanar PCBs can be atherogenic by producing an endothelial cell inflammatory response. We provide evidence that this inflammatory response is dependent on a functional AhR and that induction of CYP1A1 and activation of NF-κB are critical mechanistic mediators. Thus, our findings suggest that activation of the AhR is an underlying mechanism of endothelial cell stimulation mediated by certain environmental contaminants and that exposure to coplanar PCBs, and possibly other AhR agonists, may potentiate the pathology of cardiovascular diseases.

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