TEA FLAVONOIDS PROTECT AGAINST PCB-INDUCED VASCULAR ENDOTHELIAL INFLAMMATION

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Abstract

Tea flavonoids such as epigallocatechin gallate (EGCG) protect against vascular diseases such as atherosclerosis via their antioxidant and anti-inflammatory functions. Persistent and widespread environmental pollutants, including polychlorinated biphenyls (PCB), can induce oxidative stress and inflammation in vascular endothelial cells. Even though PCBs are no longer produced, they are still detected in human blood and tissues and thus considered a risk for vascular dysfunction. We hypothesized that EGCG can protect endothelial cells against PCB-induced cell damage via its antioxidant and anti-inflammatory properties. To test this hypothesis, primary vascular endothelial cells were pretreated with EGCG, followed by exposure to the coplanar PCB126. Exposure to PCB126 significantly increased cytochrome P450 1A1 (Cyp1A1) mRNA and protein expression and superoxide production events which were significantly attenuated following pretreatment with EGCG. Similarly, EGCG also attenuated PCB126-mediated induction of inflammatory markers such as monocyte chemotactic protein-1 (MCP-1) and vascular cell adhesion protein-1 (VCAM-1). Furthermore, EGCG decreased endogenous or base-line levels of Cyp1A1, and VCAM-1 in endothelial cells. Most of all, treatment of EGCG upregulated expression of phase II antioxidant enzymes, including glutathione S transferase (GST) and NAD(P)H:quinone oxidoreductase 1 (NQO1), in a dose-dependent manner. Pretreatment with all-trans retinoic acid, an inhibitor for antioxidant response element (ARE)-driven gene expression, resulted in an increase of Cyp1A1, MCP-1 and VCAM-1 expression, suggesting ARE-driven antioxidant enzymes play an important protective role against PCB-induced inflammatory responses in endothelial cells. These data show that EGCG may reduce inflammatory markers induced by PCB exposure via elevated expression of antioxidant genes.

Introduction

Epidemiological studies provide substantial evidence that vascular diseases are linked to exposure to environmental pollutants. For example, a recent study reported increased hospitalization rates for acute myocardial infarction and diabetes mellitus 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. We have demonstrated previously that PCBs, and in particular coplanar PCBs) can cause endothelial cell dysfunction as determined by inflammatory 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.

Our laboratory has provided strong evidence that nutrition can modulate the toxicity of environmental pollutants and thus modulate health and disease outcome associated with chemical insults. For example, we have found that specific dietary fats can further compromise endothelial dysfunction induced by selected PCBs and that antioxidant nutrients (such as vitamin E and dietary flavonoids) can protect against endothelial cell damage mediated by these persistent organic pollutants (3). Diets high in polyphenols (e.g., flavonoids) are associated with a reduced risk of chronic diseases, such as cardiovascular diseases, by affecting molecular mechanisms involved in the initiation and progression of these diseases. Flavonoids constitute a subclass of bioactive compounds rich in fruits and vegetables, soy food, legumes, tea and cocoa. Green tea consumption has been shown to be significantly greater in healthy subjects compared to those with coronary artery disease, suggesting

that green tea might be protective against coronary atherosclerosis. Catechins are the major constituents of the polyphenols in green tea, and the most abundant catechin in green tea is epigallocatechin-3-O-gallate (EGCG). Little is known about mechanisms, and in particular intracellular signaling pathways, responsible for the nutritional modulation of endothelial cell dysfunction induced by persistent environmental pollutants. In the current study we provide evidence that EGCG may reduce inflammatory markers induced by PCB exposure via elevated expression of antioxidant genes.

Materials and Methods

Cell culture and experimental media:
Primary vascular endothelial cells were isolated from porcine pulmonary arteries. Cells were cultures in M199 (Gibco, Grant Island, NY), supplemented with fetal bovine serum (FBS) (Gibco). Endothelial cells were grown to confluence, followed by incubation overnight in medium containing 5% FBS before initiation of cell treatments. Cells were pre-treated with EGCG (Cayman Chemical, Ann Arbor, MI) at 25-50 μM for 3 h and then treated with PCB126 (AccuStandard, New Haven, CT) for 16 h.

Real-time PCR:
Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Reverse transcription was performed using the AMV reverse transcription system (Promega, Madison, WI). The levels of mRNAs expression were then assessed by real-time PCR using 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA) and SYBR Green master mix (Applied Biosystems). Expressed mRNA levels were divided by the housekeeping gene β-actin. Primer sequences for SYBR Green chemistry were designed using the Primer Express Software 3.0 for real-time PCR (Applied Biosystems) and synthesized by Integrated DNA Technologies, Inc. (Coralville, IA).

Western blot analysis and ELISA:
Whole cells were lysed in RIPA lysis buffer (Cell Signaling Technology, Danvers, MA) containing protease and phosphatase inhibitor cocktails (Thermo, Waltham, MA). Following centrifugation, protein samples were separated on 10% SDS-PAGE followed by transferring onto nitrocellulose membranes. Membranes were blocked with 5% non-fat milk buffer and subsequently incubated overnight at 4°C with primary antibodies. Membranes were incubated with secondary antibodies conjugated with horseradish peroxidase, and blots were visualized by using ECL detection reagents (Thermo, Waltham, MA). Bands on film were quantified using UN-SCAN-IT software (Silk Scientific, Orem, UT) and normalized to β-actin. To measure MCP-1 protein levels, cell culture media were harvested and centrifuged. The supernatants were analyzed to measure MCP-1 protein levels using Quantikine ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer's instruction.

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, including PCB77 and PCB126, are proinflammatory and atherogenic in vascular endothelial cells (4,5). Our studies also suggest that membrane domains called caveolae play a critical role in the regulation proinflammatory events induced by PCBs (reviewed in (6). In addition to PCBs being able to induce an inflammatory response, these persistent environmental pollutants also can promote obesity and atherosclerosis (7).

Many mechanisms and signaling pathways associated with the pathology of inflammatory diseases are modulated by both dietary habits and environmental pollutants. In fact, numerous genes induced in diseases associated with vascular dysfunction such as atherosclerosis are oxidative stress-sensitive, suggesting that an imbalance in cellular oxidative stress and antioxidant status is a critical underlying factor. Evidence is emerging which suggests that antioxidant nutrients and related bioactive compounds common in fruits and vegetables

protect against environmental toxic insult to the vascular endothelium by down-regulation of signaling pathways involved in inflammatory responses associated with vascular diseases such as atherosclerosis (reviewed in 8).

Data from the current study provide further evidence of the anti-inflammatory properties of tea catechins such as EGCG. We demonstrated that EGCG can decrease PCB-induced expression of Cyp1A1 and endothelial inflammatory parameters via induction of antioxidant elements. Exposure to PCB126 significantly increased both message and protein expression of Cyp1A1 as well as superoxide production, events which were markedly attenuated when endothelial cells were first pre-enriched with EGCG. Similarly, EGCG also attenuated PCB126-mediated induction of inflammatory markers such as MCP-1 and VCAM-1. The observed reduction in inflammatory parameters may be due in part to the ability of EGCG to down-regulate the DNA-binding activity of the oxidative stress-sensitive transcription factor nuclear factor-kappa B (NF-κB) (9). Interestingly, EGCG also decreased baseline levels of Cyp1A1 and VCAM-1, suggesting that cellular protection by EGCG occurs even in the absence of a toxic stressor.

Mechanisms of protective properties of plant-derived flavonoids such as EGCG are not clear but may involve induction of phase II antioxidant enzymes (10). Indeed, in our endothelial cell system EGCG treatment markedly induced both GST and NQO1 in a dose-dependent manner. The EGCG-mediated increase in these phase II antioxidant enzymes was in part maintained even in the presence of PCB126. NQO1 and other phase II antioxidant genes, such as heme oxigenase (HO-1), are regulated by the transcription factor NF-E2-related factor (Nrf2). We have shown recently that EGCG treatment significantly induced nuclear accumulation of Nrf2 as well as enhancement of Nrf2-ARE binding at the HO-1 promoter site (11). All-trans retinoic acid has recently been shown to reduce the ability of Nrf2 to mediate induction of ARE driven genes (12). Indeed, endothelial cell pretreatment with all-trans retinoic acid further increased the PCB-mediated expression of Cyp1A1 and MCP-1, suggesting that ARE-driven antioxidant enzymes play an important protective role against PCB-induced inflammatory responses in endothelial cells. Thus, our data provide evidence that EGCG may reduce inflammatory markers induced by PCB exposure via elevated expression of antioxidant genes.

Acknowledgements

This study was supported in part by grants from NIEHS (P42ES007380), and the Kentucky Agricultural Experimental Station.

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