Dietary fat interacts with PCBs to induce changes in lipid metabolism in LDL receptor deficient mice

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INTRODUCTION

From epidemiological studies, there is substantial evidence that cardiovascular diseases are linked to environmental pollution and that exposure to polycyclic and/or polyhalogenated aromatic hydrocarbons can lead to human cardiovascular toxicity. For example, there was a significant increase in mortality from cardiovascular diseases among Swedish capacitor manufacturing workers exposed to PCBs for at least five years (reviewed in 1), and most excess deaths were due to cardiovascular disease in power workers exposed to phenoxy herbicides and PCBs in waste transformer oil. The observed cardiovascular risk factors may be associated with the ability of PCBs to modulate plasma and tissue lipids, events which can result in compromised lipid metabolism and lipid-dependent cellular signaling pathways. In a study with rhesus monkeys, a causal relationship between plasma lipid changes and PCB intake was observed following oral exposure of Aroclor 1254 (2). In addition to serum and vascular lipid changes, PCB administration also may modify liver and hepatic microsomal lipids. We have shown previously that a single injection of PCB 77 resulted in a marked change in the fatty acid composition of rat hepatic microsomal fractions (3).

A major route of exposure to PCBs in humans is via oral ingestion of contaminated food products (4). Therefore, circulating environmental contaminants derived from diets, such as PCBs, are in intimate contact with the vascular endothelium. Endothelial activation and dysfunction is an important factor in the overall regulation of vascular lesion pathology. In addition to endothelial barrier dysfunction, another functional change in atherosclerosis is the activation of the endothelium that 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. Alterations in lipid profile and lipid metabolism as a result of exposure to PCBs may be important components of endothelial cell dysfunction.

Little is known about the interaction of dietary fats and PCBs in the pathology of atherosclerosis. We have reported a significant disruption in endothelial barrier function when cells were exposed to linoleic acid (5). In the current study we aimed to demonstrate the PCB-fatty acid interaction *in vivo* and hypothesized that PCB toxicity can be modulated by the type of fat consumed.

MATERIALS AND METHODS

Animals, diets and PCB injection: The LDL-R-deficient mice used in this study were originally obtained from The Jackson Laboratory (Bar Harbor, ME; Stock Number: 002207) and bred at the University of Kentucky. Mice were divided into four groups of five mice per treatment: olive oil-rich diet; olive oil-rich diet plus PCB injection; corn oil-rich diet; and corn oil-rich diet plus PCB injection. Mice were injected intraperitoneally with PCB 77 (170 µmoles/kg mouse) or the vehicle (olive oil or corn oil; Dyets Inc., Bethlehem, PA) at two times, i.e., at weeks one and three of the four-week feeding study. This amount of PCB was based on calculated values from our *in vitro* experiments which were themselves based on levels that are usually found in humans after acute exposure. Diets were custom prepared and vacuum packed (Dyets Inc., Bethlehem, PA). Diets were based on a modified AIN-76A purified rodent diet (6) with varying sources of fat. The dietary fat content, either olive oil or corn oil, was 150 g/kg total diet. The antioxidant content of each diet and each oil was adjusted to be equal by the manufacturer with vitamin E acetate.

Immunostaining of aortic tissue: Aortic tissue from the thoracic regions was excised, immersed in OCT embedding medium, frozen at -20 °C, and 8 µm sections were cut on a cryostat. Immunocytochemistry was performed as described previously (7). Endogenous peroxidase was inactivated using hydrogen peroxide (3%) in methanol. Samples were blocked in the serum of the secondary antibody host. Primary antibodies for VCAM-1 (PharMingen, San Diego, CA) were detected using biotinylated secondary antibodies and peroxidase ABC kits (Vectastain, Burlingame, CA). Aminoethylcarbozole was used as chromogen, and sections were counterstained with hematoxylin.

Serum fatty acid analysis: Total plasma lipids were extracted using standard techniques. Internal standard (heneicosanoic acid, 5 µg in methanol) was added to the samples prior to lipid extraction. All solvents for liquid extraction contained 50 mg/L butylated hydroxytoluene (BHT) as an antioxidant. Lipids were dried under nitrogen followed by fatty acid esterification with BF_3-methanol. Fatty acid methyl esters were extracted with hexane for GC injection.

Neutral lipid staining of liver tissues: Liver sections were fixed overnight in 4% paraformaldehyde in PBS before embedding in OCT. Serial (10 mM) sections were mounted on MicroProbe slides and neutral lipids were stained with Oil Red O, as described previously (7).

Gene expression analysis: For microarray analysis, total RNA was isolated from snap frozen liver tissue using RNAeasy (Quiagen, Valencia, CA). RNA samples were pooled for analysis of two data sets per treatment group. RNA integrity analysis and biotin-labeling of cRNA was performed by the Microarray Core Facility at the University of Kentucky. Labeled RNA was spotted on Murine Genome MOE 430 chips (Affymetrix, Santa Clara, CA) and detected in the Affymetrix 428 Fluorescence Reader. Microarray data were confirmed by conventional RT-PCR. RNA was isolated from liver samples. cDNA was generated by reverse transcription and amplified by polymerase chain reaction. PCR products were separated on a 2% agarose gel and stained with SYBR gold and visualized using a phosphoimager (Fuji FLA-5000, Stamford, CT).

Statistical analysis: The data were analyzed using SYSTAT 7.0 (SPSS, Inc., Chicago, IL). Comparisons between treatments were made by one-way ANOVA with post-hoc comparisons of

the means made by Bonferroni least significance difference procedure. Statistical probability of $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

VCAM-1 expression is affected by diet and PCBs

VCAM-1 expression was negligible in mice fed the olive oil-enriched diet, whereas, corn oil-fed mice exhibited elevated VCAM-1 expression. In corn oil-fed mice, PCB treatment further increased VCAM-1 staining in aortic tissues. PCB treatment markedly increased VCAM-1 expression at the vascular surface in all animals, independent of dietary fat. Interestingly, PCB treatment increased VCAM-1 expression in smooth muscle-rich areas of the vessel in mice fed the corn oil-enriched diet. This phenomenon was not observed in mice fed the olive oil-enriched diet. These data confirm our *in vitro* data showing that PCB 77 can increase expression of cytokines, such as IL-6 and adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), in cultured endothelial cells Furthermore, PCB 77 toxicity could be amplified by cotreatment with linoleic acid (1). VCAM-1 expression is an important marker for endothelial cell dysfunction and is a critical factor in the invasion of the subendothelial space by monocytes.

PCB treatment increases diet-dependent clearance of serum fatty acid

As expected, feeding a diet enriched with olive oil resulted in elevated serum oleic acid levels. Similarly, corn oil feeding resulted in elevated levels of serum linoleic acid. PCB treatment had little effect on fatty acid patterns in animals fed the olive oil diet. In contrast, PCB treatment of corn oil-fed mice resulted in marked decreases in major serum fatty acids, with a quantitatively most significant serum clearance of serum linoleic acid. This may reflect increased uptake of linoleic acid within the vascular endothelium. Thus, our present data from plasma of LDL receptor-deficient mice support the hypothesis that linoleic acid-rich dietary oils, such as corn oil, facilitate clearance of linoleic acid from plasma into vascular tissues, events which could exacerbate fatty acid- and/or PCB-induced oxidative stress and a vascular inflammatory response. The amplified toxicity of linoleic acid and PCBs to endothelial cells could also be mediated by cellular accumulation of this fatty acid and thus its availability as a substrate for the formation of cytotoxic epoxide metabolites (8). Due to the very low basal activity of endothelial cell delta 6-desaturase, arachidonic acid is not produced from linoleic acid significantly in this type of cell (9), which can result in linoleic acid accumulation within endothelial cells (9,10).

PCBs increase neutral lipid staining in liver tissue

Baseline or control lipid staining (Oil Red O) appeared to be similar in liver tissues from both olive oil and corn oil fed mice. In contrast to the olive oil group, PCB exposure further increased neutral lipid staining only in LDL-R-/- mice fed the corn oil-enriched diet. We have previously reported an increase in liver and hepatic microsomal lipids (total lipids, phospholipids, neutral lipids and cholesterol) following PCBs administration (3,11). The liver lipid accumulation seen in our current study could be due to a PCB-mediated impairment of lipoprotein formation. In fact, the DNA Microarray data revealed a downregulation of the apolipoprotein A IV (apoAIV) gene in PCB treated animals. Because apoAIV is involved in the formation of triglyceride rich lipoproteins, a decreased expression of this gene could lead to an impaired export of lipids from the

liver. Indeed, the group (corn oil plus PCB) with the lowest expression of apoAIV had the most intense staining for neutral lipids in the liver.

PCBs modulate genes involved in fatty acid metabolism

Gene microarray analysis was used to determine global gene expression changes in livers of LDL-R-/- mice and the overall response to interactions of dietary fat and PCB treatments. PCB treatment up-regulated genes involved in fatty acid uptake and catabolism, e.g., the fatty acid uptake receptor CD36, and the enzyme carnetine-palmetoyl-CoA transferase, a critical regulatory enzyme in fatty acid beta-oxidation only in mice fed the corn oil enriched diet. On the other hand, PCB treatment down-regulated genes involved in fatty acid synthesis, such as acetyl-CoA carboxylase, in corn oil fed mice. Of interest also was the cyotochrome P450 enzyme (CYP1A1), which is critical in the metabolism of PCBs (such as PCB 77). Compared to olive oil, CYP1A1 was up-regulated by corn oil feeding. Microarray analysis of selected genes were confirmed by conventional RT-PCR. These data suggest that PCB-lipid interactions are dependent on the type of dietary fat. This suggests that dietary oils rich in linoleic acid, such as corn oil, can amplify compromised gene expression during PCB cytotoxicity.

ACKNOWLEDGEMENTS

This study was supported in part by grants from NIEHS/NIH (ES 07380), USDA (2001-35200-10675), and the Kentucky Agricultural Experimental Station.

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