Dietary Flavonoids Block PCB-Induced Proinflammatory Responses in Vascular Endothelial Cells

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Abstract:
Polychlorinated biphenyls (PCBs) are widespread environmental contaminants that can cause a wide variety of toxic effects in exposed organisms. Co-planar PCBs can induce oxidative stress and activation of pro-inflammatory signaling cascades which are associated with atherosclerosis. Vascular endothelial cells have been shown to be sensitive to chemical insult and cellular dysfunction after co-planar PCB exposure. The majority of the toxicological effects elicited by co-planar PCB exposure are associated with activation of the aryl hydrocarbon receptor (AHR) and subsequent induction of pro-inflammatory genes. Quercetin, and related dietary flavonoids, have been demonstrated to possess antioxidant and anti-inflammatory properties in various in vitro and in vivo models. Previous studies from our group have shown that flavonoids can significantly reduce PCB77 induction of the pro-inflammatory response in the aorta. In the current study, we demonstrate that quercetin and kaempferol block PCB77 and 126 induced expression of AHR responsive and pro-inflammatory genes associated with atherosclerosis, porcine endothelial cells were exposed to PCB77 or 126 in combination with quercetin and kaempferol. The expression of proinflammatory proteins was evaluated by western blot. Upon co-incubation, cells were serum deprived for 8 h, then treated with PCB77 (1 μM), quercetin (10 μM), or PCB77 plus quercetin for a period of 16 h. Quercetin co-treatment significantly blocked PCB77 induction of the pro-inflammatory and inflammatory proteins. CYP1A1 and vascular cell adhesion molecule 1 (VCAM1). Co-treatment with isorhamnetin or kaempferol altered PCB77 and 126 induced protein expression of the AHR responsive proteins; CYP1A1 and VCAM1. These results suggest that quercetin, isorhamnetin and kaempferol, can block co-planar PCB activation of the AHR pathway and induction of pro-inflammatory genes. (Supported by grants from NIH, NIEHS, P42ES07386 and the University of Kentucky AES).

Keywords: Vascular endothelium, AHR, PCB, CYP1A1, CYP1B1, VCAM1, inflammation, atherosclerosis.

Background:
• In vascular endothelial cells co-planar PCBs such as PCB77 and PCB126 increase cellular damage and activation through activation of the aryl hydrocarbon receptor (AHR) and other pro-inflammatory transcription factors such as nuclear factor kappa B (NFkB).
• Activation of these pathways leads to subsequent expression inflammatory genes such as CYP1A1, CYP1B1 and vascular cell adhesion molecule 1 (VCAM1) (Sim et al., 1999, Remedios et al., 2003).
• Induction of CYP1A1 protein expression and enzymatic activity by co-planar PCBs is associated with increased generation of reactive oxygen species.
• VCAM1 is an adhesion molecule expressed on the luminal surface of activated endothelial cells. Leucocytes bind to VCAM1 and other adhesion molecules and transmigrate through the endothelial layer.
• Flavonoids are polyphenolic compounds that are widely distributed in plants, fruits and vegetables.
• Quercetin and isorhamnetin are flavonoids commonly found in fruits and vegetables that form part of the human diet.
• Various studies have shown strong correlations between dietary intake of flavonoid rich food and decreased cardiovascular disease.
• Previous studies from our laboratory have shown that the flavonoid epigallocatechin-3-gallocatechin and quercetin can block PCB77 induction of reactive oxygen species production and AHR activation in porcine endothelial cells. However, little is known about other polyphenols that also form part of the human diet.

Objectives:
The experiments described below were designed to answer the following questions:
• Do flavonoids inhibit co-planar PCB induction of the pro-inflammatory genes; VCAM1?
• Can quercetin, isorhamnetin and kaempferol inhibit co-planar PCB induction of AHR and induction of reactive oxygen species (CYP1A1 and CYP1B1)?

Methods:
Cell culture and treatment:
Porcine aortic end arteries were isolated from post-mortem atherosclerotic aortas as described previously (Toborek et al., 2002). MCF-7 cells stably transfected with either AHR workshop-flxed or AHR workshop-flxed knock-out plasmid were obtained from Cellect Technologies (Philadelphia, USA). Cells were cultured in reduced RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO₂. Quercetin, isorhamnetin and kaempferol were added from a stock solutions prepared in DMSO. All treatment groups contained an equal volume of DMSO. Endothelial cells (ECs) were isolated from post-mortem atherosclerotic aortas as described previously (Toborek et al., 2002). MCF-7 cells stably transfected with either AHR workshop-flxed or AHR workshop-flxed knock-out plasmid were obtained from Cellect Technologies (Philadelphia, USA). Cells were cultured in reduced RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO₂. Quercetin, isorhamnetin and kaempferol were added from a stock solutions prepared in DMSO. All treatment groups contained an equal volume of DMSO. Endothelial cells (ECs) were isolated from post-mortem atherosclerotic aortas as described previously (Toborek et al., 2002). MCF-7 cells stably transfected with either AHR workshop-flxed or AHR workshop-flxed knock-out plasmid were obtained from Cellect Technologies (Philadelphia, USA). Cells were cultured in reduced RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO₂. Quercetin, isorhamnetin and kaempferol were added from a stock solutions prepared in DMSO. All treatment groups contained an equal volume of DMSO. Endothelial cells (ECs) were isolated from post-mortem atherosclerotic aortas as described previously (Toborek et al., 2002). MCF-7 cells stably transfected with either AHR workshop-flxed or AHR workshop-flxed knock-out plasmid were obtained from Cellect Technologies (Philadelphia, USA). Cells were cultured in reduced RPMI 1640 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 5% CO₂. Quercetin, isorhamnetin and kaempferol were added from a stock solutions prepared in DMSO. All treatment groups contained an equal volume of DMSO.

Results:
• Total cellular protein was extracted as previously described (Sim et al., 1999). Protein extracts were electrophoresed on SDS-PAGE and transferred to nitrocellulose membranes. CYP1A1, CYP1B1 and VCAM1 proteins were probed with commercially rabbit and goat anti-proteins. blots were used as a loading control or normalizing expression of protein of interest.

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• PCB77 was kindly provided by Larry W. Robinson PhD, University of Texas.

Conclusions:
• This study’s findings demonstrate that the dietary flavonoids quercetin, isorhamnetin and kaempferol can block the expression of pro-inflammatory proteins VCAM1, CYP1A1 and CYP1B1 caused by coplanar PCB77 and PCB126 exposure. This suggests that quercetin-like flavonoids, commonly found in fruits, vegetables, green tea and red wine are effective at inhibiting endothelial cell activation caused by co-planar PCB induction of AHR-responsive and pro-inflammatory genes.

References:

Possible mechanism for Quercetin, Isorhamnetin and Kaempferol mediated inhibition of PCB77 & 126 induced inflammation

Kaempferol (Kmp) blocks PCB77 induction of CYP1A1 and CYP1B1 protein expression

Quercetin (QE) blocks PCB126 induction of CYP1A1 protein expression after 16hrs of co-exposure

Quercetin (QE) blocks PCB126 & 77 induction of CYP1A1 and CYP1B1 protein expression

Quercetin and Isorhamnetin block PCB77 induction of VCAM1 and CYP1A1 protein expression after 16 h of co-exposure.