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

Xabier Arzuaga[1,2], Chase T. Kluemper[3], Michal Toborek[2,4] and Bernhard Hennig[1,2].
Molecular and Cell Nutrition Laboratory, College of Agriculture[1], Graduate Center for Nutritional Sciences[2], Department of Biology (Undergraduate Research Rotation)[3], Department of Surgery[4], University of Kentucky Lexington 40536-0200, USA.

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 to activation of the aryl hydrocarbon receptor (AHR) and subsequent induction of responsive genes. Quercetin, and related dietary flavonoids, have been demonstrated to possess antioxidant and anti-inflammatory properties in various *in vivo* and *in vitro* models. Previous studies from our group have shown that flavonoids can significantly reduce PCB77 induction of oxidative stress and expression of the AHR responsive gene cytochrome P450 1A1 (CYP1A1). To determine if the flavonoids quercetin, isorhamnetin and kaempferol can 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 expression of proinflammatory proteins was analyzed by western blot. Upon confluence, 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-oxidative 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 CYP1B1. These results suggest that quercetin, isorhamnetin and kaempferol, can block co-planar PCB activation of the AHR pathway and induction of responsive pro-inflammatory genes. (Supported by grants from NIEHS, NIH (P42ES07380) and the University of Kentucky AES).

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

Background:

- In vascular endothelial cells coplanar 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 (NFκB).
- Activation of these pathways leads to subsequent expression inflammatory genes such as CYP1A1, CYP1B1 and vascular cell adhesion molecule 1 (VCAM1) (Slim *et al.*, 1999; Ramadass *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 an adhesion molecule expressed on the luminal surface of activated endothelial cells. Leukocytes 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 flavonoids epigallocatechin-3-gallate and quercetin can block PCB77 induction of reactive oxygen species production and AHR activation in porcine endothelial cells. However, little is know 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: VCAM-1?*
- *Can quercetin, isorhamnetin and kaempferol inhibit co-planar PCB activation of the AHR and induction of responsive genes (CYP1A1 and CYP1B1)?*

Methods:

Cell culture and experimental media

Endothelial cells (ECs) were isolated from porcine pulmonary arteries as described previously (Toborek *et al.*, 2002). MCF-7 cells stably transfected with a luciferase gene driven by PPRE sites were utilized for selected experiments. Cells were sub-cultured in medium-199 (endothelial cells) and DMEM (MCF-7 cells) containing fetal bovine serum (FBS, HyClone Laboratories, Logan, UT) using standard techniques. PCB77, 126 and flavonoids were added from a stock solutions prepared in DMSO. All treatment groups contained an equal amount of DMSO.

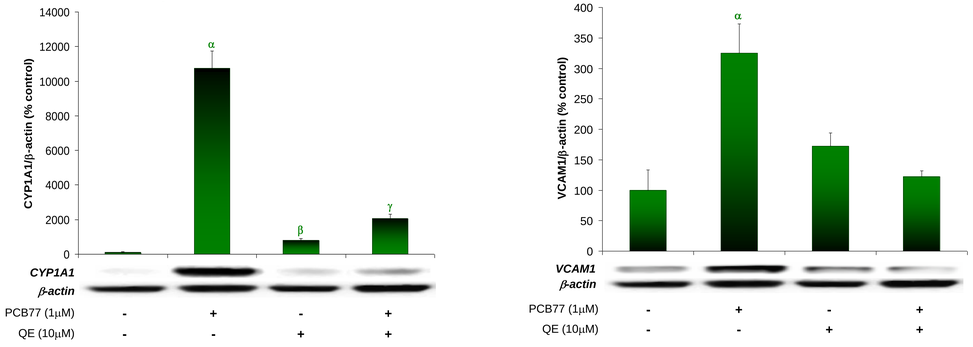
CYP1A1, CYP1B1 and VCAM1 protein expression studies

Total cellular protein was extracted as previously described (Slim *et al.*, 1999). Protein extracts were electrophoresed on SDS-polyacrilamide gels transferred to nitrocellulose membranes. CYP1A1, CYP1B1 and VCAM1 proteins were probed with commercial rabbit and goat antibodies. β-actin was used as a loading control for normalizing expression of proteins of interest.

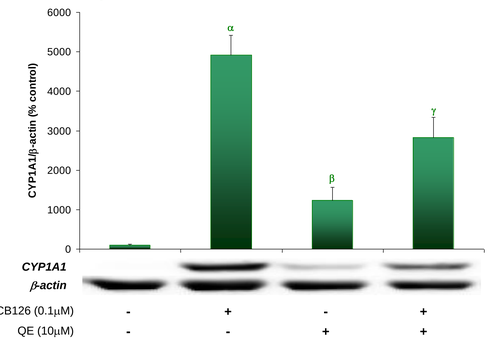
Statistics

Data were analyzed using one or two way ANOVA, depending on the number of variables used in the experiments, followed by Tukey's or Fisher's LSD test for post hoc comparisons.

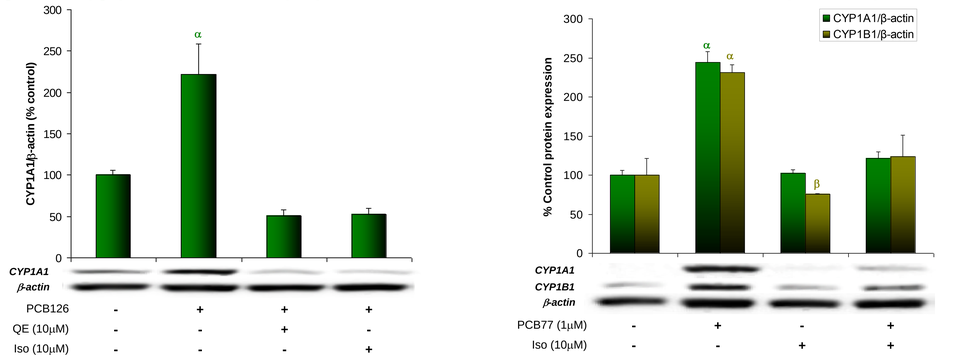
Quercetin and Isorhamnetin block PCB77 induction of VCAM1 and CYP1A1 protein expression after 16 h of co-exposure.



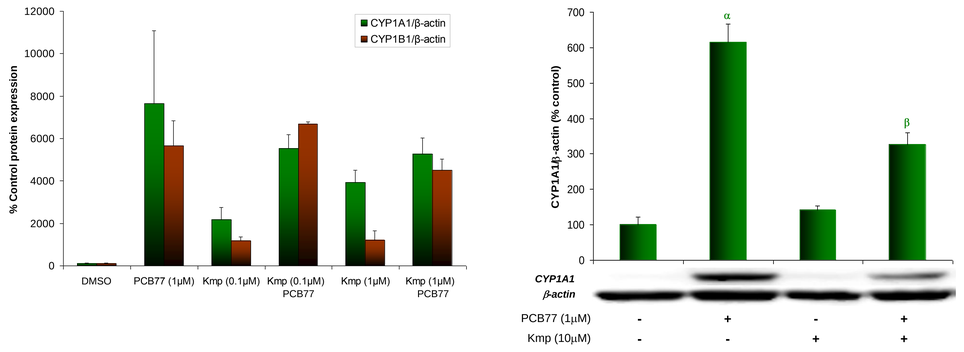
Quercetin (QE) blocks PCB126 induction of CYP1A1 protein expression after 16hrs of co-exposure



Quercetin (QE) and Isorhamnetin (Iso) block PCB126 & 77 induction of CYP1A1 and CYP1B1 protein expression



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



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

Conclusions:

In summary, our findings demonstrate that the dietary flavonoids quercetin, isorhamnetin and kaempferol can block the of 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:

Hamada, M., Satsu, H, Natsume, Y., Nishiumi, S., Fukuda, I., Ashida, H., Shimizu, M., (2006) *J. Agric. Food Chem.* **54**, 7692-7702.
Ramadass, P., Meerarani, P., Toborek, M., Robertson, L. W., and Hennig, B. (2003) *Toxicol Sci* **76**, 212-219.
Slim, R., Toborek, M., Robertson, L. W., and Hennig, B. (1999) *Toxicol Sci* **52**, 232-239.
Stegeman, J. J., Hahn, M. E., Weisbrod, R., Woodin, B. R., Joy, J. S., Najibi, S., and Cohen, R. A. (1995) *Mol Pharmacol* **47**, 296-306.
Toborek, M., Lee, Y. W., Kaiser, S., and Hennig, B. (2002) *Methods Enzymol* **352**, 198-219.

Acknowledgements:

- **This study was supported by grants from NIH (P42ES07380), the University of Kentucky AES and the University of Kentucky Lyman T. Johnson Postdoctoral Fellowship.**
- **PCB77 was kindly provided by Larry W. Robertson PhD, University of Iowa.**