Dietary DHA Promotes an Anti-oxidant Response in Mice Exposed to Environmental Pollutants

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Abstract

Consumption of fish oil is associated with improved coronary health outcomes, but the mechanism of protection is not well understood. Environmental stressors, such as polychlorinated biphenyls (PCBs), contribute to the development of cardiovascular diseases like atherosclerosis. We study the protective benefits of nutrients such as the polyunsaturated omega-3 fatty acids found in fish oil in reducing the inflammatory response to PCBs. To further our understanding of the mechanism by which the omega-3 fatty acid, docosahexaenoic acid (DHA), is protective, we fed C57BL/6 mice diets enriched in DHA (3% DHA/22% safflower oil, % kcal) or a safflower control diet for four weeks before administration of the coplanar PCB 126 via oral gavage at week five. Liver samples were analyzed by microarray and real time-PCR to examine the interactive effects of PCBs and DHA on the nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) anti-oxidant response pathway. Microarray data revealed that animals fed DHA and treated with PCBs had decreased expression of kelch-like ECH-associated protein 1 (Keap1), a cytosolic inhibitor of Nrf-2 activity. In addition, an increase was observed in the expression of peroxisome proliferator-activated receptor gamma (PPARγ), a positive co-activator of Nrf-2, and its chaperone PPARγ co-activator 1a (PPARγc1a). Real time- PCR analysis revealed increased expression levels of Nrf-2 activated anti-oxidant enzymes, NAD(P)H dehydrogenase (quinone 1) (NQO1) and hemeoxygenase 1 (HO-1) in PCB treated mice on a DHA diet. Together these data suggest that DHA promotes an anti-oxidant response in mice exposed to environmental pollutants such as PCBs.

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Experimental Design and Rationale

Experimental Design

Table 1. Experimental design of 6-week DHA feeding study with PCB 126 exposure.

WEEK	1	2	3	4	5	6
Diet [a]						
Oral Gavage [b]						
Glucose Tolerence Test [c]						
Blood Collection [d]						
Urine/Feces Collection						
ECHO MRI						
Takedown						

[a] Animals maintained on control diet 25% kcal from fat (safflower oil) or DHA diet 3% DHA/22% Safflower oil diet (% kcal).

[b] Oral gavage of 5.0 µmol/kg PCB 126 or safflower oil, vehicle control

[c] Intraperitoneal injection of 2 mg/g 20% glucose solution.

[d] Retro-orbital blood collection used at week 5 and cardiac puncture at takedown.

Rationale

- Docosahexaenoic acid (DHA) is an omega-3 fatty acid with anti-inflammatory properties (Chapkin et al. 2009).
- Coplanar PCBs, such as PCB 126, activate the aryl hydrocarbon receptor (AhR) generating reactive oxygen species (ROS) and stimulating pro-inflammatory endpoints including cytochrome P450 1A1 (CYP1A1), whose protein uncoupling is linked to higher levels of cellular ROS and the release of inflammatory cytokines via NFκB (Hennig et al. 2002, Ramadass et al. 2003).
- Nrf-2, a nuclear transcription factor, regulates anti-oxidant response and is co-regulated by PPARγ (Park et al. 2004).
- We have demonstrated *in vitro* that α-linolenic acid reduces inflammatory response to PCB stimulation (Wang et al. 2008) and that oxidized DHA promote anti-oxidant response to PCB-induced inflammation through activation of Nrf-2 (Majkova et al. 2011).

Hypothesis

- We hypothesize that dietary DHA will promote an anti-oxidant response through modulation of the transcription factors Nrf-2 and PPARγ.

Results

Microarray Transcript Analysis of Key Nrf-2 Regulators

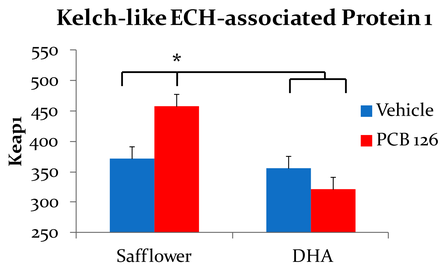

Figure 1. Dietary DHA inhibits Keap1 expression changes in PCB 126-treated mice. Microarray analysis was performed on liver RNA. Keap1 expression in Safflower oil fed, PCB-treated mice was increased over all other groups. PCB-treated, DHA fed mice did have significant changes in Keap1 expression. (* P < 0.05)

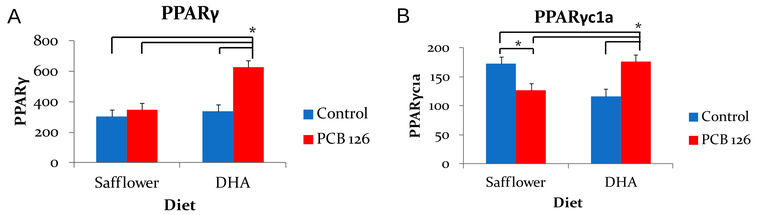

Figure 2. Dietary DHA increases PPARγ and PPARγc1a expression changes in PCB 126-treated mice. Microarray analysis was performed on liver RNA. (A) DHA fed mice have significant increases in PPARγ in response to PCB treatment. (B) PPARγc1a expression is differentially modulated by diet and PCB co-treatment with DHA:PCB co-treatment significantly increasing expression.

Anti-oxidant Response Enzymes are Up-regulated Through Modulation of Nrf-2

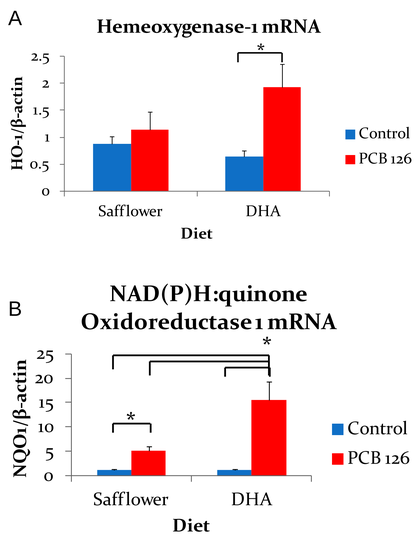

Figure 3. DHA-feeding promotes anti-oxidant gene expression in PCB 126 treated mice. (A) HO-1 and (B) NQO1 mRNA expression levels are increased in mice co-treated with DHA diet and PCB 126. (*P<0.05)

Conclusion

- Keap1 inhibition of Nrf-2 is increased in control fed mice treated with PCBs indicating an impaired anti-oxidant response.
- PCB treatment does not significantly affect Keap1 expression in DHA fed mice, which allows for robust upregulation of anti-oxidant enzymes.
- PPARγ and PPARγc1a expression is increased in PCB-treated, DHA fed mice, which promotes Nrf-2 and anti-oxidant enzyme expression.
- In response, to changes in Nrf-2 regulation anti-oxidant response genes are positively increased following PCB-treatment in DHA fed mice.

Proposed Mechanism of Protection by DHA

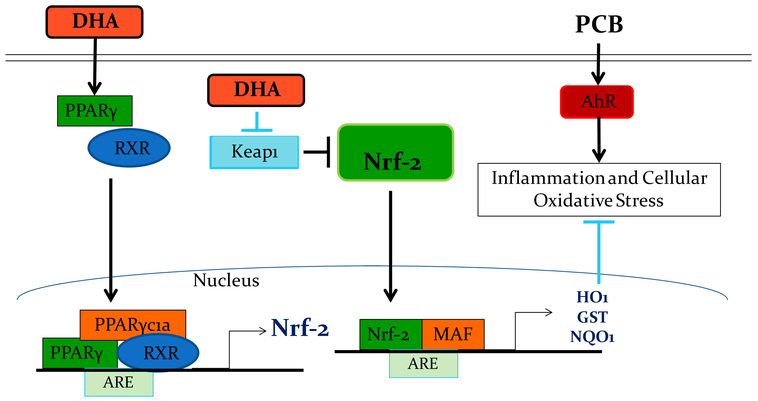

Figure 4. DHA promotes Nrf-2 expression and activation in response to PCB 126-induced ROS production. PCB 126 binding with the AhR promotes an inflammatory response. In response to PCB-induced cellular stress, the cell utilizes DHA to activate anti-oxidant enzyme systems through increased activity of the nuclear transcription factors, PPARγ and Nrf-2.

Future Directions

- Explore the mechanistic and metabolic changes induced by PCB 126 to "activate" DHA-induced protective mechanisms:
  - Investigate the formation of DHA metabolites in key tissues.
  - Assess DHA metabolizing enzymes through mRNA and protein.
- Utilize *in vitro* techniques to investigate the regulation of Nrf-2 and PPARγ by DHA and DHA metabolites.

Methods

- ***Animal Work:*** Male C57BL/6 mice were housed and handled according to a protocol approved by the University of Kentucky IACUC. After acclimation, animals were fed a humanized safflower oil control diet (25%kcal fat) or a DHA-enriched diet for 4 weeks (3%kcalDHA/22%kcal safflower). Animals were gavaged twice with 5.0 µmol/kg PCB 126 on Day 1 and received a second gavage of the same dose on Day 7. Animals were sacrificed and tissues were collected for analysis.
- ***Microarray:*** Microarray was performed in conjunction with University of Kentucky Microarray Core Facility using Affymetrix Genechip Mouse Exon 1.0 ST Arrays. RNA was isolated from liver samples and RIN values were assessed to ensure integrity. Samples from 3 mice were pooled and applied to one chip with a total of 3 chips per treatment group.
- ***Real time – PCR:*** mRNA was isolated from homogenized liver tissues using the Trizol® method. Isolated mRNA was resuspended in RN/DNAse free water and quantified using UV-vis spectroscopy at 260nM. cDNA was made using a reverse transcriptase reaction. RT-PCR of HO-1, NQO1, and β-actin was performed using SYBR® Green reagent.(Han *et al*., 2010)
- ***Statistical analysis:*** Comparisons of real time-PCR data were made by two-way analysis of variance followed by Tukey's post-hoc test using SigmaPlot 12.0 software. Statistical probability of P<0.05 was considered significant. Microarray data were analyzed using multivariate analysis in collaboration with the University of Kentucky Department of Statistics.

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