Carbaryl Exposure to *Danio rerio* Leads to Activation of the Aryl Hydrocarbon Receptor Pathway

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Abstract

Xenobiotics are foreign biological chemicals present in an environment/ecosystem. Pesticides are common examples of xenobiotics which are becoming more and more prevalent- especially in waterways. Carbaryl is a commonly used insecticide within the U.S. that acts to inhibit the acetylcholinesterase (AChE) enzyme in muscle tissues- leading to insect death. While AChE inhibition is well documented, carbaryl is also hypothesized to bind to the aryl hydrocarbon receptor (AhR) and activate expression of the *cytochrome P450* (*cyp1*) genes. Zebrafish, *Danio rerio,* were exposed to either carbaryl or 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD)-a known aryl hydrocarbon receptor agonist, and RNA was extracted after 24 hours of exposure. cDNA was synthesized and used to quantify gene expression of the *cyp1* genes using qPCR. Gene expression analysis showed that both carbaryl and TCDD exposure resulted in a comparable increase in expression of both *cyp1a* and *cyp1b* genes. TCDD, but not carbaryl, exposure lead to increased expression of *cy1c1* and *cyp1c2* genes. The data support the hypothesis that carbaryl does activate the AhR pathway and ultimately increase expression of some *cyp1* genes (*cyp1a* and *cyp1b*) in zebrafish. To confirm the increase in gene expression is AhR dependent, the next step is to use a morpholino to block AhR receptor expression to see if that eliminates the increase in *cyp1* gene expression. It is unclear why TCDD exposure, but not carbaryl, resulted in increased *cyp1c1* and *cyp1c2* expression.

Introduction

- **Carbaryl**- Found in insecticide, Sevin, and is a reversible inhibitor of AChE (reviewed in Grinnell, 1995)
  - Causes paralysis to insects and is toxic to humans and other non-target species (Behra et al., 2002)
  - Exposure causes embryonic lethality, cardiac defects, and neuronal toxicity in zebrafish (Lin et al. 2007)
- **Dioxin, TCDD** – AhR ligand shown to be a developmental toxicant in zebrafish (Carney et al. 2006)
  - Developmental toxicity manifested in zebrafish larvae include impaired heart and vascular development and arrested growth (Carney et al. 2006)
- **Cytochrome P450 genes**- induced by aryl hydrocarbon receptor ligands when the cells are exposed to exogenous chemicals (Goldstone et al. 2010)
  - *Cyp1* genes- metabolize drugs and pollutants, helping in the degradation of foreign chemicals (Goldstone et al. 2010)

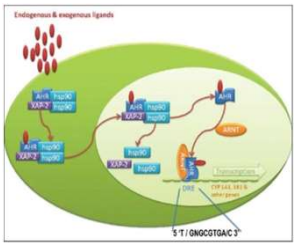


Figure 1. Schematic of AhR pathway. Upon the binding of a ligand the AhR complex translocates to the nucleus and binds to ARNT, inducing the transcription of dioxin-responsive elements (Zhang, 2011).

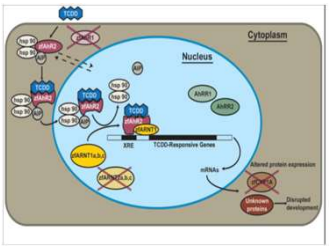
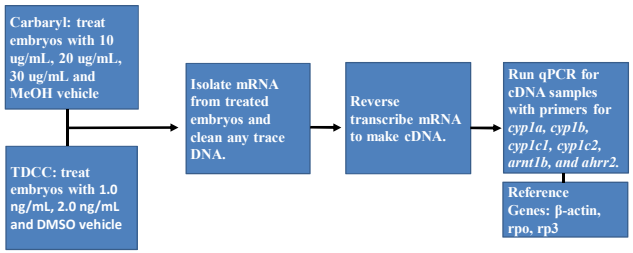


Figure 2. AhR signaling pathway when dioxin, TCDD binds to AhR2 receptor, translocating to the nucleus, forming an AhR/ARNT complex and causing the transcription of target genes (Carney et al. 2006).

Hypothesis and Prediction

Zebrafish embryos treated with both carbaryl or TCDD will show an increased expression of cytochrome P450 and AhR pathway genes. An increase in gene expression by carbaryl suggests that carbaryl is a ligand for the AhR pathway, like TCDD.

Methods



Results

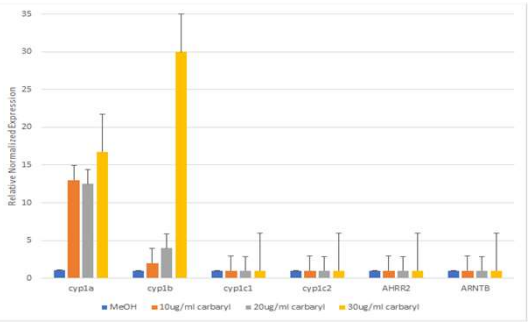


Figure 3. qPCR analysis of cytochrome P450 and AhR pathway gene expression in MeOH, 10 ug/mL, 20 ug/mL and 30 ug/mL carbaryl treated zebrafish embryos. Increased expression of *cyp1a* and *cyp1b* genes and no expression of *cyp1c1, cyp1c2, ahrr2 and arntb genes* when treated with carbaryl was observed.

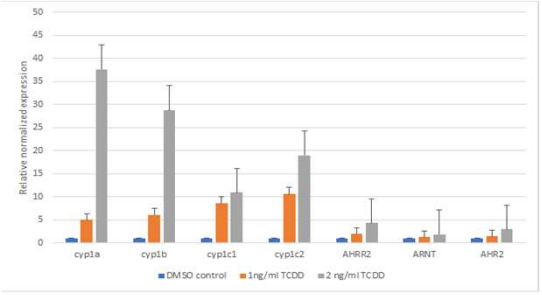


Figure 4. qPCR analysis of cytochrome P450 and AhR pathway gene expression in DMSO, 1 ng/mL, and 2 ng/mL TCDD treated zebrafish embryos. Increased expression of all genes tested (*cyp1a, cyp1b, cyp1c1, cyp1c2, ahrr2, arnt, and ahr2*) when treated with TCDD was observed.

Discussion

- *Cyp1a* and *cyp1b* gene expression is upregulated as the concentration of carbaryl and TCDD increases
- There is a direct relationship with the carbaryl and TCDD concentration used and the increase in *cyp1b* gene expression
- TCDD induced gene expression from all genes tested
- There is no change in gene expression of *cyp1c1*, *cyp1c2, ahrr2,* and *arnt* when treated with carbaryl, but when treated with TCDD there is an increase in *cyp1c1* and *cyp1c2* gene expression
- There is a direct relationship with the TCDD concentration used and the increase in *cyp1a* gene expression, that was not observed with carbaryl

Carbaryl showed an increase in gene expression for cytochrome P450 genes, which was also exhibited in TCDD. This relationship suggests that carbaryl induces expression of these genes via the AhR pathway, making it a possible ligand for this specific receptor. TCDD has already been shown, through previous research, to be a ligand for the aryl hydrocarbon receptor to induce cytochrome P450 gene expression, so by carbaryl showing an increase in these same genes implies that it too may be a ligand for this receptor.

Future Work

A morpholino oligonucleotide will be used to ablate the aryl hydrocarbon receptor. This blocks the receptor from binding to ligands. After blocking the pathway the same genes tested in this research will be tested on the morphant embryos to study how this effects gene expression when treated with carbaryl. The results of the carbaryl treated embryos will be compared to that of TCDD treated embryos, as a positive control, since TCDD has already been shown to induce expression through the AhR pathway.

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