Background

- Exposure of fishes to dioxin-like compounds can result in a wide range of adverse effects, including developmental malformation and edema, in early life stages; wasting syndrome, fibrosis, hepatotoxicity, immune and endocrine dysfunction, and carcinogenicity in juveniles and adults.
- All adverse effects associated with exposure to dioxin-like compounds are believed to result from activation of the aryl hydrocarbon receptor (AhR) and dysregulation of AhR-responsive genes.
- However, the exact molecular mechanisms that result from activation of the AhR that ultimately lead to apical adverse effects at the level of the whole organism remain unknown.
- Here, we investigated these mechanisms in white sturgeon (Acipenser transmontanus), a non-model and ecologically relevant species of fish.

Objective

1. Exposure white sturgeon to equivalent concentrations of 3 agents of the AhR, namely 2,3,7,8-TCDD (TCDD), PCB 77, and benzo[a]pyrene (BaP).
2. Compare global responses at the transcriptome and whole proteome levels in livers of white sturgeon among chemicals.
3. Compare perturbation of molecular pathways and predict similarities or differences in mechanisms of toxicity and possible apical adverse outcomes among chemicals.

Methods

- Exposure: Juvenile white sturgeon (n = 4) received one intraperitoneal injection with either 5 µg/kg bw TCDD, 5 µg/kg bw PCB 77, or 30 µg/kg bw BaP dissolved in corn oil or corn oil alone.
- Transcriptomics: RNA from each individual was pooled at equal concentrations and sequenced as 75 paired-end reads on an Illumina MiSeq at the Toxicology Centre, University of Saskatchewan. Genes were annotated using a reference transcriptome of white sturgeon containing 69,312 contigs that was generated in house.
- Proteomics: Pooled protein samples (n = 4) were sequenced on an orbitrap liquid chromatography – mass spectrometer at the University of Toronto. No proteome of white sturgeon had been sequenced. A reference proteome was created based on the translated reference transcripts and contained 24,564 contigs.

Results

- Exposure to TCDD, PCB 77, and BaP induced adverse responses in white sturgeon.
- Altered physiological pathways of the shared molecular processes among TCDD, PCB 77, and BaP and of the transcriptome (A) and proteome (B) of white sturgeon liver at exposure.
- Analysis of the transcriptome and proteome was performed in ClueGo run through Cytoscape using ontologies based on KEGG and Go Biological processes.

Discussion

- Equivalent concentrations of TCDD, PCB 77, and BaP to activate the AhR in white sturgeon were based on previous investigations and reveal comparable magnitudes of up-regulation of CYP1A gene expression (data not shown).
- A total of 674, 818, and 923 transcripts were altered by 2-fold or more by TCDD, PCB 77, and BaP, respectively (Fig 2A, B).
- At the level of the proteome, a total of 282, 359, and 370 proteins were altered by 2-fold or more by TCDD, PCB 77, and BaP, respectively (Fig 3A, B).
- The cluster of both up-regulated and down-regulated shared genes in the center of the Venn plots indicates comparable fold-change among all 3 chemicals, at equivalent doses at the level of the transcriptome (Fig 2 C-D) and proteome (Fig 3 C-D).
- Pathway analysis of shared genes at the level of the transcriptome (Fig 4A) and proteome (Fig 4B) indicates perturbations of (A) physiological processes in development, liposome homeostasis, and metabolism, and responses to both xenobiotic and estrogen stimuli.

Conclusions & Future Research

- The relatively large number of shared altered genes, as both the level of the transcriptome as well as the more physiologically relevant level of the proteome, and the anticipated disruption of their associated physiological pathways suggested that apical adverse effects associated with activation of the AhR might not be limited to a core of a few key genes, but could be due to a more widespread perturbation, either directly or indirectly mediated by the AhR.
- This indicates possible dysregulation of several critical developmental and homeostatic processes that could lead to a number of adverse effects in embryos, juveniles, or adults, including deformities, carcinogenesis, and impaired growth and reproduction.
- Ongoing studies should link perturbations at the level of physiological pathways to apical adverse effects through studies with early life-stage fish, such as embryos, which would allow development of AhR-based AOPs.

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Data Analysis: Contigs comprising each treatment group were aligned to the annotated reference transcriptome or proteome. Only contigs that had a fold-change greater than 2-fold or down-regulated relative to control were used. Density ternary plots were produced in R software.

Pathway Analysis: Pathway analysis was performed in ClueGo run through Cytoscape using ontologies based on KEGG and GO Biological processes.

Figure 2: Venn diagrams representing number of transcripts with increased(A) and decreased (B) abundance based on whole transcriptome responses in livers of white sturgeon exposed to TCDD (red), PCB 77 (green), and BaP (blue). Density ternary plots representing transcripts with increased (C) and decreased (D) abundance shared by TCDD, PCB 77, and BaP. Each dot (black) represents a gene with 129 genes represented in (C) and 57 genes represented in (D). Color gradient represents density estimation for up-regulated (red) and down-regulated (green) genes. Dashed lines border middle segment which contains genes with equal fold-change following exposure to TCDD, PCB 77, and BaP.

Figure 4: Altered physiological pathways of the shared molecular processes among TCDD, PCB 77, and BaP of the transcriptome (A) and proteome (B) in liver of white sturgeon. Clusters with a greater proportion of up-regulated processes are shaded (red), while clusters with a greater proportion of down-regulated genes are shown in (green). Degree of red or green shows relative abundance of up-regulated vs down-regulated processes in each cluster. Grey clusters consist of 50 % up-regulated processes and 50 % down-regulated processes. Size of cluster represents the relative number of genes in cluster. Intersection between pathways is represented by grey interconnecting lines. Response pathways for shared genes are representative of response pathways for each chemical, namely TCDD, PCB 77, and BaP including non-shared genes (figures not shown).

Figure 5: Juvenile white sturgeon used in project (A) and white sturgeon embryo to be used in ongoing studies linking omics perturbations to apical outcomes.

Characterization of toxicity pathways of 2,3,7,8-TCDD, PCB 77, and benzo[a]pyrene in white sturgeon using whole transcriptome and proteome analysis

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