Cellular and Molecular Modes Responsible for Induction of Birth Defects

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LTG 1 Poster 03

Science Questions

What are the cellular and molecular perturbations produced by exposure to haloacetic acids that will better define the effects produced by low dose exposure?

What are the pathways associated with the induction of cardiac and craniofacial defects produced by the HAAs in rodents and can we use this information to better define human health risk?

Research Goals

The overall goal of this research is to determine if low-dose exposure to disinfection byproducts (DBPs) poses a risk to human health. The published literature contains numerous studies that both support and refute a relationship between exposure to the chemical byproducts formed during water disinfection and adverse effects on human reproduction. However, many of the chemicals known as disinfection byproducts are rodent developmental and reproductive toxicants. The haloacetic acids (HAAs) are one class of DBPs and produce birth defects when administered to laboratory animals.

The specific aims of this research are to:

- Assess the biochemical and transcriptional effects of HAA-exposure in the mouse embryo in order to understand the pathways responsible for abnormal development
- Compare the morphological, biochemical and transcriptional effects of specific pharmacological compounds with know cellular targets to those produced by the HAAs
- Build a pathways-based model for HAA-induced malformations

The haloacetic acids and their major metabolites produce dysmorphology in embryos following exposure in whole embryo culture. The benchmark concentration ranges from 2µM to 3mM for the parent compounds. We have used Confocal microscopy to evaluate the pathogenesis focusing on cell death.

Exposure of mouse conceptuses to specific pharmacological protein kinase inhibitors produces a variety of dysmorphologies.These studies identify pathways for further assessment for induction of craniofacial defects such as MAPKinase and IP3Kinase pathways.

Findings and Conclusions

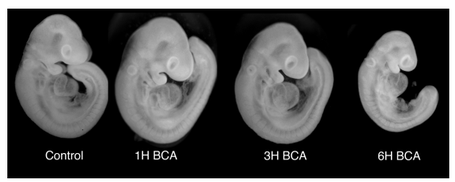
Time and chemical specific changes are produced in protein phosphorylation and transcription factor activation. The studies suggest that there are unique effects produced by the HAAs and that mechanisms of action may be different within this chemical class.

Normal development is associated with a variety of changes in gene expression during neurulation. We have identified a set of genes whose expression is changed across a variety of exposures to identify stress and compensatory responsive genes. This analysis allows us to focus pathway/network investigations on gene changes specific to the exposure.

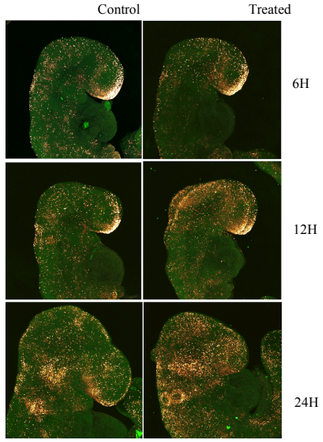
Because of the similarity in transcription factor changes produced by oxygen exposure and the HAAs, we have compared pathway changes between exposures to identify similar embryonic responses and used intervention studies to assess the role of ROS in HAA-induced maldevelopment.

Methods/Approach

Hazard Identification studies. Morphological Studies are used to characterize the effects of exposure to potential toxicants using the whole embryo culture system



Pathogenesis studies. Confocal Microscopy of Lysotracker stained

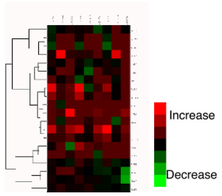


3-D reconstructions of fluorescent images documents the localization and extent of cell death in the embryo following exposure. These images show physiological cell death in controls and toxicant-induced effects in treated embryos.

Morphological studies are used to determine the relationship between selective pharmacological inhibitors and birth defects to identify pathways sensitive to perturbation. Effects of exposure to pharmacological protein kinase inhibitors

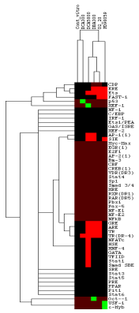
Kinase inhibited	Dysmorphology
PKC	Neural Tube Closure Pharyngeal Arch
MAPK - erk1/2	Heart Prosencephalon
PKG	Caudal Development Somitogenesis
PKA	Mesencephalon
IP3K/Akt	Heart Prosencephalon ??Pharyngeal arch??
JNK	Prosencephalon Pharyngeal arch

Effects of HAAs on protein phosphorylation in embryos in WEC



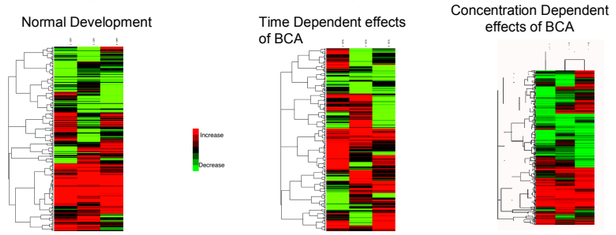
These studies demonstrate that exposure to the HAAs produces a time and protein specific change in protein phosphorylation

Effects of HAAs and model compounds on Transcription Factor activation in embryos in WEC

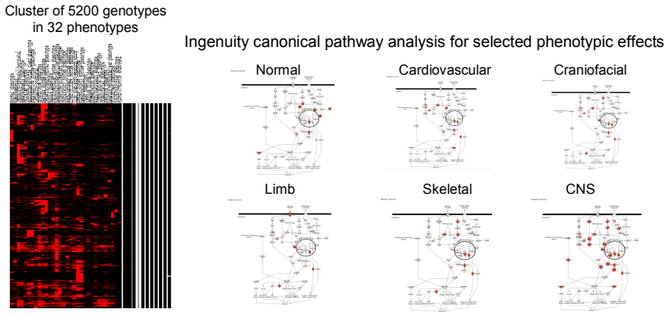


Panomics Transcription Factor (TF) arrays were used to assess which TFs were active in control and treated embryos after 24H of exposure. The increase or decrease in TFs in treated embryos relative to controls is shown clustered.

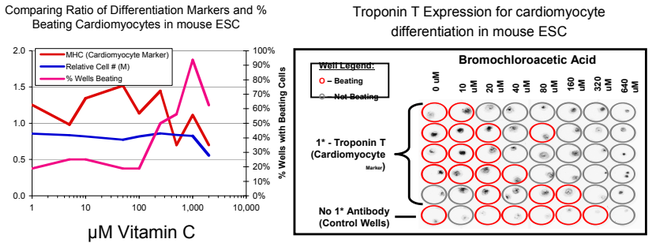
Transcriptional Effects of HAAs on Gene Expression in embryonic heads in WEC



Future Direction: Use gene knockout phenotype data to identify toxicity pathways for birth defects



Future Direction: Establish a technique to evaluate the effects of xenobiotics on embryonic stem cells



Impact and Outcomes

We identified several signaling pathways that ca be classified as Toxicity Pathways for craniofacial malformations. The phenotypic response to gene knock-out is being used to further assess these pathways. These studies will allow a comparison between rodent and human susceptibility and ultimately to understanding the effects of low-dose exposures to xenobiotics that perturb activity of these pathways

Future Directions

Identify toxicity pathways associated with craniofacial and cardiovascular birth defects. These studies will use gene array data from embryos exposed to the HAAs and model compounds to identify gene networks perturbed by exposure. Pathways will also be identified based on the gene knock out data in the Jackson Labs phenotype database.

Gene knockdown (e.g. RNAi) or over-expressing (e.g. adenoviral vector) models can be used to test the relationship between changes in toxicity pathway activity and disruption of morphogenesis using in vitro models.

Develop and assess the use of embryonic stem cells as a model of development that can be used to assess the effects of chemicals on differentiation. Understanding the similarities and differences in mouse and human stem cell response and toxicity pathways will be key to predicting human risk to xenobiotic exposure. Develop high through-put embryonic stem cell models to assess the effects of chemical exposure on selected toxicity pathways using reporter constructs.

Researchers Involved

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