USING HUMAN-DERIVED NEURAL CELLS AS AN IN VITRO MODEL FOR DEVELOPMENTAL NEUROTOXICITY FOLLOWING EXPOSURE TO PESTICIDES

Smith, Mary A.1,2,3, Henderson, W. Matthew4, Wallace, Shelley5, Majumder, Anirban2, Amosu, Mayowa1,3, Bian, Xiaoming2, Lu, Kun1,2,3, Stice, Steven2,3,5

1Department of Environmental Health Science, University of Georgia, Athens, GA, United States; 2Interdisciplinary Toxicology Program, University of Georgia, Athens, GA, United States; 3Regenerative Bioscience Center, University of Georgia, Athens, GA, United States; 4ORD/NERL/ERD, U.S. EPA, Athens, GA, United States; 5ArunA Biomedical, Inc., Athens, GA, United States.

Abstract

Agricultural, industrial and commercial use of pesticides continues to increase with an estimated usage nearing a billion lbs/year. Many of these compounds target the nervous system of nuisance animals and due to their lack of selectivity cause adverse effects in non-target species. Several classes of pesticides such as the organophosphates, carbamates and organochlorines are known to elicit neurotoxic effects in mammals. However, current testing and safety requirements do not require developmental neurotoxicity (DNT) tests for these chemicals. Understanding the consequence of pesticide exposure on fetal brain development specifically during critical windows of susceptibility is necessary to accurately predict risk. Thus, there is a critical need for in vitro models to aid in DNT screening and chemical prioritization. The objective of this study was to develop a metabolomics-based DNT assay that includes stages of neural development using progenitor (hNP) and post-mitotic neuronal cells (hN2) to delineate the adverse outcome pathways (AOP) associated with pesticide exposure. Assays were initially validated with known neurotoxic chemicals. In this study, cells were exposed to 0, 0.1, 0.3, 1, 3, 10, 30 and 100 µM of chlorpyrifos, aldicarb, and lindane for 48 hrs. Following exposure, media and cells were separated and biological reactions were quenched prior to extraction, derivatization, and analysis by GC-MS. Metabolomic profiling and subsequent multivariate analysis demonstrated separation for each pesticide class and dose dependent responses were observed at concentrations lower than those eliciting effects in cytotoxicity assays. Understanding the biochemical metabolites associated with these responses and mapping them to critical pathways of DNT will aid in predicting the risks due to pesticide exposures.

Introduction

Evidence suggests that environmental toxins play a role in the origination and progression of a host of central nervous system disorders. Such chemicals may adversely affect the nervous system in adults as well as early in development through maternal exposure. Yet no human model system can adequately predict neurotoxicity associated with toxin exposure, especially at early stages of nervous system development. More predictive human model systems must be developed to address the sheer multitude of potentially toxic chemicals that go completely unscreened or insufficiently evaluated before they reach the consumer.

Objectives

• Compare the sensitivity of a traditional viability assay to metabolic profile analysis for detecting responses of neural progenitor cells after pesticide exposure.
• Expose early neural progenitor cells (hNP) to pesticides, and use GC-MS analysis to generate metabolic profiling identities, specific metabolites disrupted and predict which pathways are affected when pesticide treated cells are compared to control cells.

Results

Figure 1: Results from viability assays following 48 hr exposure to aldicarb (A), lindane (B), or chlorpyrifos (C).

Figure 2: GC-MS scores plots from the spectra of cellular metabolites (A,C,E) and media (B,D,F) after hNP cells were exposed to aldicarb, lindane or chlorpyrifos for 48 hrs.

Understanding the Response of hNP cells to Chlorpyrifos Exposure

Table 1A: List of metabolites that were significantly altered at toxic exposure (A) and media (B).

Future Directions

• Compare the data from hNP cells to the profiles of hN2 cells to identify pathways that may adversely affect the nervous system at various stages of susceptibility.
• Add metabolic capacity to our assay using co-cultures with C3A cells.

Acknowledgements

This project is supported by Grant Number 1R43ES023530-01 from the National Institutes of Health (NIH) and PA G0212-STAR-F1 from the Environmental Protection Agency (EPA). This project is supported by the UGA Regenerative Biosciences Center.