Metabolomic and Proteomic Profiling of Organophosphorous Chemical Warfare Agent Exposure on Human Liver-on-a-Chip

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Introduction

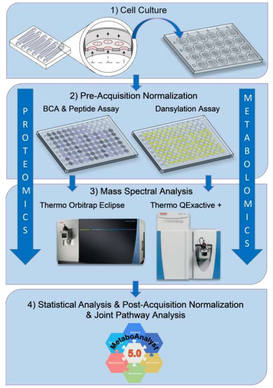
Organophosphates (OP) are molecules that cause human immune system dysfunction and are utilized as pesticides and other toxic chemicals. For many of these toxic chemicals, phosphorylation of acetylcholinesterase's catalytic site is the primary mechanism of action[1], but significant "off-target" effects disrupting the citric acid (TCA) cycle have been published regarding animals exposed to a specific toxic chemical.[2]

The work performed here aims to elucidate further "off-target" effects from different classes of toxic chemicals, and to determine if the TCA cycle or additional common pathways are affected across multiple types of toxic chemical exposures. If common pathways can be identified, this information could allow development of broad-spectrum countermeasures.

Microphysiological systems (MPS) or 'organ(s)-on-a-chip' were used to perform this work. These systems are replacing traditional cell cultures and animal models for many reasons, including increased throughput, lower cost, the ability to focus on particular organs or sub-organ systems, and the ability to expose human systems to dangerous chemicals.[3] These systems marry well with mass spectrometry-based biomarker analysis.

Figure 1 – Experimental Setup

Hepatocyte cells were seeded in CNBio plates and allowed to stabilize. Wells were then individually exposed to four different toxic chemicals or dimethyl sulfoxide (DMSO) for 24 hours. Wells were then frozen and prepared for proteomic and metabolomic analysis via mass spectrometry.[4] Data was processed by software suites including Proteome Discoverer 2.5, XGBoost, and Compound Discoverer 3.0.



References

1. Maxwell et al. (2006) Arch. Toxicol. DOI: 10.1007/s00204-006-0120-2
2. Glaros et al. (2020) Arch. Toxicol. DOI: 10.1007/s00204-020-02820-4.
3. Zhang et al. (2018) Nat. Rev. Mater. DOI: 10.1038/s41578-018-0034-7
4. Dhummakupt et al. (2022) Metabolites DOI: 10.3390/metabo12090815

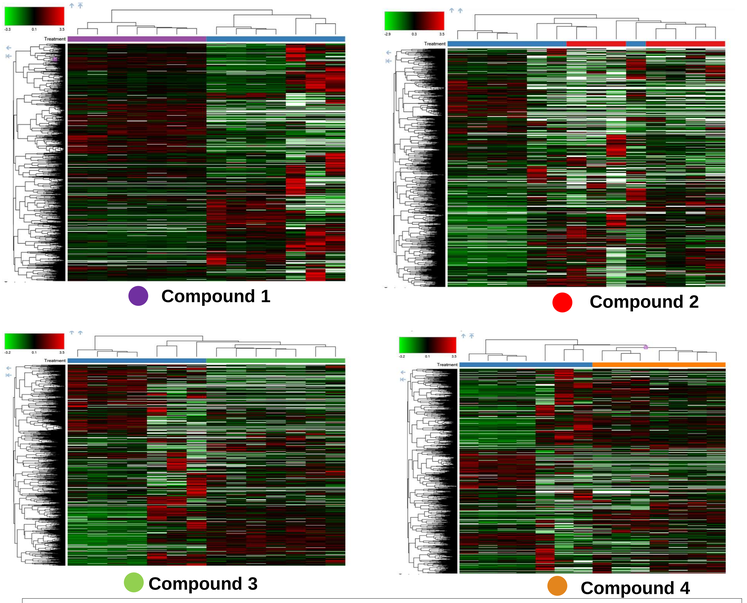


Figure 2 – Protein Heatmaps: Heatmaps of protein changes between DMSO-exposed (i.e., control) hepatocytes and toxic chemical-exposed hepatocytes. There are significant (p-value < 0.05) protein changes occurring between the exposed and control (blue) samples.

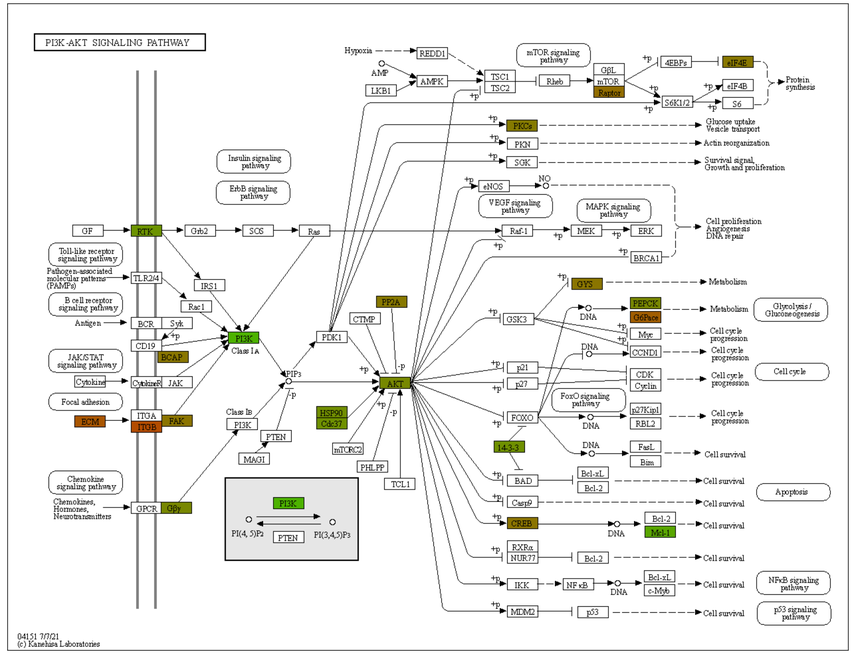
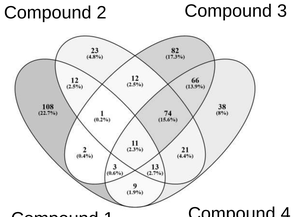


Figure 4 – PI3K-Akt Pathway: The posphoinositide-3-kinase (PI3K)-protein kinase B(Akt) pathway was identified as overlapping between different toxic chemical exposures. This pathway plays a role in cell metabolism, growth, proliferation, and survival. Several proteins, like receptor tyrosine kinase (RTK) and phophoenolypyruvate carboxykinase (PEPCK) are upregulated, while proteins like integrin beta 1 (ITGB) and glucose-6-phosphatase (G6Pase) are downregulated. Further investigation is necessary to understand the mechanism of action and identify potential druggable targets for countermeasures.

Up-regulated Metabolites



Down-regulated Metabolites

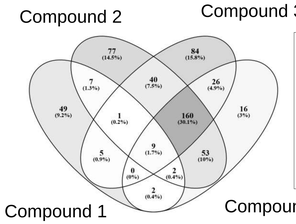


Figure 3 – Overlapping Metabolites

The numbers of overlapping metabolites that change significantly based upon toxic chemical exposure. Identification of these metabolites and their biochemical pathways could direct research into more broad-acting countermeasures.

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