Multi-omic approach to inform quantitative adverse outcome pathway development for acute organophosphorus poisoning

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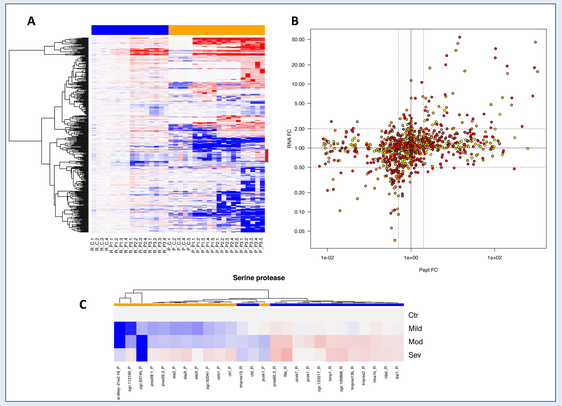
Introduction

Organophosphorus compounds constitute a class of acetylcholinesterase inhibitors used as not only pesticides but also chemical warfare nerve agents. Acute organophosphorus poisoning (acute OPP) affects 3 million people, with 300,000 deaths annually worldwide. Severe acute OPP effects include overstimulation of cholinergic neurons, hyperexcitation, seizures, status epilepticus, and brain damage. In a previous study, we developed and characterized three different chemical models of acute OPP in zebrafish larvae. To elucidate the complex pathophysiological pathways related to acute OPP, we used integrative omics (proteomic, transcriptomics and metabolomics) on these three animal models. Our results show that these stochastic, apparently disparate morphological phenotypes can result from almost linear, typically dose-response variations in molecular levels. Results from the multi-omics analysis strongly suggest that endoplasmic reticulum (ER) stress might play a central role in the pathophysiology of severe acute OPP, emphasizing the urgent need of further research to confirm with direct evidences this hypothesis. ER stress could be an important therapeutic target to be included in the treatment of patients with severe acute OPP. The multi-omics data are now being used to develop a quantitative Adverse Outcome Pathway Network (qAOPN) for AChE inhibition leading to mortality.

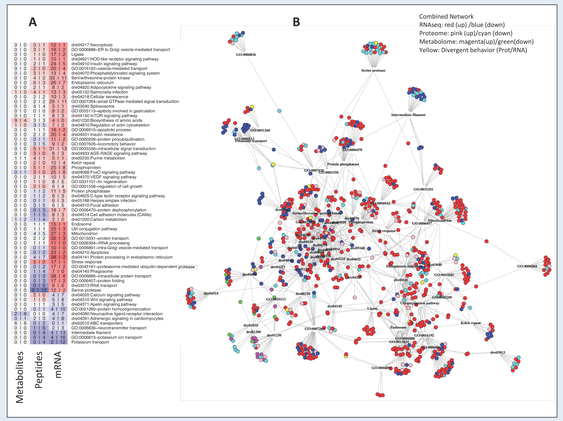
Approach

- Zebrafish (*Danio rerio*) larvae were exposed to chlorpyrifos oxon (CPO) for 24h to generate a mild (0.1 mM), moderate (1 mM), and severe (3 mM) phenotype.
- Proteomics data were generated by LC-MS/MS and analyzed using MaxQuant software.
- Transcriptomics data were generated using an Illumina HiSeq and analyzed using the *lmdme* package (see Faria et al, 2015).
- Metabolomics data were generated by LC-MS/MS (see Gomez-Canela et al, 2018)
- All omics data were integrated together. Identified markers from transcriptomic, proteomic and metabolomic data was introduced as input data into the KEGG database. Identified pathways with at least two hits were included in the network analysis, using the *reshape2* and *igraph* packages in R.
- Information was used to generate an AOPN from AChE inhibition to death.

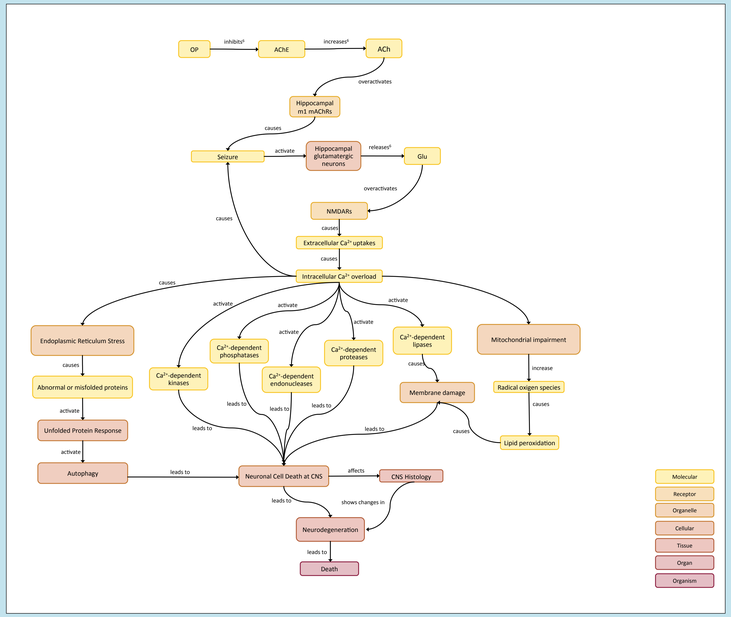
Results



Comparison between transcriptomic and proteomic data for the 371 identified DEPs. (**A**) Combined heatmap. (**B**) Correlation plot between transcriptomic (Y-axis) and proteomic (X-axis) data. Each dot corresponds to averages of the biological replicates for any given DEG at one of the three different phenotypes: Mild (yellow dots), Moderate (orange dots), or Severe (red dots). (**C**) Heatmap corresponding to transcriptomic (blue horizontal sectors at the top of the graph) and proteomic data (orange sectors) for all DEGs and DEPs included in the Ser-Protease category in the three studied phenotypes.



Functional analyses of DEGs, DEPs and Metabolites, distributed in clusters. (**A**) Distribution of DEGs, DEPs and Metabolites among the different functional modules. Cell colors indicate the ratio between the number of genes/peptides/compounds in (**B**) Network representation according to their adscription to functional modules (DAVID functional clases and KEGG pathways). Transcripts (red and blue dots), peptides (pink and cyan dots), and metabolites (magenta and green dots) are labeled by the cluster they belong (red, pink, and magenta for Cluster A, blue, cyan, and green for Cluster B). Single gene/peptide showing divergent results in RNA and proteome analyses are represented in yellow.



Adverse outcome pathway for acetylcholinesterase inhibition leading to death. OP = organophosphate; AChE = acetylcholinesterase; ACh = acetylcholine; nAChRs = nicotnic ACh receptors; mAChRs = muscarinic ACh receptors; Glu = glutamate; NMDARs = N-methyl-D-aspartate receptors; CNS: central nervous system.

Conclusions

- Whereas many different mechanisms are involved in the pathophysiology of severe acute OPP, standard therapy has not changed much over the last 50 years.
- The results from the multi-omic analysis presented here strongly suggest that that ER stress might play a central role in the secondary neurological damage in severe acute OPP.
- Multi-omics approaches using zebrafish can help develop qAOPNs by identifying potential mechanisms and providing quantifiable information.

Acknowledgements

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