Elucidating acrylamide adverse effects on zebrafish using a multi-omics approach

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Introduction

Acrylamide (AA) neurotoxicity has driven much attention after the occupational poisoning of workers injecting AA-based grouting agent during tunnel constructions in Sweden and Norway during the 1990s. A chemical model for acute AA neurotoxicity has been developed in adult zebrafish. In order to elucidate the precise mechanism by which AA elicits its neurotoxicity, we performed a multi-omic analysis (metabolomics, proteomics, transcriptomics) to describe the molecular effects of the exposure to moderate levels of AA in the zebrafish brain. We observed a cascade of molecular adverse events linked to the ability of AA to form adducts with thiol groups. These events include the depletion of glutathione, the inactivation of key components of the thioredoxin system, and the dysregulation of microtubule-related genes. As these effects are interconnected, we propose that they represent a perfect storm, blocking the normal functioning of nervous cells and explaining most, if not all, AA neurotoxic effects. Our results also suggest that neurotoxicity should be regarded as the major damage after AA exposure, and that it should be the main target for new efficient countermeasures against this toxidrome.

Approach

- Zebrafish (*Danio rerio*) adults were exposed to 0.75 mM AA for 72 h.
- Metabolomics: brain NMR spectra were recorded at 298 K on a Bruker Avance III console (Bruker Biospin, Germany) combined with a 9.7 T Oxford Instruments magnet (1H 500 MHz), equipped with a 5 mm inverse cryogenically cooled triple-resonance TCI (1H, 13C, 15N, and 2H lock) cryoprobe with a z-axis magnetic field-gradient capability. The samples were run under automation by IconNMR and the acquired data were processed using Topspin 3.6
- Transcriptomics: RNA and protein fractions were simultaneously recovered from the same samples (brain and muscle) to be used in parallel for RNA-seq and proteomics analyses. Samples weren sequenced with paired-end 150 bp (PE150, or 2x150) using the Illumina NovaSeq 6000 System (Illumina, San Diego, California, USA).
- Proteomics data were generated by LC-MS/MS and analyzed using MaxQuant software.
- Total glutathione content was determined in a Synergy 2 Multi-Mode Microplate Reader using the DTNB assay.
- Information was used to generate an AOPN from AA-thiol group conjugation to behavioral changes.

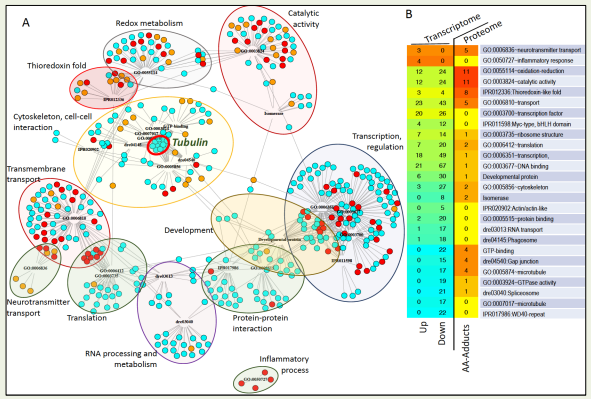

Figure 1. Functional analysis of transcripts significantly affected by acrylamide treatment (DEGs) combined with the peptides showing acrylamide adducts in more than 20% of their sequenced, Cys-containing peptides. **A)** Network representation of DEGs and peptides according to their adscription to functional modules (GO:biological process and KEGG databases, codes for each module are given as nodes). **B)** Distribution of DEGs/modified peptides among the different functional modules (rows) and their response to acrylamide (columns, "Up" and "Down" for transcripts, "ACR peptides" for peptides).

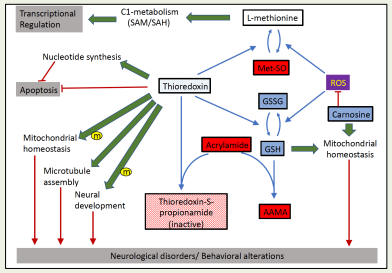

Figure 2. Functional intercorrelation of different metabolites and their relation with cellular functions linked to acrylamide-elicited brain disorders. Blue and red boxes represent metabolites that become enriched or depleted in AA-treated brain. Boxes thioredoxin and its propionamide adduct follow the same color code, indicating the formation of adducts and the decrease of free, functional thioredoxin. Blue arrows indicate direct metabolic relationships, green arrows indicate indirect functional or regulatory ones. Red lines ending with a dash indicate an inhibitory effect, red arrows indicate those cell functions whose disruption lead to neurological disorders. Small case "m" in a yellow circle indicates effects specifically described for the mitochondrial thioredoxin.

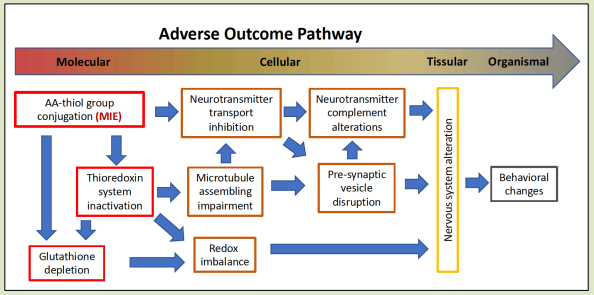

Figure 3. Adverse Output Pathway analysis of the proposed AA mode of toxicity. The graph shows the different potentially adverse effects detected at molecular (Transcriptome, metabolome), cellular (metabolome, immunohistochemistry), tissular, and organismal (behavior) levels of organization. The molecular initiating event or MIE is indicated at the upper left end corner of the scheme. Blue arrows indicate the proposed cause/effect relationships.

Conclusions

Our results unravel a new and highly relevant molecular pathway involved in AA acute neurotoxicity, the disruption of the main mechanism of the cell for controlling redox homeostasis, glutathione, and thioredoxin system, configuring a "perfect storm" that triggers a cascade of potentially catastrophic effects at cellular and tissue levels.

AA is considered as highly toxic in account of its potential carcinogenic effects; our results indicate that efforts to limit public exposure to it, or to counteract accidental, suicidal, or provoked acrylamide intoxications, should also focus on its neurotoxic effects.

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