Modelling acrylamide acute neurotoxicity in zebrafish larvae

Natàlia Garcia-Reyero[1*], Eva Prats[2], Cristian Gomez-Canela[3], Shani Ben-Lulu[4], Tamar Ziv[4], Francesc Padrós[5], Daniel Tornero[5], Romà Tauler[3], Arie Admon[4], Demetrio Raldúa[3]

[1]US Army Engineer Research & Development Center, Vicksburg MS, USA; [2]CIC-CSIC, Barcelona, Catalunya; [3]IDAEA-CSIC, Barcelona, Catalunya; [4]Techion, Israel Institute of Technology, Haifa, Israel; [5]Universitat Autonoma de Barcelona, Bellaterra, Catalunya

Abstract

Acrylamide (ACR) is a water-soluble alkene primarily used in polyacrylamides production. Exposure to ACR produces a terminalopathy characterized by ataxia, skeletal muscles weakness and numbness of the extremities. Currently, medical treatment of ACR neurotoxicity in humans is symptomatic, and only the mildly affected patients underwent complete recovery. Similar neurotoxic effects can be found in other species. In order to understand the specific mechanisms of ACR toxicity that could be linked to adverse outcomes across species, we generated a zebrafish model for ACR neurotoxicity by exposing zebrafish 5 days post-fertilization larvae to 1 mM ACR for 3 days.

Our results show that the generated zebrafish model mimics most of the pathophysiological processes described in humans and mammalian models acutely exposed to ACR. First of all, the zebrafish model exhibited altered motor function, with a significant reduction in the frequency of the swimming cycles during the escape response. The response to sudden increments or decrements in light intensity elicit was also strongly altered. The histopathological analysis identified an specific effect on the presynaptic nerve terminals at the neuromuscular junctions level, but not on the axonal tracts or myelin sheath integrity. Moreover, the specific effect of ACR on nerve terminals was confirmed by using selected transcriptional markers, as well as by the significant effect found on cholinergic and dopaminergic systems.

Results

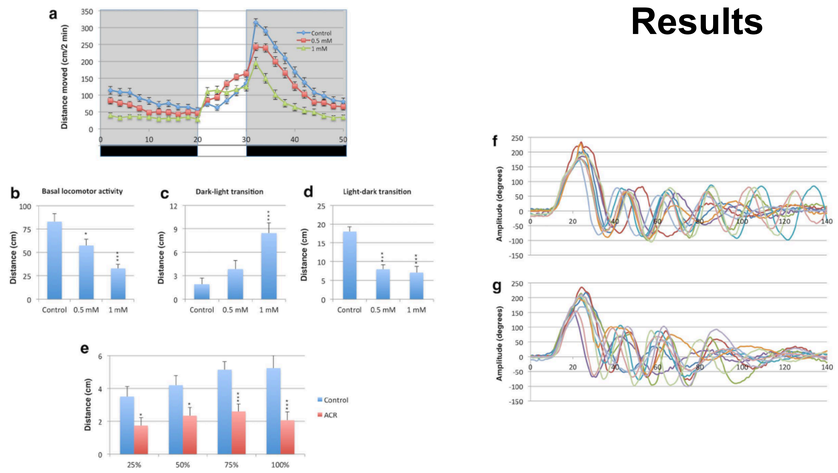

Figure 1. Motor response is strongly impaired by acrylamide (ACR) in zebrafish larvae. (a) Locomotor activity of 8 days post-fertilization zebrafish larvae control and exposed to 0.5 mM ACR and 1 mM ACR during a 20 min dark period followed by a 10 min light period and then a second cycle of 20 min of darkness. (b) Basal locomotor activity (BLA), defined as the distance moved by the larvae during the first period of 20 min in the dark, was significantly reduced by ACR; Results represent mean ± sem. (c) 1 mM ACR, but not 0.5 mM ACR, induced a period of hyperactivity in the dark to light transition (the difference in activity between the 2 first min with light and the last 2 min of the first dark period is represented); (d) Visual motor response (VMR), the hyperactivity period evoked by a sudden reduction in light intensity, is strongly reduced in larvae exposed to 0.5 and 1 mM ACR; (e) Larvae exhibiting a total abolition of the VMR after 1 mM ACR exposure exhibit a significant reduction in the acoustic/vibrational motor response evoked by a solenoid at four different intensities. (f,g) Kinematic of the touch-evoked escape response is altered in zebrafish larvae exposed to 1 mM acrylamide. Representative kinematic traces of the touch-evoked escape response of control (f) and ACR-treated (g) larvae. For each condition eight representative traces are shown from the first 140 ms of the escape response. Each trace is from a different larvae. The curvature of the body is represented in degrees, with 0 indicating straight body.

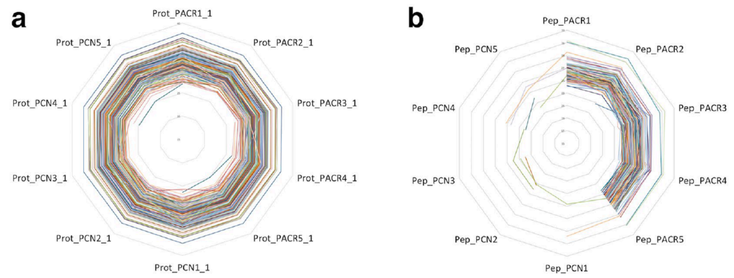

Figure 4. Proteins containing ACR-modified cysteine residues in at least 3 treated samples. (a) Expression levels of the 138 proteins containing ACR-modified cysteine residues; (b) Log-2 intensities of the peptides with ACR –adducts in cysteine residues. PCN: control pool; PACR: pool ACR.

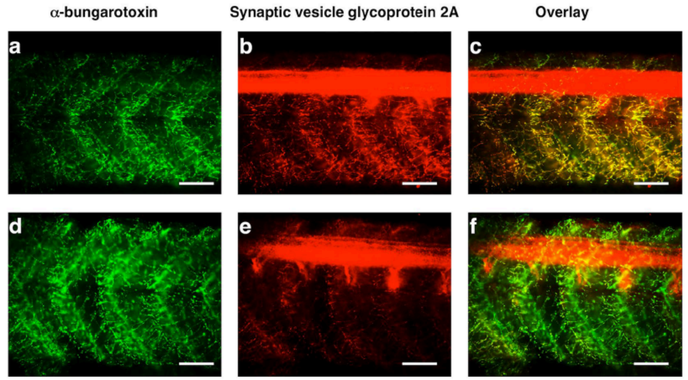

Figure 2. ACR reduces presynaptic nerve terminals in zebrafish larvae. At the neuromuscular junctions (NMJ) of the trunk, ACR-exposed larvae exhibit an strong reduction in the labelling of synaptic vesicle glycoprotein 2a (marker of synaptic terminals of the spinal motor neurons), whereas the α-bungarotoxin labelling (postsynaptic marker at the NMJ) labelling remains unaltered. Detail of the trunk, in lateral view, of control (a–c) and ACR-treated (d–f) larvae after co-labelling with α-bungarotoxin Alexa Fluor 488 conjugate (a,d) and SV2 antibody. The co-localization of the pre-synaptic and post-synaptic markers of NMJ is also showed (c,f)

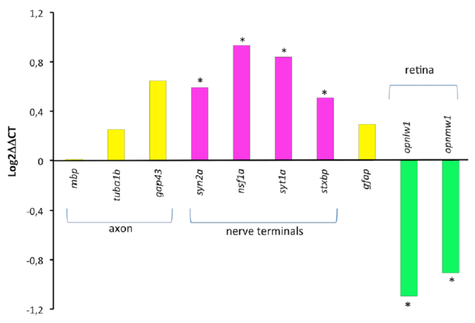

Figure 3. ACR exposure induces a significant change en the expression of transcripts related with the synaptic vesicle cycling and visual function.

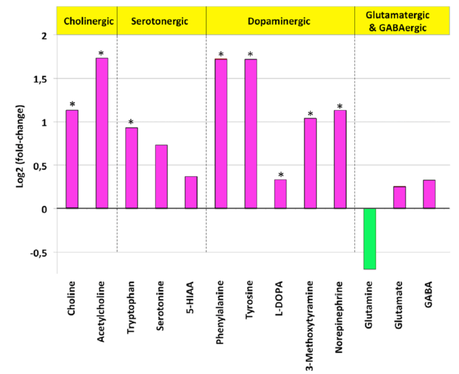

Figure 5. Changes in the profile of 13 neurochemicals (neurotransmitters, precursors, metabolites) in the zebrafish model for ACR acute neurotoxicity., *P < 0.05.

Acknowledgements

This work was supported in part by the NATO SfP project MD.SFPP 984777 (D.R., A.A. and N.G.R.), the US Army Environmental Quality and Installations Research Program (N.G.R.), the Advanced Grant ERC-2012-AdG-320737 (D.R., C.G-C, R.T.), the Spanish Government (CTM2014-51985-R; D.R.) and the I-CORE Program of the Planning and Budgeting Committee and The Israel Science Foundation (grant No. 1775/12 to A.A.)

Bibliography

Prats E, Gómez-Canela C, Ben-Lulu S, Ziv T, Padrós F, Tornero D, Garcia-Reyero N, Tauler R, Admon A, Raldúa D. Modelling acrylamide acute neurotoxicity in zebrafish larvae. Sci Rep. 2017 Oct 24;7(1):13952.

Contact: Natalia.G.Vinas@erdc.dren.mil

Conclusions

The effects of ACR in the zebrafish model mimics most of the aspects of this process in mammals, such as impaired motor function and specific effects at the nerve terminals, including altered neurotransmission. Moreover, ACR formed adducts in cysteine residues of some proteins related with the synaptic vesicle cycling in our model, which is considered as the molecular initiating event in mammalian models