rario fish, optimal model organism for ecotoxicological analysis and for the study of neurodegenerative and neurobehavioral diseases. In order to assess the toxic effects of this metal, Danio rerio embryos at shield stage were exposed to AlCl₃ at the concentrations of 50, 100 and 200 μM respectively for 72 h. We compared the swimming performances of treated larvae with those of the control larvae, assessing different parameters like Distance moved, Velocity mean, Cumulative movement, Meander and Heading using the DanioVision instrument. Collected data showed that AlCl₃ significantly affected the behavioural parameters with a trend inversely proportional to the concentrations, in fact the performances worsen at low concentrations compared to higher doses. In this light, we analysed mRNA expression level by qPCR of different marker genes of neural development and function, including c-fos, appa and appb. C-fos is an immediately early gene often used as indirect marker of neuronal activity, while appa and appb are the homolog genes of the mammalian amyloid precursor protein (APP), an essential gene for normal brain development and a key player for the Alzheimer’s disease pathogenesis. We observed that the expression of these genes was affected by AlCl₃. The results confirmed toxic effect of AlCl₃ on D. rerio larvae, suggesting the need for further experiments to uncover the mechanisms by which the aluminum exposure affects the normal developmental processes and might be at basis of neurological and behavioural disorders.

References

NOVEL APPLICATIONS OF LONG-ESTABLISHED HISTOCHEMICAL TECHNIQUES TO STUDY NANOPARTICLE-CELL INTERACTIONS AT TRANSMISSION ELECTRON MICROSCOPY

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Transmission electron microscopy (TEM) is the technique of choice to explore the effects of nanocomposites on biological systems. The high resolution of TEM allows the location and dynamic tracking of nanoparticles (NPs) inside cells and tissues, providing crucial information on their actual interactions. It is thus mandatory that NPs are unequivocally detected in the inter- and intracellular space. This is easily obtained for NPs containing electron dense components such as metal ions, but it may be difficult when they are made of organic components (e.g., polymers or lipids) whose moderate electron density makes them hardly discernible in the cytosolic milieu. We faced this situation with various types of NPs and solved the problem by setting up novel applications for long-established histochemical techniques. Chitosan-based and phospholipidic NPs were made clearly visible at TEM by labelling them with fluorochromes during their synthesis, and subsequently applying diaminobenzidine (DAB) photo-oxidation, that gives rise to a finely granular electron dense product thanks to the reactive oxygen species originating upon fluorochrome irradiation. By this method, not only the uptake mechanisms and intracellular distribution of these NPs were revealed, but also their degradation pathways, thanks to the presence of DAB precipitates on the NPs remnants inside secondary lysosomes and residual bodies. However, in the absence of fluorochrome labelling DAB photo-oxidation cannot be applied: as an alternative approach, specific histochemical methods giving rise to electron dense products may be used, such as Alcian blue staining. Hyaluronic acid-based NPs were thus visualized at TEM, and we were able to describe the very early step of their uptake as well as their degradation, which were impossible to get by conventional morphology.

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References

EFFECTS OF MOLECULES WITH NEUROTROPHIC ACTIVITY IN AN IN VITRO MODEL OF PARKINSON’S DISEASE

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Oxidative stress results from an in balance between oxidative species and scavenging antioxidant systems. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be either harmful or beneficial to the cells, depending on their concentrations. Methionine acts as amino acid precursor for glutathione that protects the cells from oxidative damage and plays pivotal role in cell detoxification from oxidative stress. The aim of this research was to study the neurotrophic and antioxidant activities of methionine and taurine on an in vitro model of PD. The methionine and taurine effects were first evaluated on an oxidative model based on H₂O₂ treatment, then followed by the set-up of a PD in vitro model by treating dopaminergic neurons with 6-OHDA. The effects of methionine and taurine were evaluated by MTS assay, Western Blotting analysis and Immunofluorescence. Methionine and taurine were both able to counteract the pro-oxidative death effects of H₂O₂ and 6-OHDA by decreasing the apoptotic markers (caspase 9, Bcl-2), by modulating oxidative stress markers (Mn-sod, catalase) and the oxidative stress index (ratio Mn-sod/catalase); by increasing the P13K/AKT survival pathway and antioxidant markers like a Nrf2. The results so far obtained confirmed the potential neuroprotective activities of methionine and taurine in the PD in vitro model. The study will continue by evaluating the possible protective activities of these molecules on mitochondrial dysfunctions using an oxidative phosphorylation uncoupling like a TMRM in live cell, JC-1 and the activation of pro-survival pathways depending on neurotrophine modulation in the PD in vitro model.

References

A NEW INSIGHT IN THE AXON AND DENDRITIC DEVELOPMENT: THE FMRP-RACK1 PARTY

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