THE USE OF roGFP2 PROBES TO EVALUATE THE MECHANISMS OF TOXICITY OF NANOPARTICLES IN *Drosophila melanogaster*

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Keywords: nanoparticles, toxicity, redox homeostasis

ABSTRACT

The excessive generation of reactive oxygen species (ROS) in biological systems is closely associated with the toxicity of metal nanoparticles. Furthermore, various pathological conditions are related to a redox imbalance caused by excessive ROS production, as seen in cardiovascular and neurodegenerative diseases. ROS are naturally generated as a byproduct of redox reactions occurring in the mitochondria. However, uncontrolled increases in ROS levels compromise the cellular biochemical environment, as these highly reactive molecules can cause DNA breaks, protein misfolding, lipid peroxidation, and mitochondrial dysfunction. Thus, understanding the ability of new substances to induce free radical generation is essential for comprehending the toxicity mechanisms associated with these compounds. Among the main *in vivo* models used in toxicology, the fruit fly, *Drosophila melanogaste*r, stands out due to its rapid life cycle, low maintenance cost, and ease of manipulation. Moreover, the binary UAS-GAL4 expression system allows for the expression of genes of interest in specific Drosophila tissues. In 2011, Albrecht et al. established a model capable of measuring mitochondrial redox homeostasis patterns in *Drosophila melanogaste*r tissues. This platform is based on the use of the UAS-GAL4 system to express mitochondrial probes called mito-roGFP2. These probes are sensitive to redox changes, undergoing conformational changes in their cysteine residues that alter the molecule's excitation spectrum, shifting from 488 nm when oxidized to 405 nm when reduced. The fusion of the roGFP2 probes to the microbial sensor Orp1 makes it particularly sensitive for evaluating changes in hydrogen peroxide levels, a molecule associated with excessive ROS generation due to its high capacity to produce free radicals. Therefore, it is possible to evaluate H_2O_2 levels in different Drosophila tissues using fluorescence microscopy. In this work, we demonstrate how this tool can be used to understand the mechanisms behind the toxicity of titanium dioxide doped with europium (TiO_2:Eu^{3+}) and simonkolleite doped with silver (Sm:Ag). Initially, a development assay was conducted to describe the toxicity of the compounds. Thus, a total of 900 first-instar larvae were placed in vials containing standard culture medium with the substances to be tested at different concentrations. After this step, subsequent developmental stages were monitored, and the effect of the substances on larval development was quantified through larval mortality and pupation rates. Next, transgenic larvae expressing the mito-roGFP2-Orp1 probe exclusively in the larval fat body were exposed to nanoparticles for 96 hours. After this period, the larvae were dissected, and the fat body was subjected to fluorescence microscopy at 405 and 488 nm channels. Subsequently, with the aid of the ImageJ software, the pixel intensity ratio of the 405/488 nm channels was calculated. The difference between the exposed groups and the control group was determined based on the mean of the ratios. As a result, we observed that the highest concentrations tested were the most toxic, showing high larval mortality rates. We then demonstrated how the mito-roGFP2-Orp1 probe successfully highlighted the increase in mitochondrial H_2O_2 levels in the fat body of animals exposed to the highest concentrations of nanomaterials, generating a state of redox imbalance and consequently oxidative stress. The way these nanomaterials generate reactive oxygen species can be explained by the ionic dissociation of the metals that compose these materials; however, underlying mechanisms of these processes remain uncertain. From this work, we conclude that using roGFP2 probes in *Drosophila melanogaster* is capable of elucidating *in vivo* the ROS-generating potential of metallic nanomaterials.