Mechanisms of toxic action of manufactured nanomaterials unraveled by means of in vitro systems

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Over the last decade, an increasing number of manufactured nanomaterials (MNs) have been incorporated into new products or processes. As a result, the risk of release of these nanomaterials to the environment is rising. However, nowadays there are analytical deficits that limit our capabilities to determine actual levels of exposure to these new substances. In addition, and although new information about the toxicity of MNs is released continuously, there is an important lack of knowledge in relation to the hazard posed by nanoparticles (NPs) to organisms. As a result the risk assessment of these materials suffers of important constraints. Establishing the mechanisms of toxic action of MNs at the organismal, tissue and cellular level is essential to better understanding their possible long-term effects necessary for an appropriate hazard assessment. Taking this into account we have done an important effort trying to unravel the mechanisms underlying the toxicity of a number of MNs to cells maintained in vitro. Most of our work has been performed with fish cells cell lines, but mammalian and human cell lines were also used as a reference. Suspensions of MNs were obtained in culture medium and characterized through a variety of techniques including dynamic light scattering (DLS; to determine frequency size distribution), transmission and scanning electron microscopy (TEM and SEM respectively; to establish frequency size distribution and shape) and inductively coupled plasma mass spectrometry (ICP-MS; to measure actual exposure concentrations) among others. Cytotoxicity was assessed through a number of techniques and the possible interference of the NPs with the readouts was estimated. In addition, levels of glutathione (GSH and GSST) and enzyme measurements gave important information about oxidative stress. By means of centrifugation or ultracentrifugation we were able to retire NPs obtaining the ionic fractions of the suspensions. The use of electron microscopy allowed observing the internalization of MNs into cells and their interaction with plasma membrane and with cellular organelles. We have been able to set up a methodology for observing through three different measurements performed simultaneously in the same plate possible disturbances on the plasma membrane, on lysosome functioning and on cellular metabolism. These approaches allowed us to estimate the contribution to the toxicity of the ionic fractions of the suspensions and to observe, with some exceptions, a limited toxicity of the particulated fraction. We reported also important variations in the toxicity of different forms of the same MN, variations that were difficult to relate with particular properties (as shape or size). We studied in depth the mechanisms of internalization of some MNs considering the possibility that they can influence the toxicity of other substances present in the medium or that they could have some applications if used as carriers. Graphene nanoplatelets exhibited a very particular behavior, with nanoplatelets causing toxicity only at high concentrations and being present in the cytoplasm without surrounding membranes. Co-exposure experiments have allowed obtaining very important information. For instance, it has been evidenced that graphene could facilitate the entrance of environmental contaminants into the cells increasing toxicity. Coincubation of CuNPs and ZnONPs also provoked striking synergistic toxicity, probably due to a mutual interaction of these MNs potentiating the entrance of particles and ions into the cells until unbearable levels. These experiments also permitted to establish some limitations to the oxidative stress paradigm applied to MNs. In this case, the simultaneous exposure to CuNPs causing an increase in reactive oxygen species (and therefore in oxidative stress) and of substances triggering the cellular defenses against oxidative stress and provoking a reduction of ROS levels did not lead to a simultaneous decrease in toxicity.