INTERACTION WITH CULTURE MEDIUM COMPONENTS, CELLULAR UPTAKE, INTRACELLULAR DISTRIBUTION, CYTOTOXICITY AND MORPHOLOGICAL TRANSFORMING POTENTIAL OF COBALT NANOPARTICLES, MICROPARTICLES AND IONS IN BALB/3T3 MOUSE FIBROBLASTS: AN *IN VITRO* MODEL

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The mechanistic understanding of nanotoxicity requires comparative investigation on nanoparticles (NP), corresponding ions and microparticles (MP). Following this approach, we carried out a comparative study in Balb/3T3 mouse fibroblasts exposed to radiolabelled Co forms (CoNP, CoMP and Co^{2+}).
In culture medium, CoNP release Co^{2+} and other uncharacterized Co forms with different behaviour (CoNP-rel). Co^{2+}firstly saturates the binding sites of molecules in the extracellular milieu (e.g., albumin and histidine) and on the cell surface, becoming unavailable to enter cells. After saturation, Co^{2+} is actively transported inside the cell. CoNP, instead, are predicted to be internalized by phagocytosis and endocytosis. As far as it concerns CoNP-rel, the mechanism of internalisation is unknown. Once inside the cell, CoNP spread to the cytosol and cellular organelles, reaching extremely high concentrations in the nucleus and mitochondria. Here they can undergo further dissolution, releasing massive amounts of Co ions and
CoNP-rel in compartments normally inaccessible to Co^{2+}.
All Co forms can induce ROS (ions with a Fenton-like reaction and NP with both Fenton and non-Fenton mechanisms) and lipid peroxidation. They can also interfere with the metabolism of essential (Mg, Zn) elements. Unlike Co^{2+} and CoNP-rel, CoNP induce morphological transformation in Balb/3T3 cells, an effect clearly linked to ROS production, as demonstrated by inhibition with an antioxidant agent, namely ascorbic acid. Morphological transformation by CoNP could be a consequence of mitochondrial toxicity and DNA adduct formation. We were unable to establish whether CoNP toxicity is due to the particles per se or to the large amount of locally released Co ions. However, when toxicity is correlated to intracellular cobalt content, ions result in the most toxic forms. CoMP resulted more efficient than NP in inducing both oxidative stress and morphological transformation. This parallels the larger cellular uptake of Co by cells exposed to CoMP.
The results of our studies allow to draw a model of the CoNP mode of action in cell cultures.