SILVER NANOPARTICLES INDUCED CELL-DEATH AND MITOCHONDRIAL DAMAGE IN EARTHWORM COELOMOCYTES

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Nanotechnology is producing large amounts of nanoparticles (NPs) that are applied in industry, daily life and health care. NPs are considered as a particle that has one or more external dimensions in the size range of 1 - 100 nm. One of the most widely used nanomaterials is the nano-silver (in clothing, wound-dressing and surgical instruments), mainly because of its wide antimicrobial activity. Because of the variety of nanotechnology applications, NPs can enter into the environment through many routes. Most toxicity and risk assessment studies of NPs focused on the human health and the environmental effects of nanoparticles are largely unexplored. It is known that silver nanoparticles (AgNPs) can interact with pathogens and immune cells. For instance silver nanoparticles are readily taken up by phagocytic cells (macrophages) of innate immunity that can lead to inflammatory processes. The relative simplicity of invertebrate immune functions offers a potentially sensitive and accessible means of monitoring nanoparticle effects and complex interactions which ultimately affect host resistance. Our current understanding of the potential impact of nanomaterials on invertebrate immunity is limited to only a handful of initial studies including those on earthworms as an ecological indicator organism.

Recently, we reported the cytotoxicity and accumulation of silver nanoparticles in immune cells (so called coelomocytes) of *Eisenia fetida* earthworms *in vitro*.

Hereby, we assessed the coelomocyte survival upon Ag+ and AgNP challenge using flow cytometry based assays. Applying Annexin V/propidium iodide staining we observed that Ag+ ions (EC20: 0.20 μg/mL; EC50: 0.60 μg/mL) and AgNPs (EC20: 1.90 μg/mL; EC50: 6.40 μg/mL) caused late apoptosis/necrosis of coelomocytes. Nanoparticles induced ROS formation lead to mitochondrial damage and cell death. Loss of mitochondrial membrane potential (demonstrated by JC-1 staining) -representing the mitochondrial damage- was observed at higher concentration of Ag+ (EC20: 0.64 μg/mL) and AgNP (EC20: 3.20 μg/mL, EC50: 7.8 μg/mL) compared to the Annexin V assays. Moreover, ionomycin evoked calcium influx of coelomocytes after AgNP treatments (EC20, EC50) were biased at early time points.

In these experiments, we demonstrated subcellular toxicological implications arising from selective uptake of nanomaterials in the coelomocytes that might lead to inter-cellular communications of various coelomocyte subpopulations during the inflammatory process.