NICKEL OXIDE NANOPARTICLES INDUCE TOXICITY IN YEASTS VIA OXIDATIVE STRESS

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The increasing use of nickel oxide (NiO) nanoparticles (NPs) raises concerns about their potential toxicity. In the present study, the yeast *Saccharomyces cerevisiae* was used, as a cell model, in order to elucidate whether the toxicity of NiO NPs is associated with the oxidative stress (OS).

In abiotic conditions (cell free), NiO NPs were unable to induce the generation of reactive oxygen species (ROS), which excludes the possibility of exerting a pro-oxidant effect. However, yeast cells exposed to NiO NPs accumulated intracellularly superoxide anions (assessed with dihydroethidium) and hydrogen peroxide (evaluated with 2',7'-dichlorodihydrofluorescein diacetate or dihydrorhodamine 123) when incubated in normal (oxygen) atmosphere. Yeast cells exposed to NiO also presented reduced cell viability (measured through a clonogenic assay). Yeasts co-exposed to NiO NPs and the antioxidants L-ascorbic acid (a scavenger of free radicals) or *N-tert*-butyl-α-phenylnitrone (a spin trapping agent) presented ROS quenching and increased cell viability, which suggests that NiO toxicity is linked to ROS production.

Wild-type (WT) yeast cells under nitrogen atmosphere or cells lacking respiratory chain (ρ^0 strain) exposed to NiO NPs displayed low level of ROS and higher resistance to NiO NPs; these facts indicate the involvement of the mitochondrial respiratory chain in the ROS accumulation.

Yeast cells exposed to NiO NPs presented decreased levels of reduced glutathione (estimated with monochlorobimane). On the other hand, the knockout mutants *gsh1*Δ and *gsh2*Δ, without or with a reduced level of GSH, respectively, comparatively to WT strain, displayed augmented levels of ROS and sensitivity to NiO NPs, which underline the central role of reduced glutathione (GSH) against OS NiO NPs-induced.

In conclusion, the results obtained strongly suggest that NiO NPs induces the loss of cell viability in *S. cerevisiae* through OS, which most likely result from the combined effect of the enhancement of intracellular ROS (due to the perturbation of the electron transfer chain in mitochondria) and the depletion of GSH pool. The present study provides new insights into the understanding of the molecular mechanisms underlying to ROS generation and the toxicity induced by NiO NPs in yeasts.