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Genotoxic effects of zinc oxide nanoparticles†

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The potential toxicity of nanoparticles has currently provoked public and scientific discussions, and attempts to develop generally accepted handling procedures for nanoparticles are under way. The investigation of the impact of nanoparticles on human health is overdue and reliable test systems accounting for the special properties of nanomaterials must be developed. Nanoparticulate zinc oxide (ZnO) may be internalised through ambient air or the topical application of cosmetics, only to name a few, with unpredictable health effects. Therefore, we analysed the determinants of ZnO nanoparticle (NP) genotoxicity. ZnO NPs (35–58 nm in diameter) were investigated at concentrations of 0.1, 10 and 100 $\mu\text{g mL}^{-1}$ using the cell line A549. Internalised NPs were only infrequently detectable by TEM, but strongly increased Zn^{2+} levels in the cytoplasm and even more in the nuclear fraction, as measured by atom absorption spectroscopy, indicative of an internalised zinc and nuclear accumulation. We observed a time and dosage dependent reduction of cellular viability after ZnO NP exposure. ZnCl_2 exposure to cells induced similar impairments of cellular viability. Complexation of Zn^{2+} with diethylene triamine pentaacetic acid (DTPA) resulted in the loss of toxicity of NPs, indicating the relevant role of Zn^{2+} for ZnO NP toxicity. Foci analyses showed the induction of DNA double strand breaks (DSBs) by ZnO NPs and increased intracellular reactive oxygen species (ROS) levels. Treatment of the cells with the ROS scavenger *N*-acetyl-L-cysteine (NAC) resulted in strongly decreased intracellular ROS levels and reduced DNA damage. However, a slow increase of ROS after ZnO NP exposure and reduced but not quashed DSBs after NAC-treatment suggest that Zn^{2+} may exert genotoxic activities without the necessity of preceding ROS-induction. Our data indicate that ZnO NP toxicity is a result of cellular Zn^{2+} intake. Subsequently increased ROS-levels cause DNA damage. However, we found evidence for the assumption that DNA-DSBs could be caused by Zn^{2+} without the involvement of ROS.

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Introduction

Zinc oxide nanoparticles (ZnO NPs) are used in a variety of applications, including cosmetics and coatings.^{1–4} The common utilization of ZnO NPs results in an increasing

environmental release (estimated ~500 tons in 2009 in the U.S.) and may lead to adverse health effects.⁵ The inflammatory potential of inhaled metal oxides like ZnO has been well known for decades as acute metal fume fever syndrome.⁶ Recent studies demonstrated that ZnO NPs induce multiple toxic responses in mammalian cells, bacteria, algae, fish, nematodes and plants.^{7–10} Several studies have been reported with the aim to decipher the role of solid ZnO NPs, dissolved Zn^{2+} and triggered intracellular metabolites and pathways. Whereas some authors found an effect from both solid ZnO NPs as well as dissolved Zn ions, for the toxic responses of exposed cells^{11–17} other studies have suggested that ZnO NP toxicity is mainly attributable to the intake of the particles by endocytosis, their dissolution and subsequently generated reactive oxygen species (ROS) in mitochondria.¹⁸ The release of ROS has been reported in cells exposed to ZnO NPs,^{12,18,22–25} suggesting the destructive oxidation of cellular components and structures as the possible downstream mechanism of ZnO NP exposure. More recently, DNA-double strand breaks (DSBs) have been observed after ZnO NP

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†Electronic supplementary information (ESI) available: (1) NP characterization: representative TEM images of 35–58 nm ZnO NPs and XRD patterns of as synthesized ZnO NPs. (2) Analysis of viability using ZnO NP supernatants. See DOI: 10.1039/c5nr01167a

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