Genotoxicity of zinc oxide nanoparticles in human bronchial epithelial cells and mesothelial cells *in vitro*

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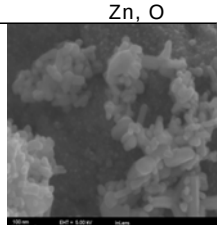
Introduction

Engineered nanomaterials have often unique properties when compared with similar materials of larger size, which may result in differential toxicity. ZnO nanoparticles are used, e.g., in cosmetics. ZnO nanoparticles are cytotoxic and genotoxic in various *in vitro* systems, but the underlying mechanism is not well understood. ZnO is partly soluble, and its solubility is increased in acidic conditions and in the presence of chelators. The genotoxic effects of ZnO *in vitro* may be caused by ZnO dissolving inside the cell, after particle uptake. A treatment with soluble zinc compounds at similar ion concentrations could then be expected to result in a differential effect.

Materials and methods

The cells were exposed to nanosized ZnO (Table 1) and $ZnCl_2$ (Sigma-Aldrich, product no. 211273). Cytotoxicity was assessed by cell count using Trypan blue and CellTiter-Glo Luminescent Assay (Promega). Only doses showing 55±5% cytotoxicity were included in the genotoxicity assays. The induction of DNA damage was studied by single-cell gel electroforesis assay (comet) with 4-h, 24-h, and 48-h exposures in human bronchial epithelial BEAS 2B cells and mesothelial MeT 5A cells. H_2O_2 (20 mM) was used as a positive control. The percentage of DNA in tail was analysed from 100 cells/ sample. The cytokinesis-block micronucleus (CBMN) assay and fluorescence *in situ* hybridization (FISH) were used to study the induction of micronuclei (MN) and the mechanism of micronucleus formation in BEAS 2B cells. Mitomycin C was used as a positive control. Dublicate cultures were included in all the assays.

Table 1. **Characterization of the ZnO nanoparticles.**

Vendor	Umicore
Product number	ZANO
Particle size	30-35 nm
Specific surface area	22 m^2/g
Composition	Zn, O
Morphology by SEM	

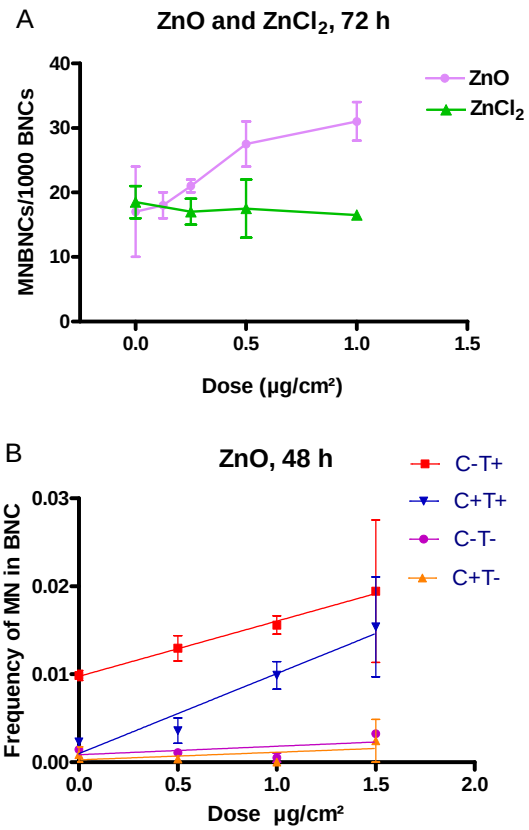


Fig. 1. **Cytokinesis-block micronucleus assay.**

A. Micronucleus induction in BEAS 2B cells after a 72-h exposure to ZnO and $ZnCl_2$. Micronucleated binucleate cells (MNBNC) were scored from 1000 binuclear cells (BNC) / culture. ZnO significantly increased the number of micronucleated BEAS 2B cells at 0.5 and 1 µg/cm², and the effect was dose-dependent. $ZnCl_2$ did not increase the frequency of micronucleated BEAS 2B cells at any of the tested doses. Error bars show SEM.

B. Micronucleus (MN) content analysis by centromeric (C) and telomeric (T) FISH after 48-h exposure to ZnO, linear regression. Total micronucleus frequency was scored from 1000 BNC/ culture and MN content was analysed from 25 MN/ culture. Micronuclei with only telomeric signal (C-T+) were assumed to contain chromosome fragments resulting from a clastogenic effect, whereas micronuclei with both centromeric and telomeric signals (C+T+) were interpreted as containing whole chromosomes resulting from an aneugenic effect. Both types of MN were induced by ZnO with a dose-response, although there was a higher induction of C+T+ MN than C-T+ MN. Error bars show SEM.

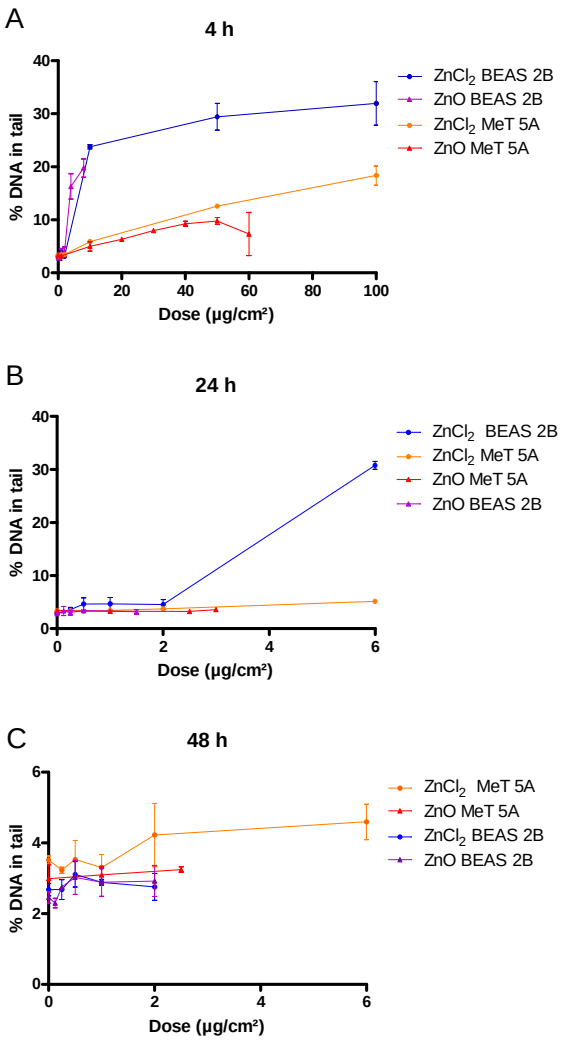


Fig. 2. **Comets assay. Mean DNA damage (% DNA in tail) after 4-h (A), 24-h (B) and 48-h (C) exposure to ZnO or $ZnCl_2$ in BEAS 2B and Met 5A cells. DNA damage was induced in the 4-h exposure by both ZnO and $ZnCl_2$, in the 24-h exposure by $ZnCl_2$, and in the 48-h exposure by $ZnCl_2$, but only in Met 5A cells. Doses showing more than 50% cytotoxicity were excluded. Error bars show SEM.**

Conclusions

- **ZnO is clearly more cytotoxic than $ZnCl_2$ in both BEAS 2B and Met 5A cells**
- **ZnO induces DNA damage and produces micronuclei via aneugenic and clastogenic mechanisms**
- **The genotoxic effects of nanosized ZnO are not simply explained by Zn^{2+} ions released from the particles to the culture medium**
- **These findings probably reflect a fast uptake and intracellular solubilisation of the ZnO nanoparticles.**

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