

# Cytotoxicity of Metallic Oxide Nanoparticles in Human Neural and Non-Neural Cells

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## ABSTRACT

The use of nanomaterials in industrial applications (e.g., drug delivery, additives to drugs and cosmetics) has escalated in the last decade, leading to the possibility of their becoming environmental pollutants. However, these cytotoxic and other health effects of exposure to nanoparticles have not been systematically investigated. We have developed a series of cell models to facilitate a systematic investigation of cytotoxicity of metallic oxide nanoparticles. In this study, we tested the hypothesis that metallic oxide nanoparticles exert differential cytotoxic effects on human neural and non-neural cell types. We exposed human neural (SK-N-SH) and non-neural (HepG2 and BJ) cell lines to zinc oxide and magnesium oxide nanoparticles (0.1-100 µg/mL) for 48 hours and then determined their survival. The results of our on-going studies indicate that, in general, human neural cells are more susceptible to the cytotoxicity of metallic oxide nanoparticles studied compared with human non-neural cells. Moreover, the metallic oxide nanoparticles so far investigated also exert dissimilar effects depending on the cell type under study. Our results provide some support for our hypothesis and may have implications in nanotoxicity and health risks involved with exposure to metal oxide nanoparticles.

**Keywords:** metal oxide nanoparticles, nanotoxicity, titanium oxide, magnesium oxide, zinc oxide

## 1 INTRODUCTION

The use of nanomaterials in industrial applications (e.g., drug delivery, additives to drugs and cosmetics) has escalated in the last decade, leading to the possibility of their becoming environmental pollutants [1]. Because of their wide use, occupational and other environmental exposure to nanomaterials, especially nanoparticles, may pose as health risks [2-5]. There are some recent studies suggesting that exposure to nanoparticles may induce as yet ill-defined cytotoxicity in some mammalian cell types [2]. However, these cytotoxic and other health effects of exposure to nanoparticles have not been systematically investigated [2-5].

We have therefore developed a series of cell models to facilitate a systematic investigation of cytotoxicity of metallic oxide nanoparticles and the underlying molecular mechanisms [4,5]. In this study, we tested the hypothesis that metallic oxide nanoparticles exert differential cytotoxic effects on human neural and non-neural cell types.

We exposed human neural (SK-N-SH) and non-neural (HepG2 and BJ) cell lines to a range of concentrations (0.1-100 µg/mL) of zinc oxide and magnesium oxide nanoparticles for 48 hours because we had previously established that this is an optimal exposure time to determine cytotoxicity of metallic oxide nanoparticles on a comparative basis [see 5 and references therein]. Then we determined the survival of the cells exposed to the

nanoparticles and compared them to those in untreated (i.e., control) cells from the corresponding cell lines using a modified MTT assay, which has been extensively employed to assess cell survival and cell proliferation.

## 2 MATERIALS AND METHODS

### 2.1 Materials

Dulbecco's minimum essential medium (DMEM) for cell growth and other chemicals (usually of analytical grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Magnesium oxide (MgO) nanopowder (from Sigma-Aldrich, St. Louis, MO, USA; Cat. #549649; nanopowder, <50 nm particle size) and zinc oxide (ZnO) nanopowder (from Sigma-Aldrich, St. Louis, MO, USA; Cat. #544906; nanopowder, <100 nm particle size) were suspended in 100 mL of sterile saline in a sealed conical flask and the suspension stirred at ambient temperature overnight before being used to be diluted to the specified concentrations for treatment of cells (see below).

### 2.2 Cells and Culture Conditions

Human astrocytoma SK-N-SH (Neurons-like) cells and normal human fibroblasts (BJ cells) were obtained from ATCC (Manassas, VA, USA) and were cultured in DMEM, supplemented with 10% (v/v) fetal bovine serum and were incubated at 37°C and 5% (v/v) CO<sub>2</sub> as described previously [6].

### 2.3 Cellular Viability Assay

Cellular viability was determined using the MTT assay [6]. Cells were seeded with a density of 2,500 or 3,000 cells per well in 96-well plates and allowed to attach to the bottom of each well. Cells were then treated with specified concentrations of MgO, or ZnO nanoparticles for 48 hours at 37°C. MTT dye (0.5% (w/v) in phosphate-buffered saline) was added to each well and the plates (set one) were incubated for another 4 or 12 hours at 37°C. Purple-colored insoluble formazan crystals in viable cells were dissolved using dimethyl sulfoxide (DMSO, 100  $\mu$ L per well). The absorbance of the content of each well in each plate was then measured at 567 nm using the multi-detection microplate reader (Bio-Tek Synergy HT, Winooski, VT, USA). To prevent MgO and ZnO nanoparticles from interfering with this assay (data not shown), the formazan material dissolved in DMSO in each well of each plate was quantitatively transferred to an empty well in another plate (set two) while the material in DMSO from a well with nanoparticles only (i.e., without cells) served as the corresponding control. The absorbance of the contents of each well in each plate (set two) was again measured after transfer using the same method as depicted above [5].

### 2.4 Cellular Morphology

The changes in the morphology of SK-N-SH cells treated with specified concentrations of nanoparticles for 48 hours at 37°C as described previously were compared to that of corresponding untreated cells by light microscopy. Bright field images of cells were acquired using a Leica light microscope (Leica DM IRB, Bannockburn, IL, USA) equipped with a digital camera (Leica DFC 300 FX, Bannockburn, IL, USA) [7].

### 2.5 Statistical Analysis of Data

Results are presented as mean  $\pm$  standard error of the mean (S.E.M.) of 6-9 determinations in each experiment. Each experiment was performed at least three times. Data analysis was carried out by one-way ANOVA, followed by Tukey test for multiple comparisons using the software KaleidaGraph version 4 (Synergy Software, Reading, PA, USA). Significance level was set at  $p < 0.05$ .

## 3 RESULTS AND DISCUSSION

The limited literature on nanotoxicity of nanomaterials suggests that metal oxide nanoparticles may induce cytotoxic effects on mammalian cell types [see 2-5 and references therein]. However, there have been few systematic studies that focus on nanotoxicity in human cells and mainly non-neural cells have been studied [2,5,8]. We have therefore launched a series of systematic studies to elucidate the cytotoxicity of metallic oxide nanoparticles in human neural and non-neural cells.

Our initial studies demonstrated that titanium dioxide, magnesium oxide, and zinc oxide nanoparticles induced decreases in cell survival in both human astrocytoma U87 (astrocyte-like) and HFF-1 (normal human fibroblasts) cells [5,8]. Moreover, the effects of those nanoparticles varied between the two cell types and between the types of nanoparticles investigated. This study further elucidates the effects of metallic oxide nanoparticles on human neural and non-neural cells.

Although magnesium oxide and zinc oxide nanoparticles induced a decrease in cell survival in SK-N-SH (neurons-like) cells, zinc oxide nanoparticles exerted the greater decreases in cell survival in SK-N-SH cells with an IC<sub>50</sub> of  $\sim 14$   $\mu$ g/mL (Figure 1). The effect of zinc oxide nanoparticles was detected at concentrations higher than 1  $\mu$ g/mL while at concentrations of 50  $\mu$ g/mL or higher, few SK-N-SH cells survived (Figure 1). On the other hand, magnesium dioxide nanoparticles exerted decreases in SK-N-SH cell survival with an IC<sub>50</sub> of  $\sim 60$   $\mu$ g/mL, approximately five times that of zinc oxide nanoparticles, and their effect was only detected at concentrations higher than 10  $\mu$ g/mL (Figure 1).

Survival of SK-N-SH Cells Treated with Zinc Oxide and Magnesium Oxide Nanoparticles

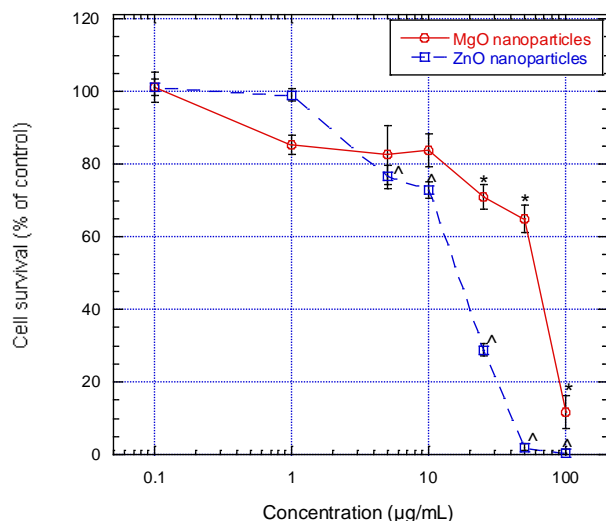


Figure 1: Treatment of neuroblastoma (Neurons-like) SK-N-SH cells with magnesium oxide (MgO) and zinc oxide (ZnO) nanoparticles induced differential decreases in cell survival. SK-N-SH cells were treated with increasing concentrations of magnesium oxide, or zinc oxide nanoparticles (0.1-100 µg/mL) for 48 hours. MTT assay was used in determining cell survival. Values were then normalized with respect to control mean (mean of untreated cells) and given as means  $\pm$  SEM of 6-9 replicates. (^ and \* p<0.05 versus corresponding control means)

In general, the results of the morphological observations with bright field light microscopy on the effects of zinc oxide and magnesium oxide nanoparticles on SK-N-SH cells (data not shown) were consistent with results on cell survival obtained with MTT assays (Figure 1).

Treatment of BJ cells (normal human fibroblasts) with zinc oxide and magnesium oxide nanoparticles showed somewhat similar trends as those of SK-N-SH cells treated with these nanoparticles. Treatment of BJ cells with zinc oxide nanoparticles for 48 hours induced the greater decreases in cell survival in comparison with magnesium oxide nanoparticles, similar to the effects of zinc oxide nanoparticles on SK-N-SH cells, with an IC<sub>50</sub> of ~14 µg/mL (Figure 2). The effect of zinc oxide nanoparticles on BJ cells were detected at concentrations higher than 1 µg/mL (Figure 2).

In contrast with the effect of zinc oxide nanoparticles, exposure of BJ cells to magnesium oxide nanoparticles did not significantly decrease BJ cell survival at any of the concentrations examined (Figure 2).

Survival of BJ Cells Treated with Zinc Oxide and Magnesium Oxide Nanoparticles

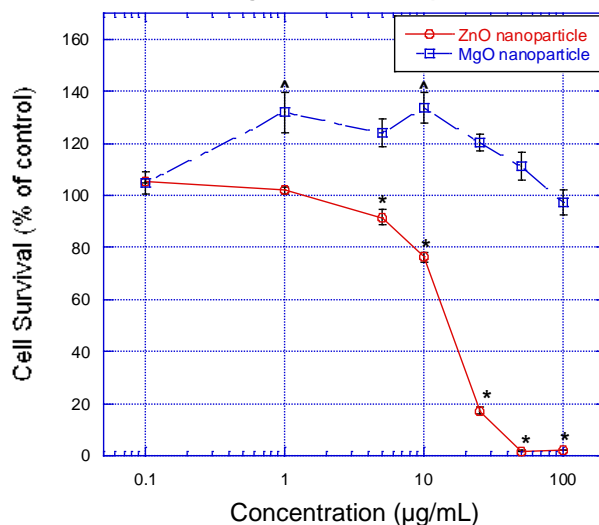


Figure 2: Treatment of BJ cells (normal human fibroblasts) with magnesium oxide and zinc oxide nanoparticles induced differential decreases in cell survival. BJ cells were treated with increasing concentrations of magnesium oxide or zinc oxide nanoparticles (0.1-100 µg/mL) for 48 hours. MTT assay was used in determining cell survival. Values were then normalized with respect to control mean (mean of untreated cells) and given as means  $\pm$  SEM of 6-9 replicates. (^ and \* p<0.05 versus corresponding control means)

Our results indicated treatments with zinc and magnesium oxide nanoparticles employed demonstrate a dose-related decreases in cell survival in both (i.e., SK-N-SH and BJ) cell types, although the dose-related decreases varied between these cell types. Overall, in both cell types used (SK-N-SH and BJ), zinc oxide nanoparticles was the more effective (Figure 1 and 2).

## 4 CONCLUSIONS

Our study is the first to report on the comparative cytotoxic effects of zinc oxide and magnesium oxide nanoparticles on the human neural SK-N-SH cells that are neurons-like. Furthermore, we have compared the effects of these metal oxide nanoparticles on BJ cells (i.e., normal human fibroblasts). Our results are consistent with the hypothesis that magnesium oxide and zinc oxide nanoparticles exert differential cytotoxic effects on human neural and non-neural cells.

The results of our studies completed to date may have pathophysiological implications in human exposure to these metal oxide nanoparticles: as such these effects we have demonstrated merit further systematic investigation. Moreover, our results also suggest that our cellular approach may be modeled and gainfully employed for general cytotoxicity studies of nanoparticles and other nanomaterials.

We are continuing to further elucidate the cell death and other molecular mechanisms underlying the cytotoxic effects of metallic oxide nanoparticles on human neural and other cell types as part of our systematic program to elucidate the cytotoxicity and the underlying molecular mechanisms of metallic oxide nanoparticles [4,5,8,9]. Our systematic, interdisciplinary research program continues to provide the needed data that are not currently present in the literature of metallic oxide nanoparticles [5,8,9].

## 5 ACKNOWLEDGMENTS

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