Differential Cytotoxicity of Metallic Oxide Nanoparticles in Mammalian Cells

Maria B. Lai[*], Sirisha Jandhyam[**], Vikas V. Dukhande[***], Alok Bhushan[****], Christopher K. Daniels[*****], Solomon W. Leung[******], and James C.K. Lai[*******]

[*]Department of Biomedical & Pharmaceutical Sciences, College of Pharmacy, Idaho State University, Pocatello, ID 83209, USA, biqlai@hotmail.com
[**]Department of Biomedical &Pharmaceutical Sciences, College of Pharmacy, Idaho State University, Pocatello, ID 83209, USA, sirisha@pharmacy.isu.edu
[***]Department of Biomedical &Pharmaceutical Sciences, College of Pharmacy, Idaho State University, Pocatello, ID 83209, USA, vikas.dukhande@gmail.com
[****]Department of Biomedical & Pharmaceutical Sciences, College of Pharmacy, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, abhushan@otc.isu.edu
[*****]Department of Biomedical & Pharmaceutical Sciences, College of Pharmacy, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, cdaniels@otc.isu.edu
[******]Civil and Environmental Engineering, College of Engineering, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, leunsolo@isu.edu
[*******]Department of Biomedical & Pharmaceutical Sciences, College of Pharmacy, and Biomedical Research Institute, Idaho State University, Pocatello, ID 83209, USA, lai@otc.isu.edu (corresponding author)

ABSTRACT

Because the putative cytotoxic effects of metal oxide nanoparticles on human cell types have not been fully elucidated, we investigated the hypothesis that titanium oxide, magnesium oxide, and zinc oxide nanoparticles exert differential cytotoxic effects on human astrocytoma (U87) cells and human fibroblasts (HFF-1 cells). Treatment with 5 µg/mL of titanium oxide (but not magnesium or zinc oxide) nanoparticles induced significant decreases in survival of U87 but not that of HFF-1 cells. However, treatment with zinc oxide nanoparticles at higher concentrations exerted greater decreases in survival of U87 cells more so than in HFF-1 cells, compared to the corresponding effects of titanium and magnesium oxide nanoparticles. By contrast, treatment of both cell types with magnesium oxide nanoparticles did not lower cell survival below 50% even at higher concentrations. Thus, these results are consistent with our hypothesis and may have implications in health risks involved with exposure to metal oxide nanoparticles.

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

1 INTRODUCTION

Nanomaterials have been increasingly used in industrial applications (e.g., drug delivery, additives to drugs and cosmetics) [1]. Because of their ubiquitous applications, occupational exposure to nanoparticles and other nanomaterials may pose as health risks. Recent studies have suggested that exposure to nanoparticles may induce cytotoxic effects in some mammalian cell types although these effects have not been systematically investigated [1].

Few studies have focused on systematic investigation of cytotoxic effects of metal oxide nanoparticles, though some have been conducted on titanium dioxide nanoparticles. Stearns et al (2001) showed that titanium dioxide nanoparticles (50 nm diameter) could be taken up into human lung epithelial (A549) cells via endocytosis [2]. Other studies indicated exposure of these human lung epithelial cells to titanium dioxide nanoparticles induced inflammatory responses and cellular damage [3-5]. Nevertheless, not many human cell types other than lung epithelial cells have been studied with respect to the putative cytotoxicity of metal oxide nanoparticles.

The minimal number of studies on different human cell types notwithstanding, even fewer studies have elucidated the effects of metal oxide nanoparticles on mammalian cells other than the effects of titanium oxide nanoparticles. To gain a better understanding of the effects of metal oxide nanoparticles on human cell types, we utilized U87 (astrocyte-like) cells as model for human astrocytes and HFF-1 (normal human fibroblasts) cells and treated both cell types with titanium dioxide, zinc oxide, and magnesium oxide nanoparticle at the same concentration range to investigate the hypothesis that the metal oxide

nanoparticles induce differential cytotoxicity in human cells.

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). Titanium dioxide (TiO_2) anatase nanoparticles (from Sigma-Aldrich, St. Louis, MO, USA; Cat. #637254; nanopowder, <25 nm particle size, 99.7% (metal basis)), 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 U87 (astrocytes-like) cells and normal human fibroblasts (HFF-1 cells) were obtained from ATCC (Manassas, VA, USA) and were cultured in DMEM, supplemented with 10% (v/v) (and in the case of HFF-1 cells, 15% (v/v)) fetal bovine serum and were incubated at 37°C and 5% (v/v) CO_2 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 cells per well in 96-well plates and allowed to attach to the bottom of each well for 60-90 minutes. Cells were then treated with specified concentrations of TiO_2, 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 hours at 37°C. Purple-colored insoluble formazan crystals in viable cells were dissolved using dimethyl sulfoxide (DMSO, 100 µL 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 TiO_2 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.

2.4 Cellular Morphology

The changes in the morphology of U87 cells treated with specified concentrations of TiO_2 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 ± 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

Previous studies have suggested that metal oxide nanoparticles may induce cytotoxic effects on mammalian cell types [3-5]. The putative cytotoxic effects of metal oxides have been studied on human lung epithelial cells using titanium dioxide nanoparticles as the test metal oxide nanoparticles [1-5]. On the other hand, our results 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 with increasing concentrations as determined by the MTT assay (Figure 1 and 2). Although titanium dioxide, magnesium oxide, and zinc oxide nanoparticles induced decreases in cell survival in U87 and HFF-1 cells, the effects varied between the two cell types and between the types of nanoparticles investigated.

Though titanium dioxide induced a decrease in cell survival in U87 cells, zinc oxide nanoparticles exerted the greatest decreases in cell survival in U87 cells with an IC_{50} of ~11 µg/mL (Figure 1). Titanium dioxide nanoparticles exerted decreases in cell survival with an IC_{50} of ~50 µg/mL, almost five times that of zinc oxide nanoparticles (Figure 1). Interestingly, upon exposure to magnesium oxide nanoparticles for 48 hours, U87 cell survival did not decrease below 50% with respect to that in control (i.e., untreated cells) (Figure 1). Moreover, treatment at the highest concentration of magnesium oxide nanoparticles used (100 µg/mL) decreased cell survival only by ~30%, whereas treatment at the same concentration (100 µg/mL) with titanium dioxide nanoparticles decreased U87 cell survival by ~70% and with zinc oxide nanoparticles decreased U87 cell survival by ~95% (Figure 1).

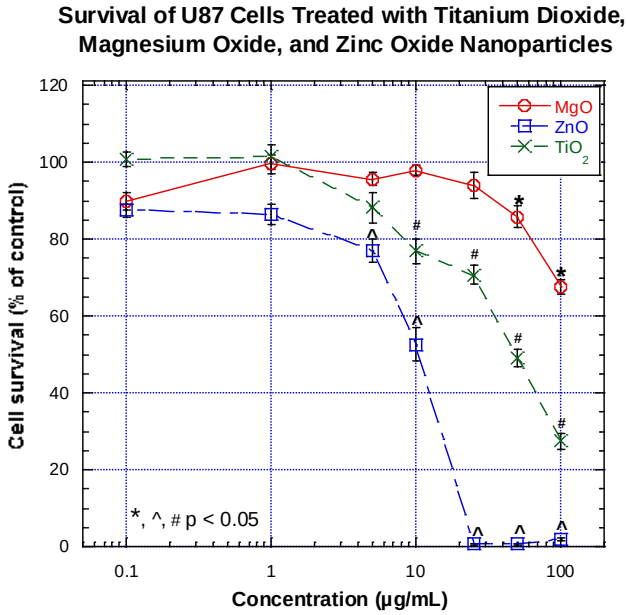

Figure 1: Treatment of astrocytoma (astrocytes-like) U87 cells with titanium dioxide, magnesium oxide, and zinc oxide nanoparticles induced differential decreases in cell survival. U87 cells were treated with increasing concentrations of titanium dioxide, magnesium oxide, or zinc oxide nanoparticles (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 ± SEM of 6-9 replicates.

In general, the results of the morphological observations with bright field light microscopy on the effects of titanium dioxide nanoparticles on U87 cells (data not shown) were consistent with results on cell survival obtained with MTT assays (Figure 1).

Treatment of HFF-1 (normal human fibroblasts) cells with zinc oxide, titanium dioxide, and magnesium oxide nanoparticles showed somewhat similar trends as those of U87 cells treated with these nanoparticles. Treatment of HFF-1 cells with zinc oxide nanoparticles for 48 hours induced the greatest decreases in cell survival in comparison with the other two nanoparticles, similar to the effects of zinc oxide nanoparticles on U87 cells, with an IC_{50} of ~25 µg/mL (Figure 2).

Exposure of HFF-1 cells to titanium dioxide nanoparticles exerted a 50% decrease in cell survival at ~40 µg/mL, whereas a concentration of ~75 µg/mL of magnesium oxide nanoparticles was needed to attain the same effect (Figure 2). Yet, at the highest concentrations of both titanium and magnesium oxide nanoparticles employed (100 µg/mL), HFF-1 cell survival decreased similarly, to less than 30% of that of untreated cells (Figure 2). However, at lower concentrations of the nanoparticles used (10 µg/mL), titanium dioxide induced greater decreases in cell survival compared to zinc or magnesium oxide nanoparticles.

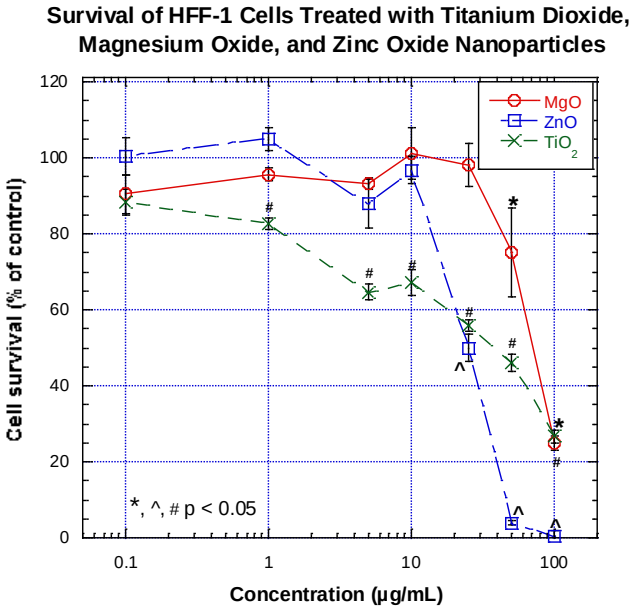

Figure 2: Treatment of HFF-1 cells (normal human fibroblasts) with titanium dioxide, magnesium oxide, and zinc oxide nanoparticles induced differential decreases in cell survival. HFF-1 cells were treated with increasing concentrations of titanium dioxide, magnesium oxide, and zinc oxide nanoparticles (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 ± SEM of 6-9 replicates.

Magnesium oxide nanoparticles exerted a significant difference in decreasing cell survival in U87 cells compared to that in HFF-1 cells with ~30% and ~70% decrease, respectively, at a concentration of 100 µg/mL (Figure 1 and 2). These results suggest that HFF-1 (normal human fibroblasts) cells are more sensitive towards magnesium oxide nanoparticle cytotoxicity than U87 (astrocyte-like) cells. On the other hand, lower concentrations of zinc oxide nanoparticles (10 µg/mL) induced greater decreases in U87 cell survival than in HFF-1 cell survival (Figure 1 and 2). This observation suggests that zinc oxide nanoparticles are more cytotoxic in U87 (astrocyte-like) cells more so than in HFF-1 (normal human fibroblasts) cells. Interestingly, titanium dioxide nanoparticles exerted similar effects in

decreasing cell survival in both U87 and HFF-1 cell types (Figure 1 and 2).

Our results indicated treatments with all three metal oxide nanoparticles employed demonstrate a dose-related decreases in cell survival in both (i.e., U87 and HFF-1) cell types, though the dose-related decreases varied between these cell types. Overall, in both cell types used (U87 and HFF-1), zinc oxide nanoparticles was the most effective, followed by titanium dioxide nanoparticles, and magnesium oxide nanoparticles were the least effective (Figure 1 and 2). Similar cytotoxic studies have been noted using titanium dioxide nanoparticles in human lung epithelial (A549) cells [2]; however, prior to our study, very few studies have been conducted on the cytotoxic effects of metal oxide nanoparticles on other human cell types (especially human neural cells).

4 CONCLUSIONS

Our study is the first to report on the comparative cytotoxic effects of zinc oxide, titanium dioxide, and magnesium oxide nanoparticles on human neural cells (i.e., U87). Furthermore, we have compared the effects of these metal oxide nanoparticles on normal human fibroblasts. Our results are consistent with the hypothesis that titanium oxide, magnesium oxide, and zinc oxide nanoparticles exert differential cytotoxic effects on human astrocytoma (U87) cells and normal human fibroblasts (HFF-1 cells).

Other studies are ongoing in our laboratories to further elucidate the cell death and other molecular mechanisms underlying the cytotoxic effects of metal oxide nanoparticles on human neural and other cell types. Nevertheless, 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.

5 ACKNOWLEDGMENTS

Our studies were supported by an USAMRMC Project Grant (Contract #W81XWH-07-2-0078) and NIH Grant #P20 RR016454 from the Idaho INBRE Program of the National Center for Research Resources.

REFERENCES

[1] Wang JJ, Sanderson BJ, Wang H, "Cyto-and genotoxicity of ultrafine TiO_2 particles in cultured human lymphoblastoid cells," Mutat Res, 628, 99-106, 2007.

[2] Stearns RC, Paulauskis JD, Godleski JJ, "Endocytosis of ultrafine particles by A549 cells," Am J Respir Cell Mol Biol, 24, 108-115, 2001.

[3] Singh S, Shi T, Duffin R, et al, "Endocytosis, oxidative stress and IL-8 expression in human lung epithelial cells upon treatment with fine and untrafine TiO_2: role of the specific surface area and of surface methylation of the particles," Toxicol Appl Pharmacol, 222, 141-151, 2007.

[4] Monteiller C, Tran L, MacNee W, et al, "The pro-inflammatory effects of low-toxicity low-solubility particles, nanoparticles and fine particles, on epithelial cells in vitro: the role of surface area," Occup Environ Med, 64, 609-615, 2007.

[5] Park S, Lee YK, Jung M, et al, "Cellular toxicity of various inhalable metal nanoparticles on human alveolar epithelial cells," Inhal Toxicol, 19 Suppl 1, 59-65, 2007.

[6] Malthankar GV, White BK, Bhushan A, et al, "Differential lowering by manganese treatment of activities of glycolytic and tricarboxylic acid cycle enzymes in neuroblastoma and astrocytoma cells is associated with manganese-induced cell death," Neurochem Res, 29, 709-717, 2004.

[7] Dukhande VV, Malthankar-Phatak GH, Hugus JJ, et al, "Manganese induced neurotoxicity is differentially enhanced by glutathione depletion in astrocytoma and neuroblastoma cells," Neurochem Res, 31, 1349-1357, 2006.