Silicon Dioxide Nanoparticles Exert Dissimilar Cytotoxic Effects on Mammalian Cell Types

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ABSTRACT

Applications of nanomaterials (e.g., silicon dioxide (SiO_2) nanoparticles) are ubiquitous. Thus, environmental and occupational exposure to these nanoparticles could pose potential health risk. SiO_2 microparticles are toxic in animals *in vivo* and in some mammalian cell types *in vitro* although studies using human cell types are scarce. We have compared their effects on human astrocytoma U-87 (astrocytes-like) and neuroblastoma SK-N-SH (neurons-like) cells, and on normal human fibroblasts.

Treatment with SiO_2 nanoparticles (0.1-100 μg/ml) induced dose-related, differential cytotoxic effects on the human cell types studied, the effect being least pronounced in fibroblasts. Observations of the treated cells using bright-field light microscopy were compatible with the results noted using MTT assay. Thus, our results may have implications in risk assessment of environmental and occupational exposure to these nanoparticles.

Keywords: Silicon dioxide nanoparticles, nanotoxicity of nanoparticles, human cell types, cytotoxicity

1 INTRODUCTION

Applications of metal oxide nanoparticles like silicon dioxide (SiO_2) nanoparticles in food additives, cosmetics, paints, drug delivery agents are increasing. Because of these increasing applications in diversified fields, occupational and accidental exposure of humans to these nanoparticles is escalating [1]. This exposure to SiO_2 nanoparticles may pose potential health risks to humans as well as other species [2]. Therefore, it is important to systematically elucidate the toxicity profiles of these nanoparticles. Though previous studies have shown that these nanoparticles may induce toxicity in humans as well as other species, their cytotoxic effects have not been fully elucidated.

Our goal is to investigate the putative cytotoxicity of SiO_2 nanoparticles on different human cell types. We hypothesized that SiO_2 nanoparticles exert differential cytotoxic effects on various human cell types. To test the hypothesis, we employed two human neurotumor cell lines (SK-N-SH, neurons-like & U-87, astrocytes-like) and normal human fibroblasts (HFF-1 cells) to determine the effects of SiO_2 nanoparticles on cell survival and on cell death mechanism.

2 MATERIALS AND METHODS

2.1 Materials:

Silicon dioxide (SiO_2) nanoparticles (50 mg) (0.012 micron, from STREM Chemicals, Newburyport, MA, USA) were dissolved in 100 ml of sterile saline under sterilized conditions in a conical flask and the flask was sealed: the suspension was stirred at room temperature for at least one hour before being added to the cells. Different concentrations (0.1 to 100 μg/mL) were prepared to treat the cells. Dulbecco's minimum essential medium (DMEM) and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2 Cell culture and conditions:

Human astrocytoma U-87 and neuroblastoma SK-N-SH cells were obtained from ATCC (Manassas, VA, USA) and were cultured in Dulbecco's minimum essential medium (DMEM), supplemented with 10% (v/v) fetal bovine serum and were incubated at 37ºC and 5% (v/v) CO_2. Normal human fibroblasts (HFF-1 cells) were also obtained from ATCC (Manassas, VA, USA) and cultured under similar conditions as those for U87 cells except 15% (v/v) fetal bovine serum were used [4].

2.3 Cell Viability Assay:

Cellular viability was determined by the MTT assay [4]. Cells were added at equal numbers in each well of a 96-well plate and incubated for 60-90 min at 37ºC to allow them to attach to the bottom of the well. Cells were then treated with specified concentrations of SiO_2 nanoparticles for 48 hrs. At the end of the incubation period, the MTT dye (0.5% (w/v) in phosphate-buffered saline) was added to each well and the plates were incubated for 4 hrs at 37º C. Purple colored insoluble formazan crystals in viable cells were dissolved using dimethyl sulfoxide and the absorbance of the contents of the wells was measured at 567 nm using a multi-detection microplate reader (Bio-Tek Synergy HT, Winooski, VT, USA).

2.4 Determination of Cell Damage and Necrosis:

Cell damage was assessed by measuring lactate dehydrogenase (LDH) release from cells into the culture medium (a marker of necrotic cell damage and cell death). U-87 and SK-N-SH cells were cultured in DMEM in 75 cm^2 flasks until they were ~70% confluent and then treated with different concentrations of SiO_2 nanoparticles for 48 hrs at 37º C. Subsequently, the culture medium from each flask was removed and kept at -70º C until they were used for assaying LDH activity therein. LDH activity released by cells into the culture medium was measured by using a spectrophotometer by the Clark and Lai procedure [3].

2.5 Cellular Morphology

Changes in the morphology of U-87 and SK-N-SH cells and normal human fibroblast treated with SiO_2 nanoparticles were compared to that of the untreated (control) 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 300FX, Bannockburn, IL, USA) [5].

2.6 Statistical Analyses of Data:

Results are presented as mean ± standard error of the mean (S.E.M.). Data analysis was carried out by one-way ANOVA, followed by post-hoc Tukey's HSD test for multiple comparisons. Significance level was set at $p< 0.05$.

3 RESULTS AND DISCUSSION

There are very few studies demonstrating the cytotoxic effects of silicon dioxide nanoparticles on human neural cells [6]. We therefore employed human astrocytoma (astrocytes-like) U-87 and neuroblastoma (neuron-like cells) SK-N-SH cells and normal human fibroblasts (HFF-1 cells) to investigate the putative cytotoxic effects of silicon dioxide (SiO_2) nanoparticles.

3.1 Effect of treatment with SiO_2 nanoparticles on inhibition of cellular proliferation:

Exposure of U-87 cells to SiO_2 nanoparticles for 48 hrs induced dose-related decrease in cell survival of these cells (Figure 1). Treatment with 0.1μg/mL to 10μg/mL of SiO_2 nanoparticles showed approximately 20% decrease in the survival of U-87 cells whereas at concentrations of SiO_2 nanoparticles higher than 10 μg/mL, the dose-related decreases in cell survival was more pronounced with a 75% decrease in cell survival at the maximum concentration of nanoparticles employed (Fig 1). The concentration of SiO_2 nanoparticles that induced 50% decrease in the cell survival of U-87 cells (i.e., the IC_{50} value) was ~ 38 μg/mL (from Figure 1).

Additionally, exposure of SK-N-SH and HFF-1 cells to SiO_2 nanoparticles also induced dose-related decreases in cell survival as determined by the MTT assay (Figures 2 & 3). However, the effects of SiO_2 nanoparticles on SK-N-SH and HFF-1 cells differed from that on U-87 cells: their effects on SK-N-SH and HFF-1 were only noted at the highest concentration employed (100 μg/mL) (Figures 2 & 3). Thus, both SK-N-SH and HFF-1 cells were less susceptible to the cytotoxic effect of SiO_2 nanoparticles than U-87 cells.

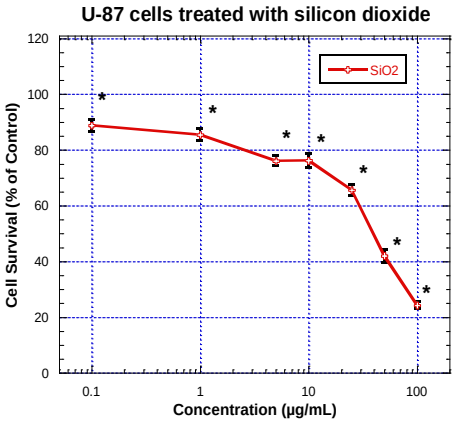

Figure 1. Treatment of U-87 cells with silicon dioxide nanoparticles induced decrease in cell survival.

U-87 cells were treated with different concentrations of silicon dioxide (0.1-100 µg/mL) for 48 hrs. Then MTT assay was employed to determine the survival of cells. Values were normalized with respect to the control mean (i.e., mean of untreated cells). The experiment was repeated three times. *p<0.05 versus control.

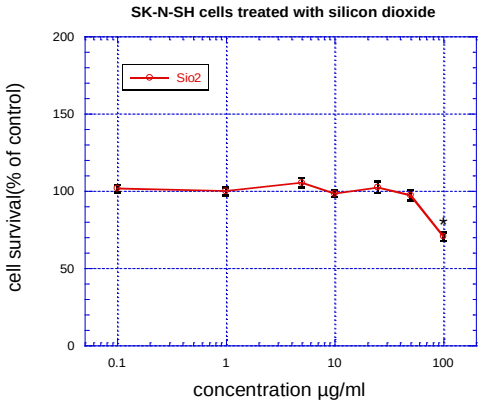

Figure 2. Treatment of SK-N-SH cells with silicon dioxide nanoparticles induced decrease in cell survival.

SK-N-SH cells were treated with different concentrations of silicon dioxide (0.1-100 µg/mL) for 48 hrs. Then MTT assay was employed to determine the survival of cells. Values were normalized with respect to the control mean (i.e., mean of untreated cells). The experiment was repeated three times. *p<0.05 versus control.

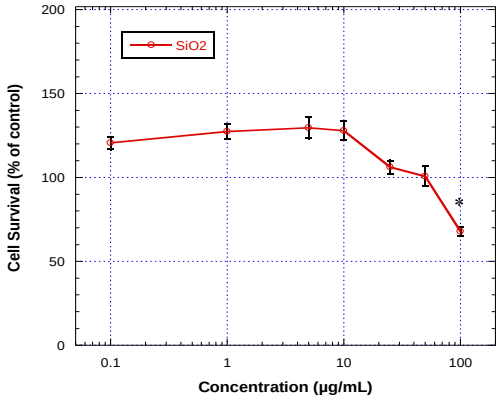

Figure 3. Treatment of HFF-1 cells with silicon dioxide nanoparticles induced decrease in cell survival.

HFF-1 cells were treated with different concentrations of silicon dioxide (0.1-100 µg/mL) for 48 hrs. Then MTT assay was employed to determine the survival of cells. Values were normalized with respect to the control mean (i.e., mean of untreated cells). Experiment was repeated three times. *p<0.05 versus control.

3.2 Alterations in cellular morphology of U-87 and SK-N-SH cells on exposure to SiO_2 nanoparticles:

We hypothesized that SiO_2 nanoparticles enter mammalian cells by endocytosis mechanism. Thus, we anticipated that exposure of these cells to SiO_2 nanoparticles at different concentrations may alter their cellular morphology [7]. Results from bright field light microscopy shows that these nanoparticles were surrounding the cells and some of them appeared to have penetrated into the cells (data not shown).

3.3 Exposure of U-87 and SK-N-SH cells to SiO_2 nanoparticles at higher concentrations increased Lactate Dehydrogenase (LDH) Release from these cells into the culture medium:

Lactate dehydrogenase release from cells is a marker for necrotic cell damage and cell death. We determined LDH activity in the medium to investigate whether treatment with SiO_2 nanoparticles induced LDH release from U-87 and SK-N-SH cells. Treatment of U-87 cells with different concentrations of SiO_2 nanoparticles showed a dose-related increase in the release of LDH into the culture medium. At treatment concentrations of 10, 50, and 100 µg/mL, their LDH release was 200, 870, and 1050 %, respectively

NSTI-Nanotech 2008, www.nsti.org, ISBN 978-1-4200-8504-4 Vol. 2

compared to LDH levels in medium of control (i.e., untreated) U-87 cells. Our results indicated that treatment with SiO_2 nanoparticles, at concentrations from 10 to 100 μg/mL, induced necrotic damage and cell death in U87 cells. Thus, our results from the LDH release into the medium correlated with the data obtained using the MTT assay, thereby suggesting that the cell death type induced by treatment with SiO_2 nanoparticles included at least necrosis. Our morphological data where swelling was noted in the treated cells were also consistent with this conclusion.

SK-N-SH cells treated with SiO_2 nanoparticles at 10, 50, and 100 μg/mL showed LDH release into the medium at the levels of 61, 160, and 320 %, respectively. Thus, our results indicated that U87 cells are more susceptible than SK-N-SH cells to the cytotoxic effect of these nanoparticles.

4 CONCLUSIONS

Taken together, our findings may have pathophysiological implications in human health risks in that upon entering the brain, SiO_2 nanoparticles could induce differential cytotoxic effects on different neural cell types although they clearly demonstrate that these nanoparticles do exert differential cytotoxic effects on various human cells types. Nevertheless, this interesting area certainly merits further systematic investigation to elucidate the molecular mechanisms underlying the cytotoxicity of these nanoparticles.

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.

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