Silicon Dioxide Nanoparticles Can Exert Cytotoxic Effects on Neural Cells

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

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

ABSTRACT

Nanomaterials have been increasingly employed in diverse industrial applications. Because of their ubiquitous use, exposure to nanoparticles and other nanomaterials may pose health risks. However, the cytotoxic effects of nanoparticles are unknown. We hypothesized that silicone dioxide nanoparticles can traverse the plasma membranes of cells via endocytosis and thereby exert their cellular and cytotoxic effects on cells. To test this hypothesis, we have investigated the effects of such nanoparticles on two human neurotumor cell lines. Our results indicate exposure to silicon dioxide nanoparticles led to cytotoxic damage and decreased cell survival and may have implications in nanotoxicity and health risk of exposure to metallic oxide nanoparticles.

Keywords: silicon dioxide nanoparticles; nanotoxicity; Cytotoxicity; cell survival/death; health risk

1 INTRODUCTION

Diverse nanomaterials have been increasingly employed in a wide variety of industrial application including drug delivery, fabrications of medical devices, and the manufacture of sensors [1,2]. Because of their ubiquitous use in such multiple industries, occupational exposure to nanoparticles and other nanomaterials may pose some as yet undefined health risks to workers in that environment [3,4]. Nevertheless, the health risk and cytotoxic effects of nanoparticles and other nanomaterials are almost unknown [3,4].

We have launched an interdisciplinary research program to systematically investigate the toxicity of nanoparticles. An initial observation prompted us to hypothesize that silicon dioxide nanoparticles can traverse the plasma membranes of cells through the endocytosis mechanism and thereby exert their cellular and cytotoxic effects on cells. To test this hypothesis, we have investigated the effects of silicon dioxide nanoparticles on two human brain tumor cell lines (SK-N-SH, a neuroblastoma line and U87, an astrocytoma line) employing light microscopy, the MTT assay (an indicator of cell survival), and lactate dehydrogenase (LDH) release into the culture medium (an indicator of cell damage and necrosis) [5-7].

2 MATERIALS AND METHODS

U-87 and SK-N-SH cells were obtained from ATCC (Manassas, VA, USA). Modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bicinchoninic acid protein assay kit from Pierce (Rockford, IL, USA) was used to determine protein levels in cells [5].

U-87 (Human Astrocytoma) and SK-N-SH (Human Neuroblastoma) cells were cultured in modified Eagle's medium supplemented with 10% (v/v) fetal bovine serum and were incubated at 37ºC and 5% (v/v) CO_2. Cells were seeded with equal density in each well of 96 well plates. Silicon dioxide nanoparticles (0.012 micron, from STREM Chemicals, Newburyport, MA) were then added at different concentrations and cells were incubated for

48 hours or for other specified periods [5,6]. Various assays were performed on the treated and untreated cells at the end of the incubation.

Cellular morphology was determined by light microscopy. Bright field images of cells were captured using a Leica light microscope (Leica DM IRB, Bannockburn, IL, USA) equipped with a digital camera (Leica DFC 300 FX, Bannockburn, IL, USA) [6].

Cellular viability was determined using the MTT assay [5,6]. Cells were set up and treated in 96-well plates as described in the previous section. At the end of the incubation period, MTT dye (0.5% (w/v) in phosphate-buffered saline) was added to each well and the plates were incubated for 4 hours at 37º C. Purple colored insoluble formazan crystals in viable cells were dissolved using dimethyl sulfoxide and the subsequent absorbance of the content of each well was measured at 570 nm using a multi-detection microplate reader (Bio-Tek Synergy HT, Winooski, VT, USA).

Cellular damage was monitored by release of lactate dehydrogenase (LDH) into the culture medium: LDH release from cells is a marker of necrotic cell damage and cell death [7]. U87 and SK-N-SH cells were cultured in DMEM until ~ 70% confluency in 75 cm^2 flasks and then treated with different concentrations of silicon dioxide nanoparticles for 48 hours at 37^0 C. Then the culture medium from each flask was decanted and kept frozen at -70^0 C until they were employed for determining the activity of LDH therein. LDH activity in the medium was determined using the procedure of Clark and Lai [7].

Data are presented as mean ± standard error of the mean (S.E.M.). Data analysis was performed by one-way ANOVA with Student-Newman-Keuls test for multiple comparisons. Significance level was set at $p < 0.05$.

3 RESULTS AND DISCUSSION

As shown in Fig. 1, exposure of SK-N-SH cells to silicon dioxide nanoparticles induced decreases in cell survival (as determined by the MTT assay). This effect was concentration- and time-dependent (data not shown).

To further determine that exposure of cells to silicon dioxide nanoparticles may induce cell damage and cell death, we studied the effect of exposure of SK-N-SH cells to various concentrations of the nanoparticles for 48 hours. As shown in Fig. 2, at concentrations of 5 µg/ml or higher, treatment of the cells with silicon dioxide nanoparticles induced them to release LDH into the medium: this effect of the nanoparticles was particularly pronounced at levels of 50 and 100 µg/ml. These results (Fig. 2) indicate that treatment of SK-N-SH cells with silicon dioxide nanoparticles led to concentration-related cell damage and necrotic cell death at higher treatment levels.

Treatment of U87 astrocytoma cells with silicon dioxide nanoparticles also induced effects similar to those detected in SK-N-SH cells (data not shown). Moreover,

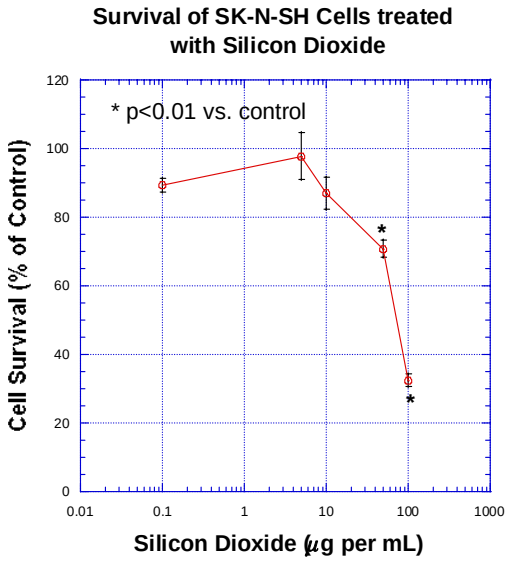

Figure 1. Treatment of SK-N-SH cells with silicon dioxide induced decreases in cell survival.

SK-N-SH cells were treated with different concentrations of silicon dioxide (0.1-100 µg per ml) for 48 hours. 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) and are the means ± SEM of six replicates.

the morphological changes in these two cell types upon exposing them to the nanoparticles, as observed by bright field light microscopy, were also consistent with the cytotoxicity data (Figs. 1 & 2). Thus, taken together, our results (Figs. 1 & 2) indicate that treatment of both neural cell types with silicon dioxide nanoparticles gave rise to concentration-related cell damage and cell death.

There have not been any systemic studies that address the cellular toxicity of silicon dioxide nanoparticles. The literature suggests that silicon nanoparticles are not toxic and only induce mild inflammatory responses to lung cells [see 1 & references therein]. Contrary to these assumptions that are not supported by experimental data, the results of our studies clearly demonstrate that silicon dioxide nanoparticles of 0.012 micron do exert cytotoxic effects on human brain tumor (SK-N-SH and U87) cells. The cytotoxic effects of silicon dioxide nanoparticles on these two cell types are dose- and time-related (Figs 1 & 2 and data not shown). Thus, our results suggest that it is imperative to further elucidate the mechanisms underlying the cytotoxic effects of silicon dioxide nanoparticles on these two types of neural cells. They also suggest that exposure to silicon dioxide nanoparticles may pose some health risk, which needs to be further and more systematically investigated.

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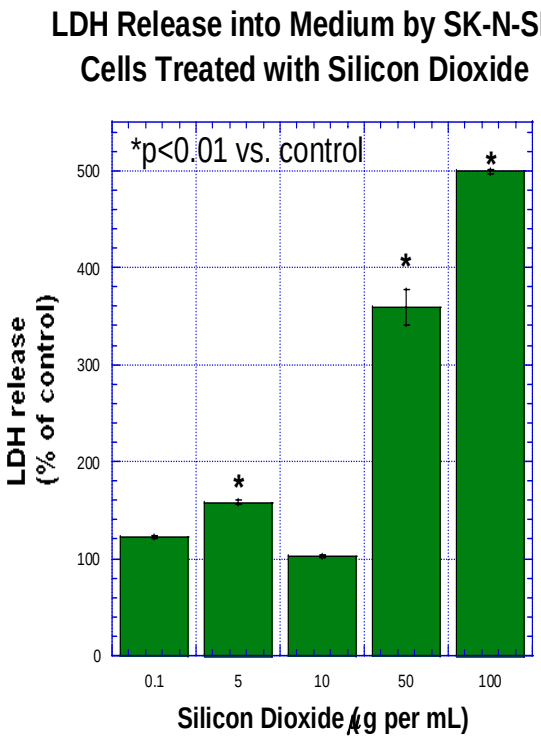

Figure 2. Treatment of SK-N-SH cells with silicon dioxide induced cell damage/death as determined by LDH release into the medium.

SK-N-SH cells were treated with different concentrations of silicon dioxide (0.1-100 µg per ml) for 48 hours. Then the activities of lactate dehydrogenase (LDH) in the cell cultured medium were determined to assess cell damage and cell death induced by silicon dioxide treatment. Values were normalized with respect to the control mean (i.e., mean of untreated cells) and are the means ± SEM of three separate determinations. (Note that in some cases, the error bars were too small to be visible.)

4 CONCLUSIONS

Silicon dioxide nanoparticles exert cytotoxic effects on both human neuroblastoma (SK-N-SH) and human astrocytoma (U87) cells in a dose- and time-related manner as determined by cell survival (i.e., MTT) assay, release of lactate dehydrogenase (LDH) into the medium, and by light microscopy. Consequently, our results demonstrate that, contrary to current assumptions in the nanomaterials literature, silicon dioxide nanoparticles do induce cytotoxic effects on neural cells. Our results also have clear implications in nanotoxicity and health risks of exposure to nanoparticles. They suggest it is imperative to systematically investigate the putative cytotoxic effects of nanoparticles and other nanomaterials.

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