Schwann Cells and Dorsal Root Ganglion Neurons Are Differentially Susceptible to Oxidative Stress Induced by Silicon Dioxide Nanoparticles

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ABSTRACT

Silicon dioxide (SiO_2) particles, including nanoparticles, have been increasing employed in diverse industrial and biomedical applications including those in drug formulations, food, and cosmetics as SiO_2 has been generally regarded as a non-toxic substance. However, the environmental safety and health impact of SiO_2 particles have not been elucidated. This study investigated the hypothesis that SiO_2 nanoparticles exert differential cytotoxic effects on DRG neurons and Schwann cells. Treatment with SiO_2 nanoparticles induced dose-related decreases in survival of DRG neurons and Schwann cells with Schwann cells being more susceptible. SiO_2 nanoparticles induced concentration-related decreases in glutathione (GSH) in Schwann cells and such decreases were related to their decreases in survival. SiO_2 nanoparticles also induced some decreases in GSH in DRG neurons. Expression of manganese superoxide dismutase (Mn-SOD) in Schwann cells showed concentration-related decreases when treated with SiO_2 nanoparticles. However, expression of Mn-SOD in DRG neurons was increased. Thus, our findings may have pathophysiological implications in the biocompatibility and health hazard of SiO_2 nanoparticles and may be critically relevant to toxicological studies prior to clinical trials of drugs formulated with and/or delivered employing agents containing such nanoparticles.

Key words: silicon dioxide nanoparticles, cytotoxicity of silicon dioxide nanoparticles, Schwann cells and dorsal root ganglion neurons, oxidative stress & glutathione, biocompatibility, nanotoxicity

1 INTRODUCTION

Silicon dioxide (SiO_2) particles, including nanoparticles, have been increasing employed in diverse industrial and biomedical applications including those in drug formulations, food, and cosmetics as SiO_2 has been generally regarded as a non-toxic substance [1-4]. Nevertheless, there have been reports that SiO_2 particles of different sizes down to nanoparticles are not as harmless as they were assumed to be [4]. Furthermore, the environmental safety and health impact of SiO_2 particles have not been elucidated [2-4].

There is evidence that the blood-brain barrier is inadequate to restrict entry of nanoparticles into the central

nervous system [4]. Additionally, there are reports of nanoparticles penetrating through the skin and then entering peripheral nerve terminals and moving by retrograde transport from the nerve terminals to neuronal perikarya (or cell bodies) [4]. Consequently, it is pathophysiologically important to investigate how nanoparticles may exert their putative cytotoxicity upon entry into the nervous system and neural cells either through the blood-brain barrier or via retrograde transport upon entry through the skin [4].

For over a decade, we have been developing various neural cell models *in vitro* to facilitate the systematic investigation of the putative cytotoxicity of nanoparticles and other nanomaterials [3, 5-10]. More recently, we have developed neural cell models employing physiologically important neural cell types derived from the peripheral nervous system (PNS), namely dorsal root ganglion (DRG) neurons and Schwann cells [8-10]. We have employed these versatile models *in vitro* to elucidate the putative cytotoxicity of nanoparticles of metallic and non-metallic oxides and of other nanomaterials and the underlying cellular and molecular mechanisms [4, 8-10]. This study was therefore initiated to test the hypothesis that SiO_2 nanoparticles exert differential cytotoxic effects on DRG neurons and Schwann cells.

2 MATERIALS AND METHODS

2.1 Materials

Silicon dioxide (SiO_2) nanoparticles (STREM Chemicals, Newburyport, MA, USA; Cat. #93-1434; <12 nm particle size, 99+ % colloidal silica) were dispersed in 100 ml of sterile saline in a sealed conical flask and the suspension stirred at ambient temperature overnight before being diluted to the specified concentrations for treatment of cells. The immortalized DRG neurons were a gift from Dr. Ahmet Höke's Laboratory at Johns Hopkins University School of Medicine. The rat Schwann cell line (R3) was obtained from ATCC (Manassas, VA, USA).

2.2 Cells and Culture Conditions

DRG neurons or Schwann cells were cultured in DMEM, supplemented with 10% (v/v) fetal bovine serum, 1% (w/v) sodium pyruvate, 0.292 g/l L-glutamine, and 1.5 g/l sodium bicarbonate and were incubated at 37°C and 5% (v/v) CO_2.

2.4 MTT Assay

Cellular viability was determined using the modified MTT assay [5]. DRG neurons or Schwann cells were seeded per well in a 48-well plate in DMEM with specified concentrations of SiO_2 nanoparticles. After incubation at 37^{o}C for 36 hours, 10% of MTT (5 mg/ml in PBS) reagent was added to each well. After incubation for another 4 hours, the medium was removed gently and the cellular reaction product was solubilized in 200 µl DMSO. Then the optical density of the contents of each well was measured at 570 nm using a plate reader [5]. The absorbance corresponds to live cells present in each well [5].

2.5 Other Assays

The assay of total cellular content of glutathione (GSH) was carried out as we had described previously [11, 12]. The expression of manganese superoxide dismutase (Mn-SOD, a key antioxidant enzyme) in DRG neurons and Schwann cells treated with or without silicon dioxide nanoparticles was determined employing Western blot analysis as described previously [6, 7, 11].

2.6 Statistical Analysis of Data

Statistical significance of experimental results was analyzed with one-way ANOVA followed by Dunnett's post-hoc test with a minimum significance level set at $p< 0.05$ using the SPSS 17 software package.

3 RESULTS AND DISCUSSION

Results of our ongoing studies indicate that, consistent with our hypothesis, treatment with SiO_2 nanoparticles induced dose-related decreases in survival of DRG neurons and Schwann cells once the nanoparticles had entered both cell types. It is noteworthy that, in comparison with DRG neurons, Schwann cells were more susceptible to the cytotoxicity of the nanoparticles, especially at the lower treatment concentrations (data not shown).

To further elucidate the molecular mechanisms underlying the cytotoxic effects of SiO_2 nanoparticles in Schwann cells and DRG neurons, we tested the hypothesis that these nanoparticles induce oxidative stress on these two neural cell types to a greater or lesser extent.

We found that SiO_2 nanoparticles induced concentration-related decreases in the cellular content of glutathione (GSH) (Figure 1), an important antioxidant, in Schwann cells and such decreases were closely related to the nanoparticles-induced decreases in their survival. By contrast, even though SiO_2 nanoparticles also induced decreases in the cellular content of GSH in DRG neurons (Figure 2), the magnitude of the decreases was less compared to those induced by the nanoparticles in Schwann cells, especially at the highest treatment concentration of nanoparticles (Figure 1).

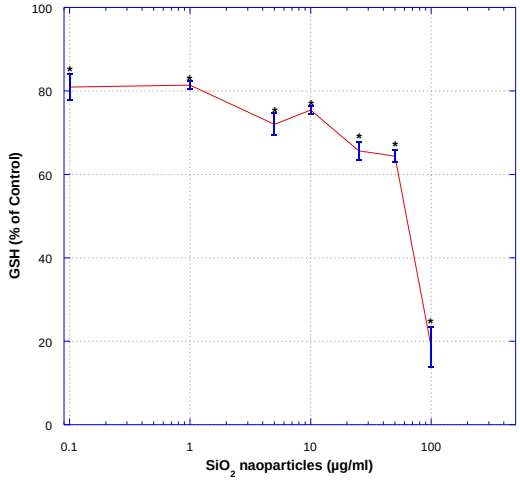

Figure 1. Effects of treatment with silicon dioxide nanoparticles on glutathione (GSH) content in Schwann cells. Schwann cells were treated with silicon dioxide nanoparticles at the specified concentrations for 36 hours and their GSH content was determined. Values are the mean ± S.E.M. of at least three separate determinations and are expressed as % of control; *p<0.05 versus control.

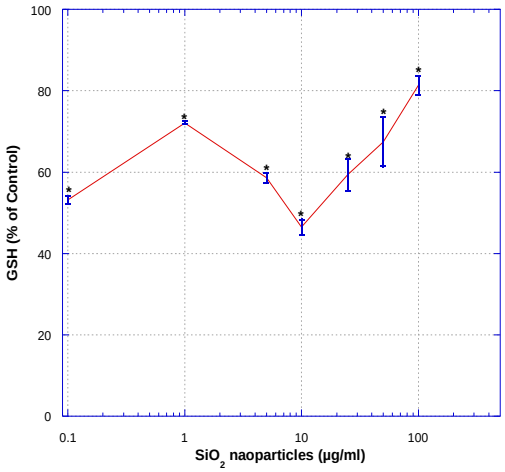

Figure 2. Effects of treatment with silicon dioxide nanoparticles on glutathione (GSH) content in DRG neurons. DRG neurons were treated with silicon dioxide nanoparticles at the specified concentrations for 36 hours and their GSH content was determined. Values are the mean ± S.E.M. of at least three separate determinations and are expressed as % of control; *p<0.05 versus control.

Consistent with the decreases in GSH in Schwann cells, their expression of manganese superoxide dismutase (Mn-SOD, a key antioxidant enzyme) also showed concentration-related decreases when treated with SiO_2 nanoparticles (data not shown). On the other hand, when DRG neurons were treated with SiO_2 nanoparticles, their expression of Mn-SOD was increased (data not shown). The latter observation suggested that the increased Mn-SOD expression in DRG neurons treated with SiO_2 nanoparticles might be a compensatory mechanism elicited by the treatment of the neurons with the nanoparticles. Indeed, compatible with this conclusion was the trend of increases in GSH content in DRG neurons treated with the nanoparticles at concentrations higher than 10 µg/ml (Figure 2). Thus, these findings prompted us to conclude that Schwann cells are more susceptible than DRG neurons to oxidative stress induced by SiO_2 nanoparticles.

4 CONCLUSIONS

Treatment with silicon dioxide (SiO_2) nanoparticles induced concentration-related decreases in survival of both Schwann cells and DRG neurons, the Schwann cells being more susceptible to the cytotoxicity of the nanoparticles. In parallel with the dose-related decreases in the survival of Schwann cells there were corresponding decreases in the content of glutathione (GSH), an important antioxidant, in Schwann cells treated with increasing concentrations of SiO_2 nanoparticles. On the other hand, even though treatment of DRG neurons with increasing concentrations of these nanoparticles also lowered their survival, their decreases in survival did not closely parallel the decreases in their GSH content induced by the nanoparticles. Furthermore, while treatment of Schwann cells with SiO_2 nanoparticles induced decreases in their expression of manganese superoxide dismutase (MnSOD), such treatments induced increases in the expression of MnSOD in DRG neurons. The latter increases in MnSOD expression in DRG neurons also coincided with the trend of increased GSH content suggesting that the concurrent increases in MnSOD expression and GSH content might be the consequence of compensatory mechanisms elicited in the DRG neurons by treatment with the higher levels of SiO_2 nanoparticles. These results strongly suggested that SiO_2 nanoparticles exerted differential cytotoxic effects on Schwann cells and DRG neurons, the two key neural cell types derived from the peripheral nervous system. Thus, our results may assume pathophysiological importance in determining how exposure to SiO_2 nanoparticles may impact on the structure and function of neural cells in the peripheral nervous system. Additionally, our findings may be critically relevant to toxicological studies prior to clinical trials of drugs formulated with and/or delivered employing agents containing such nanoparticles.

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