Molecular Effects of Silicon Dioxide Nanoparticles on Cell Survival Signaling of Dorsal Root Ganglion (DRG) Neurons and Schwann Cells

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ABSTRACT

The lack of good model systems in understanding molecular mechanism(s) underlying peripheral neuropathies has led us to develop unique peripheral nervous system (PNS) neural cell culture models *in vitro* consisting of immortalized Dorsal Root Ganglion (DRG) neurons and Schwann cells. In this study we have investigated the expression of proteins involved in cell survival in these two cell models and the effects of treatment with SiO_2 nanoparticles thereon. We hypothesized that SiO_2 nanoparticles induce differential cytotoxicity in DRG neurons and Schwann cells. Our results suggest one mechanism underlying the cytotoxicity of SiO_2 nanoparticles in PNS neural cell types may be via disturbing their cell survival signaling. These findings have functional and pathophysiological implications in cell survival signaling in PNS neural cells and in chemically-induced peripheral neuropathy.

Keywords: peripheral nervous system, cell signaling, immortalized DRG neurons, Schwann cells, SiO2 nanotoxicity

1 INTRODUCTION

Peripheral neuropathy is a condition of severe damage to the nerves which constitute the peripheral nervous system. Factors like trauma, stress, chemical toxins and diabetes can lead to peripheral nerve damage [1]. The incidence of diabetic peripheral neuropathy is as high as 60%-70% in patients with diabetes [2]. Such neuropathies are associated with impaired sensations, pain, muscle weakness and functional abnormalities. A wealth of knowledge has been generated underlying the pathophysisology of the disease but studies directed at the molecular level are still lacking [3, 4].

Dorsal root ganglion neurons (DRG) and Schwann cells form a major part of the peripheral nervous system and are subjected to injury or stress associated damage, leading to several neuropathies [4]. Model systems utilizing primary cultures of DRG neurons and Schwann cells are unsuitable for studying chronic diseases like peripheral neuropathies. Limited life span of *in vitro* cultures and inaccessibility to animal models necessitate development of new model systems for understanding the molecular mechanism of diabetic and chemically induced neuropathies.

To overcome the limitations of primary cultures of DRG neurons and Schwann cells as *in vitro* models, we have developed unique peripheral nervous system neural cell culture models *in vitro* consisting of immortalized DRG neurons and Schwann cells, which can allow the probing of molecular mechanisms underlying peripheral nerve damage and dysfunction.

Cell signaling pathways involving several kinases are known to participate in cell differentiation, proliferation and growth. Some studies have postulated a role of extracellular signal-regulated kinase (ERK), a member of mitogen activated protein kinase (MAPK) pathway in peripheral nerve disorders associated inflammation and pain [5, 6]. To shed more light on cell signaling in both DRG neurons and Schwann cells, especially when cultured *in vitro*, we have determined the expression of proteins involved in cell survival in these two cell models and the effects of treatment with SiO_2 nanoparticles thereon.

Nanotoxicity due to SiO_2 is well documented, though the underlying molecular mechanism is yet to be fully elucidated [7, 8]. We have previously demonstrated the cytotoxic effects of SiO_2 nanoparticles in neurons-like SK-N-SH neuroblastoma and astrocytes-like U87 astrocytoma cells [9]. However, the putative cytotoxicity of SiO_2 nanoparticles on PNS is unknown. Therefore, in this study we have hypothesized that SiO_2 nanoparticles induce differential cytotoxicity in DRG neurons and Schwann cells and analyzed the expression of proteins involved in cell signaling pathways in the two cell types with and without treatment with SiO_2 nanoparticles. This study will not only elucidate how exposure to such nanoparticles may impact on the structure and function of the PNS but also will shed light on pathophysiological and related molecular mechanisms underlying chemically-induced peripheral neuropathy.

2 MATERIALS AND METHODS

2.1 Cell lines

The immortalized DRG neurons were a gift from Dr. Ahmet Hoke's Laboratory at Johns Hopkins University. Schwann cells were obtained from ATCC (Manassas, VA, USA). The cells were maintained in Dulbecco's Modified Eagles Medium (DMEM: Sigma-Aldrich, St. Louis, MO), supplemented with 10 % (v/v) fetal bovine serum (Atlanta Biologicals Inc., Lawrenceville, GA) and were incubated at 37°C and 5 % CO_2 (v/v) in a Nuair tissue culture incubator (Plymouth, MN).

2.2 Nanoparticle stock solution

Silicon dioxide nanoparticles were purchased from STREM Chemicals, Newburyport, MA. A stock suspension of 50 mg nanoparticle in 100 ml of sterile saline was prepared. The solution was continuously stirred overnight with the help of a magnetic stirrer in a sealed conical flask. Dilutions were made from the stock solution to attain different concentrations.

2.2 Western blot analysis

DRG neurons and Schwann cells were grown in T-75 tissue culture flasks to 70%-80% confluency. Cells were then treated with different concentrations of Silicon dioxide nanoparticle (1 μg/mL, 25 μg/mL and 50 μg/mL) for 48 hours. For western blot analysis, drug treated or untreated (control) cells were harvested at the end of 48 hours and cell lysates were prepared as described previously [10]. 30 μg of cell lysate proteins were separated by sodium dodecyl sulfate poly acrylamide gel electrophoresis (10% SDS-PAGE) and analyzed for the protein expression of ERK, p-ERK, AKT, p-AKT using chemiluminescence kit. β-actin was used as a loading control. The western blot scans were digitized using Unscan-it-gel 6.1 software.

3 RESULTS AND DISCUSSION

Diseases of the peripheral nervous system like diabetic neuropathy are well studied. However, the molecular mechanism underlying the disease still needs to be explored. In this study we have utilized a unique *in vitro* culture model of immortalized DRG neurons and Schwann cells to understand cell survival signaling in 25 mM glucose culture conditions. In accord with other studies highlighting the role of ERK/p-ERK in such diseased conditions [5], our protein expression analysis revealed significant amount of ERK and its active form p-ERK in both the cells (Figure 1a and 1b). These proteins have been associated with different symptoms of diabetic neuropathy and thus could be employed in studies in the future as markers of peripheral nerve damage.

Silicon dioxide nanoparticles find numerous applications in many industries and in everyday living. Recent studies have listed harmful effects of these nanoparticles on the human body although silicon dioxide, include its microparticles have traditionally regarded as being harmless to humans and other mammalian species [7]. We have previously shown that SiO_2 nanoparticles induce cytotoxicity in human neural cells [9]. In this study we have analyzed the effect of SiO_2 nanoparticles on the two cell models employed for studying peripheral neuropathies. Different exposure of cells to SiO_2 nanoparticles (1, 25, 50 μg/mL) for 48 hours, resulted in inhibition of p-ERK protein expression in both DRG neurons and Schwann cells, cultured and treated separately (Figure 1a and 1b). No detectable change was observed in the protein expression of ERK (Figure 1a and b), AKT (Figure 2a and 2b) and p-AKT (data not shown).

The results from this study suggest that SiO_2 differentially alters survival signaling in cells comprising PNS and a decrease in the expression of p-ERK by SiO_2 nanoparticles could have therapeutic implications in signs and symptoms related to peripheral nerve diseases. Moreover, these findings have functional and pathophysiological implications in cell survival signaling in PNS neural cells and in chemically-induced peripheral neuropathy [9,11]. Evidently, based on our observations of the differential changes induced by treatment with SiO_2 nanoparticles in the expression of the signaling molecules ERK/p-ERK and AKT/p-AKT in DRG neurons and Schwann cells, it is timely to employ other functional biomarkers to further elucidate the molecular mechanisms underlying the pathologically-induced and toxicologically-induced peripheral neuropathies. Indeed, as the results of this and our previous study [11] have demonstrated, our cell model systems *in vitro* of PNS neural cells provide unique opportunities to delve into those mechanistic issues that are of fundamental importance in peripheral neuropathies. After all, the pathogenesis of such neuropathies are still largely undefined.

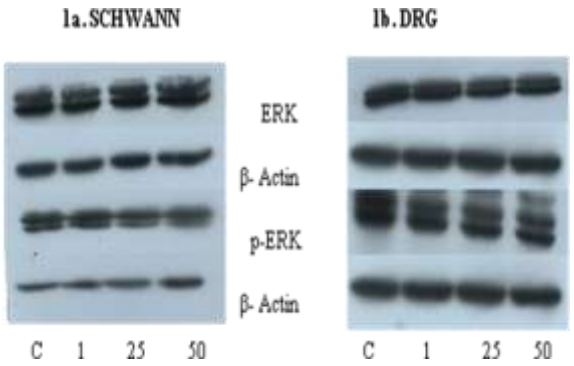

Figure 1a and 1b. Western blot analysis of SiO_2 (in μM) treated Schwann cells and DRG neurons. SiO_2 in increasing concentrations inhibited the expression of p-ERK in both the cells.

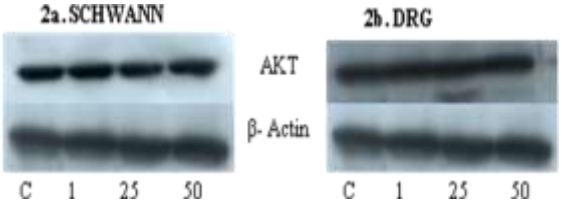

Figure 2a and 2b. Protein expression of AKT in SiO_2 (in μM) treated Schwann cells and DRG cells, as analyzed by western blot analysis.

4 CONCLUSIONS

In this study we have investigated the expression of proteins involved in cell survival in two cell models of PNS and the effects of treatment with SiO_2 nanoparticles

thereon. We hypothesized that SiO_2 nanoparticles induce differential cytotoxicity in DRG neurons and Schwann cells. Our study to investigate the hypothesis has yielded interesting results that suggest one mechanism underlying the cytotoxicity of SiO_2 nanoparticles in PNS neural cell types may be via the disturbances and/or disruption of their cell survival signaling. Evidently, this mechanistic possibility deserves further study because we have observed differential changes induced by treatment with SiO_2 nanoparticles in the expression of the signaling molecules ERK/p-ERK and AKT/p-AKT in DRG neurons and Schwann cells. More importantly, our findings are relevant to the notion that because these signaling proteins have been associated with different symptoms of diabetic neuropathy, they, and other signaling proteins with similar functions, could be employed in future studies as markers of peripheral nerve damage. Studies in our laboratories are in progress to further investigate these exciting possibilities.

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