Cytotoxic Effects of Short Multi-Wall Carbon Nanotubes in Dorsal Root Ganglion (DRG) Neurons

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ABSTRACT

Because of their unique physico-chemical properties, carbon nanotubes (CNTs) have found their way into diverse industrial and biomedical applications. Their escalating uses in multiple industries result in enhanced occupational human exposure to CNTs. However, the human health hazard of exposure to CNTs has not been assessed. Furthermore, the effects of CNTs on the nervous system and/or neural cells are virtually unknown. Because the putative neurotoxicity of CNTs has not been reportedly studied, we have investigated the putative cytotoxic effects of both funtionalized (i.e., carboxylated and hydroxylated) and non-functionalized short multi-wall carbon nanotubes (SMWCNTs) on dorsal root ganglion (DRG) neurons, an excellent model *in vitro* of neurons derived from the peripheral nervous system (PNS). We found SMWCNTs induced concentration-related decreases in survival and growth of DRG neurons, the non-functionalized ones being more cytotoxic than the functionalized ones, especially at the higher treatment concentrations. Treatment of DRG neurons with non-functionalized SMWCNTs induced necrotic damage and/or cell death in the neurons and decreased their expression of phospho-Akt, a cell survival and proliferation signal. Thus, our results may have pathophysiological implications in how exposure to SMWCNTs impacts on the structure and function of the PNS.

Key words: carbon nanotubes, cytotoxicity of carbon nanotubes, peripheral nervous system, DRG neuron, biocompatibility, nanotoxicity

1 INTRODUCTION

Due to their unique physico-chemical properties, carbon nanotubes (CNTs) have found their way into diverse industrial and biomedical applications, including but not limited to fabrication of sensors, electrodes, catalysts, actuators, transistors and capacitors [1-3]. Their escalating uses result in humans being more and more exposed to CNTs, especially in the workplace where they are found [1-4]. However, the human health hazard of exposure to CNTs has not been assessed even though there have been recent studies employing animal models indicating that various types of CNTs can induce toxicity to different organs and tissues [2,4]. Nonetheless, the effects of CNTs on the nervous system and/or neural cells are virtually unknown [3].

We have initiated a series of studies to systematically investigate the putative cytotoxicity of a variety of nanomaterials, including CNTs [3,5-15]. Because the putative neurotoxicity of CNTs has not been reportedly studied [3,4], we have developed several neural cell models *in vitro* to facilitate such cytotoxicity studies [5-15]. In this study, we have investigated the putative cytotoxic effects of both funtionalized and non-functionalized short multi-wall carbon nanotubes (SMWCNTs).

We have examined the effects two functionalized, namely carboxylated and hydroxylated, and non-functionalized SMWCNTs on dorsal root ganglion (DRG) neurons, which constitute an excellent model *in vitro* of neurons derived from the peripheral nervous system (PNS) [13-15]. We have investigated the hypothesis that treatment with CNTs induces a dose-related decrease in viability of DRG neurons and functionalization of CNTs modulates their cytotoxicity.

2 MATERIALS AND METHODS

2.1 Materials

50B 11 DRG neurons were kind gifts from Dr Hoke's Laboratory at Johns Hopkins School of Medicine. Thiazolyl blue tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St Louis, MO, USA). Fetal bovine serum (FBS) was obtained from Atlanta Biologicals (Lawrenceville, GA, USA). All chemicals were of analytical grade unless otherwise stated.

2.2 Cell Culture

Doral root ganglion (DRG) neurons were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum.

2.4 MTT Assay

Cell survival and growth was determined using the MTT assay [8,9]. DRG neurons were cultured as described in the cell culture subsection and were treated with specified concentrations of carbon nanotubes for specified times. At the end of the treatment periods, MTT dye (0.5%, w/v, in PBS) was added to each well and the plates were incubated for an additional 4 hours at 37°C. Purple-color insoluble formazan crystals in viable cells were dissolved using DMSO, and the subsequent absorbance of the content of each well was measured at 570 nm using a Bio-Tek Synergy HT Plate Reader (Winooski, VT, USA) [8,9].

2.5 Other Methods

The assay of lactate dehydrogenase (LDH) release from cells (a marker of necrotic damage or necrotic cell death) and Western blot analysis for detection of protein expression were carried out as we had described previously [9,12].

2.6 Statistical Analysis of Data

Results are presented as mean ± standard error of the mean (S.E.M.) of 6 determinations in each experiment. Replicate experiments showed essentially the same patterns of results.

3 RESULTS AND DISCUSSION

Employing the MTT assay, we systematically compared the effects of non-functionalized short multi-wall carbon nanotubes (SMWCNTs) with those of two functionalized, namely hydroxylated and carboxylated, SMWCNTs on dorsal root ganglion (DRG) neurons (Figures 1-3). As shown in Figure 1, exposure of DRG neurons to non-functionalized SMWCNTs induced a concentration- and time-related decreases in survival, proliferation, and/or growth of the neurons (Figure 1).

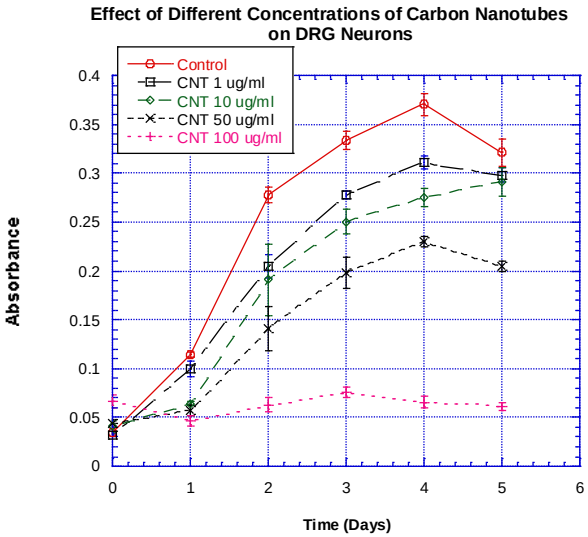

Figure 1: Effect of different concentrations of non-functionalized short multi-wall carbon nanotubes (SMWCNTs) on dorsal root ganglion (DRG) neurons. Circles: untreated, i.e., control; Squares: 1 µg/mL; Diamonds: 10 µg/mL; Crosses: 50 µg/mL; and Pluses: 100 µg/mL; at the end of the specified time, cell survival and growth was determined using the MTT assay. Values are the mean ± S.E.M.

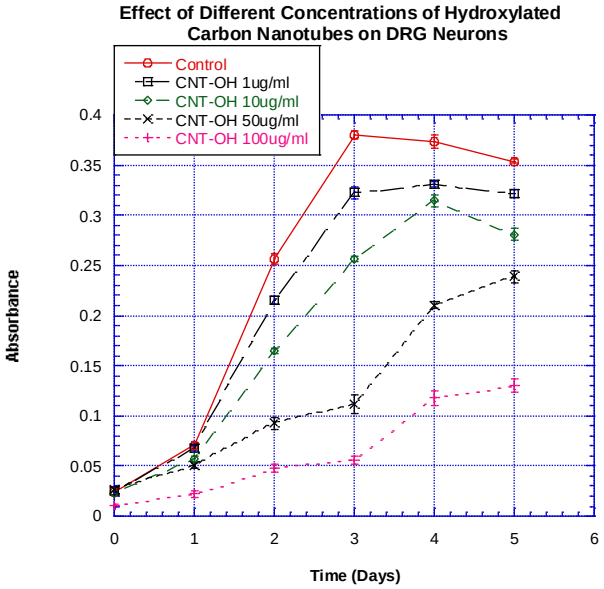

Figure 2: Effect of different concentrations of hydroxylated short multi-wall carbon nanotubes (SMWCNTs) on dorsal root ganglion (DRG) neurons. Other details were the same as those in Figure 1 except hydroxylated SMWCNTs were used.

Exposure of DRG neurons to hydroxylated (Figure 2) or carboxylated (Figure 3) SMWCNTs also induced a concentration- and time-related decreases in survival, proliferation, and/or growth of the neurons. However, while the effects of the functionalized SMWCNTs were somewhat similar, the effects of the non-functionalized SMWCNTs were generally more pronounced than those of the functionalized SMWCNTs, especially at the higher treatment concentrations of SMWCNTs (compare Figure 1 with Figures 2 and 3). Taken together, these results suggest that non-functionalized SMWCNTs are generally more cytotoxic to DRG neurons compared to the functionalized SMWCNTs.

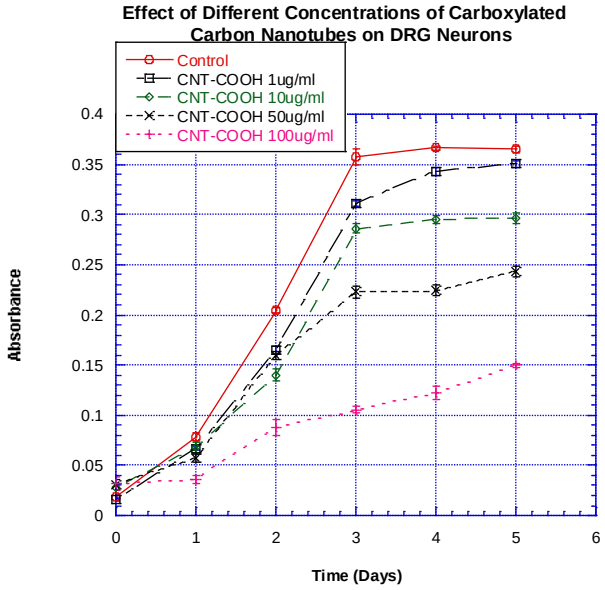

Figure 3: Effect of different concentrations of carboxylated short multi-wall carbon nanotubes (SMWCNTs) on dorsal root ganglion (DRG) neurons. Other details were the same as those in Figure 1 except carboxylated SMWCNTs were used.

To further determine some of the putative mechanisms underlying the cytotoxic effects of non-functionalized SMWCNTs on DRG neurons, we monitored the effect of the nanotubes on lactate dehydrogenase (LDH) release (a marker of necrotic damage and/or necrotic cell death) by DRG neurons into the medium when the neurons were treated with the non-functionalized SMWCNTs. We found that when DRG neurons were treated with 100 µg/mL of non-functionalized SMWCNTs, they showed time-related increases in the release of LDH into the medium (data not shown), suggesting that necrosis may at least be one cell death mechanism underlying the cytotoxicity of the non-functionalized SMWCNTs.

As necrosis could account for the effect of non-functionalized SMWCNTs in lowering the survival of DRG neurons, we further ascertained the possibility that another mechanism whereby the nanotubes could induce in the DRG neurons may be via decreasing their survival signaling pathway(s). Results of our ongoing experiments suggest that the expression Akt and phospho-Akt was decreased in DGR neurons treated with 100 µg/mL of non-functionalized SMWCNTs. Because Akt and phosphor-Akt are important signaling protein involved in cell survival and/or proliferation [see 12 and references therein], our observation suggests that another mechanism whereby the non-functionalized SMWCNTs induced in DRG neurons in lowering their survival and/or proliferation was through decreasing signaling mechanisms such as those involving Akt and phospho-Akt.

4 CONCLUSIONS

Our ongoing studies have clearly demonstrated that SMWCNTs induced time- and concentration-related decreases in survival, proliferatio and/or growth of DRG neurons, the non-functionalized ones being more cytotoxic than the functionalized ones, especially at the higher treatment concentrations. Treatment of DRG neurons with non-functionalized SMWCNTs induced necrotic damage and/or cell death in the neurons, as indicated by the enhanced lactate dehydrogenase release (an established marker of necrotic cell damage or necrotic cell death) by the treated DRG neurons into the surrounding medium. Furthermore, results from our ongoing studies suggest that DRG neurons treated with a high concentration of non-functionalized SMWCNTs exhibited decreased expression of phospho-Akt, a cell survival and proliferation signal. Thus, our results may have pathophysiological implications in how exposure to SMWCNTs impacts on the structure and function of the PNS. Clearly, this is an important area that merits further investigation so that ultimately the cytotoxicity of carbon nanotubes in neural as well as non-neural cells can be more fully understood.

5 ACKNOWLEDGMENTS

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