Cytotoxic Effects of Four Metallic Oxide Nanoparticles on Dorsal Root Ganglion (DRG) Neurons

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ABSTRACT

Metallic oxide nanoparticles are extensively used in diverse industries. Consequently, the environmental exposure of humans to these nanoparticles is accelerating. However, the health impact of exposure to such nanoparticles is poorly understood. We found nanoparticles of several metallic and non-metallic oxides exerted differential cytotoxic effects on human U87 astrocytoma and SK-N-SH neuroblastoma cells. Because effects of such nanoparticles in neural cells in the peripheral nervous system (PNS) are unknown, we developed PNS neural cell models *in vitro* (e.g., dorsal root ganglion (DRG) neurons) to investigate their effects. Our results suggested that the nanoparticles investigated induced concentration- and time-related decreases in survival of DRG neurons and exerted differential effects on their expression of p-AKT and p-ERK. Our findings may have pathophysiological implications in the impact of exposure to such nanoparticles on the PNS.

KeyWords: peripheral nervous system, nanoparticles, nanotoxicity, dorsal root ganglion (DRG) neurons, cytotoxicity, cell culture models

1. INTRODUCTION

Nanoparticles of metals and metallic oxides (e.g., iron, titanium oxide, alumina) have diverse applications in a variety of industries because of their unusual but exploitable physico-chemical and other properties [1]. Titanium dioxide is known to be extensively employed in the production of paper, plastics, cosmetics and paints. Titanium- and zinc-based nanoparticles are used as UV light absorbing component in sun-screen. Due to their ubiquitous and accelerating uses in industrial applications, exposure of humans to nanparticles and other nanomaterials is increasing, especially in the occupational and environmental settings.

Even though the health impact of exposure to such nanoparticles has not been fully assessed, there is recent

evidence, based on studies employing animal models, suggesting that such exposure may induce differential toxicity in various organs [see 2,3 and references therein]. Nevertheless, little is known about effects of metallic oxide nanoparticles in the nervous system. On the other hand, many metal ions, including aluminum, iron, and zinc, are known to be neurotoxic and can induce neurodegeneration when taken in excess [4-7]. Furthermore, elevated brain levels of these and other heavy metals have been implicated in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease [4-7].

Until recently, little is known about the effects of metallic and non-metallic oxide nanoparticles in neural cells. We therefore designed several cell models *in vitro* and have systematically investigated the effects of nanoparticles of metallic and non-metallic oxides [see 2,3 and references therein]. For example, we were the first to demonstrate that treatment with TiO_2 micro- or nanoparticles induced cell death in human astrocytes-like U87 astrocytoma cells in a time- and concentration-related manner [2].

Because as far as we are aware, the putative cytotoxic effects of anatase and rutile TiO_2 nanoparticles, Fe_2O_3 nanoparticles and ZnO nanoparticles in peripheral nervous system (PNS) neural cells have not been studied, we have initiated studies to systematically investigate the hypothesis that these nanoparticles can exert differential cytotoxic effects on PNS neural cells.

2. MATERIALS AND METHODS

2.1 Materials

Dorsal root ganglion (DRG) neurons were a kind gift from Dr. A. Hoke of Johns Hopkins University School of Medicine. Anatase titanium dioxide (nanopower, <25 nm particle size) rutile titanium dioxide (nanopower, <100 nm particle size) Fe_2O_3 (nanopower, <50 nm particle size), ZnO (nanopower, <100 nm particle size), thiazolyl blue tetrazolium bromide (MTT), and Dulbecco's Modified Eagle's Medium (DMEM) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Antibodies were purchased from Abcam (Cambridge, MA). Fetal bovine serum (FBS) was from Atlanta Biologicals (Lawrenceville, GA, USA). Other chemicals were of analytical grade.

2.2 Cell Survival (MTT) Assay

Nanoparticles were freshly suspended in 100 mL sterile saline in a sealed conical flask and diluted with DMEM to the specified concentration before use.

Cells were treated with specified concentrations of nanoparticles for 24, 36, 48 or 72 hours after they attached to the bottom of the well. MTT dye (0.5%, (w/v) in PBS) was added to each well and the plates were incubated for another 4 hours. Plates containing no seeded cells but only culture medium with and without nanoparticles served as the controls in each experiment. The absorbance of the material in each well in the plates was read in a plate reader as described previously [2].

2.3 Western Blot Analysis

DRG neurons were seeded in T-75cm^2 culture flasks. After they reached 50-60% confluent, DRG neurons were treated with nanoparticles for 24 or 48 hours. Then cells were collected and homogenized. Protein concentrations were determined using the BCA protein assay kit. Equal amounts of protein were separated by SDS-PAGE on 10% polyacrylamide gels and then transferred to PVDF membranes. Finally, separated proteins identified with respective antibodies were visualized with an ECL detection system as described previously [3].

2.4 Statistical Analysis

Results are presented as mean ± standard error of the mean (SEM) of twelve determinations. Statistical significance of experimental results was analyzed with one-way ANOVA followed by the post-hoc Tukey test using the Kaleidagraph 4.0 software package. The minimum significance level was set at $p< 0.05$

3 RESULTS AND DISCUSSION

In recent years, we have launched a series of studies to systematically investigate the cytotoxic effects of nanoparticles of metallic and non-metallic oxides in human and other mammalian neural and non-neural cell types. We found that such nanoparticles exerted differential cytotoxic effects on human astrocytes-like U87 astrocytoma and human neurons-like SK-N-SH neuroblastoma cells [2,3]. However, little is known regarding the putative cytotoxicity of such nanoparticles in neural cells derived from the peripheral nervous system (PNS). We have therefore investigated the putative cytotoxic effects of four metallic oxide nanoparticles in DRG neurons.

As shown in Figures 1 to 4, treatment with nanoparticles of all four types of metallic oxides induced time- and concentration-related decreases in survival of DRG neurons. These results suggest that these four types of nanoparticles exerted differential effects on the survival of DRG neurons. The relative potencies of the nanoparticles in lowering the survival of DRG neurons are in the rank order: ZnO nanoparticles > antase TiO_2 nanoparticles > Fe_2O_3 naniparticles > rutile TiO_2 nanoparticles (compare Figures 1 through 4).

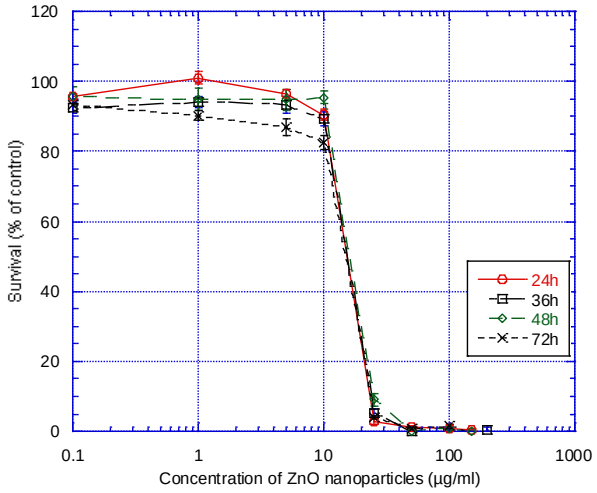

Figure 1. Effect of ZnO nanoparticles on survival of DRG neurons. Results are mean ± SEM of 12 determinations. The results of two other experiments showed essentially the same results.

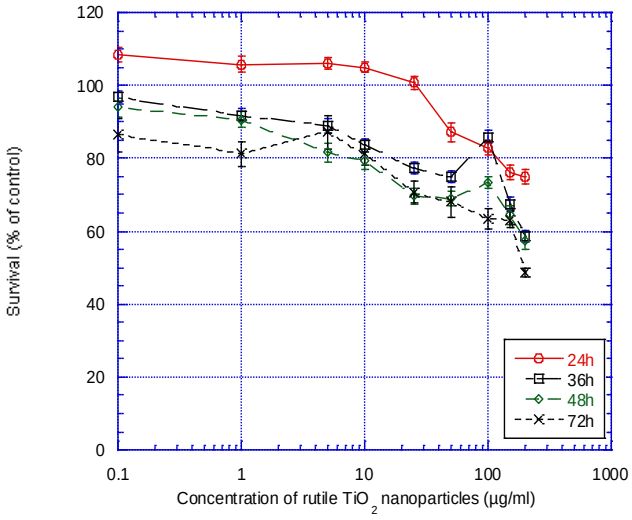

Figure 3. Effect of rutile TiO_2 nanoparticles on survival of DRG neurons. Results are mean ± SEM of 12 determinations. The results of two other experiments showed essentially the same results.

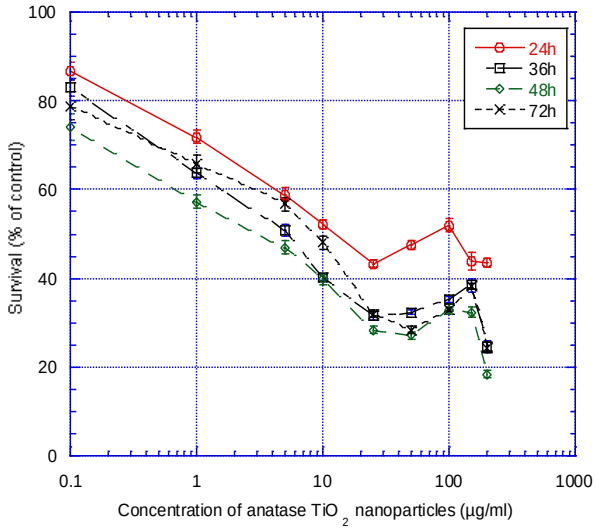

Figure 2. Effect of anatase TiO_2 nanoparticles on survival of DRG neurons. Results are mean ± SEM of 12 determinations. The results of two other experiments showed essentially the same results.

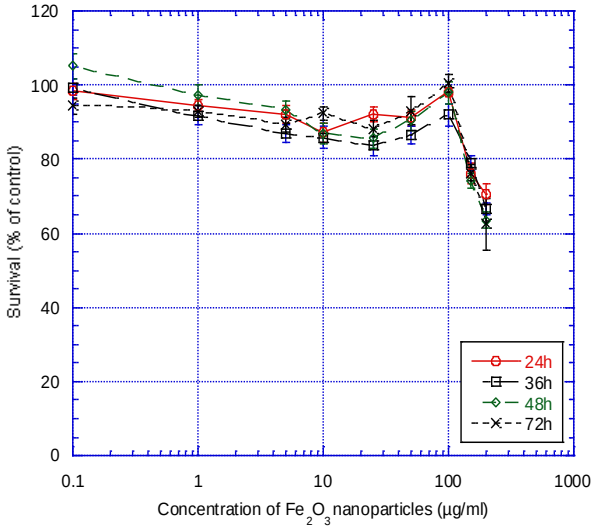

Figure 4. Effect of Fe_2O_3 nanoparticles on survival of DRG neurons. Results are mean ± SEM of 12 determinations. The results of two other experiments showed essentially the same results.

In order to elucidate the molecular mechanisms underlying the cytotoxic effects of the four types of nanoparticles investigated, we have begun to examine the effects of the four types of nanoparticles on signaling pathways associated with cell survival and/or proliferation. Results of our ongoing studies suggest the four types of nanoparticles exerted differential effects on expression of p-AKT and p-ERK by DRG neurons (data not shown). Because both AKT and ERK are cell signals that regulate cell survival and proliferation, our findings suggest that one mechanism by which the four types of nanoparticles lowered the survival of DRG neurons may be through the modulation of the survival and proliferation signaling of the neurons.

4. CONCLUSIONS

Results of our ongoing studies revealed that the nanoparitcles investigated induced concentration- and time-related decreases in survival of DRG neurons. The rank order of the potency of the four types of metallic oxide nanoparticles in inducing decreases in survival of DRG neurons was: ZnO nanoparticles > anatase TiO_2 nanoparticles > Fe_2O_3 nanoparticles > rutile TiO_2 nanoparticles. The four types of nanoparticles investigated also exerted differential effects on the expression of p-AKT and p-ERK proteins in DRG neurons. Other ongoing studies are to further elucidate these and other related molecular mechanisms underlying the cytotoxic effects of the four types of nanoparticles. Thus, our findings may have pathophysiological implications in the impact of exposure to such nanoparticles on the structure and function of the peripheral nervous system.

5. ACKNOWLEGDEMENTS

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