Differential Cytotoxic Effects of Titanium Oxide Nanoparticles on Peripheral Nervous System Neural Cells

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ABSTRACT

The health impact of exposure to TiO_2 nanoparticles is poorly understood. We recently found treatment with TiO_2 micro- or nanoparticles induce cell death in human astrocytes-like U87 astrocytoma cells and in normal human fibroblasts in a concentration-related manner. Because the cytotoxic effects of these nanoparticles in peripheral nervous system (PNS) neural cells have not been elucidated, we have developed two PNS neural cell models *in vitro* consisting of dorsal root ganglion (DRG) neurons and Schwann cells to facilitate the systematic investigation of such effects. We noted treatment with TiO_2 nanoparticles lowered the survival of both DRG neurons and Schwann cells in a dose- and time-related manner, DRG neurons being more sensitive than Schwann cells to the effects. Thus, our findings may have pathophysiological implications in the impact of exposure to TiO_2 nanoparticles on the structure and function of the PNS.

Keywords: peripheral nervous system (PNS), TiO_2 nanoparticles, dorsal root ganglion neurons (DRG) Schwann cells, cell culture models, cytotoxicity

1 INTRODUCTION

Titanium dioxide, in the form of micro- and nanoparticles, is widely used as an important industry material for its good photocatalytic activity and other features [1]. Titanium dioxide is known to be employed, although not exclusively, in the production of paper, plastics, cosmetics and paints.

The increasing use of TiO_2 particles in a variety of industrial applications raise the possibility of elevating occupational and other environment exposure of TiO_2 particles to humans and other species. Consequently, such exposures to the nanoparticles could lead to their entry into their bodies through transdermal penetration or inhalation. Wu and colleagues demonstrated that TiO_2 nanoparticles penetrated the skin of pigs and mice, and eventually reaching their systemic circulation [2]. Andersson and colleagues recently observed the uptake and distribution of five types of anatase and rutile TiO_2 in A549 lung epithelial cells by static light scattering, Raman microspectroscopy and transmission electron spectroscopy [3].

The health hazard of TiO2 particles has not been fully elucidated. Some studies reported that exposure to TiO_2 micro- and nanoparticles induced inflammatory responses in lung tissue [4,5] and another study reported that genotoxicity induced by TiO_2 particles in human epidermal cells was mediated via ROS generation. Nevertheless, the effects of TiO_2 particles on the nervous system have not been systematically investigated.

Our nervous system consists of two parts, the central nervous system (CNS) and the peripheral nervous system (PNS). The CNS, comprising of brain and spinal cord, is in charge of integrating the information that it receives from PNS and peripheral organs. There have been few studies that examined the effects of TiO_2 particles on the CNS or neural cells. One study by Takeda and co-workers noted that subcutaneous injection of TiO_2 nanoparticles into pregnant mice led to decreased testicular function and increased number of cells exhibiting CASPASE-3 activities in the olfactory bulb in their offsprings [7]. Their findings strongly suggest that the nanoparticles had crossed the placental barrier from the dam's circulation into the fetal circulation and subsequently crossed the blood-testis and blood-brain barriers, ultimately resulting in inducing pathological changes in the testis and olfactory bulb of the offsprings of the dams adminstered with the nanoparticles [7]. Our recent finding that treatment with TiO_2 micro- or nanoparticles induced cell death in human astrocytes-like U87 astrocytoma cells and in normal human fibroblasts in a concentration-related manner constituted the first study that reported the cytotoxicity of TiO_2 nanoparticles on human neural cells [8].

Because as far as we are aware the cytotoxic effects of TiO_2 nanoparticles on peripheral nervous system (PNS) neural cells have not been reportedly studied, we have initiated studies to systematically investigate the hypothesis that TiO_2 micro- and nanoparticles can exert cytotoxicity effects on the peripheral nervous system neural cells. To facilitate the investigation of our hypothesis, we have developed two PNS neural cell models *in vitro* consisting of dorsal root ganglion (DRG) neurons and Schwann cells.

2 MATERIALS AND METHODS

2.1 Materials

Dorsal root ganglion (DRG) neurons were a gift from Dr. A. Hoke of Johns Hopkins University School of Medicine. Schwann cells were obtained from ATCC (Manassas, VA, USA). Titanium dioxide (Cat #637254, nanopower, <25 nm particle size, 99.7%), Thiazolyl blue tetrazolium bromide (MTT) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma-Aldrich. Fetal bovine serum (FBS) was from Atlanta Biologicals (Lawrenceville, GA, USA). Other chemicals were of analytical grade.

2.2 Cell Culture of DRG Neurons and Schwann Cells

DRG neurons and Schwann cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), 1% (w/v) sodium pyruvate, 0.292 g/L L-glutamine, 1.5 g/L sodium bicarbonate and 4.535 g/L glucose. The cells were incubated at 37°C in a 5% CO_2 humidified environment.

2.3 Cell survival (MTT) Assay

Cells were seeded in 96-well plates at a density of 3.0×10^3 cells per well.

TiO_2 nanoparticles were freshly suspended in 100 mL sterile saline in a sealed conical flask and diluted to appropriate concentration with DMEM to the specified concentration before use.

Cells were exposed to TiO_2 nanoparticles after they had attached to the bottom of plates for 2-3 hours. Then cells were treated with specified concentrations of nanoparticles for 24, 36, 48 or 72 hours. 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 TiO_2 nanoparticles served as the controls in each experiment. The formazan product formed in each well of each plate by live cells was dissolved in DMSO and then transferred to an empty well in another plate gently to reduce the interference of nanoparticles. The absorbance of the material in each well in the plates was read in a plate reader as described previously [8].

2.4 Statistical Analysis of Results

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 with a minimum significance level set at $p< 0.05$ using the Kaleidagraph 4.0 software package.

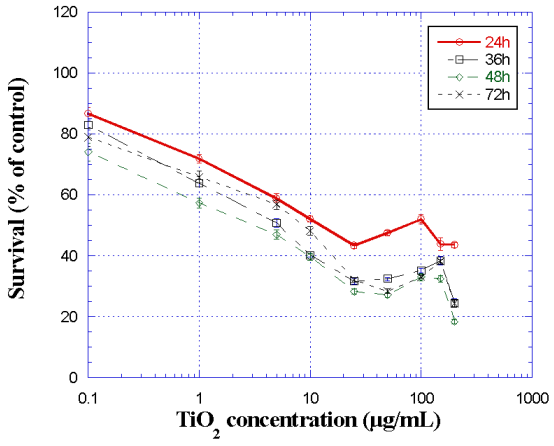

Figure 1: Effect of TiO_2 nanoparticles on survival of DRG neurons. Results are mean ± SEM of 12 determinations. All treatment groups were significantly different from the control group ($p<0.05$). The results of two other experiments showed results almost identical to those shown in this Figure.

3 RESULTS AND DISCUSSION

DRG neurons and Schwann cells are two main kinds of cells in the PNS. DRG neurons are the excitable cells that receive and transmit signals. Schwann cells, which normally are in direct contact with PNS neurons and often surround them, not only provide the neurons with physical support but also supply them with metabolites and nutrients, and protect them from a variety of pathophysiological assaults [9].

After they were exposed to TiO_2 nanoparticles, the survivals of both cell types decreased as the concentrations of the nanoparticles increased (Figures 1 and 2). The dose-related effects in both cell types induced by treatment with the nanoparticles for different treatment times showed similar trends (Figures 1 and 2). The IC_{50} of TiO_2 nanoparticles in lowering the survival of Schwann cell was approximately 25 µg/mL and this value varied somewhat depending on the treatment time (Figure 2). On the other hand, the IC_{50} of TiO_2 nanoparticles in lowering the survival of DRG neurons was approximately 5 µg/mL and this value also varied somewhat with treatment time (Figure 1).

That the IC_{50} of TiO_2 nanoparticles in lowering the survival of DRG neurons was lower than that of these nanoparticles in lowering the survival of Schwann cells (~5 µg/mL versus 25 µg/mL) strongly suggests that DRG neurons are more susceptible than Schwann cells to the cytotoxicity of TiO_2 nanoparticles. This conclusion becomes more evident when we compared the effects of TiO_2 nanoparticles on both cell types after treatment for 72 hours with various concentrations of the nanoparticles (Figure 3). Indeed, as shown in Figure 3, the two plots of cell survival versus concentration of TiO_2 nanoparticles do not overlap. All in all, our results strongly suggest that treatment with TiO_2 nanoparticles exerts differential cytotoxic effect on DRG neurons and Schwann cells. Thus, the results of this study on PNS neural cells are also compatible with our previous study where we had demonstrated that treatment with TiO_2 nanoparticles induces differential cytotoxic effects on neural cells from the central nervous system [8].

Our results may have some pathophysiological implications in the impact of exposure to TiO_2 nanoparticles on the structure and function of the peripheral nervous system. For example, our observation that DRG neurons are more susceptible than Schwann cells suggests that when the PNS neural cells are exposed to the nanoparticles, the DRG neurons and perhaps other PNS neurons may become dysfunctional first prior to Schwann cells showing any signs of dysfunction. Because as far as we are aware, ours is the first report on the cytotoxicity of TiO_2 nanoparticles in DRG neurons and Schwann cells, additional studies are needed to not only further characterize these cytotoxic effects on the PNS neural cells but also elucidate the underlying molecular mechanisms.

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 with a minimum significance level set at $p< 0.05$ using the Kaleidagraph 4.0 software package.

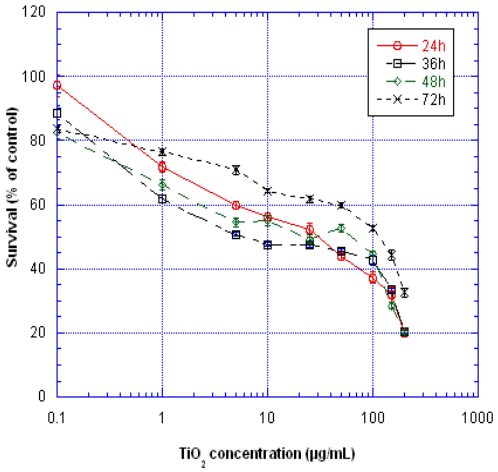

Figure 2: Effect of TiO_2 nanoparticles on survival of Schwann cells. Results are mean ± SEM of 12 determinations. All treatment groups were significantly different from the control group ($p<0.05$). The results of two other experiments showed results almost identical to those shown in this Figure.

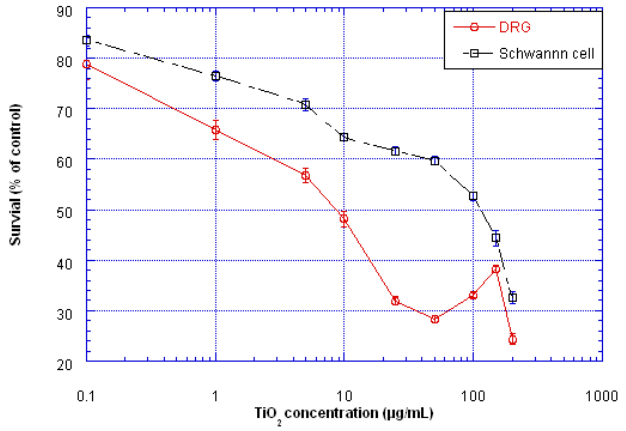

Figure 3: Comparison of the survival of DRG neurons and Schwann cells exposed to various concentrations of TiO_2 nanoparticles for 72 hours.

4 CONCLUSIONS

Because the cytotoxic effects of TiO_2 nanoparticles on peripheral nervous system (PNS) neural cells have not been reportedly studied, this study is the first to report on the differential cytotoxic of TiO_2 nanoparticles on DRG neurons and Schwann cells. Our results demonstrated treatment with TiO_2 nanoparticles lowered the survival of both DRG neurons and Schwann cells in a dose- and time-related manner, DRG neurons being more susceptible to their effect compared to Schwann cells. Thus, our findings may assume pathophysiological importance in the impact of exposure to TiO_2 nanoparticles on the structure and function of the neural cells of the peripheral nervous system. Our studies are in progress in our laboratories to further elucidate the cellular and molecular mechanisms underlying the cytotoxicity of TiO_2 nanoparticles.

5 ACKNOWLEGDEMENTS

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