Cytotoxicity of Magnesium Oxide Nanoparticles in Schwann Cells

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Abstract

Diverse applications of metal oxide nanoparticles in various industries have been accelerating in the last decade. Magnesium oxide (MgO) nanoparticles have been employed in ceramics and as adhesive and additive in chemical raw materials, leading to their increased human exposure, thereby posing potential health hazards. Because cytotoxicity of MgO nanoparticles in neural cells derived from the peripheral nervous system is unknown, we have investigated the effects of MgO nanoparticles on Schwann cells. Our results indicated treatment with MgO nanoparticles for 24 hours induced dose-related cytotoxicity in Schwann cells. Moreover, such treatment also induced distinct morphological changes in Schwann cells leading us to investigate the hypothesis that these nanoparticles elicit inflammatory responses in Schwann cells. Consistent with this hypothesis was our finding that the nanoparticles induced enhanced expression of cytokines such as IL-6. Thus, our findings may have pathophysiological implications in toxicity of MgO nanoparticles in neural cells derived from the peripheral nervous system.

Keywords: magnesium oxide nanoparticles, Schwann cells, cytotoxicity, nanotoxicity, peripheral nervous system, inflammatory response.

1. Introduction

Nanotechnology has emerged as a science over the past decade and its applications have found their way into many consumer goods, including cosmetics, clothes and sporting goods. Because of the ubiquitous applications of nanomaterials in diverse industries and the escalating numbers of such applications, humans and other species are increasingly exposed to nanomaterials. However, the regulatory control of the use of nanomaterials is not presently in place. More importantly, the environmental and health impact of exposure to most nanomaterials has

not been assessed. On the other hand, evidence has been accumulating that many nanomaterials, including nanoparticles, are not as harmless as they have been assumed to be [1-3]. We have therefore developed several neural and non-neural cell models *in vitro* and employed them to systematically investigate the putative cytotoxicity of the nanoparticles of metallic and non-metallic oxides [see 1-3 and references therein].

Nanoparticles in the atmosphere may enter the body via ingestion, inhalation and/or dermal exposure and repeated exposure may result in accumulation of significant amounts of the nanoparticles in various organs [1-5]. More importantly the blood-brain barrier does not provide adequate protection to the brain to prevent the penetration of nanoparticles from the blood into brain [see 2 and references therein]. Additionally, inhaled nanoparticles can reach the brain via retrograde transport in the olfactory nerve [4]. Nanoparticles are also known to penetrate through the skin and enter the peripheral circulation [5].

Our previous and ongoing studies have demonstrated that nanoparticles of metallic and non-metallic oxides exert differential cytotoxic effects in neural cells derived from the central nervous system [see 1-3 and references therein]. Nonetheless, as the putative toxic effects of such nanoparticles in neural cells derived from the peripheral nervous system (PNS) are largely unknown, we have initiated studies to systematically investigate the putative cytotoxicity of nanoparticles of metallic and non-metallic oxides in neural cells derived from the PNS [7-9] As metal oxide nanoparticles are also known to induce inflammatory responses in various cell types [11,12], in this study we have investigated the hypothesis that MgO nanoparticles elicit inflammatory responses in Schwann cells .

2. MATERIALS AND METHODS

2.1 Materials

R3 (neuronal Schwann cell; immortalized with SV40 large T antigen) cells were obtained from ATCC (Manassas, VA, USA). Magnesium Oxide Nanoparticles (Cat #549649-5G, nanopowder, <50 nm particle size, 99.7%), Thiazolyl blue tetrazolium bromide (MTT) and Dulbecco's Modified Eagle's Medium (DMEM) were purchased from Sigma-Aldrich (St. Louis, MO).. Fetal bovine serum (FBS) was from Atlanta Biologicals (Lawrenceville, GA, USA), protease inhibitor cocktail tablets from Roche diagnostics, antibodies to Il-6 were from Santa-Cruz (Santa Cruz, California) and antibodies to peroxisome-proliferator activator receptor γ (PPARγ) and β-actin were from Abcam (Cambridge, MA). Other chemicals were of analytical grade and were usually obtained from Sigma-Aldrich (St. Louis, MO).

2.2 Cell culture of Schwann cells

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 and humidified environment.

2.3 Cell Survival (MTT) assay

MgO 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 seeded on 96-well plates at a density of 4.0×10^3 cells per well. Cells were exposed to MgO nanoparticles after they had attached to the bottom of the wells for 2-3 hours. Then cells were treated with specified concentrations of nanoparticles for 24 hours. MTT dye (20 µl of 0.5%, (w/v) in PBS) was added to each well and the plates were incubated for another 4 hours. The formazan crystals formed in each well of each plate by live cells was dissolved in DMSO after aspirating the medium in the wells and then transferred gently to an empty well in another plate to reduce the interference of absorbance measurement by nanoparticles. The absorbance of the material in each well in the plates was read in a plate reader as described previously [1-3]. Plates containing no seeded cells but only culture medium with and without MgO nanoparticles served as the controls in each experiment.

2.3 Western Blot Analysis:

Schwann cells were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) until they were 70% confluent and then treated with freshly prepared magnesium oxide nanoparticle suspension in saline. After 24 hours, the cells were washed with ice-cold PBS and cell lysates were prepared using 0.32 M sucrose, 5 mM HEPES-Tris, pH 7.4 containing protease inhibitors. Protein analysis for lysates was performed using Bicinchonic assay (BCA) kit employing 96 well plate reader at 562 nm.

Western blot analysis was performed as described previously [3, 9] using 12.5% gel at 150 constant volts after electrophoresis; the separated proteins were transferred to a PVDF membrane at 100 volts for 90 minutes. Once the transfer was complete, the membranes were incubated in 5% (w/v) fat-free milk for 3-4 hours at room temperature. The blocking solution was then washed and incubated in solutions containing primary antibodies against respective protein overnight, followed by their respective peroxidase-conjugated secondary antibodies. The protein-antibody complexes were visualized using

Chemiluminescent solution [3,9].

2.4 Bright Field Microscopy:

The morphology of Schwann cells treated or not treated with MgO nanoparticles for 24 hours were monitored with light microscopy using a Leica DM IRB microscope after at 400X magnification as described previously [1-3].

2.5 Statistical Analysis

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

3 RESULTS AND DISCUSSION

Schwann cells are the supportive cells for neurons in the peripheral nervous system (PNS): they also produce the myelin sheath and play important roles in axonal function and regeneration [6,7]. Consequently, they are frequently employed to elucidate pathophysiological mechanisms in peripheral neuropathy. We have therefore employed Schwann cells to investigate the putative cytotoxic effects of MgO nanoparticles on neural cells derived from the PNS.

As shown in Figure 1, MgO nanoparticles induced dose-related decreases in survival of Schwann cells when they were exposed to MgO nanoparticles for 24 hours. The IC_{50} of MgO nanoparticles in lowering the survival of Schwann cell was ~ 100 µg/ml. That initial observation prompted us to monitor the effects of MgO nanoparticles on the morphology of Schwann cells.

We found that as the concentration of MgO nanoparticles was increased, more and more morphological differences between the treated and untreated (i.e., control) Schwann cells were noted (Figure 2). When the Schwann cells were treated with the lower levels of the nanoparticles (i.e., 0.1-5 µg/ml), the polarity of the cells were more pronounced and they appeared to send out more processes (Figure 2; circled cells). Additionally, at the higher treatment levels (i.e., 5-100 µg/ml), the nanoparticles induced the cells to swell and round off, suggesting that those cells were possibly undergoing necrosis (Figure 2; circled cell(s)).

Morphological changes induced by MgO nanoparticles led us to investigate the possibility of an inflammatory response elicited by the Schwann cells. Inflammatory response is a protective mechanism that most cells exhibit towards an obnoxious stimulus. We therefore examined the expression of inflammatory markers such as interleukin-6 (IL-6) and peroxisome-proliferator activator receptor γ (PPARγ). Il-6 is a pro-inflammatory cytokine that protects the cells by eliciting immune response and regulating the transcription of anti-apoptotic genes. On the other hand, PPARγ is a member of nuclear receptor super-family important in regulating many genes relating to glucose metabolism; it has also been shown to have anti-inflammatory actions by regulating cytokine expression.

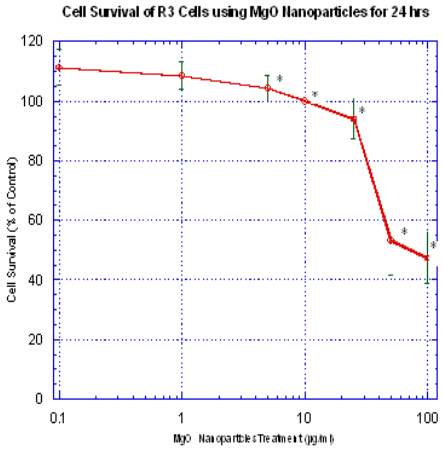

Figure 1: Survival of R3 Schwann cells exposed to MgO nanoparticles for 24 hours (*p<0.05 versus control, as determined by ANOVA with post-hoc Tukey test).

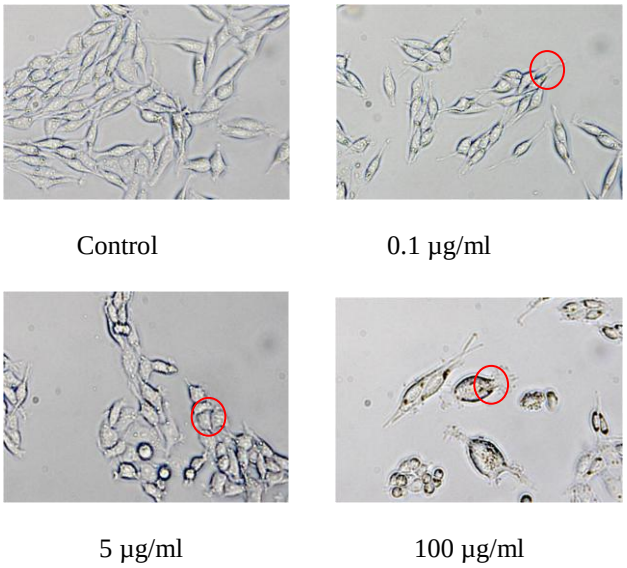

Figure 2: Schwann cells treated with or without (i.e., the control) MgO nanoparticles for 24 hours were monitored with bright field microscopy using a Leica DM IRB microscope at a magnification of 400X. Distinct morphological changes are circled.

Our western blot analysis shows Schwann cells treated with MgO nanoparticles for 24 hours exhibited increased expression of both of the markers (data not

shown). However, the simultaneous increase in expression of both pro-inflammatory and anti-inflammatory markers in the treated cells is intriguing. This observation prompted us to further examine the expression of these markers in Schwann cells after exposing them to the nanoparticles for 48 hours. The cells so treated did not show any difference in expression of IL-6 and PPARγ compared to those in respective control cells (data not shown), suggesting that the anti-inflammatory effect of PPARγ might have counter-balanced the effects of IL-6.

4. CONCLUSIONS

Our results confirm that the Schwann cells we employed in this study constitute a good cell model *in vitro* for investigating the pathophysiological mechanisms of agents that induce peripheral neuropathy [6-9]. Our results also indicate that exposure of Schwann cells to MgO nanoparticles lowered their survival in a dose-related manner. In parallel with these effects, the treated Schwann cells also exhibited progressive changes in morphology as the treatment level was increased. At the higher treatment doses, their morphological changes were reminiscent of cells undergoing necrosis. Furthermore, our findings also strongly suggest MgO nanoparticles induced inflammatory responses in Schwann cells.

Our results may have some pathophysiological implications in the impact of exposure to MgO nanoparticles on the structure and function of the peripheral nervous system. Our on-going and future studies aim to further elucidate the mechanism(s) underlying the cytotoxicity and inflammatory effects of MgO nanoparticles in Schwann cells.

5. ACKNOWLEGDEMENTS

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