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Cytotoxic effects of aggregated nanomaterials ☆

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Abstract

This study deals with cytotoxicity assays performed on an array of commercially manufactured inorganic nanoparticulate materials, including Ag, TiO_2, Fe_2O_3, Al_2O_3, ZrO_2, Si_3N_4, naturally occurring mineral chrysotile asbestos and carbonaceous nanoparticulate materials such as multiwall carbon nanotube aggregates and black carbon aggregates. The nanomaterials were characterized by TEM, as the primary particles, aggregates or long fiber dimensions ranged from 2 nm to 20 μm. Cytotoxicological assays of these nanomaterials were performed utilizing a murine alveolar macrophage cell line and human macrophage and epithelial lung cell lines as comparators. The nanoparticulate materials exhibited varying degrees of cytoxicity for all cell lines and the general trends were similar for both the murine and human macrophage cell lines. These findings suggest that representative cytotoxic responses for humans might be obtained by nanoparticulate exposures to simple murine macrophage cell line assays. Moreover, these results illustrate the utility in performing rapid in vitro assays for cytotoxicity assessments of nanoparticulate materials as a general inquiry of potential respiratory health risks in humans.

Keywords: Cytotoxicity assessment; Nanoparticulates; TEM analysis

1. Introduction

There is now compelling evidence that ultrafine or nanoparticulate matter (with mean or geometric diameters <100 nm) is associated with an increased prevalence of respiratory and cardiovascular disease and mortality [1–10]. This includes TiO_2, black carbon (BC) and silica [2,11], as well as a wide range of natural mineral nanoparticles [12]. Recent cytotoxicological assays of a wide range of manufactured nanoparticulate materials, utilizing a murine lung macrophage cell line, have illustrated varying degrees of cytotoxicity, with nanoparticulate silver, chrysotile asbestos, multiwall carbon nanotubes and BC exhibiting particularly acute cytotoxicity [13,14]. Similarly, recent in vivo studies in rats have shown lung lining inflammation, dermal inflammation and even death in response to such materials [15–17]. While the toxicological mechanisms of in vitro and in vivo responses are poorly understood [2,18], there seems to be mounting evidence that nanoparticles in particular exert their toxic effects through the formation of reactive oxygen species (ROS) which cause oxidative stress [18–20].

Although chrysotile asbestos has been demonstrated to be morphologically identical to many forms of multiwall carbon nanotubes [21], and its short-term cytotoxic response for murine macrophage exposure has been demonstrated to be identical to multiwall carbon nanotubes [13,14], there is no long-term in vivo evidence that multiwall carbon nanotubes would pose the same health risks as asbestos. Nonetheless, short-term cytotoxic responses should be regarded as a first alert. Indeed, since the inception of the US National Nanotechnology Initiative in 2000, cautions of nanoparticulate risks in particular have persisted and the implementation of nanotechnology innovations seem linked to biological issues, especially human

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health effects. It would seem unrealistic to repeat the failures of the asbestos industry, which largely ignored the product dangers for more than 2000 years.

Although simple, short-term cytotoxicity assessments have been utilized to evaluate a wide range of nanoparticulate materials [13,14], the implications for humans may not be convincing unless a strong correlation is established between animal cell assays and human cell assays. In this study we compare cytotoxicity assays for a range of manufactured nanoparticulate materials utilizing both a murine lung macrophage cell line and a human lung macrophage cell line. In addition, we compare the human macrophage cell line assay results with a series of human lung epithelial cell line assays to provide a more comprehensive, short-term lung function cytotoxicity assessment.

2. Materials and methods

2.1. Characterization of nanoparticulate materials

It has been evident that ultrafine and particulate materials (mean diameter <100 nm) in the atmosphere are toxic and pose considerable health risks, such as asthma complications, chronic bronchitis and respiratory tract infections [18,27,28]. The assessment of health effects, especially the pulmonary toxicity of particulates, is often a complex issue, in particular for nanoparticulate materials. Although many, like Lam et al. [15,29] and Warheit et al. [16], have demonstrated single-wall carbon nanotubes (SWCNTs) to be toxic, there have been no detailed microscopic examinations to determine the particle morphologies or aggregation. The shape and size of micron-sized particulates is a complicating issue in assessing pulmonary toxicity and especially their airway deposition. Fibers, fiber bundles or aggregates, or other nanoparticulate composites have a variety of airstream response and deposition behaviors. Correspondingly, biological assays to evaluate the function of alveolar macrophages upon exposure to nanoparticulate materials must include their characterization at the nano-scale.

A wide spectrum of commercially manufactured nanoparticulate materials and carbonaceous nanoparticulates were examined as an extension of previous work [13]. Table 1 provides a very general description of the nanomaterials examined in this study. In addition, nanoparticulate materials were characterized by transmission electron microscopy, as described in detail previously [13].

2.2. Viability assays

Cytotoxicity assessments of the manufactured nanoparticulates in Table 1 were performed using a murine alveolar macrophage cell line (RAW 264.7) (courtesy of Kenneth S.K. Tung at the University of Virginia Health Science Center), which was used as a standard against a human alveolar macrophage cell line (THB-1) (The American Type Culture Collection ATTC, Manassas, VA) as well as a human epithelial cell line (A549) (ATCC). Viability assessments and the culturing of the murine alveolar macrophages are described in detail elsewhere [14].

All the nanoparticulate materials were suspended in a stock solution at 5 μg ml^{-1} in dimethyl sulfoxide (DMSO), a solvent which assures suspension of even hydrophobic substances. Pourahmad and O'Brien [26] have demonstrated that DMSO is an effective antioxidant and is capable of inhibiting cellular death at concentrations ranging from 140 to 280 mM. In this investigation the DMSO concentration ranged from 0.0344 mM to 35.25 nM, or the concentration in which DMSO did not function as a scavenger of ROS. The effect of DMSO was determined on background production of ROS and hydrogen peroxide induction of ROS. In both cases DMSO concentrations up to 35.25 nM did not inhibit ROS formation [13]. The murine and human macrophages, as well as the human epithelial cells, were cultured in a 96-well flat-bottom plate (50,000 cells $well^{-1}$) starting with a concentration of 10 μg ml^{-1} followed by 11 doubling dilutions. Controls were incubated with equivalent dilutions of vehicle (DMSO) and with neither vehicle nor compound. The human alveolar macrophage cell line THB-1 was cultured in RPMI 1640, supplemented with 10% FCS, 5×10^5 M 2-Me penicillin/streptomycin, 2 mM L-glutamine adjusted to contain 1.5 g l^{-1} sodium bicarbonate, 4.5 g l^{-1} glucose, 10 mM HEPES and 1.0 mM sodium pyruvate, and supplemented with 0.05 mM 2-mercaptoethanol. The human

Table 1
Description of manufactured nanoparticulate materials

Nanoparticulate material	Primary particle size range (nm)	Aggregate size range	Specific surface area BET (m^2/g)
Chrysotile asbestos	15–40	Fiber bundles 0.5 μm–15 μm	1.3
Black carbon (BC)	2–50	0.1 μm–1 μm	239
Multi-wall carbon nanotube-R	10–30	0.1 μm–3 μm	16
Multi-wall carbon nanotube-N	5–30	0.1 μm–3 μm	218
(Ag-1)	3–100	25 nm–1 μm	15
Al_2O_3	4–115	0.5 μm–1 μm	54
Fe_2O_3	5–140	0.5 μm–0.9 μm	39
ZrO_2	7–120	0.5 μm–1 μm	88
TiO_2-anatase	5–40	1 μm–2 μm	55
TiO_2-rutile	2–60	0.5 μm–1.5 μm	125