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Measurement of reactive species production by nanoparticles prepared in biologically relevant media

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Abstract

Exposure to nanoparticles may pose a risk to health and this hypothesis is currently being investigated by toxicologists. Although the mechanism of nanoparticle toxicity has been shown to be mediated, in part, by oxidative stress, the precise mechanism and molecules involved are still unknown. In light of this, the evaluation of the oxidative potential of nanoparticles is an important consideration in measuring their toxicity. The aim of this study was to examine the use of a fluorogenic probe, 2′,7′-dichlorofluorescin (DCFH), in a cell-free assay system and to assess the relationship between the results obtained with this method and with the reactive species formation observed in cells. In order to obtain a well-dispersed nanoparticle suspension, bovine serum albumin (BSA) and dipalmitoyl phosphatidyl choline (DPPC) addition in suspension medium was investigated. Both 1% BSA and 0.025% DPPC added to the medium significantly improved the stability of the nanoparticle suspension, decreasing the extent of particle agglomeration and settling over time. In a cell-free system, reactive oxygen species (ROS) production by 14 nm carbon black particles (CB) suspended in DPPC was higher than that measured with the other suspensions (saline or 1% BSA). A greater ROS production was observed in MonoMac 6 cells (MM6) following treatment with 14 nm CB suspended in medium containing BSA and/or DPPC compared to medium alone. In conclusion, 1% BSA and 0.025% DPPC solution was the most efficient for the preparation of a nanoparticle suspension and to measure their oxidative potential.

Keywords: 2′,7′-Dichlorofluorescein; Nanoparticles; Carbon black; Dipalmitoylphosphatidyl choline; Albumin; Horseradish peroxidase

1. Introduction

Human exposure to nanoparticles (or ultrafine particles) has increased significantly over the last century via anthropogenic combustion-derived air pollution. In addition, during recent decades the development of nanotechnology has generated a source of new engineered nanoparticles for use in a diverse array of applications including medicine, food, clothes, personal care products, IT and construction materials, resulting in a wide range of exposure scenarios. The hazard of these newly engineered nanoparticles needs to be considered in order to determine the potential risk to health. Nanotoxicology, an emerging discipline, assesses the different characteristics of these nanoparticles and their interactions with biological systems, in order to define common principles explaining toxicity (Oberdorster et al., 2005). One of the most significant characteristics of nanoparticles is their relatively large surface area

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and the number of particles per unit mass. Both of these properties may result in increased interactions between nanoparticles and biological tissue compared to larger particles (Donaldson et al., 2001). The relationship between particle surface area and biological effect has been demonstrated by the induction of lung inflammation following intra-tracheal instillation of fine and ultrafine titanium dioxide (TiO_2) particles in the rat lung. When results were expressed as particle surface area, the same dose response was observed for both the fine and ultrafine TiO_2 (Oberdorster et al., 2000). A similar linear relationship between the surface area of polystyrene particles and resulting inflammation has also been demonstrated following installation into the rat lung (Brown et al., 2001). Many studies have demonstrated the ability of nanoparticles to generate reactive oxygen species (ROS) in a cell-free environment (Brown et al., 2001; Stone et al., 1998; Wilson et al., 2002). Moreover a relationship has recently been established between the surface area and ROS-generating capability of carbon black nanoparticles (Koike and Kobayashi, 2006a,b) suggesting that ROS generation and oxidative stress could explain the toxic effects of nanoparticles. Studies have demonstrated that carbon nanoparticles can induce glutathione depletion indicative of oxidative stress in epithelial cell lines (Stone et al., 1998) and in the rat lung (Li et al., 1999). This oxidative stress has been linked to the induction of signalling pathways (Stone et al., 2000) which lead to pro-inflammatory gene expression in macrophages (Brown et al., 2004). Therefore, the evaluation of the oxidative potential of nanoparticles is an important parameter to evaluate their toxicity.

To accurately study this oxidative potential, stability and dispersion of nanoparticles has to be considered regarding compatibility with *in vitro* and *in vivo* systems. The use of the major components of the lung lining fluid, i.e. proteins and phospholipids, appeared to be the most appropriate (Kendall et al., 2004). For example, dipalmitoyl phosphatidyl choline (DPPC) is a phospholipid that is found in surfactant of the lung, and therefore is likely to come into contact with inhaled particles. The combination of particles with phospholipids could increase their dispersion and their bioavailability. Indeed, diesel exhaust particles (DEP) dispersed in aqueous mixtures of DPPC had a positive mutagenic response equivalent or higher than a dichloromethane extract of the same sample (Wallace et al., 1987). Further investigations suggested that the genotoxicity activity associated with DEP inhaled into lung might be made bioavailable by dispersion properties of pulmonary surfactant components (Keane et al., 1991). More recently, DPPC (0.04%) was used to suspend 95 nm CB and diesel exhaust particles (Baulig et al., 2003). A study by Sager and collaborators suggests that phosphate buffer containing protein plus DPPC is an appropriate medium to suspend particles although this medium appeared less effective that bronchoalveolar lavage fluid (Sager et al., 2007). Moreover interaction between DPPC and silica dust has been suggested to modulate the toxicity of these particles (Murray et al., 2005).

Fluorogenic probes are a convenient and sensitive means to monitor oxidative activity. Among these, 2′,7′ dichlorofluorescin diacetate (DCFH-DA) has been widely used as a marker for oxidative stress, and has been suggested to be a good indicator of the overall oxidative status of the cell (Wang and Joseph, 1999). This hydrophobic non-fluorescent molecule penetrates rapidly into the cell and is hydrolyzed by intracellular esterases to give the dichlorofluorescin (DCFH) molecule which can be oxidized to its fluorescent 2-electron product 2′,7′-dichlorofluorescein (DCF). DCFH-DA can also be chemically or enzymatically hydrolyzed to allow the use of DCFH in cell-free assays (Rota et al., 1999; Wilson et al., 2002).

The aim of this study was to investigate the oxidative potential of 14 nm carbon black particles (CB) suspended in different media containing DPPC or bovine serum albumin (BSA), respectively or in combination. The use of DCFH-DA in a cell-free assay system was first characterized in the presence of horseradish peroxidase (HRP) and BSA in order to compare the results to those obtained by flow cytometry, following treatment of cells with 14 nm CB suspended in different biologically relevant media.

Our data demonstrate that the oxidative potential of 14 nm CB is modified by the medium used. Medium containing DPPC and BSA resulted in stable dispersion of the 14 nm CB particles which induced a greater oxidation of DCFH than 14 nm CB suspended in saline solution. These observations may be related to an increase in exposed surface area of 14 nm CB in the different test media.

2. Materials and methods

2.1. Chemicals and solutions

All materials were obtained from Sigma, UK, unless otherwise stated.

Foetal bovine serum (FBS), L-glutamine (200 mM) and penicillin–streptomycin (10,000 u/ml–10,000 μg/ml) were obtained from Invitrogen Corporation (UK). Sterile water