Toxicology Letters 224 (2014) 147–156

Contents lists available at ScienceDirect

Toxicology Letters

journal homepage: www.elsevier.com/locate/toxlet

Inflammation and gene expression in the rat lung after instillation of silica nanoparticles: Effect of size, dispersion medium and particle surface charge

David M. Brown*, Nilesh Kanase, Birgit Gaiser, Helinor Johnston, Vicki Stone

School of Life sciences, Heriot-Watt University, Riccarton, Edinburgh EH14 4AS, UK

HIGHLIGHTS

- Silica particles (50 nm) caused significantly more inflammation than 200 nm in the lung.
- Phagocytosis of some types of particles was reduced using BSA as a dispersant.
- Particle size was important in determining Nrf2 expression.
- Inflammatory effects of silica can be modulated by the dispersion medium.

ARTICLE INFO

Article history:
Received 16 August 2013
Received in revised form 16 October 2013
Accepted 17 October 2013
Available online 26 October 2013

Keywords:
Silica nanoparticles
In vivo
Inflammation
Gene expression

ABSTRACT

We investigated the effects of silica particles and nanoparticles (NPs) (50 nm and 200 nm) with a neutral and positively charged surface when dispersed in saline, bovine serum albumin (BSA) or lung lining fluid (LLF) 24 h post instillation into the lungs of rats. There was a significant increase in the recruitment of neutrophils in animals instilled with 50 nm plain and aminated NPs compared with 200 nm particles when dispersed in saline or BSA, but not when dispersed in LLF. There was no evidence of toxicity or an increase in the albumin content of the bronchoalveolar lavage fluid. Immunostaining for the transcription factor Nrf2 in BAL cells indicated that there was a significant increase in nuclear colocalisation in animals treated with plain and aminated 50 nm NPs compared with plain and aminated 200 nm particles when dispersed in saline, but no difference was observed between 50 nm and 200 nm aminated particles when dispersed in BSA. There was no difference in nuclear colocalisation with any of the particle types dispersed in LLF. This study suggests that low dose intratracheal exposure to silica nanoparticles can produce an acute inflammatory response and that the dispersion medium may influence the magnitude of this response.

© 2013 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The use of engineered NPs in various manufacturing industries continues to increase worldwide and it is estimated that this trend will continue. Apart from manufacturing, and industrial applications, NPs are widely used in medical and pharmaceutical settings, such as drug delivery and in medical imaging procedures (Gao et al., 2004). The potential risks to workers or consumers when exposed to these materials have not been fully evaluated (Balbus et al., 2007). The manufacture of NPs on an industrial scale may produce a significant exposure to workers involved in the production of these materials. A major concern is the exposure route, as particles may enter the body *via* the lungs, skin, the bloodstream (during medical procedures) or through ingestion (Oberdorster et al., 2002; Jani et al., 1990; Schleh et al., 2012). Thus, NPs may ultimately become sequestered in different sites throughout the body (Lipka et al., 2010).

We have demonstrated previously in *in vitro* studies that NPs are easily coated with proteins, consequently this has implications for particles which enter the body *via* different routes (Brown et al., 2000, 2010). Interaction of particles with protein present in serum or in lung lining fluid (LLF) may result in a change in the physicochemical properties of the particle surface encountered by cells (Monopoli et al., 2011). It has been suggested that the size and surface properties of nanoparticles determine the protein corona composition (Lundqvist et al., 2008). The bound protein may undergo a conformational change in structure and become inactivated, or suffer a loss in functionality (Brown et al., 2010).

* Corresponding author at: School of Life Sciences, Heriot-Watt University, Nanosafety Research Group, Edinburgh EH14 4AS, UK. Tel.: +44 131 4514561; fax: +44 131 4513009.
E-mail addresses: d.brown@hw.ac.uk (D.M. Brown), n.kanase@hw.ac.uk (N. Kanase), b.gaiser@hw.ac.uk (B. Gaiser), h.johnston@hw.ac.uk (H. Johnston), v.stone@hw.ac.uk (V. Stone).

0378-4274/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.10.019

Conversely, the structure of the protein may be altered in such a way that its biological effects are increased, or the protein may be delivered to the cell by a different mechanism (Brown et al., 2010). The persistence of NPs in the lung may produce inflammation, and therefore particle clearance is an important issue. In this regard, macrophages play a key role and macrophage migration to sites of inflammation and phagocytosis of foreign material depends on a variety of factors including coordination between cytokines and other biological molecules (Ishii et al., 2005).

A major concern in the field of nanoparticle toxicology has been how particles are treated so that they are either monodispersed, or dispersed in a more environmentally relevant fashion. In the context of exposure of particles to cells both *in vitro* and *in vivo*, factors such as surface area (Brown et al., 2001; Duffin et al., 2007), size (Donaldson et al., 2000) and particle number (Oberdorster et al., 1994) are of particular importance. We were concerned in this study that the dispersion of particles prior to instillation should utilise media which is biologically relevant to the *in vivo* situation. We chose albumin as a dispersant because of its relevance with respect to exposure to blood–borne particles, and lung lining fluid due to its relevance to exposure of particles *via* the lung. Although albumin is not relevant in the lining fluid of the healthy lung, other studies have used this as a dispersant (Bihari et al., 2008), so it was included for comparison.

Lung surfactant, and hence lung lining fluid (LLF, obtained from bronchoalveolar lavage) consists of a mixture of proteins and lipids with a range of functions. For example, the phospholipids such as dipalymitoylphosphatidylcholine (DPPC) are important in reducing the surface tension of the lung, therefore preventing the lungs from collapsing. Schleh et al. (2013) describe pulmonary surfactant as consisting of phospholipids and the four surfactant proteins (SP) A, B, C, and D. In some studies hundreds of different proteins have been identified in LLF. Bartlett et al. (2013) identified 674 porcine-specific proteins in bronchoalveolar lavage including proteins involved in host defence (for example surfactant proteins A and D), molecular transport, metabolic function and cell communication. These results also have implications for the design of *in vitro* studies in which LLF can be used as a dispersant to better represent exposure *via* the lung (Schleh et al., 2013).

We used a rat model to study exposure to particles *via* the respiratory system using a low dose of particles. It is considered that long-term inhalation studies are the most relevant way to model this exposure route, but the disadvantages to studies of this type are that they require specialist equipment, are costly to run, and for this reason, alternative exposure methods mirroring the *in vivo* situation have been developed. One of the most common exposure techniques is intratracheal instillation, or intranasal instillation and most studies of this nature examine lung inflammation as an end point, as inflammation is the precursor to many particle-induced lung diseases (Donaldson et al., 2000; Borm et al., 2006; Warheit et al., 2005).

For the present investigation, we chose silica particles (200 nm) and NPs (50 nm) with two different surface charges (positive and neutral) since previous studies have highlighted the influence of surface charge of NPs with regard to the uptake of NPs into cells and to their cytotoxic consequences (Stayton et al., 2009; Chung et al., 2007). Different forms and compounds of silica have been used in medical imaging techniques such as MRI, exploiting silica's properties of being easily bio-functionalised, and their suitability as carriers of high contrast imaging dyes. The surface charge of particles has been reported to influence the binding of biological molecules and consequently how efficiently the particles are phagocytosed and cleared (Nel et al., 2009).

We measured the influx of neutrophils into the lungs 24 h post exposure as an index of inflammation, and also examined the expression of pro-inflammatory genes in bronchoalveolar (BAL) cells. These cells were also used in immunostaining studies to determine the colocalisation of the transcription factor Nrf2, which regulates antioxidant gene expression (Kaspar et al., 2009). The albumin and LDH activity of the BAL fluid was also assessed to identify injury to the lung. Finally, to investigate effects distal to the initial exposure route, we measured the GSH concentration in liver homogenates as this has been shown to be affected by instillation of other NPs (Kermanizadeh et al., 2012). This study was designed to investigate whether particle size and charge can influence particle induced pro-inflammatory effects *in vivo*, and which may be influenced by the dispersant and agglomeration state of the particles.

2. Materials and methods

2.1. Particle characterisation (dynamic light scattering and zeta potential measurements)

Silica NPs were obtained from Kisker Biotech, Steinfurt, Germany. We used silica particles (50 and 200 nm diameter), one type with a neutral surface charge and a second type with a positive (NH_2 modification) charge. Silica NPs were suspended in RPMI medium at a concentration of 125 μg/ml and sonicated for 10 min. Dispersants were diluted in saline to give concentrations of BSA 0.2%, LLF 2 μg/ml (see below for preparation method) and serum 0.2%. Prior to addition of the treatments to the cells, an equal volume of particle suspension and dispersant were mixed together and vortexed briefly. The silica NP suspensions were serially diluted in the appropriate dispersant at half the concentrations referred to above, to give a range of dilutions from 62.5 μg/ml to 3 μg/ml. These dilutions corresponded to 1–16 μg/cm^2 particles in wells of a 96 well plate. The hydrodynamic diameter and zeta potential of the NPs were determined using a Malvern nano ZS zetasizer, according to the manufacturer's protocol.

2.2. Preparation of silica particles for TEM

Silica particles and NPs were suspended in each of the dispersants described previously at a concentration of 31.25 μg/ml. Five microlitres of each suspension were pipetted onto the surface of 200 mesh Formvar coated copper grids (Agar Scientific) and allowed to dry at 37 °C for 24 h. The grids were coated with 5 nm of evaporated carbon prior to imaging. A FEI Tecnai TF20 FEGTEM microscope fitted with a Gatan Orius SC600 CCD camera was used to image the particles.

2.3. Lung lining fluid (LLF) preparation

LLF was obtained from rat lungs in accordance with the method of Baughman et al. (Baughman et al., 1987). Male Sprague Dawley rats, approximately 3 months old, were used to obtain a stock of lung lining fluid. Rats were euthanised by single intraperitoneal injection of pentobarbitone, the lungs cannulated and removed, and lavaged with 4× 8 ml volumes of sterile saline. The lavageate was pooled into a single tube. Tubes were centrifuged at 258 × *g* for 5 min at 4 °C, and the supernatant collected for LLF preparation. The supernatant was transferred into a 50 ml centrifuge tube and centrifuged at 258 × *g* for 10 min to remove remaining debris. The supernatant was transferred to a 50 ml ultracentrifuge tube and centrifuged at 60,000 × *g* for 45 min. At the end of the centrifugation period, the supernatant from each tube was discarded and the remaining pellet resuspended in 10 ml sterile PBS. This surfactant-enriched fraction, termed LLF, was aliquoted into 1 ml volumes and stored at −80 °C in cryotubes until required. The protein content of the LLF was determined using Biorad reagent, according to the manufacturer's instructions, and used at a concentration of 1 μg/ml in medium to disperse the NPs.

Specific proteomics screening was carried out as part of a commercial service by the Moredun Institute, Edinburgh to determine the main components of the LLF.

In order to test for the presence of endotoxin in the LLF and in the silica suspensions, a Pyrostate (Associates of Cape Cod, Falmouth. MA, USA) kit was used according to the manufacturer's instructions. This kit detects endotoxin by the presence or absence of a clot in the reagent tube.

2.4. Experimental design

Before instillation, silica particles were suspended in sterile saline at a concentration of 120 μg/ml and sonicated for 5 min. Immediately after sonication, 1 ml of the particle suspension was mixed with 1 ml of dispersant at twice the required final concentration (BSA 0.2%, LLF 2 μg/ml serum 0.2%) and vortexed for 30 s. This provided the stock suspensions for instillation into the rat lung.

2.5. Silica particle instillations

Male Sprague Dawley rats approximately 3 months old were used throughout. Ethical approval was obtained from Edinburgh Napier University ethics committee prior to commencement of the experiments. Animals were allowed free access to

Download English Version:

https://daneshyari.com/en/article/2599125

Download Persian Version:

https://daneshyari.com/article/2599125

Daneshyari.com