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The Importance of Surface Area and Specific Reactivity in the Acute Pulmonary Inflammatory Response to Particles

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A range of different particle types cause excessive lung inflammation that is thought to play a role in the various types of pathology they produce. Recently attention has been focused on ultrafine particles and the phenomenon of rat lung overload. The authors and their collaborators have shown previously that the surface area metric drives the overload response. Acute inflammatory response following instillation of particles has also been used to evaluate hazard but has been criticized because of the non-physiological delivery and the problems of local overload. We have instilled a number of low-toxicity dusts of various particle sizes and assessed neutrophil influx into the lung at 18–24 h. The extent of inflammation has been shown to be a function of the surface area instilled. Since ultrafine particles present a 'special' case of high surface area, they are relatively inflammogenic. There is no evidence that ultrafine particles of carbon black, titanium dioxide or latex have any special reactivity in addition to their large surface area. We tested whether we could use this approach to model the reactivity of highly toxic dusts. Rats were instilled with either DQ12 quartz or aluminium lactate-treated DQ12 and, as anticipated, the high specific surface toxicity of DQ12 meant that it was much more inflammogenic than was predicted using the relationship described for low-toxicity dusts. By contrast, aluminium lactate-treated DQ12 fell on to the line of 'low-toxicity' dusts. This approach presents the possibility of modelling potential toxicity for nuisance dusts based on the inflammatory response of a given instilled surface area dose.

Keywords: surface area; surface reactivity; ultrafine; quartz; inflammation; macrophage inflammatory protein-2

INTRODUCTION

A range of different poorly soluble particle (PSP) types can cause excessive lung inflammation that may then play a role in the resultant pathologies. For PSPs, the particle surface has always been assumed to be the 'dose' that interacts with the lung, leading to inflammation. Quartz is an example of a particle with a highly reactive surface that has been implicated in its toxicity (Donaldson and Borm, 1998; Fubini, 1998; Duffin *et al.*, 2000). Recently attention has been focused on ultrafine (uf) particles and the phenomenon of lung overload. Onset of overload inflammation and impairment of clearance has been found to be related to lung burden dose expressed as surface area, rather than mass or volumetric dose (Oberdorster, 1996; Tran *et al.*, 2000). To study the pro-inflammatory effects of particle surfaces in the lung, we have developed a rat instillation model of acute inflammation. Whilst inhalation provides a more physiological route of delivery, we show here that instillation can yield concordant results for short-term inflammatory responses and can therefore be utilized to study the effects of different particles in the lung. We have hypothesized that: (i) surface area alone drives the acute pulmonary response to 'low-toxicity' PSPs such as titanium dioxide, carbon black and polystyrene (latex), suggesting that the higher the surface area instilled, the greater the inflammatory response; (ii) for quartz, a low surface area with a highly reactive surface produces inflammation and

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treatments which decrease its reactivity and should make it act like a 'low-toxicity' dust; (iii) for specific ultrafine particles with a highly reactive surface, inflammogenicity is related to both surface area and surface reactivity; and finally (iv) macrophage inflammatory protein-2 (MIP-2) levels in lavage should be related to inflammatory cell influx.

MATERIALS AND METHODS

Instillation

Male Wistar rats ~4 months old were used in all experiments. The animals were anaesthetized with halothane, cannulated with a laryngoscope to expose the trachea, and 0.5 ml of a 500 μg/ml particle suspension in saline instilled into the lungs. Animals instilled with 0.5 ml of saline were used as controls. Animals were killed either 4 or 18 h after instillation.

Bronchoalveolar lavage

Rats were killed with a single intraperitoneal injection of pentobarbital and the lungs cannulated, removed and lavaged with 1×8 ml volume of sterile saline. This first lavage was retained in a separate tube for analysis of bronchoalveolar lavage fluid (BALF) MIP-2 levels (4 h instillations only). Subsequently, the lungs were lavaged with a further 3×8 ml of sterile saline. All samples were centrifuged at 180 *g* for 5 min at 4°C. The supernatant was removed and the cell pellet from the first lavage was combined with the cells from the subsequent lavage before resuspension in 1 ml of phosphate-buffered saline. Total cells numbers were counted and Cytocentifuge smears were prepared and stained with Diff-Quik for assessment of differential cell counts. Three hundred cells per slide were counted and the results are expressed as total number of neutrophils in the lung lavage.

Expression of PMN data

Data in Fig. 1 are expressed as polymorphonuclear leukocyte (PMN) number in millions. For Figs 2 and 4a, the data are expressed as PMN per microgram of particle instilled, by way of adjusting for the bolus mass effect observed with an acute instillation model (this is particularly apparent in the case of the surface-modified quartz, where mass doses of up to 5 mg were instilled to achieve the desired surface areas). For Fig. 4b, data are expressed as PMN/μg/cm^2 by way of evaluating the 'specific' particle response, i.e. equalizing for both mass and surface area.

Macrophage inflammatory protein-2 assay

BALF MIP-2 concentration was assessed using an ELISA kit, which uses peroxidase and tetramethylbenzidine as a detection method (Biosource International, Camarillo, CA). The absorbance was quantified at an emission of 450 nm and BALF samples were compared with a standard curve of rat MIP-2.

Statistical analysis

One- or two-way analysis of variance was used to determine significance of treatment effects.

RESULTS

For 'low-toxicity' dusts, the extent of inflammation is not related to the mass of particles instilled (Fig. 1a). However, when the mass dose was expressed as surface area, it dictated the inflammatory response for a variety of very different particles of low toxicity (Fig. 1b). The fact that the line passes through zero indicates that it is surface area alone that elicits inflammation.

Very low surface areas of quartz were sufficient to cause large-scale inflammation as measured by PMN number per microgram of particle instilled (by way of adjusting for the bolus mass effect observed with instillation models—see Materials and Methods), in BALF 18 h after instillation. However, quartz which has had its surface modified by treatment with aluminium lactate, or quartz from workplaces where the surface has been affected by the industrial processes

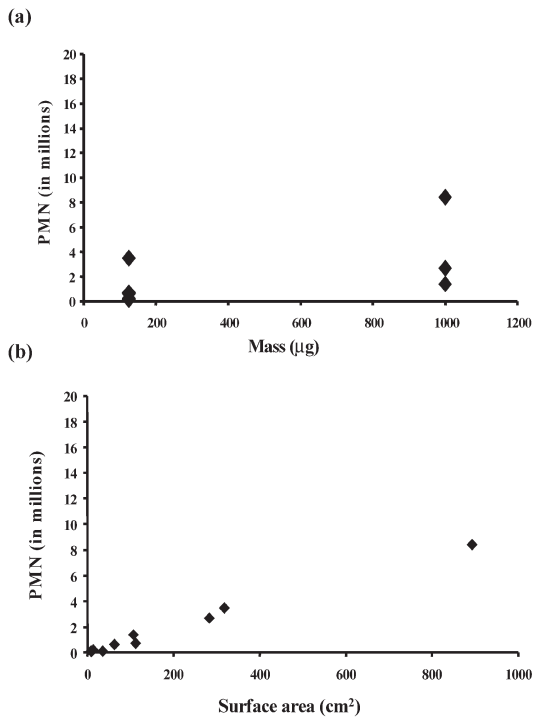

Fig. 1. (a) Relationship between mass and number of neutrophils in BALF 18 h after instillation with low-toxicity dusts. (b) Relationship between surface area and number of neutrophils in BALF 18 h after instillation with low-toxicity dusts.

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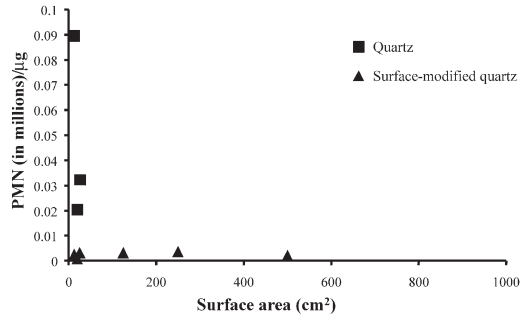

Fig. 2. The effect of surface area on neutrophil number/µg particle instilled, in BALF 18 h after instillation with quartz and surface-modified quartz.

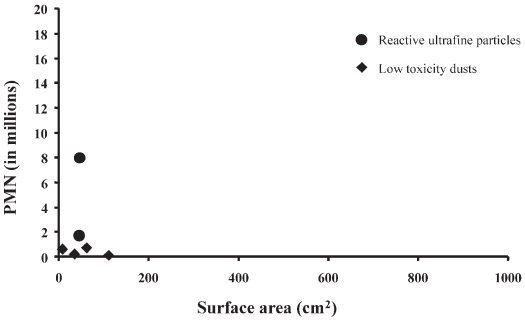

Fig. 3. Relationship between surface area and neutrophil number in BALF 18 after instillation with reactive ultrafine particles.

encountered, showed relatively low toxicity and in fact these samples fall into the category of 'low-toxicity' dusts (Fig. 2).

Reactive ultrafines, such as uf-Co and uf-Ni, induced an inflammatory response that fell midway between the highly toxic quartz and the low-toxicity dusts, suggesting that surface reactivity as well as surface area are important in driving the inflammation observed 18 h after instillation (Fig. 3).

Figure 4a shows the relationship between the various particles instilled and the PMN number per microgram of particles. The specific response of each particle type, expressed as PMN/µg/cm^2 is shown in Fig 4b. This clearly divides the particles into three categories: 'low-toxicity' dusts (including uf-TiO_2, carbon black and the aluminium lactate-treated/workplace quartz), high-reactivity ultrafines (uf-Co and uf-Ni) and quartz.

Figure 5 shows the levels of MIP-2 in BALF after instilling the various particles at various surface area doses.

DISCUSSION

We have demonstrated, using an instillation model, that particle surface area is the dose metric for the acute inflammatory response seen 18–24 h after instillation of a wide range of 'low-toxicity' PSPs in

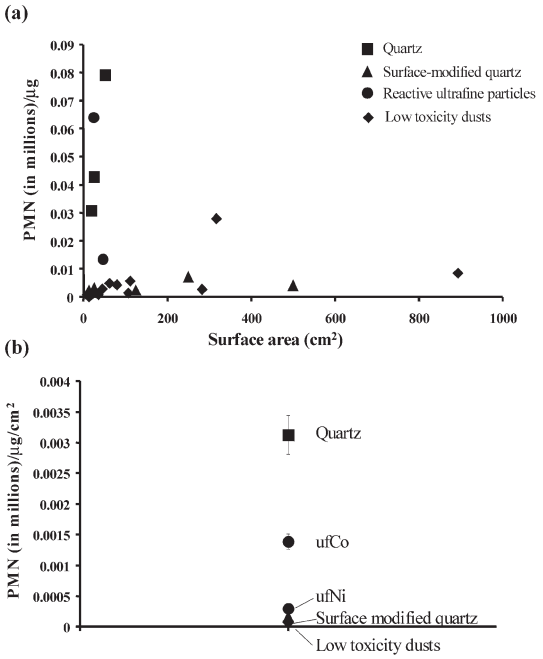

Fig. 4. (a) Relationship between the various particle surface areas and the neutrophil number/µg particle instilled, in BALF 18 h after instillation. (b) The specific response of each particle type expressed as PMN/µg/cm^2.

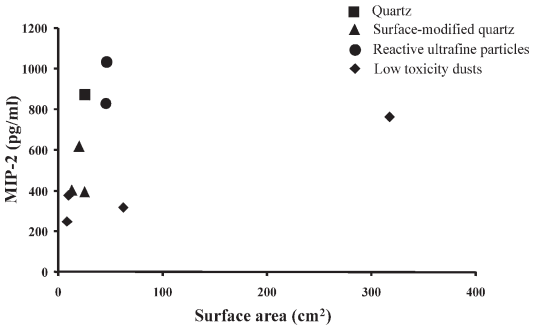

Fig. 5. Relationship between surface area and MIP-2 concentrations in BALF 4 h after instillation with the various particle types.

rats. Quartz is know to have a highly reactive surface (Fubini, 1998; Duffin *et al.*, 2001). Thus, it is expected that a severe inflammatory reaction can result from a comparatively low surface area dose. Moreover, if this surface reactivity is neutralized, then quartz is expected to behave like a 'low-toxicity dust'. Interestingly, workplace samples of quartz exhibited much lower toxicity (per unit surface area dose), indicating a variability in surface reactivity for different quartz samples as reported by Clouter *et al.* (2001). For ultrafine particles, we have shown that the toxicity of uf-TiO_2 and ultrafine carbon black can be attributed to their large surface area. However, metal particles, such as cobalt and nickel in the ultrafine form, had both a high surface area and a

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reactive surface, and this resulted in highly inflammogenic particles that had more ability to cause inflammation than the 'low-toxicity' particles, including the ultrafines. These particles are potentially more inflammogenic than quartz, on a mass dose basis.

MIP-2 measured in BALF, 4 h after instillation, showed a correlation between MIP-2 and PMN levels. This is not surprising, as MIP-2 is a key pro-inflammatory chemokine for recruiting PMN to the site of lung injury (Driscoll, 1994).

As a marker for the potential of particles to cause inflammation, measurement of MIP-2 has the advantage of being measurable across both instillation and inhalation models and may have potential as a valid *in vitro* screen for rapidly classifying particles into the categories described here, namely (i) active quartz; (ii) low-toxicity particles, surface-modified quartz; (iii) ultrafines with surface reactivity.

In conclusion, we have shown, in a simple instillation model, the dosimetric relationship between particle dose and the inflammatory reaction. Our model offers a simple model for ranking inflammogenic potency of PSPs according to their surface area and surface reactivity. We have also demonstrated an alternative assay for inflammation that has the potential for extrapolation between *in vitro* and *in vivo* results.

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