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Evaluation of particle translocation across the alveolo-capillary barrier in isolated perfused rabbit lung model

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Abstract

Particulate air pollution is associated with respiratory and cardiovascular morbidity and mortality. It has been suggested that ultrafine particles are able to translocate from the airways into the bloodstream in vivo. We have investigated this in an isolated perfused and ventilated rabbit lung preparation lacking pulmonary lymphatic flow. Fluorescent polystyrene particles of different diameters (24, 110 or 190 nm) and surface chemistry (carboxylate or amine modified) were injected either intratracheally (i.t.) or intravascularly (i.v.) and, after a period of 2 h, their presence in the perfusion liquid or in the bronchoalveolar lavage (BAL) fluid, was assessed by spectrofluorimetry. Vascular pressures and lung weights were monitored. Following the i.t. administration, no particle translocation was observed from the alveoli into the vascular compartment. Similarly, no particle translocation was found after i.v. administration of particles. However, when microvascular permeability was pharmacologically increased by administering histamine (10^{-4} M) in the vascular compartment, inducing a positive driving force provided by fluid filtration, a fluorescent signal in BAL was recorded ($2.5 \pm 1\%$ of the dose of particles administered), suggesting a translocation of particles through the alveolo-capillary barrier. We conclude that ultrafine polystyrene particles cannot significantly diffuse from lung into the vascular compartment in our model, but they are able to translocate in the opposite direction when the microvascular permeability is increased by histamine. The relevance of these ex vivo findings for the in vivo translocation of inhaled ultrafine particles remains to be established.

Keywords: Air pollution; Polystyrene particles; Isolated perfused lung

1. Introduction

Epidemiological studies have reported close and consistent associations between exposures to particulate matter with a diameter $\leq 10\,\mu m$ (PM_{10}) and

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respiratory and cardiovascular mortality and morbidity (Brunekreef and Holgate, 2002; Samet et al., 2000; Schwartz, 1997).

The toxicity of particles is related to their chemical components, such as endotoxins or transition metals, and their physical properties, i.e. mainly their size and solubility (Oberdörster et al., 1994). In this context, it has been reported that high lung toxicity of particles is related to both their size and their solubility (Toya et al., 2001; Zhang et al., 2003). Significant quantities of water-soluble transition metals may be present in fine ambient PM, and these metals may affect human health (Fubini, 1997). Metal-containing PM have been recovered from the lungs of human subjects (Churg and Brauer, 1997).

Inhaled ultrafine particles (UFPs), i.e. particles with diameter below 0.1 μm, are able to penetrate deeply in the lungs and they have a large specific surface area, which contributes to their marked inflammatory potential (Oberdörster et al., 1994; Oberdörster, 2001).

The mechanisms underlying the cardiovascular effects of particles are not well understood. It has been suggested that inhaled particles may lead to pulmonary inflammation and subsequent release of soluble mediators that may influence blood coagulation parameters (Donaldson et al., 2001). The autonomic nervous system may also be a target for the adverse effects of air pollution (Gold et al., 2000). We (Nemmar et al., 2001, 2002a) and others (Kreyling et al., 2002; Oberdörster et al., 2002; Takenaka et al., 2001) have reported extrapulmonary translocation of UFPs after intratracheal instillation or inhalation, suggesting an alternative and/or a complementary explanation for the cardiovascular effects of particles. However, the pathways and mechanisms for this translocation remain to be established. After their translocation through the alveolar epithelium and reaching the interstitial pulmonary tissues, particles can be cleared by lymphatic flow (Ferin et al., 1992) but they also seem to diffuse directly into the microvascular bed (Kato et al., 2003; Nemmar et al., 2001, 2002a).

The aim of the present study was to investigate the translocation of fluorescent UFPs across the alveolocapillary barrier in an isolated perfused rabbit lung model.

2. Material and methods

The experiments described below were reviewed and approved by the Institutional Review Board of the University of Liège. These results have not been previously reported, but they were obtained from (a proportion of) experiments on the effects of particles on microvascular permeability, which have been previously published (Hamoir et al., 2003).

2.1. Particle characterization

Particles were carboxylate-modified (approximately $pK_a = 5$) or amine-modified (approximately $pK_a = 10$) polystyrene particles loaded with red dye, which maximally emits a fluorescent signal at 605 ± 5 nm when excited at 580 nm (FluoSpheres® fluorescent microspheres, Molecular Probes, Eugene, Oregon, USA). They were supplied as aqueous suspensions containing 2% solids. The main physical characteristics of these particles were provided by the manufacturer. The geometric diameters were determined by electron microscopy. We used particles with diameters of 24, 110, and 190 nm and individual surface areas of 1.81×10^{-11} cm^2, 3.80×10^{-10} cm^2, and 1.13×10^{-9} cm^2, respectively. The number of particles administered to each rabbit varied from 1.06×10^{12} to 5.2×10^{14}. The total surface area was calculated as the product of the particle number and individual surface area. Particles were suspended in saline (0.5 ml). To minimize particle aggregation, the suspensions were vortexed and sonicated for 10 min, immediately prior to their administration at zero-time (T_0) in the trachea or in the perfusion circuit.

2.2. Preparation of isolated, ventilated and perfused rabbit lungs

New Zealand rabbits aged from 10 to 14 weeks (2.63 ± 0.43 kg) were used. The animals were deeply anesthetized and then the heart–lung block was rapidly removed from the chest (Delaunois et al., 1994b; Hamoir et al., 2003). Both ventricles were opened and glass cannulae were secured into the pulmonary artery and left atrium via the corresponding ventricle. The heart–lung block was weighed and connected to a recirculating perfusion circuit and perfused with a constant flow (15 ml/kg/min). This flow