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Serum protein oxidation by diesel exhaust particles: Effects on oxidative stress and inflammatory response *in vitro*

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ABSTRACT

Considerable evidence shows a key role for protein modification in the adverse effects of chemicals; however, the interaction of diesel exhaust particles (DEP) with proteins and the resulting biological activity remains unclear. DEP and carbon black (CB) suspensions with and without bovine serum albumin (BSA) were used to elucidate the biological effects of air pollutants. The DEP and CB samples were then divided into suspensions and supernatants. Two important goals of the interaction of DEP with BSA were as follows: (1) understanding BSA modification by particles and (2) investigating the effects of particles bound with BSA and the corresponding supernatants on cellular oxidative stress and inflammation. We observed significant free amino groups production was caused by DEP. Using liquid chromatography–mass spectrometry (LC–MS), we observed that BSA was significantly oxidised by DEP in the supernatants and that the peptides ETYGDMADCCEK, MPCTEDYLSLILNR and TVMENFVAFVDK, derived BSA-DEP conjugates, were also oxidised. In A549 cells, DEP-BSA suspensions and the corresponding supernatants reduced 8-hydroxy-2′-deoxyguanosine (8-OHdG) production and increased interleukin-6 (IL-6) levels when compared to DEP solutions without BSA. Our findings suggest that oxidatively modified forms of BSA caused by DEP could lead to oxidative stress and the activation of inflammation.

1. Introduction

Epidemiological and clinical studies show that respiratory allergies, deaths due to cardiovascular and pulmonary diseases and lung cancer development are all associated with chronic exposure to particulate air pollution, such as diesel exhaust particles (DEP) [1–4]. The human health effects of DEP have been studied over several years; however, an investigation of the chemical components responsible for the macromolecular effects is lacking, and a detailed understanding of the underlying mechanisms remain unclear [5]. DEP is a complex mixture of hundreds of components, consisting of a central core of elemental carbon and adsorbed organic compounds such as polycyclic aromatic hydrocarbons (PAHs) and nitro-PAHs as well as small amounts of sulfate, nitrate and

Abbreviations: TNBS, 2,4,6-trinitrobenzene sulphonic acid; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; CB, carbon black; DEP, diesel exhaust particle; DLS, dynamic light scattering; ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6; NF-κB, nuclear factor-kappa beta; PBS, phosphate buffered saline; PAHs, polycyclic aromatic hydrocarbons; RSLC, rapid separation liquid chromatography; ROS, reactive oxygen species; TEM, transmission electron microscopy; TNF-α, tumour necrosis factor-alpha.

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metals (including trace elements) [6,7]. DEP is used to evaluate the potential effects on vasoactive function and the inflammatory response because the nano-scaled fractions are more capable of being inhaled into the deep lung environments and entering the circulation [8–10]. Traffic-related DEP have been identified as a common air pollutant associated with reactive oxygen species (ROS) formation. Certain chemicals in DEP, such as organic carbon, PAHs and metals, are associated with particle bioreactivity, and organic and metal compounds in particles account for oxidative and inflammatory effects [11].

Protein interactions with particles are associated with some degree of protein unfolding, which affects the biological function of the protein [12]. An increasing number of studies have investigated the interaction of carbon-based nanoparticles with blood carrier protein molecules and the effects on the biological activity of the proteins [13–15]. Particle surface chemistry has shown a considerable influence on the amount of protein binding to distinct ligands, leading to alterations in oxidative stress and inflammation [16]. Serum albumin is a globular protein synthesised by the liver in mammals and is the most abundant protein in serum (approximately 60% of the total globular protein in blood plasma) [17]. Albumin maintains the colloidal osmotic blood pressure and is essential for the transportation of several endogenous and exogenous compounds, including proteins and fatty acids, to specific targets [18]. Bovine serum albumin (BSA), a globular, protein consisting of 583 amino acid residues, is commonly used to investigate protein-particle interactions. In terms of its secondary structure, 67% of the protein is composed of alpha helices, and the protein contains 17 disulfide bridges, which increase its stability [18]. Previous studies have investigated the interactions of nanoparticles with serum protein; however, the chemical effects of DEP on serum protein remain unclear.

Increasing evidence suggests that the oxidative damage of proteins is an important mechanism in aging and multiple diseases [19]. The important effects of protein oxidation on cellular homeostasis derive from the fact that proteins play critical roles in regulating cell structure, cell signalling and various enzymatic processes of the cell. There are many different modes of protein oxidation such as amino acid oxidation and metal-catalysed oxidation. However, there is a paucity of literature investigating the interaction of DEP with proteins and the resulting oxidative stress and inflammatory responses in biological systems. To pursue this investigation, we examined BSA modification (in supernatants of suspensions and in BSA-particle conjugates) following interactions with DEP, as a model chemical-rich carbonaceous particle, and with carbon black (CB), as a model chemical-less carbonaceous core. We also compared the effects of exposure of human alveolar epithelial Type II (A549) cells to DEP and CB suspensions and the corresponding supernatants, either with or without BSA. These experiments allowed us to investigate the effects of DEP solutions with and without BSA on oxidative stress [measured by 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels] and on the pro-inflammatory cytokine levels [measured by interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)] in cellular systems.

2. Materials and methods

2.1. Cell culture

A549 cells were obtained from the American Type Culture Collection and cultured in RPMI containing 10% fetal bovine serum, penicillin and streptomycin. Cells were incubated in air at 37 °C, 95% humidity and 5% CO_2. All chemicals used in this study were reagent grade and were obtained from Sigma Aldrich (UK), unless stated otherwise.

2.2. Sample preparation and characterisation

In this study, we investigated protein modification as well as oxidative and inflammatory responses after DEP and CB exposure. The DEP were the Standard Reference Material 2975 obtained from the National Institute of Standards and Technology (NIST, Gaithersburg, USA). DEP are chemical-rich particles, and their chemical characteristics have been described previously [20]. DEP were generated by a heavy-duty forklift diesel engine and collected using a filtering system designed for diesel forklifts [5]. Near-pure, manufactured, CB, with an average diameter of 65 nm (Monarch 120; Cabot Corporation, UK), was selected as a control particle. CB is an industrial carbon produced by the thermal decomposition of hydrocarbons. The chemical characteristics of CB have been described previously [21,22]. A solution of 5% BSA was prepared with sterile phosphate-buffered saline (PBS) and then filter-sterilised. DEP and CB were suspended in 5 ml of PBS with (+) or without 5% BSA (−) (PBS only) after 15 min of sonication. Aliquots of the DEP and CB suspensions were then combined with the 5% BSA solution or the BSA-free PBS (final volume of 10 ml) to yield final particle concentrations of 0, 50, 150 and 1000 μg/ml. The DEP and CB samples (±5% BSA) were vortexed and incubated at 37 °C for 2 h under constant shaking at 500 rpm to ensure thorough mixing. Five milliliters of the samples (0, 50, 150 and 1000 μg/ml DEP and CB) were then separated into the following two fractions by centrifugation (3500 rpm) and filtration: supernatant and pelleted particles. The pelleted particles were washed thoroughly by repeated resuspension in deionised water and centrifugation [23,24]. The DEP and CB supernatants and the corresponding pelleted particles were used to investigate protein modification, whereas the DEP and CB suspensions and their corresponding supernatants were used for cell-based oxidative stress and inflammation analyses. Particle-free solution ±5% BSA in PBS were used as negative controls and were treated in the same manner as the test samples. Dynamic light scattering (DLS; Malvern Zetasizer Nano-ZS, UK) was used to determine the hydrodynamic diameters of 50 and 150 μg/ml DEP and CB (±5% BSA) suspensions. Transmission electron microscopy (TEM; Philips CM12) was used to investigate the morphology of DEP and CB suspended in 5% BSA. Samples were loaded onto aurum TEM grids (Agar, UK) and investigated at an accelerating voltage of 80 kV with a spot size 1. The samples were kept at 4 °C and used immediately upon removal from storage.

2.3. 2,4,6-Trinitrobenzene sulphonic acid assay

A 2,4,6-trinitrobenzene sulphonic acid (TNBS) assay was used to determine the free amino groups produced in proteins by free radicals, as described previously [25]. Briefly, the concentrations of amino groups in the BSA before and after incubation with CB and DEP was directly quantified with a PowerWave microplate reader (BioTek, USA) monitoring the absorbance at 335 nm. CB and DEP suspensions at 0, 50, 150 and 1000 μg/ml were mixed with 100 μg/ml BSA in 0.1 M $NaHCO_3$ (pH 8.5). Then, 0.5 ml of a 0.01% (w/v) TNBS solution was added to the particle-BSA samples and incubated at 37 °C for 2 h. To relate the absorbance of the samples to the amino acid standard solution (2.5 μmol/ml L-alanine, 2.5 μmol/ml ammonium chloride, 2.5 μmol/ml L-arginine, 2.5 μmol/ml L-aspartic acid, 1.5 μmol/ml L-cystine, 2.5 μmol/ml glutamic acid, 2.5 μmol/ml glycine, 2.5 μmol/ml L-histidine, 2.5 μmol/ml L-isoleucine, 2.5 μmol/ml L-leucine, 2.5 μmol/ml L-lysine, 2.5 μmol/ml L-methionine, 2.5 μmol/ml L-phenylalanine, 2.5 μmol/ml L-proline, 2.5 μmol/ml L-serine, 2.5 μmol/ml L-threonine, 2.5 μmol/ml L-tyrosine and 2.5 μmol/ml L-valine), a calibration curve of amino acid levels ranging from 0.008 to 2.5 μmol/ml was generated. All TNBS assay data are presented as the free