

Intratracheal instillation of air pollution nanoparticles into rats is associated with system inflammation and damage to the vascular endothelium

R Adams¹, H Al-Bulushi¹, A Al-Mosawi¹, O Alabi¹, J Hawke¹, P Foley¹, S Hicks¹, S Potter¹, K Bérubé², T Jones³ and S-A Evans¹

¹ Cardiff School of Health Sciences, UWIC, Llandaff Campus, Western Avenue, CF5 2YB, UK

² School of Biosciences, Cardiff University, Museum Avenue, CF10 3US, UK

³ School of Earth and Ocean Sciences, Cardiff University, Park Place, CF10 3YE, UK

Summary

Exposure to air pollution is associated with increased cardiovascular risk but the mechanisms are poorly understood. The aim of this investigation was to assess the effect, in rats, of intratracheal instillation of particulate air pollution on leucocyte activation and damage to the vascular endothelium. Rats were exposed to particulate air pollution by intratracheal instillation of PM₁₀. Blood was collected 3 days and 6 weeks post instillation. Plasma neutrophil elastase, von Willebrand factor (vWF) and ICAM-1 were determined by ELISA. Plasma neutrophil elastase increased from baseline. There was a statistically significant increase in both von Willebrand factor ($p=0.006$, ANOVA) and sICAM-1 ($p=0.009$, ANOVA). We conclude that lung exposure to particulate air pollution causes systemic inflammation, activation of blood leucocytes and damage to the vascular endothelium. Inflammatory changes persist long after the initial instillation and exposure to even short term episodes of high levels of particulate air pollution could increase cardiovascular risk.

Introduction

In epidemiological studies in humans, increased exposure to airborne particulate matter with an aerodynamic diameter of less than 10 microns (PM₁₀) has been implicated in increasing asthma symptoms, cardiovascular malfunction, hospital admissions and morbidity and mortality rates (Peters *et al.*, 2001; Pope *et al.*, 2004; Miller *et al.*, 2007). The mechanisms by which exposure increases risk is poorly understood. Our research group reported an increase in plasma viscosity in rats which correlates with the degree of inflammatory changes in the lung after instillation of nanoparticles (Evans *et al.*, 2006). Inflammatory changes to leucocytes and damage to the vascular endothelium are associated with atherosclerosis which is an inflammatory disease of the arteries. Damage to the vascular endothelium is associated with an increase in blood vWF and sICAM-1 concentration and increases in these markers are independent risk factors for cardiovascular disease (Malik *et al.*, 2001; Varughese *et al.*, 2007). Inflammatory changes associated with damage to the vascular endothelium could, therefore, be the cause of the association between particle exposure and cardiovascular disease. The aim of the study described here was to test the hypothesis that exposure to airborne particulate pollution causes inflammatory activation of blood leucocytes which leads to damage to the vascular endothelium. This endothelial damage may be the mechanism by which air pollution increases cardiovascular risk.

Methods

Animals and experimental protocol

All animals were treated humanely under guidelines provided by Cardiff University and the local ethical committee. Study approval was obtained from the local ethical committee and by a Home Office animal project licence. The animal and experimental protocols used were as previously reported (Evans *et al.*, 2006). Briefly, male Sprague Dawley rats (200 g, $n=10$ per group) were purchased from Charles River (UK) and were acclimatised within the animal holding facility for one week prior to instillation. The animals were kept on wire-bottom cages with pelleted food and tap water *ad libitum*. The animals were lightly anaesthetised with Halothane before receiving intratracheal instillations. Controls were instilled with saline only whilst test animals were instilled with saline containing PM₁₀. Animals were harvested 3 days and 6 weeks after dust instillation.

Collection and preparation of PM₁₀

Airborne dust was collected within and proximal to Park Slip West opencast coal mine using mobile Negretti PM₁₀ heads and polycarbonate Millipore collection filters (Greenwell *et al.*, 2002) in a co-operation with the operators, Celtic Energy. For instillation, particles were suspended in 0.15M saline and 'wetted' (suspended in solution) by sonication for 15 minutes.

Blood Samples

Blood samples were collected, by cardiac puncture, into sufficient tri-potassium EDTA to give a final concentration of 1.5 mg/ml. Within four hours of collection samples were centrifuged at 1500g for 10 minutes (Sanyo Harrier 18/80) and plasma was removed and stored at -80°C (Sanyo VIP series) until analysis.

Measurement of plasma concentration of soluble markers of inflammation.

Plasma α -1-trypsin neutrophil elastase inhibitor complex was measured by ELISA using sheep anti-human neutrophil elastase and peroxidase-conjugated sheep anti-human α -1-antitrypsin (The Binding Site, Birmingham, UK) and PMN leucocyte elastase calibrator (Merck Ltd, UK). vWf was measured by ELISA using

polyclonal anti-von Willebrand Factor primary antibody (Dako,UK) and HRP conjugated polyclonal anti-von willebrand Factor secondary antibody (Dako,UK) and vWF standard (NIBSC,UK). Rat sICAM-1 was measured by commercially available ELISA (R&Dsystems, UK).

Results

Instillation of particulate air pollution into the lung of rats resulted in changes in markers of inflammation in the systemic blood circulation. There was a significant increase in neutrophil elastase in the plasma post instillation. This increase in neutrophil elastase continued for at least 6 weeks post instillation (Figure 1). There was a statistically significant increase in both markers of damage to the vascular endothelium. von Willebrand Factor increased at 3 days post instillation. At 6 weeks post instillation the concentration of vWF had started to reduce but was still considerably higher than that recorded at baseline (Figure 2). The plasma concentration of the soluble adhesion molecule sICAM-1 increased significantly 3 days post instillation and continued to rise and at 6weeks post instillation was substantially higher than baseline (Figure 3).

Figure 1 Increase (p=0.06) in Neutrophil elastase level after 3days of dust instillation with increase even after 6 weeks incubation

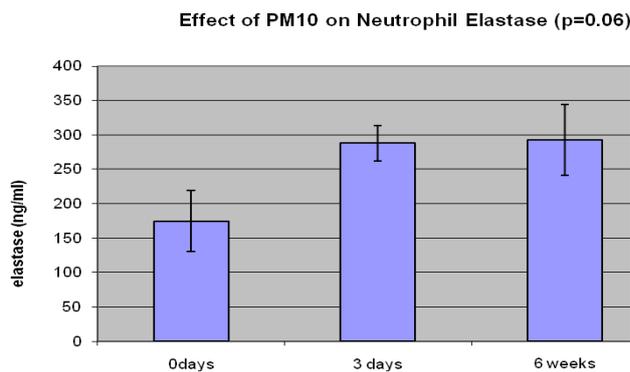


Figure 2 Significance increase (p=0.006) in vWF level after 3days of dust instillation and it remained increased even after 6 weeks incubation.

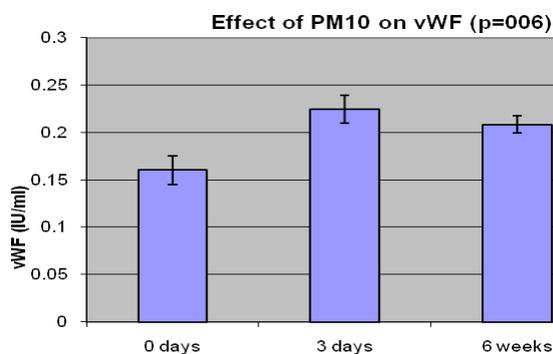
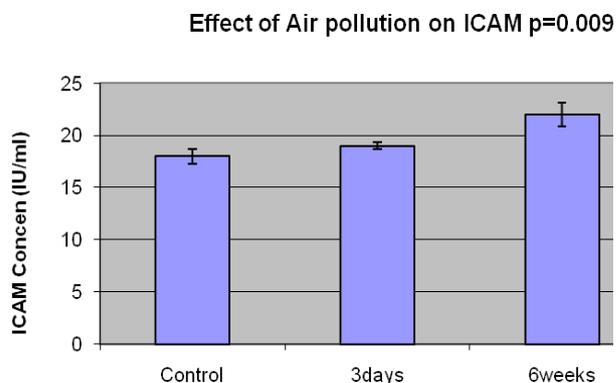


Figure 3 Significance increase (p=0.009) in ICAM level after 3days of dust instillation and it continued increasing even after 6 weeks incubation.



Discussion

The increase in neutrophil elastase observed here is an indication that air pollution particles in the lung of rats results in activation of leucocytes in systemic blood. Activation of neutrophils results in degranulation of leucocytes and release of the proteolytic enzyme elastase into the blood. The release of this potent proteolytic enzyme can result in damage to surrounding tissues. Here we report that this increase in elastase is associated with damage to the vascular endothelium. Von Willebrand factor and sICAM-1 levels are indicators of endothelial damage and increased levels are associated with a significant increase the risk of cardiovascular disease (Blann *et al.*, 1996; Blann *et al.*, 2001).

The increased level of soluble ICAM-1 reported here not only reflect damage to the endothelium but indicates that there is increased expression of ICAM-1 on the surface of the endothelium. This increase ICAM-1 on the endothelium will allow the activated neutrophils to adhere to the endothelium. Adhesion of neutrophils will increase the damage they can cause to the endothelium as attached activated neutrophils will produce free radicals which will cause further damage. Once adhered these neutrophils can extravasate and thus migrate into the surrounding tissues. These neutrophils may then take with them any particles which may have phagocytosed. This could be a mechanism of entry of particles into tissues distant from the site of exposure. This movement of particles around the body has important implications for the potential toxicity of particles.

Interestingly the inflammatory changes reported here persist for weeks after the initial instillation of air pollution particles. Exposure to even short term episodes of high levels of particulate air pollution could cause systemic inflammatory changes which increase the risk of developing cardiovascular disease. Exposure to even short episodes of poor air quality could not only precipitate myocardial infarctions but could also be associated with long term increases in cardiovascular risk.

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