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The Mechanism of Lung Injury Caused by PM_{10}

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1 Adverse Health Effects of PM_{10}

This article addresses the mechanisms of the adverse health effects of PM_{10}, *i.e.* the mass of particulate air pollution collected by a convention that has 50% efficiency for particles with an aerodynamic diameter of 10 μm. Epidemiological studies, not discussed here but reviewed elsewhere (*e.g.* Pope *et al.*,[1]), have demonstrated a clear relationship between the levels of PM_{10} and exacerbations of asthma and COPD (chronic obstructive pulmonary disease), as well as deaths from cardiovascular causes, *i.e.* heart attacks and strokes.

2 Target Tissues for the Adverse Health Effects of PM_{10}

The range of mortality and morbidity described above indicates that there is a wide variety of tissues that are affected by PM_{10} in ways that lead to disease and these are described in this section.

Airways

Both asthma and COPD are inflammatory diseases of the airways. The defences of the pulmonary airways comprise the mucociliary escalator, where mucus-secreting cells release mucus which traps deposited particles. Mucus with its trapped particles is then propelled upwards by ciliated cells to be either spat out or swallowed. In addition, the epithelial cells themselves are capable of responding with the release of inflammatory mediators to particle stimulation (*e.g.* Driscoll *et al.*[2]). Macrophages are also present in the airway walls and on the surfaces of the airways and these can phagocytose particles and release mediators. Within the airway walls are smooth muscle cells and mesenchymal cells which could also be targets for particles.

[1] C. A. Pope, D. V. Bates and M. E. Raizenne, *Environ. Health Perspect.*, 1995, **103**, 472.
[2] K. E. Driscoll, J. M. Carter, D. G. Hassenbein and B. Howard, *Environ. Health Perspect.*, 1997, **105**, 1159.

Terminal Airways and Proximal Alveoli

Particles deposit in large numbers beyond the ciliated airways in the terminal airways and proximal alveoli[3] where the net flow of air is zero and where, for very small particles, deposition efficiency increases because of the high efficiency of deposition by diffusion.[4] In this region it is the macrophages that play the most important role in removing particles. Macrophages phagocytose particles and eventually they migrate to the start of the mucociliary escalator and leave the lung with their cargo of particles, bound for the gut. Although some adverse effects associated with PM_{10} are clearly focused on the larger airways, effects beyond the ciliated airways could be important in the cardiovascular effects and in holding up neutrophils in the pulmonary vasculature which may be significant in causing lung injury (see later).

The Pulmonary Interstitium and Lymph Nodes

If particles cross the epithelium and enter the lung interstitium they are no longer likely to be cleared by the normal processes and will either remain in the subepithelial regions close to key responsive cell populations, such as interstitial macrophages, fibroblasts and endothelial cells, or be taken to the draining lymph nodes. Interstitial inflammation is likely to be more potentially harmful than inflammation in the alveolar spaces. The effects of dust in the lymph node are not known but adjuvant effects might be anticipated.

The Liver

Cardiovascular deaths are an important aspect of the adverse health effects of PM_{10}.[1] Classically, these are caused by the production of clots in the coronary vessels in the case of heart attacks and in the brain microvasculature in the case of stroke. Whilst intuitively an effect of inhaled particles on the lungs might be understandable, a link between the deposition of particles in the airways and effects that increase the likelihood of clots is more problematic. However, we have hypothesized that the inflammation arising in the lungs of persons inhaling PM_{10} could impact on the coagulation systems *via* the local production of pro-coagulant factors in the lungs or effects of mediators from the lungs on the liver, acting to increase the level of pro-coagulant factors. This is demonstrated diagrammatically in Figure 1. Research is underway to test this hypothesis experimentally, but there is epidemiological evidence to show that there is increased blood viscosity in a population during high pollution episodes;[5] fibrinogen is a major contributor to plasma viscosity. In this study there was an increase in the number of individuals in the highest quintile of blood viscosity, during a period of high air pollution.

[3] A. R. Brody, D. B. Warheit, L. Y. Chang, M. W. Roe, G. George and L. H. Hill, *Ann. N. Y. Acad. Sci.*, 1984, **428**, 108.

[4] P. J. Anderson, D. J. Wilson and A. Hirsch, *Chest*, 1990, **97**, 1115.

[5] A. Peters, A. Doring, H. E. Wichmann and W. Koenig, *Lancet*, 1997, **349**, 1582.

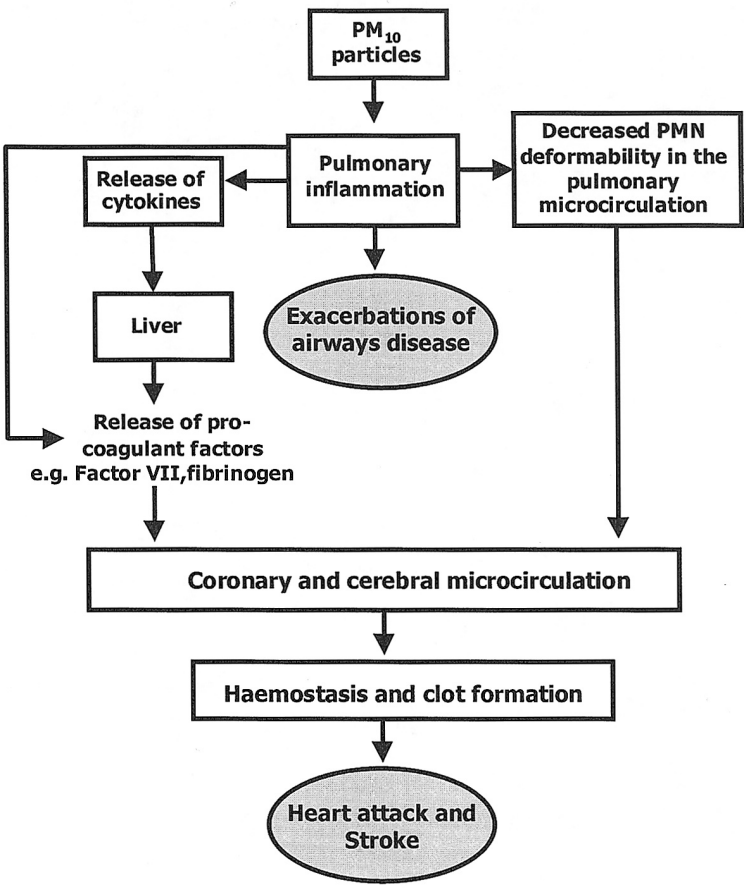

Figure 1 Diagram of the hypothetical scheme of events leading from deposition of PM_{10} to morbidity and mortality outcomes (shaded ellipses)

PMN in the Pulmonary Microvasculature

PMN (polymorphonuclear neutrophil leukocytes) are important mediators of lung injury in chronic inflammation, as a result of their ability to release injurious substances such as proteases and reactive oxygen species. These toxic products of the PMN could be released whilst the PMN are in the vascular space as well as when they extravasate into tissue. Neutrophils are known to be held up (or sequester) in the pulmonary microcirculation under normal circumstances owing to the fact that they have to deform because of their larger size in order to negotiate the smaller pulmonary capillary segments.[6] PMN deformability is therefore a critical initiating factor, prior to increased PMN-endothelial adhesion in PMN sequestration in the pulmonary microvasculture.[7] Any factors which change cell deformability will change PMN sequestration in the pulmonary circulation.[7] Airway inflammation, such as occurs in exacerbations of COPD, causes decreased PMN deformability, and hence increased PMN sequestration

[6] C. Selby and W. MacNee, *Exp. Lung Res.*, 1993, **19**, 407.
[7] C. Selby, E. Drost, P. K. Wraith and W. MacNee, *J. Appl. Physiol.*, 1991, **71**, 1996.

in the pulmonary microvasculature,[8] associated with evidence of oxidant stress.[9] These events are mediated by oxidative stress from acute smoking,[10] causing decreased neutrophil deformability.[11,12] As discussed below, oxidative stress from PM_{10} could also cause decreased PMN deformability, which could have important consequences for haemostasis and coagulation in the coronary and cerebral microvasculature.

3 Toxic Potency of PM_{10}

The levels of PM_{10} associated with adverse effects are very low and indeed some studies have shown no increase in PM_{10} levels that does not have an associated adverse health effect (*e.g.* Pope *et al.*,[1]), *i.e.* no threshold. The relative toxicity of PM_{10} is illuminated by considering the regulation of low toxicity or 'nuisance' particles in workplaces in the UK. The average PM_{10} level in UK urban areas is below 50 $\mu g\,m^{-3}$ and levels are even less in country areas. By contrast, nuisance dusts are regulated in UK workplaces to 4 $mg\,m^{-3}$ of respirable dust. Since PM_{10} appears to have adverse health effects without a threshold in some studies even at very low levels as measured by mass, this suggests that PM_{10} is a highly toxic material. However, the components of PM_{10} taken on their own are not particularly toxic at the levels present in ambient air, containing typically large proportions of carbon, salts and metals, as well as organic components.[13] The potential role of transition metals and very small particles in mediating the effects of PM_{10} are discussed below at length.

4 Ultrafine Particles

Toxicity of Ultrafine Particles

Research on ultrafine particles, *i.e.* those in the size range < 100 nm in diameter, provides a possible explanation of the toxicity of PM_{10}. Ultrafine particles are highly toxic to the lung, even when the particles are formed from materials that are not toxic as larger but still respirable particles, *e.g.* titanium dioxide and carbon. For example, the study of Ferin *et al.*[14] reported the bronchoalveolar lavage inflammatory profile in rats exposed to the same airborne mass concentration of TiO_2 as fine particles, approximately 250 nm in diameter (fine particles are hereafter defined as respirable particles that are larger than ultrafine particles), or ultrafine particle, 20 nm in diameter. Although rats were exposed to aerosols of 23 $mg\,m^{-3}$ of both ultrafine TiO_2 and fine TiO_2, there was a marked

[8] C. Selby, E. Drost, E. Lannan, P. K. Wraith and W. MacNee, *Am. Rev. Respir. Dis.*, 1991, **143**, 1359.
[9] I. Rahman, D. Morrison, K. Donaldson and W. MacNee, *Am. J. Respir. Crit. Care Med.*, 1996, **154**, 1055.
[10] W. MacNee, B. Wiggs, A. S. Belzberg and J. C. Hogg, *New Eng. J. Med.*, 1989, **321**, 924.
[11] E. M. Drost, C. Selby, S. Lannan, G. D. O. Lowe and W. MacNee, *Am. J. Respir. Cell Mol. Biol.*, 1992, **6**, 287.
[12] E. Dorst, C. Selby, M. M. E. Bridgeman and W. MacNee, *Am. Rev. Respir. Dis.*, 1993, **148**, 1277.
[13] P. S. Gilmour, D. M. Brown, T. G. Lindsay, P. H. Beswick, W. MacNee and K. Donaldson, *Occup. Environ. Med.*, 1996, **53**, 817.
[14] J. Ferin, G. Oberdorster and D. Penney, *Am. J. Respir. Cell Mol. Biol.*, 1992, **6**, 535.

inflammatory response seen with the ultrafine TiO_2 but little effect of the fine TiO_2. Thus the same material as ultrafine particles and as fine particles showed a dramatic difference in pathogenicity. We have reported a similar finding with fine (260 nm diameter) and ultrafine (14 nm diameter) carbon particles.[15] This suggests that ultrafine particles have a toxicity that is the result of their small size, as opposed to their chemical composition. The potential mechanisms of the toxicity of ultrafine particles has recently been reviewed.[16]

Deposition of Ultrafine Particles

The deposition fraction is high for ultrafine particles, approaching 50% for 20 nm particles, and interestingly the deposition efficiency is greater in a susceptible population (COPDs) than in normals.[4] This could be explained by the slower breaths of COPD patients that allow a longer residence time for the particles which would favour deposition that depends largely on Brownian motion, as is the case for these very small particles.[4]

Evidence that PM_{10} Contains Ultrafine Particles

There is ample evidence that PM_{10} contains an ultrafine component,[17] and indeed, there is one report that decrements in the evening peak flow in a group of asthmatics were best associated with the ultrafine component of the airborne particles during pollution episodes.[18] Diesel exhaust is composed of singlet particles of around 50 nm diameter[19] and several studies have described a substantial component, in number terms of particles in the ultrafine range, although these represent a relatively small fraction of the total mass.[18]

Classical Particle Overload

The rat has been shown to be a high responder to particles, and indeed, dusts considered to be 'non-toxic' in humans (*e.g.* carbon, diesel particles, TiO_2) can cause severe lung injury, culminating in fibrosis, epithelial hyperplasia, metaplasia and cancer in rats, if exposure is to high airborne concentrations such that a high lung dose is attained.[20] This phenomenon of *overload* lung injury appears to be confined to rats and is not seen in other rodents even at similar lung doses (see papers in Mauderly and McCunney).[21] The phenomenon of overload is associated with slowed clearance from the deep lung (macrophage-mediated clearance) and subsequent rapid accumulation of dose with ongoing exposure, culminating in the effects described above. The original hypothesis for the mechanism of overload was focused on the volume of particles within the lung

[15] X. Y. Li, P. S. Gilmour, K. Donaldson and W. MacNee, *Thorax*, 1996, **51**, 1216.
[16] K. Donaldson, X. Y. Li and W. MacNee, *Aerosol Sci.*, 1998, in press.
[17] G. Oberdorster, R. Gelein, J. Ferin and B. Weiss, *Inhalation Toxicol.*, 1995, **71**, 111.
[18] A. Peters, H. E. Wichmann, T. Tuch, J. Heinrich and J. Heyder, *Respir. Crit. Care Med.*, 1997, **155**, 1376.
[19] R. L. Maynard and R. E. Waller, *Thorax*, 1996, **51**, 1174.
[20] J. L. Mauderly, *Inhalation Toxicol.*, 1996, **8** (suppl.), 1.
[21] J. L. Mauderly and R. J. McCunney. Proceedings of a conference held at the Massachusetts Institute of Technology, March 1995, *Inhalation Toxicol.*, 1996, **8** (suppl.).

and specifically the load volume of particles inside macrophages.[22] This hypothesis suggested that when the macrophages had phagocytosed a volume of particle equivalent to 6% of their internal volume, they began to show impaired ability to move and carry their particle burden to the start of the mucociliary escalator for removal from the lungs. Morrow[22] also calculated that by the time the average volume of particles inside macrophages reaches 60% of the total macrophage volume, their ability to move, and hence clearance, is completely inhibited; this has been confirmed.[23] Recently, however, new data have allowed a revision of this hypothesis, concluding that overload is best correlated to the metric of *surface area* of particles in the rat lung, not mass, volume or number of particles.[24,25] A role for surface area appears intuitively likely for *toxic* particles since the interaction between particles and biological systems will occur with the surface, not the internal mass, of the particle. However, it is not immediately apparent why *non-toxic* particles might mediate their effects *via* their surface. However, leaching of soluble components, including metals, will be greater from a large surface area and the potential role that this might play is described below.

Ultrafine Particles and Overload

As the particle diameter reduces for a constant mass of monodispersed (single diameter) particles, then the surface area increases dramatically.[17] It would appear, for this reason, that ultrafine particles may be more likely to cause overload at any given mass burden in the lungs because of their large surface area per unit mass. Amongst the most active of the 'low toxicity' dusts in causing lung overload tumours are the ultrafine particles,[25] presumably because they represent the biggest surface area per unit mass. Macrophages appear to be more adversely affected by loading of ultrafine TiO_2 than fine TiO_2. This is shown by the fact that the retention time of a radioactive marker particle in the lungs of rats exposed to ultrafine TiO_2 following inhalation exposure was inceased about eightfold compared to controls; however, lung burden data indicated that the macrophages were loaded with particles to a calculated volume of only 2.6%.[23] By contrast, the calculated macrophages load volume was 9% for fine TiO_2 and this caused only a doubling of the retention time of the test particle, in keeping with Morrow's prediction on the volumetric index of dose. This supports the contention that volume is not the dose metric that best predicts impairment of macrophage function caused by ultrafine particles; particle number or particle surface area may be the most important index for ultrafine particles. Macrophage functions associated with clearance are substantially impaired when the cells contain small load volumes of ultrafine particles, although this could be a large surface area.

The term overload should not be applied to the effect of ultrafine TiO_2 in inhibiting clearance, since it occurs at low lung burden. In relation to human risk assessment it should be noted that the phenomenon of overload occurs in rats

[22] P. E. Morrow, *Fundam. Appl. Toxicol.*, 1988, **10**, 369.
[23] G. Oberdorster, J. Ferin and B. E. Lehnert, *Environ. Health Perspect.*, 1994, **102** (suppl. 5), 173.
[24] G. Oberdorster, *Inhalation Toxicol.*, 1996, **8** (suppl.), 73.
[25] K. E. Driscoll, *Inhalation Toxicol.*, 1996, **8** (suppl.), 139.

and it is not clear whether it occurs in humans. In rats it occurs only at very high airborne mass concentrations, whereas PM_{10} toxicity occurs in humans at remarkably low airborne mass concentrations. Classical overload is not, therefore, the mechanism of lung injury caused by PM_{10}. Whereas the most important factors contributing to slowed clearance in classical overload is the high macrophage burdens of particles, this is clearly not the case with ultrafines and toxicity to the macrophages may be more important. Ultrafine particles can be highly toxic to the lungs, as shown by high levels of LDH in the lungs of exposed rats,[15,26] and toxic particles may cause slowed clearance by a mechanism involving frank toxicity to macrophages.

Particle Numbers

Macrophages attempting to phagocytose a large number of ultrafine particles could be stimulated, by the high particle load, to release inflammatory mediators. In addition, the large numbers of particles may exceed the ability of the macrophages to phagocytose them, resulting in sustained stimulation of epithelial cells. This could cause the release of chemokines such as IL-8/MIP-1α that would contribute to inflammation. Increased production of these chemokines has been demonstrated in rats inhaling ultrafine carbon black[27] and also ultrafine particles of perfluoropolymer.[28]

Transfer of Particles to the Interstitium

Anything that interferes with the normal process of phagocytosis and macrophage migration to the mucociliary escalator can lead to the adverse outcome of *interstitialization*.[22] Interstitialization is an adverse outcome because interstitial particles cannot now be cleared *via* the normal pathways and must either remain in the interstitium, where they can chronically stimulate interstitial cells, or transfer to the lymph nodes. Interstitialization of particles was a prominent correlate of the onset of inflammation for ultrafine TiO_2 in the study of Ferin *et al.*,[14] and interstitialization of particles was found to arise concomitantly with overload inflammation.[29] Interstitialization is likely to occur when there is failed clearance, which could result from (a) particle-mediated macrophage toxicity or impairment of macrophage motility or (b) overload. Both of these events would allow increased interaction between particles and the epithelium that would favour interstitialization, and this could be further enhanced by increased epithelial permeability. In studies with rats it is clear that ultrafine particles and PM_{10} can cause increased epithelial permeability.[15]

[26] Q. Zhang, Y. Kusake, K. Sato, A. Morita, K. Nakakuki, B. Li, K. Okada and K. Donaldson, *Toxicol. Appl. Pharmacol.*, 1998, in press.

[27] K. E. Driscoll, J. M. Carter, B. W. Howard, D. G. Hassenbein, W. Pepelko, R. B. Baggs and G. Oberdorster, *Toxicol. Appl. Pharmacol.*, 1996, **136**, 372.

[28] C. J. Johnston, J. N. Finkelstein, R. Gelein, R. Baggs and G. Oberdorster, *Toxicol. Appl. Pharmacol.*, 1996, **140**, 154.

[29] J. H. Vincent and K. Donaldson, *B. J. Ind. Med.*, 1990, **47**, 302.

5 Transition Metals

Transition Metals and Free Radicals in Particle Toxicity

Production of free radicals in the lung has been seen as a general mechanism mediating the biological activity of a number of different pathogenic particles[30,31] such as quartz,[32,33] coalmine dust,[34] residual oil fly ash,[35] asbestos[36] and synthetic mineral fibres.[37] The oxidative stress is considered to arise first from the particles themselves, normally by localized release of high concentrations of transition metals supplemented by the inflammatory cell influx that results from the primary interaction between lung cells and particles. Oxidative stress is a general signalling mechanism within cells that produces the transcription of a number of pro-inflammatory genes for cytokines, anti-oxidant enzymes, receptors and adhesion molecules.[38] Under the influence of oxidative stress, NF-κB separates from its inhibitor IκB and translocates to the nucleus to bind to the promoter region of key genes, allowing their transcription.[39] The ultrafine component of PM_{10}, with its large surface area, could generate free radicals that would be a substantial stimulus to transcription.

Free Radical Production by PM_{10}

To test this free radical hypothesis in the case of PM_{10} we have collected PM_{10} in Edinburgh and London. PM_{10} was found to have the ability to generate hydroxyl radical activity as shown in a supercoiled plasmid DNA scission assay[13] and by the ability to form the hydroxylated derivative of salicyclic acid (2,3-dihydroxybenzoic acid).[40] PM_{10} contained a large proportion of iron and the generation of hydroxyl radicals was blocked with iron chelators, confirming that Fenton chemistry is indeed the source of the hydroxyl radicals.[13] The majority of the available iron was in the form of Fe^{3+}, but the presence in the lung of reductants such as superoxide anion and glutathione (GSH) would be able to initiate the reaction by reducing Fe^{3+} to Fe^{2+}. Following the instillation of PM_{10} into the lungs of rats there was evidence of inflammatory neutrophil influx and oxidative stress, as shown by depletion of GSH in lung lining fluid.[15] Importantly, PM_{10} caused significantly more inflammation than a similar mass

[30] T. P. Kennedy, R. Dodson, N. V. Rao, H. Ky, C. Hopkins, M. Baser, E. Tolley and J. R. Hoidal, *Arch. Biochem. Biophys*., 1989, **269**, 359.
[31] K. Donaldson, P. H. Beswick and P. S. Gilmour, *Toxicol. Lett*., 1996, **88**, 293.
[32] V. Castranova, V. Vallyathan, D. M. Ramsey, J. L. McLaurin, D. Pack, S. Leonard, M. W. Barger, J. C. Ma, N. S. Dalal and A. Teass, *Environ. Health Perspect*., 1997, **105**, 1319.
[33] K. Zay, D. Devine and A. Churg, *J. Appl. Physiol*., 1995, **78**, 53.
[34] N. S. Dalal, M. M. Suryan, V. Vallyathan, F. Y. Green, B. Jafari and R. Wheeler, *Ann. Occup. Hyg*., 1989, **33**, 79.
[35] K. L. Dreher, R. H. Jaskot, J. R. Lehmann, J. H. Richards, J. K. Mcgee, A. J. Ghio and D. L. Costa, *J. Toxicol. Environ. Health*, 1997, **50**, 285.
[36] P. S. Gilmour, P. H. Beswick, D. M. Brown and K. Donaldson, *Carcinogenesis*, 1995, **16**, 2973.
[37] D. M. Brown, C. Fisher and K. Donaldson, *J. Toxicol. Environ. Health Part A*, 1998, **53**, 101.
[38] I. Rahman and W. MacNee, *Thorax*, 1998, in press.
[39] M. Meyer, H. K. Pahl and P. A. Bauerle, *Chem. Biol. Interact*., 1994, **91**, 91.
[40] K. Donaldson, D. M. Brown, C. Mitchell, M. Dineva, P. H. Beswick, P. Gilmour and W. MacNee, *Environ. Health Perspect*., 1997, **105** (suppl. 5), 1285.

(125 μg) of carbon black that was 260 nm in diameter, *i.e.* not in the ultrafine size range; the inclusion of a mass bolus control is vital to interpreting this type of data.

ROFA (residual oil fly ash) has been used as a surrogate for PM_{10}, although it is very different in many respects from PM_{10}. ROFA has been found to cause pulmonary inflammation after instillation, *via* a transition metal-mediated mechanism.[35] Furthermore, in rats instilled with ROFA, an intraperitoneal injection of the free radical scavenger DMTU lowered the amount of PMN influx to the lung.[41] ROFA particles cause increased transcription of cytokine genes by human bronchial epithelial cells *in vitro via* a transition metal-mediated mechanism,[42] as shown by the fact that the effect could be blocked with the metal chelator deferoxamine. Interestingly, the stimulation of cytokine production could be mimicked by vanadium salts in solution but not by iron or nickel sulfate, pointing towards the possible importance of vanadium.

6 Hypothetical Mechanism for the Cardiovascular Effects of PM_{10}

The effect of PM_{10} in increasing the numbers of cardiovascular deaths (*i.e.* from strokes and heart attacks) is one of the most puzzling of its adverse effects. We have suggested that the local pulmonary inflammation could be translated into increased pro-coagulant status and conditions that could favour haemostasis in the coronary and cerebral microvasculature; these conditions would promote heart attack and stroke, respectively. Two mechanisms could be operative: (a) increased production of pro-coagulant by the liver or the lungs; (b) decreased deformability of PMNs.

Lung and Liver as Sources of Pro-coagulant Factors

We have shown that following deposition of ultrafine particles in the lungs, there is inflammation, oxidative stress[15,43] and up-regulation of oxidative stress-responsive genes such as TNF, SOD and iNOS.[15,44] In preliminary studies we have shown that inhalation of ultrafine particles in the rat increases the levels of factor VII in plasma (unpublished data). Factor VII has been shown to be a risk factor for cardiovascular disease in population studies.[45,46] This association is supported by two recent studies. The first showed that polymorphisms in the factor VII gene influence both the plasma concentration of factor VII antigen and activity, and the risk of myocardial infarction;[47] secondly, the use of warfarin to lower factor VII plasma concentrations, to those associated with low vascular risk in epidemiological studies, is accompanied by significant protection from fatal vascular events.[48] The principal site of synthesis of factor VII is the

[41] J. A. Dye, K. B. Adler, J. H. Richards and K. Dreher, *Am. J. Respir. Cell Mol. Biol.*, 1997, **12**, 625.
[42] J. D. Carter, A. J. Ghio, J. M. Samet and R. B. Devlin, *Toxicol. Appl. Pharmacol.*, 1997, **146**, 180.
[43] X. Y. Li, W. MacNee and K. Donaldson, unpublished results.
[44] X. Y. Li, P. S. Gilmour, K. Donaldson, W. MacNee and A. Churg, *Am. J. Respir. Crit. Care Med.*, 1998, in press.
[45] T. W. Meade, V. Ruddock, R. Chakrabarti and G. J. Miller, *Lancet*, 1993, **342**, 1076.
[46] T. W. Meade, S. Mellows, M. Brozovic *et al.*, *Lancet*, 1986, **2**, 533.
[47] L. Iacovelli, A. Di Castelnuovo, P. de Knijff *et al.*, *New Engl. J. Med.*, 1998, **338**, 79.
[48] The Medical Research Council's General Practice Research Framework, *Lancet*, 1998, **351**, 233.

hepatocyte, but mRNA for both factor VII and tissue factor, which is also involved in coagulation, have been demonstrated in alveolar macrophages,[49] and these cells are capable of synthesis of factor VII *in vitro*.[50] Thus local inflammation in the lungs, and the activation of alveolar macrophages, could result in local and generalized release of pro-coagulant factors, which may enter the blood stream and have a systemic effect.

Local inflammation in the lungs could also result in increased pro-coagulant activity from the liver. There are at least two possible mechanisms by which these effects can occur following inhalation of particulate air pollution: (1) through the release of cytokines from inflammatory cells in the lungs; (2) as a result of the development of systemic oxidant stress. Both of these could up-regulate the genes for pro-coagulant factors in hepatocytes. We have shown clear evidence of systemic oxidant stress, measured as a decrease in the anti-oxidant capacity of the plasma following installation of PM_{10} particles, which was not apparent following instillation of the same mass of fine carbon particles.[43] Candidate cytokines which may result in up-regulation of pro-coagulant factors in the liver are TNFα and IL-6.

Alterations in Blood Rheology as a Cause of Increased Haemostasis

One unique feature of the pulmonary microcirculation is the close proximity of the distal airspace to the circulating blood, across the alveolar–capillary membrane, allowing easy access for inflammatory mediators in the airspaces to reach the blood. A recent study has shown increased plasma viscosity during an episode of air pollution, confirming that a 'signal' from the lungs following exposure to particles can affect plasma indices.[5] We have shown that the ability of neutrophils to deform in transit in the pulmonary capillaries is the factor which initiates neutrophil sequestration in the lungs, as the precursor of migration into the airspaces.[7] We have also shown that lung inflammation, which occurs during smoking or acute exacerbations of chronic obstructive pulmonary disease, increases neutrophil sequestration in the lungs,[8,10] very likely *via* an oxidant-induced decrease in cell deformability.[11] Thus oxidant-generating ultrafine environmental particles depositing in the distal airspaces may also produce increased neutrophil sequestration in the lungs, and so contribute to the initiation of lung inflammation. In addition, the decrease in neutrophil deformability induced in cells in transit in the pulmonary microcirculation, or the release of less deformable cells from the bone marrow in response to particle-induced lung inflammation,[51] may result in sequestration of these cells in the microcirculation of other organs, such as the heart and the brain, so contributing to local haemostasis and thrombotic events.

7 Activation of NF-κB in the Lungs after Inhalation of Ultrafine Particles as a Central Initiating Event

The transcriptional activator NF-κB is a nuclear factor of the Rel family that is

[49] M. P. McGee, R. Devlin, G. Saluta and H. Koren, *Blood*, 1990, **75**, 122.
[50] H. A. Chapman, C. L. Allen and O. L. Stone, *J. Clin. Invest*., 1985, **75**, 2030.
[51] T. Terashima, B. Wiggs, D. English, J. C. Hogg and S. F. van Eeden, *Am. J. Respir. Crit. Care Med*., 1997, **155**, 1441.

translocated to the nucleus to permit expression of a wide range of pro-inflammatory genes.[39] The NF-κB heterodimer, comprising p65 and p50 proteins, is found in resting cells bound to its inhibitor IκB, which masks the nuclear translocation signal and so prevents its translocation to the nucleus. Under oxidative stress or a range of other stimuli such as TNF, the IκB is phosphorylated and then degraded *via* the ubiquitin proteosome system, allowing the NF-κB to relocate to the nucleus.[52] Genes that have a κB binding site in their promoter include cytokines, growth factors, chemokines, and adhesion molecules and receptors.[38] In addition, the genes for tissue factor contain an NF-κB binding site[53] and so may be susceptible to transcriptional activation during oxidative stress. We have demonstrated activation of NF-κB by oxidative stress in airspace epithelial cells.[54] The deposition of particles that deliver an oxidative stress to the lungs cause activation of NF-κB, and possibly other oxidative stress-responsive transcription factors, that initiate a cascade of gene expression which lead to a pro-coagulant and haemostatic state.

8 Implications of an Oxidative Stress-mediated Mechanism of Action of PM_{10} for Susceptibility in Patients with Airways Disease

The Central Role of the Epithelium

Because of the deposition of particles on the epithelium, prior to phagocytosis, it seems likely that the epithelium is a target for the PM_{10} in leading to increased asthma and COPD attacks. Antigens for asthma are present in most atmospheres and to trigger an asthma attack the antigen need only gain access to the subepithelial lymphoid tissue. There is evidence that various kinds of environmental particles such as ROFA,[41] PM_{10}[15] and also ultrafine carbon black[55] can compromise the epithelium by causing injury or oxidative stress. This presents the possibility that increased production of inflammatory mediators and increased permeability to antigens may be a mechanism for the induction of asthma attacks, additional to the fact that the underlying inflammation in the airways of asthmatics means that they are in a 'primed' state for the further oxidative stress caused by depositing PM_{10}.

Existing Oxidative Stress in Susceptible Populations

It should be noted that the principal pulmonary effects of PM_{10} are seen in susceptible populations, such as those with airways disease. If, as hypothesized here, the PM_{10} has its effect mainly by a mechanism that involves oxidative stress, then these susceptible populations might be susceptible because of pre-existing oxidative stress. We have utilized an assay (Trolox Equivalent Antioxidant Defence; TEAC) that detects the global anti-oxidant defence in the plasma, and

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have demonstrated depleted anti-oxidant defences in patients with airways disease.[9] Plasma samples from patients with asthma and COPD were found to have significantly lower TEAC values than normal patients and these were found to be further lowered during asthma attacks and exacerbations of COPD. Clearly these patients would be susceptible to an oxidative insult such as that hypothesized here to emanate from PM_{10}.