The role of oxidative stress in the cardiovascular actions of particulate air pollution

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Abstract

Air pollution has been estimated to be responsible for several millions of deaths worldwide per year, the majority of which have been attributed to cardiovascular causes. The particulate matter in air pollution has been shown impair vascular function, increase blood pressure, promote thrombosis and impair fibrinolysis, accelerate the development of atherosclerosis, increase the extent of myocardial ischaemia, and increase susceptibility to myocardial infarction. The pathways underlying these effects are complex and poorly understood; however, particulate-induced oxidative stress repeatedly emerges as a potential mechanism in all of these detrimental cardiovascular actions. The present mini-review will use diesel exhaust as an example of a pollutant rich in combustion-derived nanoparticles, to describe the potential by which oxidative stress could drive the cardiovascular effects of air pollution.

Introduction

The detrimental health effects of air pollution have been known for many centuries. Despite this knowledge, it is only recently that we are beginning to appreciate the scale and complexity of this issue. Estimates of the magnitude of the health effects of air pollution are alarming, suggesting that air pollution is responsible for up to 7 million deaths worldwide per year {[1]; World Health Organization (2011–2014) Air Quality and Health, WHO Factsheets (number 313), http://www.who.int/mediacentre/factsheets/fs313/en/; World Health Organization (2014) 7 million premature deaths annually linked to air pollution, http://www.who.int/mediacentre/news/releases/2014/air-pollution/en/}. A compelling investigation by Lim et al. [2] estimated the risk factors for all-cause disease in different populations across the world. Both indoor and outdoor air pollution were ranked in the top ten risk factors for mortality. Furthermore, the additional hospitalization, morbidity and lost work days have been estimated to represent a cost of ∼£16 billion per year in the U.K. alone [Department for Environment, Food and Rural Affairs, U.K. Government (2013), https://www.gov.uk/air-quality-economic-analysis]. Although the pulmonary effects of air pollution are widely recognized, it is only in the last three decades that the wider health effects of air pollution have become apparent. As the study by Lim et al. [2] nicely demonstrated, if the cause of mortality is categorized by class of disease, then (due to the high prevalence of cardiovascular disease) the cardiovascular effects of air pollution far outweigh those directly attributable to the lung.

Associations of the health effects of air pollution are strongest for the airborne PM (particulate matter) rather than gaseous pollutants (see [1]). PM is categorized into three groups according to sampling conventions: coarse particles (PM_{10}, particles with a diameter of 10 μM or less), fine particles ($PM_{2.5}$, particles with a diameter of 2.5 μM or less) or ultrafine particles ($PM_{0.1}$, particles with a diameter of 100 nM or less), commonly referred to as nanoparticles. The toxicity of PM is greatly dependent on the composition of the particulate; however, in general the small size fractions exert greater effects due to their large reactive surface area for a given mass, and their ability to penetrate deep into the lungs. Urban PM is usually derived from numerous sources, and subsequently has a varied chemical composition and size distribution. Diesel exhaust is recognised as an important contributor to urban air pollution, in that it is especially rich in CDNPs (combustion-derived nanoparticles) in comparison with emissions from other fuel sources [3]. DEP (diesel exhaust particulate) is far from a simple soot. Although it has a central carbon core, the surface of the particle is coated with a diverse range of reactive transition metals and organic carbon species (e.g. quinones, polyaromatic hydrocarbons and alkanes). This complex chemical composition of DEP engenders it with the potential to initiate redox reactions and this is believed to be a key feature influencing its biological toxicity.

The cardiovascular effects of DEP

Controlled exposures to diesel exhaust in man have demonstrated that this pollutant has multiple detrimental actions on the cardiovascular system. Our group has demonstrated that a 1–2 h exposure to diesel exhaust, at a level

Key words: air pollution, atherosclerosis, cardiovascular, diesel exhaust, oxidative stress, superoxide.

Abbreviations: ApoE, apolipoprotein E; CDNP, combustion-derived nanoparticle; DEP, diesel exhaust particulate; PM, particulate matter; $PM_{2.5}$, particulate matter with a diameter of less than 2.5 μm; PM_{10}, particulate matter with a diameter of less than 10 μm.

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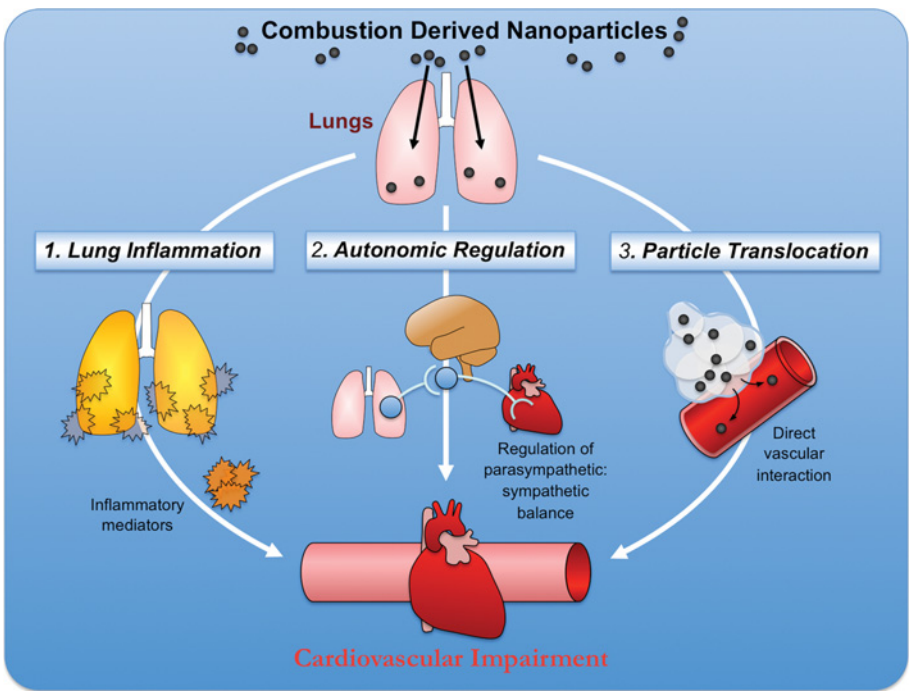
Figure 1 | **Three hypotheses to explain the cardiovascular actions of inhaled particles**

1. Inhaled particles induce an inflammatory response in the lung, the mediators of which enter the circulation to indirectly affect the cardiovascular system. 2. Particles stimulate sensory receptors on the alveolar surface leading to changes in the function of the autonomic nervous system, which regulates cardiovascular haemostasis, especially cardiac function. 3. The small size of particles allows them to translocate from the lung into the circulation and directly impair cardiovascular function.



representative of roadsides in heavily polluted cities, causes impaired relaxation of blood vessels [4], increased arterial stiffening [5], promotes blood clotting [6], inhibits the release of endogenous factors that prevent excessive clotting [4] and exacerbates myocardial ischaemia [7]. Importantly, filtering of the particulate constituents from the exhaust prevents these effects [8,9], demonstrating the importance of CDNPs on the cardiovascular system. Parallel animal studies have shown that CDNPs also increase blood pressure [10], promote the vascular disease atherosclerosis [11], induce arrhythmia (irregular beating of the heart) and increase the susceptibility of the heart to injury [12].

Oxidative stress is a prominent feature of almost all cardiovascular diseases and conditions. While there is debate as to whether an oxidative insult actually initiates the disease process, it is almost universally accepted that oxidative stress will contribute to, and worsen, the cardiovascular disease. A plethora of studies have clearly demonstrated a clear relationship between cardiovascular disease and the oxidative stress induced by particulate air pollution (reviewed in [13,14]). Furthermore, studies are emerging that demonstrate that antioxidant supplementation may reduce the cardiovascular effects of particulates in rodents *in vivo* [15–17] and in man [18,19]. This mini-review will discuss the potential mechanism for these observations, by providing examples of how inhaled particles can promote oxidative stress in different aspects of the cardiovascular system.

From lung to cardiovascular system

The means by which inhaled particles enter and deposit in the alveoli of the lungs have been well characterized. But how then do inhaled particles have cardiovascular actions: what is the 'signal' that links the lung to the heart and blood vessels? This question has hindered research in this field for many years, although three hypotheses have emerged (Figure 1). The traditional ('inflammation') hypothesis is that inhaled particles are ingested by alveolar macrophages, activating these cells to an extent that induces a marked pulmonary inflammation. Inflammatory mediators then 'spill-over' into the circulation and indirectly alter cardiovascular function [20]. Alternatively, particles can stimulate sensory receptors on the alveolar surface providing a neural stimulus that results in changes in autonomic function, altering cardiovascular homoeostasis, particularly that of the heart [21,22]. More recently it has been hypothesized that the minute size of nanoparticles allows them to cross ('translocate') into the blood themselves and directly interact with the vasculature [23].

There is evidence for and against each hypothesis. Inflammation is known to play an important role in numerous cardiovascular conditions, through pathways common to those associated with exposure to air pollutants. However, whereas many studies have demonstrated evidence of systemic inflammation following exposure to PM (e.g. increased levels of blood leucocytes and presence of cytokines

or acute-phase proteins) there is a lack of consistency across markers of inflammation, both within and between studies (even from the same laboratories). Furthermore, there is often a dissociation between the extent and time course of inflammation, with that of the other biological actions of particles, especially between different classes of particulate. Thus inflammation alone cannot fully account for the diverse cardiovascular effects of inhaled particles. Arguably the most robust hypothesis for the cardiovascular effects of inhaled particles is that mediated by the autonomic nervous system. However, whereas this pathway clearly provides a mechanism for rapid regulation of cardiac function (e.g. changes in heart rate variability: although again there is often a lack of consistency between the parameters assessed [24]), it is less apparent how other aspects of the cardiovascular system are significantly influenced by these alterations in autonomic function and what the consequences of these alterations are long-term.

Particle translocation offers an exciting alternative mechanism. However, demonstrating particle translocation has been a significant obstacle in this field of research for many years. Although particles clearly have the capacity to penetrate across cellular barriers, it is extremely difficult to detect translocated particles in systemic tissues, due to their small size and number, the leaching of labels from particles required to detect/image translocation, and the difficulty in distinguishing particles from biological structures. Nevertheless, it has become accepted that various 'model' nanoparticles can translocate across biological barriers and between organs [23,25,26]. What remains, however, are the questions as to whether all nanoparticles (especially CDNPs) can translocate, and whether they reach susceptible sites in sufficient numbers to induce pathophysiological effects. The present mini-review will approach this question from a different perspective, by asking 'if CDNPs do gain access to the circulation, do they have the capacity to affect cardiovascular function'? And if so, then what is the role of oxidative stress in this pathway?

Generation of free radicals from particulate

Many types of particulate have the ability to cause cytotoxicity in cell cultures, although the dose required tends to be excessively high for many carbon-centred particles [13]. Nevertheless, changes in cell function/phenotype are readily seen at non-cytoxic doses [27], and oxidative stress has been shown to be a prominent mediator of many of these effects (see [13]). Interestingly though, particles have the capacity to generate oxygen-derived free radicals without the presence of cells/tissue. Previously we have used electron paramagnetic resonance (a sensitive technique that is not compounded by the black colour of carbon particle suspensions) to demonstrate that DEP generates superoxide free radicals in physiological solutions [28]. The use of different spin-trap reagents and free radical scavengers can provide further information as to the role of other free radicals. Hydroxyl radicals have also been implicated in the action of PM, especially PM from urban environments [13,29]; however, our own studies failed to show generation of hydroxyl radicals from DEP in the absence of tissue [8]. Many studies supplement exogenous H_2O_2 to particles to generate hydroxyl radicals, suggesting a role for Fenton reactions catalysed by transition metals on the particle surface. Nevertheless, these reactions may be of biological importance in cells already under oxidative stress.

Direct impairment of vascular function by particles

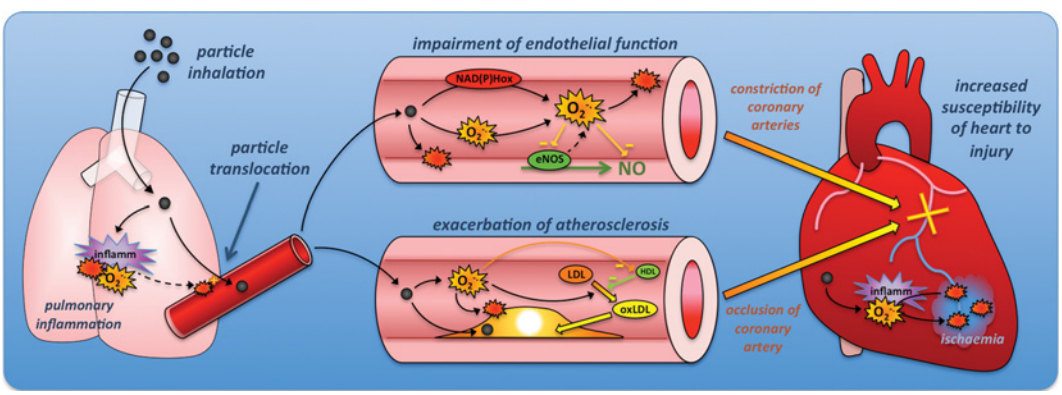
Superoxide is especially pertinent to the cardiovascular system due to its ability to scavenge endothelial nitric oxide (NO). The vascular endothelium is a single layer of cells lining the inner surface of blood vessels. As well as controlling barrier function, it also synthesises and releases a number of active mediators. NO is one of the most important endothelium-derived mediators, due to the multiple protective roles it performs, including relaxation of the underlying vascular smooth muscle to control blood flow through arteries and blood pressure, inhibition of smooth muscle proliferation and remodelling, regulation of blood clotting, and inhibition of circulating inflammatory cells. Thus the scavenging of NO by superoxide not only generates harmful peroxynitrite as a product of the reaction, but also, importantly, leads to a loss in the 'bioavailability' of these diverse actions of NO.

Numerous studies have demonstrated that pulmonary administration of particles impairs endothelial/NO-mediated relaxation of blood vessels *in vivo* (comprehensively reviewed in [30]). Interestingly, although direct application of DEP to isolated functioning segments of arteries also impairs the ability of these arteries to relax to agents which act through NO. Co-incubation with the superoxide scavenging enzyme, superoxide dismutase, prevents the inhibition of vascular function caused by the particles. Thus CDNPs can directly impair vascular function through generation of superoxide, without prior interaction with the pulmonary system, or the need for inflammatory cells.

Particles and atherosclerosis

The *in vitro* properties of particulates in many ways emulate those of *in vivo* models in which particles are administered by a physiological relevant route (i.e. via the lungs). The multiple detrimental actions of CDNPs (free radical generation and endothelial impairment together with the pro-inflammatory and pro-thrombotic actions) provide a combined insult on the cardiovascular system to drive cardiovascular disease. This raises the question as to whether CDNPs promote the development of atherosclerosis: the formation of fatty lipid-rich lesions within arteries that obstruct blood flow and, potentially, rupture leading to thrombotic occlusion of the vessel, an event that underlies a heart attack, stroke or limb ischaemia.

Figure 2 | Mechanisms through which particle translocation may impair cardiovascular function via the generation of oxidative stress
Particles may translocate across the alveolar barrier into the circulation, a process that is likely to be aided by generation of pulmonary inflammation/oxidative stress. Particles accessing the circulation can directly impair vascular function through generation of superoxide ($O_2^{\bullet -}$) free radicals that scavenge endothelial cell-derived nitric oxide (NO). In diseased arteries, particles may promote the development of atherosclerosis by accumulating in plaques, through increased oxidation of blood-borne lipids and promoting plaque vulnerability to rupture. Vasospasm and plaque atherothrombosis may occlude coronary artery flow, causing ischaemia of downstream regions of the heart. Ischaemic injury will be exacerbated by a local inflammation/oxidative stress in the myocardium, leading to further myocardial injury and, potentially, a heart attack. eNOS, endothelial nitric oxide synthase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NAD(P)H oxidase, nicotinamide adenine dinucleotide phosphate oxidase: oxLDL, oxidized low-density lipoprotein.



It has been demonstrated that pulmonary administration of PM promotes the development of atherosclerosis in rabbits [31], a finding that was then extended to inhalation studies in mice [32] and epidemiological studies in man [33]. Subsequently, it has been demonstrated that vehicle emissions in particular exacerbate atherosclerosis [34], although there remains a degree of uncertainty as to the relative roles of PM as opposed to gaseous co-pollutants [35,36]. We have employed a commonly used transgenic mouse model of atherosclerosis to specifically address the action of DEP on atherosclerotic plaques [11]. ApoE (apolipoprotein E)-knockout mice were fed on a high-fat 'Western' diet for 8 weeks to accelerate the development of atherosclerosis. For the final 4 weeks of feeding, mice were instilled with DEP or saline vehicle, twice per week at a dose representing the upper range a person may be exposed to in a heavily polluted city over 24 h. This short period of DEP exposure increased the coverage of lipid-rich lesions across the aortic surface, and almost doubled the size of plaques, as assessed by histological quantification of serial sections of key arteries (to provide a surrogate of plaque volume). Interestingly, the atherosclerotic burden correlated with the degree of lung inflammation after the final exposure, although there was no evidence of a systemic inflammation. Surprisingly, many of the mechanisms commonly associated with atherosclerosis (lipid levels, endothelial function, altered fibrinolysis, proportion of detrimental plaque constituents and matrix-metalloproteinase levels) were unchanged by DEP in this model. However, we found an increased expression of several antioxidant proteins [haemoxygenase-1, NAD(P)H-quinone oxidoreductase 1 and NF-E2-related factor-2] in the liver of DEP-treated mice, especially the ApoE$^{-/-}$ mice. These results suggest that despite the lack of a systemic inflammation, there was evidence of a response to a systemic oxidative insult associated with the action of DEP in atherosclerosis. Evidence from other groups using similar models confirms a role for oxidative stress, showing that DEP, and other types of PM, increased urinary isoprostanes [37], increased oxidation in the vascular wall [32], caused greater nitrotyrosine staining within plaques [35], increased peroxidation of arachonic acid/linoleic fatty acids [38], resulted in higher numbers of NAD(P)H oxidase subunits [39] and caused a loss of the antioxidant capacity of high density lipoprotein in the blood [40].

Interventions

PM in the air clearly has the capacity to have multiple detrimental effects on the cardiovascular system. But, given that levels of PM in the air have dramatically fallen over the last 50 years, does this really matter? First, although PM levels have fallen, WHO/EU limits for particulate air pollution are regularly breached in even relatively clean cities. Furthermore, recent studies suggested that PM has significant cardiovascular effects at levels below the limits set in the European Union and U.S.A. [41,42]. Another concern is that policies are based on metrics of particle mass that are currently limited to PM_{10} and $PM_{2.5}$ outside of the laboratory. These metrics are not suitable to assess the number of nanoparticles that have exponentially increased with the number of vehicle on the roads in many countries. Mass does not provide an accurate assessment of the large reactive surface area of

nanoparticles or the toxicity provided by differences in the composition of surface chemicals.

On a more reassuring note, the use of technical innovations to reduce the numbers of harmful particle emissions, is becoming ever more common-place. 'Particle traps' on exhausts have been shown to be extremely effective in reducing both particle mass and number. We have demonstrated that the use of a typical retrofit particle trap completely prevents the vascular and thrombotic effects of diesel exhaust inhalation [9]. Furthermore, addition of a cerium oxide nanoparticle to diesel fuel improves burning efficiency and produces a more condensed particulate in exhaust fumes, an effect that dramatically reduces the pro-atherosclerotic effects of these emission in ApoE$^{-/-}$ mice [43].

A concern though is that, although particle emission itself may be reduced by these interventions, there is a risk that co-pollutants (e.g. gases) may be raised, or that the particles that do remain have a greater toxicity. Thus novel interventions are still needed. A long-term aim of researchers in this area is to identify the harmful chemical species within CDNPs, with the hope that these can be selectively removed from either the exhaust or fuel. However, even identifying the composition of the many thousand chemical species in DEP is a daunting task, especially as this can vary dramatically with the use of different fuels, engines and engine running conditions. However, even relatively crude separation of the constituents through 'washing' in solvents of different polarity may be informative. Indeed, preliminary data from our laboratory show that removal of aqueous soluble constituents reduces the harmful capacity of DEP to impair vascular function and promote atherosclerosis (M.R. Miller, unpublished work). Findings of this kind may be useful for the design of a new generation of technology that specifically reduces the toxicity of particulate emissions, in addition to reducing particle mass/numbers.

Conclusions

The cardiovascular effects of particulate air pollution represent an enormous burden on public health. The mechanisms underlying how inhaled particles affect the cardiovascular system are poorly understood. Although several hypotheses have been suggested, no single theory adequately explains the wide-ranging actions of particles on the cardiovascular system; in all likelihood multiple pathways will be involved, acting in concert to detrimentally affect cardiovascular function. Oxidative stress is one mechanism that appears to play a central role at multiple stages of each pathway (Figure 2). Particles appear to have the ability to translocate from the lung into the circulation. This is a worrying prospect considering the high capacity that particles such as DEP have to induce oxidative damage, especially if particles can access susceptible areas of the circulation already under oxidative stress from cardiovascular disease. Identification of these mechanisms, as well as the physiochemical properties of CDNP that drive the detrimental actions, will have an immediate impact on public health policy and the development if interventions to limit the detrimental actions of air pollution. In particular, removal of groups of redox-active chemical species within CDNPs may be a useful means to limit the cardiovascular actions of vehicle emissions.

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