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Commentary

Evaluation of atherosclerosis as a potential mode of action for cardiovascular effects of particulate matter

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ABSTRACT

Epidemiology studies have consistently reported associations between $PM_{2.5}$ exposure and cardiovascular (CV) morbidity and mortality, but the epidemiology evidence for associations between $PM_{2.5}$ and subclinical measures of atherosclerosis is unclear. We critically reviewed the experimental studies of $PM_{2.5}$ and effects associated with acceleration and exacerbation of atherosclerosis and evaluated whether they support a biologically plausible, human-relevant mode of action (MoA) for the associations between $PM_{2.5}$ exposure and adverse CV outcomes reported in epidemiology studies. We focused on outcomes related to atherosclerotic plaque development, thrombosis, and coagulation, and we examined whether these outcomes were correlated with measures of oxidative stress and systemic or pulmonary inflammation, to evaluate whether these processes are likely to be key early events for atherogenic effects of PM. While the current experimental evidence indicates that the acceleration and exacerbation of atherosclerosis is a biologically plausible MoA in experimental animal models, we found that the human relevance of the key events in the proposed MoA is unclear and not well supported by the existing data. Further studies are needed to fill several important data gaps before the human relevance of this MoA can be established.

1. Introduction

Particulate matter (PM) is a heterogeneous mixture of solid and liquid particles that comprises the particle phase of air pollution. Because it is derived from numerous sources, including fossil fuel combustion, industrial processes, crustal material, and burning of natural materials, PM has a variable chemical composition and particle size distribution. PM is classified according to the aerodynamic diameter of the particles it contains. Thoracic particles have a diameter of less than 10 μm (PM_{10}), coarse particles have a diameter of 2.5–10 μm ($PM_{10\text{-}2.5}$), fine particles have a diameter less than 2.5 μm ($PM_{2.5}$), and ultrafine particles (UFPs), also called nanoparticles, have a diameter of less than 0.1 μm. The toxicity of PM within these categories depends largely on particle composition rather than size, although the toxicity of smaller particles may be enhanced by their larger, reactive surface area-to-mass ratio and the fact that they can be inhaled more deeply into the lung and deposited in the alveoli, compared to larger particles which show greater deposition in the upper airways (Araujo and Nel, 2009; Miller, 2014).

Over the last two decades, the epidemiology literature has provided evidence for associations between PM exposure and cardiovascular disease (CVD) morbidity and mortality, with stronger associations for $PM_{2.5}$ compared to PM_{10}. Multiple studies have reported that short-term (*i.e.*, hours to days) exposure to $PM_{2.5}$ is associated with CVD-related morbidity, including increased hospital admission rates for ischemic coronary events such as heart failure and myocardial infarction (MI), as well as increased CVD-related mortality (*e.g.*, Brook et al., 2010; Franchini and Mannucci, 2007; Mustafic et al., 2012; Pope and Dockery, 2006). Similarly, many studies of long-term (*i.e.*, months to years) exposure to $PM_{2.5}$ have

Abbreviations: ABI, ankle-brachial index; apo, apolipoprotein; aPTT, activated partial thromboplastin time; CAP, concentrated ambient particle; CIMT, carotid intima-media thickness; CV, cardiovascular; CVD, cardiovascular disease; DEP, diesel exhaust particle; ETP, endogenous thrombin potential; FVII, Factor VII; HDL, high-density lipoprotein; HEC, human equivalent concentration; IL, interleukin; IPCS, International Programme on Chemical Safety; LDL, low-density lipoprotein; LDLr, low-density lipoprotein receptor; MI, myocardial infarction; MoA, mode of action; NAAQS, National Ambient Air Quality Standard; NIST, National Institute of Standards and Technology; PAH, polycyclic aromatic hydrocarbon; PAI, plasminogen activator inhibitor; PFA, Platelet Function Analyzer; PM, particulate matter; PT, prothrombin time; ROS, reactive oxygen species; SRM, standard reference material; TAT, thrombin-antithrombin; TF, tissue factor; TFPI, tissue factor pathway inhibitor; tPA, tissue plasminogen activator; UFP, ultrafine particle; US EPA, United States Environmental Protection Agency; VLDL, very low-density lipoprotein; WHHL, Watanabe heritable hyperlipidemic; WoE, weight of evidence; vWF, von Willebrand factor.

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reported associations with adverse cardiovascular (CV) events and CVD-related deaths (*e.g.*, Brook et al., 2010; Chen et al., 2008; Pope and Dockery, 2006). These increased risks are related to temporal variations in $PM_{2.5}$ and are typically reported as percent increases in rates of each outcome per 10 or 20 $\mu g/m^3$ increase in ambient $PM_{2.5}$ concentrations averaged over a specified time period. The associations with adverse CV events are greater in sensitive populations, including the elderly and individuals with pre-existing CVD or compromised CV function, such as diabetes or hypertension (Costa et al., 2014). Based on this evidence, US EPA concluded that there is a causal relationship between both short-term and long-term exposure to $PM_{2.5}$ and CVD morbidity and mortality in its last PM review (US EPA, 2009).

Potential mechanisms related to the effects of PM on CVD are not well understood, and a mode of action (MoA) has not been established. Proposed MoAs include PM-induced alterations to the control of the heart by the autonomic nervous system, effects on ion channel function in myocardial cells, ischemic responses in the myocardium, and acceleration or exacerbation of atherosclerosis, the precursor to CVD (Costa et al., 2014; Emmerechts and Hoylaerts, 2012; Shannahan et al., 2012; Utell et al., 2002).

Atherosclerosis is a progressive disease that begins as early as childhood, and is characterized by the build-up of plaques in the arteries over a prolonged period (Viles-Gonzalez et al., 2004). The biological pathway to atherosclerosis is complex and involves factors related to inflammation, oxidative stress, coagulation, and blood lipids. The endothelial cells lining the inner surface of arteries are subject to injury from insults such as oxidative stress, hemodynamic forces, and modified lipoproteins (Zakynthinos and Pappa, 2009). Injured endothelial cells express adhesion molecules that facilitate their attachment to monocytes, and they also secrete chemoattractant cytokines that mediate the migration of monocytes into the intima, or subendothelial space, of the artery (Libby et al., 2011; Moore et al., 2013). Monocytes within the artery wall differentiate into macrophages that engulf normal and oxidized low-density lipoprotein (LDL) particles and transform into lipid-laden foam cells (Libby et al., 2011; Moore et al., 2013). Foam cells constitute the first recognizable progenitor lesion of an advanced atherosclerotic plaque, known as the fatty streak (Zakynthinos and Pappa, 2009). The foam cells produce tissue factor procoagulants, reactive oxygen species, and cytokines that recruit inflammatory cells, resulting in further uptake of LDL, stimulation of smooth muscle cell proliferation, and the development of a collagenous fibrous cap over the plaque core (Libby et al., 2011).

Atherosclerotic plaques cause narrowing of the artery lumen, resulting in ischemia (*i.e.*, reduced blood flow to tissues). Plaques can also be physically disrupted, and this process can be hastened by the presence of inflammatory cells (Libby et al., 2011). Once disrupted, the procoagulant material within the core of the plaque is exposed to coagulation proteins in the circulating blood; this triggers thrombosis (*i.e.*, blood clot formation) that can block the artery or embolize and lodge in distal arteries (Libby et al., 2011). When blockage of an artery leads to prolonged cardiac ischemia, the result is a myocardial infarction (MI; *i.e.*, a heart attack).

Several epidemiology studies have examined associations between long-term $PM_{2.5}$ exposure and subclinical measures of atherosclerosis. Small, but statistically significant, increases (generally 1–4%) in carotid intima-media thickness (CIMT) have been associated with increases in annual or 20-year average $PM_{2.5}$ concentrations in some studies (Bauer et al., 2010; Diez-Roux et al., 2008; Kunzli et al., 2005) but not others (Kunzli et al., 2010; Lenters et al., 2010). Similarly, increases in CIMT were significantly associated with the proximity of participants' residences to major roadways in one study (Kunzli et al., 2010) but not others (Bauer et al., 2010; Lenters et al., 2010). There were no statistically significant associations between long-term $PM_{2.5}$ levels and ankle-brachial index (ABI) (Diez-Roux et al., 2008; Hoffman et al., 2009), coronary artery calcification (Diez-Roux et al., 2008; Hoffman et al., 2007), or abdominal aortic calcification (Allen et al., 2009), although ABI and coronary artery calcification were associated with residential proximity to major roadways in one study each (Hoffman et al., 2007, 2009). All of these studies are limited by relatively small sample sizes, potential confounding, and a high likelihood of exposure measurement error. Considering these limitations and the inconsistent results, it is difficult to discern from these studies whether $PM_{2.5}$ has any effect on subclinical measures of atherosclerosis.

Because of the more consistent evidence for associations between PM exposure and CVD in the epidemiology literature, and the uncertainties with the epidemiology studies of atherosclerosis, we sought to evaluate whether the proposed MoA for $PM_{2.5}$-associated CVD *via* acceleration or exacerbation of atherosclerosis is supported by experimental data (Fig. 1). If short-term exposure to $PM_{2.5}$ induces CVD, it is unclear whether acute responses would lead to cumulative effects that manifest as chronic disease, or whether they provoke adverse outcomes such as plaque rupture and thrombosis in underlying chronic disease states. It has been proposed that short-term exposure to $PM_{2.5}$ increases the risk of arterial thrombosis, including MI (*i.e.*, exacerbates atherosclerosis), and that long-term exposure to $PM_{2.5}$ enhances atherosclerotic plaque formation (*i.e.*, accelerates atherosclerosis) (Emmerechts and Hoylaerts, 2012).

The proposed MoA for the effects of PM on CVD through acceleration and/or exacerbation of atherosclerosis includes both direct and indirect key events. Direct events would result from the translocation of UFPs and/or $PM_{2.5}$ into the circulation, leading to direct damage to endothelial cells, induction of oxidative stress and/or inflammation, and stimulation of thrombosis. Although systemic translocation of synthetic nanoparticles from the lungs has been demonstrated in humans and experimental animals, it has not been shown for inhaled ambient particles (Araujo and Nel, 2009). The most recent studies indicate that only a small fraction of nanoparticles translocate from the alveoli into the circulation in humans, and it is unclear whether they could reach the arteries most susceptible to atherosclerotic plaque formation in sufficient numbers to induce adverse effects (Miller, 2014; Moller et al., 2011; Nemmar et al., 2013).

Indirect events would result from an inflammatory response in the lungs with secondary systemic inflammation, or the generation of oxidative products from the reaction of PM in the lung that are released into the circulation, both contributing to atherosclerosis. The role of oxidative stress and systemic inflammation as proposed key events on the causal pathway to atherosclerosis resulting from exposure to $PM_{2.5}$ has been reviewed extensively (Araujo and Nel, 2009; Miller et al., 2012; Mills et al., 2007; Nelin et al., 2012). Multiple studies in humans and experimental animals indicate that PM exposure induces pulmonary inflammation and oxidative stress (*e.g.*, Grahame and Schlesinger, 2010; Nelin et al., 2012; Shannahan et al., 2012). The mechanisms for how these processes in the lung translate to effects on atherosclerosis is unclear.

In this paper, we critically review experimental animal and *in vitro* mechanistic studies of $PM_{2.5}$ and effects associated with acceleration and exacerbation of atherosclerosis and we evaluate whether they support a biologically plausible MoA for associations between $PM_{2.5}$ and adverse CV outcomes such as MI and CVD mortality reported in epidemiology studies. We focus on outcomes directly related to atherosclerosis, such as atherosclerotic plaque development, thrombosis, and coagulation, and we examine whether these outcomes are correlated with measures of oxidative stress and systemic or pulmonary inflammation in the same