Beware the Air! : Why Particulate Matter Matters

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Editorial

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Why Particulate Matter Matters

Daniel J. Conklin

The "Six Cities Study," as it is affectionately known by epidemiologists, was published in 1993. This landmark study laid the groundwork for an association between ambient air particulate matter (ie, fine particulate matter [PM] or $PM_{2.5}$ [particulate matter of 2.5 μm aerodynamic diameter or less]) and the risk of all cause mortality in the United States.[1] Simply put, the message of the study was: "Air pollution kills." Since then, a steady stream of studies has grown into a river of reports that collectively have swelled the banks of this initial association and have further specified that ischemic heart disease (cardiovascular disease [CVD]) is the single-most abundant cause of morbidity and mortality in this association.[2–4] Analysis of more than 100 studies (>100 million people in 119 cities in the United States and Europe) show that for each 10 $\mu g/m^3$ acute or chronic increase in $PM_{2.5}$, there is a significant increase in relative risk of cardiovascular mortality (chronic relative risk, 1.06 to 1.76), indicating that PM, even at ambient levels, has negative health consequences.[4] Moreover, chronic exposure to each 10 $\mu g/m^3$ $PM_{2.5}$ increase is associated with a 4 to 6% increase in CVD deaths, which translates to 800 000 deaths annually in the world (according to the World Health Organization), making PM exposure the 13th leading cause of CVD deaths[4] and, thus, deserving of urgent scientific and social attention. Currently, researchers in the field of environmental cardiology[5] are addressing at least 1 of 2 major unanswered questions regarding this association: (1) What constituent of inhaled $PM_{2.5}$ is responsible for the association?; and (2) What is the mechanism by which inhaled $PM_{2.5}$ can specifically affect cardiovascular disease risk?

In this issue of *Circulation Research*, Kampfrath et al address the latter "mechanistic question" and provide, for the first time, a biologically plausible mechanism that connects $PM_{2.5}$ exposure with vascular inflammation and dysfunction.[6] To do so, the group tracked oxidative changes induced in the lungs following chronic exposure to concentrated ambient $PM_{2.5}$ (CAPs) to the terminal changes of vascular inflammation and dysfunction in the aortic wall and in several other systemic vascular beds as well, which ultimately increases risk for CVD (eg, promoting atherosclerosis, thrombosis). Although it is nearly cliché to read that inhaled particles ($PM_{2.5}$, nanoparticles) induce "oxidative stress and/or lipid peroxidation" in the lungs and systemically and that this leads to cardiovascular inflammation and dysfunction, one finds in the present study of Kampfrath et al that "oxidative stress" has a molecular identity in the derivatives of oxidized phospholipid 1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine (ox-PAPC), including 1-palmitoyl-2-(5′-oxo-valeroyl)-*sn*-glycero-3-phosphocholine (POVPC), a well-known constituent of oxidized low-density lipoprotein, which can directly activate endothelial cells and stimulate the release/production of interleukin-8 (KC in mice) via Toll-like receptor (TLR)4 and an associated protein.[7,8] Nonetheless, POVPC (or a related lipid peroxidation product) generated in the lungs after $PM_{2.5}$ exposure appears to touch off a positive-feedback response in the lungs that also involves TLR4 and NADPH oxidase, leading to enhanced superoxide production and mobilization of mononuclear cells (eg, $F4/80^+$, $LyG6^{+high}$, $c\text{-}fms^+$ cells) from bone marrow into the circulation, lungs, and vascular wall, specifically into the perivascular adipose (Figure). The emphasis on this latter step whereby mononuclear (inflammatory) cells home to perivascular adipose is timely and, more importantly, provides a plausible link between a disparate number of stimuli (eg, CAPs, high-fat diet–induced obesity,[9,10] metabolic syndrome and diabetes,[11] angiotensin II infusion, and hypertension[12]) and vascular inflammation and vascular/endothelial dysfunction. Once a sufficient number of F4/80 ($c\text{-}fms^+$ or $LyG6^{+high}$) cells has homed to the vascular wall, there is a significant increase in NADPH oxidase– and perivascular-derived superoxide. Both the TLR4 and NADPH oxidase dependences of vascular inflammation were elegantly shown by Kampfrath et al, through chronic CAPs exposure of mice deficient in either the TLR4 or the NADPH oxidase subunit Nox2 ($gp91phox^{-/-}$) proteins.[6] It is worth noting, however, that various mechanistic steps were shown in different mouse strains.

The significance of these findings extends beyond the important mechanistic insights. Exposure of normal, healthy mice to real world particle (CAPs) pollution demonstrates that this level of pollution is alone sufficient to induce systemic vascular inflammation/dysfunction without any additional stressor required (eg, angiotensin II infusion, high fat diet, "second hit"). Moreover, the level of $PM_{2.5}$ exposure is relevant to human exposures. Mice were exposed for 6 h/d, 5 d/wk for 20 weeks using a versatile aerosol concentration enrichment system (VACES) to an average level of 92 $\mu g/m^3$ $PM_{2.5}$. This CAPs level is ≈9 times higher than the average ambient $PM_{2.5}$ of 10 $\mu g/m^3$ in Columbus, OH, whereas, the annual mean US National Ambient Air Quality Standards (NAAQS) for $PM_{2.5}$ is 15 $\mu g/m^3$. Because mice were exposed

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for only 6 h/d and not on weekends, overall exposure level is approximately equivalent to the annual US NAAQS and the World Health Organization annual mean $PM_{2.5}$ of 10 $\mu g/m^3$. The significance of this exposure condition is best appreciated when assessing daily and annual levels of $PM_{2.5}$ in major cities around the globe, as in China,[13,14] India,[15] and eastern Europe.[16] For example, daily average $PM_{2.5}$ in Shanghai, China, is 56.4 $\mu g/m^3$,[13] with levels regularly exceeding 100 $\mu g/m^3$ because of a confluence of traffic, coal burning, industry, and meteorologic conditions.[14] Similarly, during a winter inversion event in the Wasatch Valley of Utah, $PM_{2.5}$ significantly increased with peak levels reaching 150 $\mu g/m^3$.[17] Importantly, exposure to this elevated $PM_{2.5}$ level significantly decreases the number of circulating endothelial progenitor cells[17] in healthy young adults, indicating that acute $PM_{2.5}$ exposure (as well as chronic) also initiates systemic vascular injury.[18] Whether this striking acute event involves similar mechanisms as presented by Kampfrath et al[6] (ie, lung oxidation, POVPC generation, TLR4 activation, and upregulation of NADPH oxidase) remains to be seen but provides, at minimum, evidence that circulating endothelial progenitor cells could be a useful "$PM_{2.5}$ bellwether" for predicting vascular insult/dysfunction during $PM_{2.5}$ exposures.

Like all good research, this study raises more questions than it answers. For example, in what cells does pulmonary oxPAPC or POVPC stimulate TLR4 (eg, lung macrophage, lung microvascular endothelial cells)? Similarly, it is not only possible but likely that additional products of oxPAPC, namely free, unsaturated aldehydes, such as 4-hydroxy-nonenal (HNE), are involved in inflammatory signaling. HNE is generated in equal molar amounts with POVPC following β-scission of lipid peroxides. Whereas POVPC is membrane-bound and saturated, HNE is far more reactive (α,β-unsaturated), has greater diffusive mobility, and induces a variety of proinflammatory, proliferative, and cytotoxic effects in vascular cells.[19–22] In any case, the role of specific oxidized products generated in the lungs following $PM_{2.5}$ exposure in activation of TLR4-dependent pathways remains an important question. Systemically, the signals that induce homing of mononuclear cells into the perivascular adipose and induce conversion of perivascular fat from benign regulator of vascular tone[23] to an inflammatory organ[24,25] are being identified and perivascular cytokines/chemokines appear to provide the requisite stimuli (eg, superoxide, NO, interleukin-6, tumor necrosis factor-α, vascular endothelial growth factor) for endothelial dysfunction, endothelial activation, adhesion molecule expression, and mononuclear cell retention. Finally, the specific component of $PM_{2.5}$ that is responsible for "jump starting" the whole concerted activity

Non-standard Abbreviations and Acronyms

CAP	concentrated ambient particulate
CVD	cardiovascular disease
HNE	4-hydroxy-nonenal
oxPAPC	1-palmitoyl-2-arachidonyl-*sn*-glycero-3-phosphorylcholine
PM	particulate matter
$PM_{2.5}$	particulate matter of 2.5 μm aerodynamic diameter or less
POVPC	1-palmitoyl-2-(5′-oxo-valeroyl)-*sn*-glycero-3-phosphocholine
TLR	Toll-like receptor
VACES	versatile aerosol concentration enrichment system

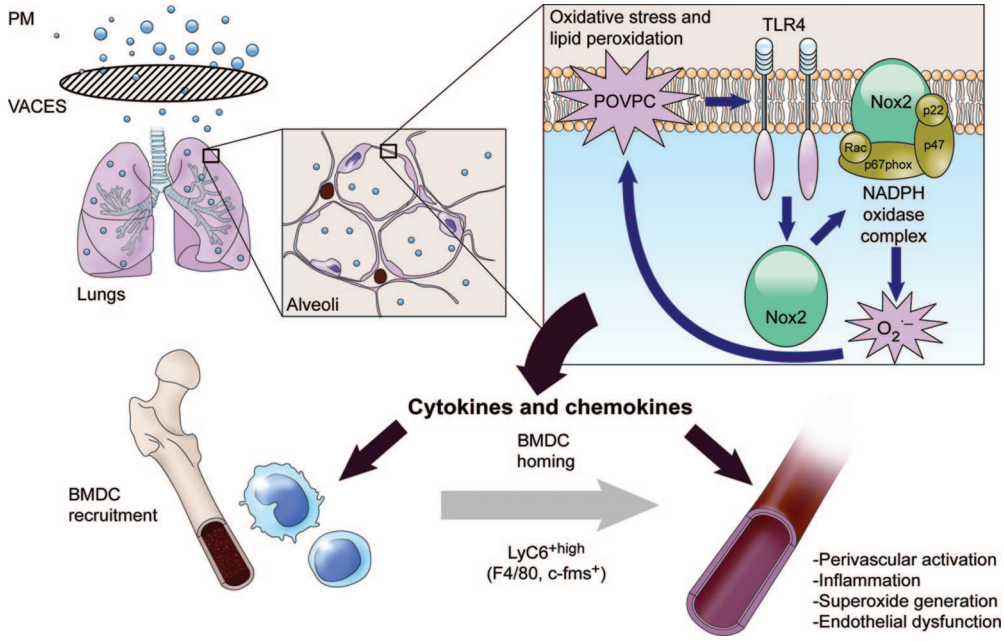

Figure. Mechanism of $PM_{2.5}$ induction of vascular inflammation. Ambient $PM_{2.5}$ is selectively concentrated by the VACES and then inhaled. $PM_{2.5}$ interacts with pulmonary cells and initiates lipid oxidation, generating oxPAPC and POVPC, which activate TLR4-dependent pathways including increased NADPH oxidase expression. Released cytokines and chemokines stimulate bone marrow–derived mononuclear cells (BMDCs especially $Ly6C^{+high}$) to home to the perivascular adipose and increase vascular formation of NADPH oxidase–dependent superoxide, which contributes to endothelial activation and dysfunction. (Illustration credit: Cosmocyte/Ikumi Kayama.)

is unidentified and remains an area of controversy. Many inhalation studies indicate that pulmonic and systemic effects of $PM_{2.5}$ (or whole combustion exhaust) can be attributed to a particle fraction much smaller than 2.5 μm (eg, ultrafine or $PM_{0.1}$ [$<$0.1 μm diameter] or nanoparticles)[26,27] or even to nonparticulates/volatiles.[28–31] Similarly, lipopolysaccharide can be a constituent of PM and can stimulate TLRs. In the present study, a VACES concentrates only ambient particles $\leq$2.5 μm diameter but not the ambient gases/volatiles, and, thus, the particulate fraction is clearly the trigger (noteworthy is that lipopolysaccharide levels were similar in lungs of air- and PM-exposed mice), but whether the $PM_{2.5}$ or a smaller $PM_{0.1}$ fraction (and what chemical constituent) is responsible remains a question.

Is the mystery solved regarding the mechanism how inhaled $PM_{2.5}$ exposure stimulates vascular inflammation and injury? Well, probably not completely, but the present scenario laid out by Kampfrath et al[6] connects findings from their study with many disparate human and animal epidemiological/exposure studies into a plausible story. Future studies will be required to elucidate exactly what constituent(s) of $PM_{2.5}$ is (are) so prooxidant/toxic in the lung, how POVPC (or a related product) targets important cells, what specific signals regulated bone marrow–derived mononuclear cell trafficking to perivascular adipose, and whether these steps are ultimately activated in humans exposed chronically to $PM_{2.5}$. One thing is clear: particulate matter matters!

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