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Is atherosclerotic disease associated with organic components of ambient fine particles?

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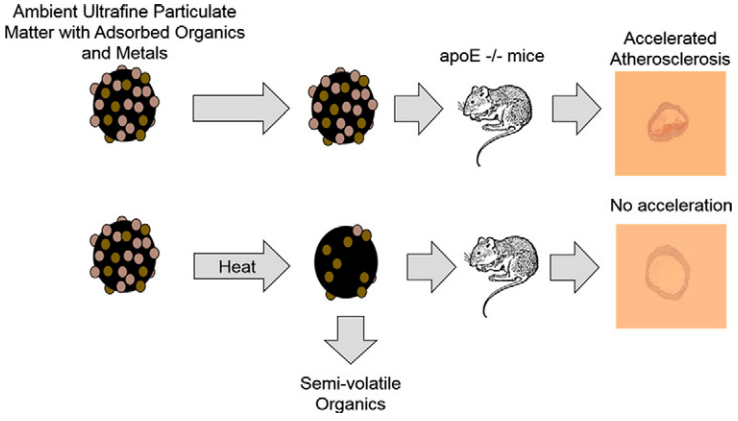
HIGHLIGHTS

- ApoE −/− mice were exposed for 8 weeks to concentrated ultrafine ambient particles (CAP) and thermally denuded concentrated ambient particles (deCAP).
- CAP-exposed mice exhibited accelerated development of atherosclerotic plaques and a progressive reduction of heart function.
- Responses in deCAP exposed mice were not statistically different from controls.
- Removing organic constituents from ultrafine particles by thermal denuding significantly reduced particle toxicity and greatly reduced effects on the cardiovascular system.

GRAPHICAL ABSTRACT



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ABSTRACT

Heart disease is a major killer in western societies; coronary artery disease and atherosclerosis are important contributors to this mortality. Atherosclerosis in mice with a deleted apoE gene (apoE −/−) is accelerated by exposure to ambient ultrafine particles (UFP) which are particles smaller than 180 nm in diameter. UFP contain organic components that are pro-oxidant and may cause or aggravate heart disease. Could removal of these organic constituents mitigate adverse cardiovascular effects? ApoE −/− mice were exposed to concentrated UFP (CAP), CAP from which organic constituents were removed by thermal denuding (deCAP) or purified air (controls) for 5 hr/day, 4 days/week for 8 weeks. Heart rate (HR), heart rate variability (HRV), biomarkers of oxidative stress and the sizes of arterial plaques were measured. Adverse effects were seen in CAP-exposed mice (increased size of arterial plaque, increased oxidative stress and decreased HRV, compared to controls). Adverse effects were not observed in deCAP-exposed mice. Removal of organic constituents from ambient particles resulted in significant reduction of toxic cardiovascular effects of air pollution exposure.

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1. Introduction

Exposures to elevated concentrations of ambient fine particulate matter ≤2.5 μm in diameter ($PM_{2.5}$) have been associated with cardiovascular hospital admissions and mortality in cohort and time series epidemiological studies (Pope and Dockery, 2006). The underlying causal chemical components, including elemental carbon, metals, and organic components of inhaled PM and the sources of those components that might be responsible for the adverse effects have been documented in human studies (Bell et al., 2009; Belleudi et al., 2010; Delfino et al., 2009, 2010) and with rodent models of human-like diseases (Araujo et al., 2008; Araujo and Nel, 2009; Cascio et al., 2007). The finding that quasi-ultrafine particles (UFP, <0.18 μm in aerodynamic diameter) were more pro-atherogenic activity than $PM_{2.5}$ (Araujo et al., 2008; Araujo and Nel, 2009), led to the current investigation of the characteristics and toxicity of UFP.

Unlike $PM_{2.5}$, UFP are not regulated by the EPA, yet the particles in this size fraction may have a very high toxic potential compared to larger particles that dominate $PM_{2.5}$ mass because they are present in the atmosphere at much higher number concentrations and carry disproportionately higher concentrations of redox active or otherwise toxic organic chemicals (Borm et al., 2006; Brown et al., 2001; Ntziachristos et al., 2007; Oberdorster, 2001). Deposition of these particles in the respiratory tract could lead to a cascade release of mediators and free radicals related to oxidative stress and inflammation and some of the deposited UFP and/or mediators can translocate to extrapulmonary organs (Delfino et al., 2005). We had previously reported that organic constituents of PM near heavily trafficked roads were associated with adverse airway effects (Kleinman et al., 2005, 2007; Li et al., 2010). This led us to extend our research to examine the role that organic constituents of UFP play in the development or pathophysiology of atherosclerosis and heart disease. We hypothesized that the pro-atherogenic effects of ambient particles in apoE −/− mice could be attenuated by the removal of semi-volatile components from the aerosol.

2. Approach

Genetically modified apoE −/− mice wereexposed to concentrated UFP (CAP) and to CAP from which organic constituents were removed (deCAP) for 5 hr/day, 4 days/week for 8 weeks. Controls were exposed to purified air. ApoE −/− mice have abnormal lipid metabolism and very high levels of circulating very low-density lipoproteins that make them susceptible to developing atherosclerotic plaques in their arteries. Heart rate, heart rate variability (HRV), biomarkers of oxidative stress and extent of arterial plaques were measured.

3. Materials and Methods

3.1. Exposures

ApoE −/− mice were exposed to concentrated ambient $PM_{0.18}$ (CAP) and to CAP which had been stripped of its semivolatile organic constituents using a thermal denuder (deCAP). Particles were concentrated with a Versatile Aerosol Concentration Enrichment System (VACES) that had been adapted for animal exposure studies in real world environments (Kleinman et al., 2005, 2007; Li et al., 2010). Exposures were performed in Los Angeles in a location proximal to two heavily trafficked roadways for 5 hr/day, 4 days/week for 8 weeks, an exposure regimen similar to previous studies that found ultrafine PM accelerated atherosclerosis in mice (Araujo et al., 2008; Araujo and Nel, 2009). Control apoE −/− mice were exposed to purified, filtered air at the same time at the same field location. Particle mass and number concentrations were measured during the exposures real-time using a DataRAM (ThermoFisher Scientific, Waltham, MA) and a Condensation Particle Counter (TSI, Shoreview, MN), respectively. Filter samples were collected during the exposures for chemical characterizations and gravimetric determination of particle mass.

A Dekati thermal denuder was used to remove volatile/semivolatile compounds from the CAP (Verma et al., 2011). The thermal denuder heats aerosols up to a temperature sufficient to volatilize semi-volatile organic compounds. The volatilized compounds are subsequently collected in an activated charcoal adsorber section. The particles have much slower diffusional deposition rates than the vaporized compounds (for 10 nm particles < 1/100), allowing the vaporized semivolatile constituents of the particles to be collected efficiently in an annular flow-through activated carbon adsorber, while the denuded aerosol particles follow the gas streamlines and exit the denuder.(Verma et al., 2011) For this study, the thermal denuder was used at 120 °C because at that temperature there was no nucleation of new particles downstream of the heated zone of the denuder, which was important for a subsequent study of the effects of particle free organic constituents. (Pakbin et al., 2009)

Methods used for the chemical analyses of the CAPs and the collected semivolatile organics that had been stripped from the particles were reported in detail (Verma et al., 2011). Briefly, samples of particles were collected on fluorocarbon and quartz filters. One aliquot of the fluorocarbon filter was extracted with a mixture of hydrofluoric, hydrochloric and nitric acids which was evaporated to a known volume and analyzed for trace element composition using inductively-coupled plasma mass spectrometry (ICP-MS). A second aliquot of the fluorocarbon filter was extracted with aqueous carbonate-bicarbonate buffer and analyzed for cationic and anionic inorganic components using ion chromatography (IC). The quartz filter was sectioned, extracted with a mixture of organic solvents and analyzed for OC constituents using high resolution gas chromatography with mass spectrometric detection (GC/MS/MS).

3.2. Vascular Histology and Bioassays

All morphological assessments were done blind, i.e. without knowledge of the treatment group. At sacrifice, the mice were euthanized with an overdose of pentobarbital (65 mg/kg body weight). Segments of the thoracic aorta and the right bracheocephalid (A1) artery were removed and embedded in Optimal Cutting Temperature compound (OCT; Tissue-Tek, Sakura Finetek USA Inc, Torrance, CA) for frozen sectioning and Oil Red-O staining (Sun et al., 2005). Each cross-section was digitized and then analyzed using Image-J software (National Institutes of Health; NIH) to determine total atherosclerotic lesion area and lesion lipid content. Lesion areas were normalized by calculating them as a percentage of the total area of the cross-section of the lumen of the vessel. The lipid deposition in the arterial wall was measured and normalized by the total cross-sectional area of the arterial wall tissue to control for animal-to-animal differences in arterial geometry and wall thickness.

Blood serum samples were collected from the posterior vena cava and used to determine total cholesterol and LDL using previously described methods (Sukhova et al., 2003). Serum protein carbonyl content of the serum was measured using a fluorescein-5-thiosemicarbazide assay (Cayman Chemical Company, Ann Arbor, MI). Serum lipid peroxidation was determined via malondialdehyde assay (Gerard-Monnier et al., 1998). This assay, using 1-methyl-2-phenylindole to form an MDA chromophore, was performed in a hydrochloric acid-based medium, which enables the specific measurement of MDA in the presence of 4-hydroxyalkenals. Protein carbonyl and lipid peroxidation concentrations were normalized by total protein concentration which was measured using the Pierce bicinchoninic acid colorimetric assay (Pierce Biotechnology, Thermo Scientific, Rockford IL).

3.3. ECG Measurement and Analysis

12 male 7 week old mice from Jackson Laboratories (four per group) were surgically implanted with DSI TA-ETAF20 electrocardiographic