Systemic and Cardiovascular Effects of Airway Injury and Inflammation: Ultrafine Particle Exposure in Humans

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The concentration of particles in the ambient air is associated with deaths from cardiovascular disease, and determining the biologic mechanisms involved has been identified as a high-priority research need. Hypotheses have focused on the possibility of direct cardiac effects, or indirect effects related to inflammatory responses, including increased blood viscosity or increased blood coagulability. Ultrafine particles (UFPs; those smaller than 100 nm) may be important in cardiovascular effects because of their very high deposition efficiency in the pulmonary region, and their high propensity to penetrate the epithelium and reach interstitial sites. We have initiated human clinical studies of the health effects of UFPs using a mouthpiece exposure system. Healthy, nonsmoking subjects 18–55 years of age are exposed at rest for 2 hr to 10 μg/m^3 carbon UFPs and to filtered air as a control. Preliminary findings indicate a relatively high overall deposition fraction (0.66 ± 0.12 by particle number) consistent with model predictions and an absence of particle-associated symptoms or changes in lung function. Planned studies examine responses in susceptible subject groups, and the effects of particles of varying composition. Human clinical studies using model particles will complement other approaches such as epidemiologic, animal exposure, and *in vitro* studies in determining the mechanisms for heath effects related to ambient particle exposure. *Key words*: air pollution, cardiovascular, health effects, human, inflammation, ultrafine particles. — *Environ Health Perspect* 109(suppl 4):529–532 (2001).
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A growing body of evidence indicates that inhalation of particles in the air, especially particles from combustion-related sources, has potential health effects for susceptible people. A strong and consistent association has been observed between adjusted mortality rates and ambient particle concentrations, with an increase in the concentration of particulate matter with a mass median aerodynamic diameter less than 10 μm (PM$_{10}$) of 50 μg/m^3 associated with a 3–8% increase in relative risk of death (1). The strongest associations are seen for respiratory and cardiovascular deaths, particularly among the elderly. Associations with mortality have been observed at mean PM$_{10}$ concentrations well below the current National Ambient Air Quality Standard (NAAQS) for PM$_{10}$ of 150 μg/m^3 as a 24-hr average (1,2). Because of concern about the potential public health implications of these findings, the U.S. Environmental Protection Agency (U.S. EPA) promulgated a new standard for particulate matter with a mass median aerodynamic diameter of 2.5 μm (PM$_{2.5}$). Determining the biologic mechanisms involved has been identified as a high-priority research need by the U.S. EPA and the National Academy of Sciences (3,4).

The observed links between ambient particle concentrations and deaths from cardiovascular disease appear particularly puzzling (5). The overall mortality risk associated with an increase in PM$_{10}$ levels of 10 μg/m^3 has been estimated at about 1% (2). The highest risk is among patients with chronic obstructive pulmonary disease (COPD)—about 3.4%. The relative risk of cardiovascular mortality is lower (1.4%), but because cardiovascular deaths outnumber COPD deaths by a wide margin, pollution-related cardiovascular deaths actually outnumber COPD deaths. The risk for cardiovascular events does not appear to be explained by hypoxemia (6).

Recent studies in healthy and compromised animals from the laboratories of the U.S. EPA (7) and from Harvard University (8) have suggested that inhalation of particulate pollutants may induce changes in cardiac rhythm or repolarization. Panel studies have shown that particle exposure is associated with increases in heart rate among the elderly (6) and decreases in heart rate variability in active Boston residents (9).

Seaton et al. (10) have proposed that pollutant exposure induces a transient increase in blood coagulability as part of the acute-phase response associated with inflammation. This hypothesis is supported by the recent finding that plasma viscosity is increased on high-pollution days relative to low-pollution days in men and women 25–64 years of age (11). Blood markers of the acute-phase response, including C-reactive protein (CRP), fibrinogen, and interleukin (IL)-6 are associated with cardiovascular events and adverse outcomes (12,13). The combined effect of these processes, i.e., effects on myocardial function, increased blood viscosity, and increased blood coagulability, could precipitate adverse cardiac events in individuals with critical coronary lesions.

What are the specific particle characteristics contributing to cardiovascular effects? Hypotheses proposed to explain which particles are responsible have focused on issues related to particle acidity (14), particle content of transition metals (15), bioaerosols (16), and ultrafine particles (UFPs; those smaller than 100 nm) (10,17). Ambient UFPs may be important with regard to respiratory health effects for several reasons: *a*) UFPs are biologically more reactive than larger-sized particles and elicit effects at low concentrations. *b*) UFPs at the same mass concentration in the air have a much higher number concentration and surface area than larger particles. To achieve a low airborne concentration of 10 μg/m^3, 2.4×10^6 20 nm particles/cm^3 are needed; in contrast, only one 2.5 μm particle/cm^3 is needed to reach the same concentration (17). *c*) Inhaled singlet UFPs have very high deposition efficiency in the pulmonary region (e.g., 20-nm particles have up to 50% deposition efficiency (18). *d*) UFPs have a high propensity to penetrate the epithelium and reach interstitial sites (19). This raises the intriguing possibility that UFPs could enter the systemic circulation and induce direct effects on myocardium or coronary vasculature, although there is no direct evidence to support this possibility.

Our proposed pathophysiology for pollutant-related cardiovascular events is shown in Figure 1, and involves the following sequence of events:

a) Injury to epithelial cells by reactive oxygen species, accompanied by activation of nuclear regulatory factors, leading to elaboration of proinflammatory cytokines, including IL-8 and IL-6.

b) Activation of vascular endothelium and circulating polymorphonuclear leukocytes,

eosinophils, lymphocytes, and monocytes. Emigration of inflammatory cells from blood to tissue sites involves upregulation of adhesion molecules on vascular endothelium [E-selectin, P-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1] and on circulating leukocytes (L-selectin, lymphocyte function associated antigen-1, Mac-1, very late activation antigen-4, ICAM-1) (20). The events in the process of leukocyte–endothelial binding include increased expression followed by shedding of adhesion molecules as cells "tether and roll," cell activation, stable adhesion, and transmigration through the epithelium (21). Endothelial activation may contribute to the increase in exhaled nitric oxide (NO) concentrations seen with airway inflammation.

c) Increased release of IL-6 and tissue factor by activated blood mononuclear cells.

IL-6 initiates hepatic synthesis of acute-phase proteins, including CRP, serum amyloid A (SAA), fibrinogen, and plasminogen activator inhibitor-1. Monocyte tissue factor and endothelial cell activation initiate the coagulation cascade, as reflected by the presence of fibrinopeptide A (FPA) and $prothrombin_{1+2}$.

Each step in this pathogenic sequence can be assessed in human clinical studies, using minimally invasive markers from blood, exhaled air, induced sputum, and the continuously recorded electrocardiogram.

Clinical Studies of Exposure to UFPs

A strategy for human clinical studies of UFPs must assure subject safety, employ particle concentrations and composition relevant to ambient exposures, incorporate control exposures to clean, filtered air, and include determinations of particle retention for purposes of dosimetry. For safety reasons, our studies have been initiated with healthy subjects, with the goal of studying susceptible subject groups if there are minimal effects in healthy subjects. Also for safety reasons, we have chosen to use particles of carbon, which have been used in inhalation studies in animals,
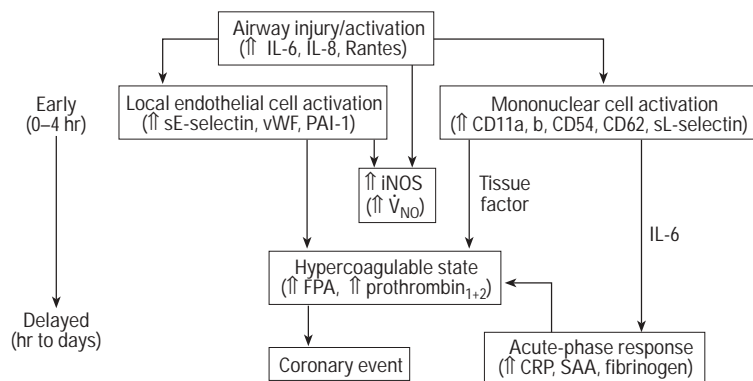
Figure 1. Hypothesized relationships between airway and cardiovascular responses to UFPs. iNOS, inducible nitric oxide synthase; sE-selectin, soluble E-selectin; sL-selectin, soluble L-selectin; V_{NO}, airway production of nitric oxide; vWF, von-Willebrand factor.
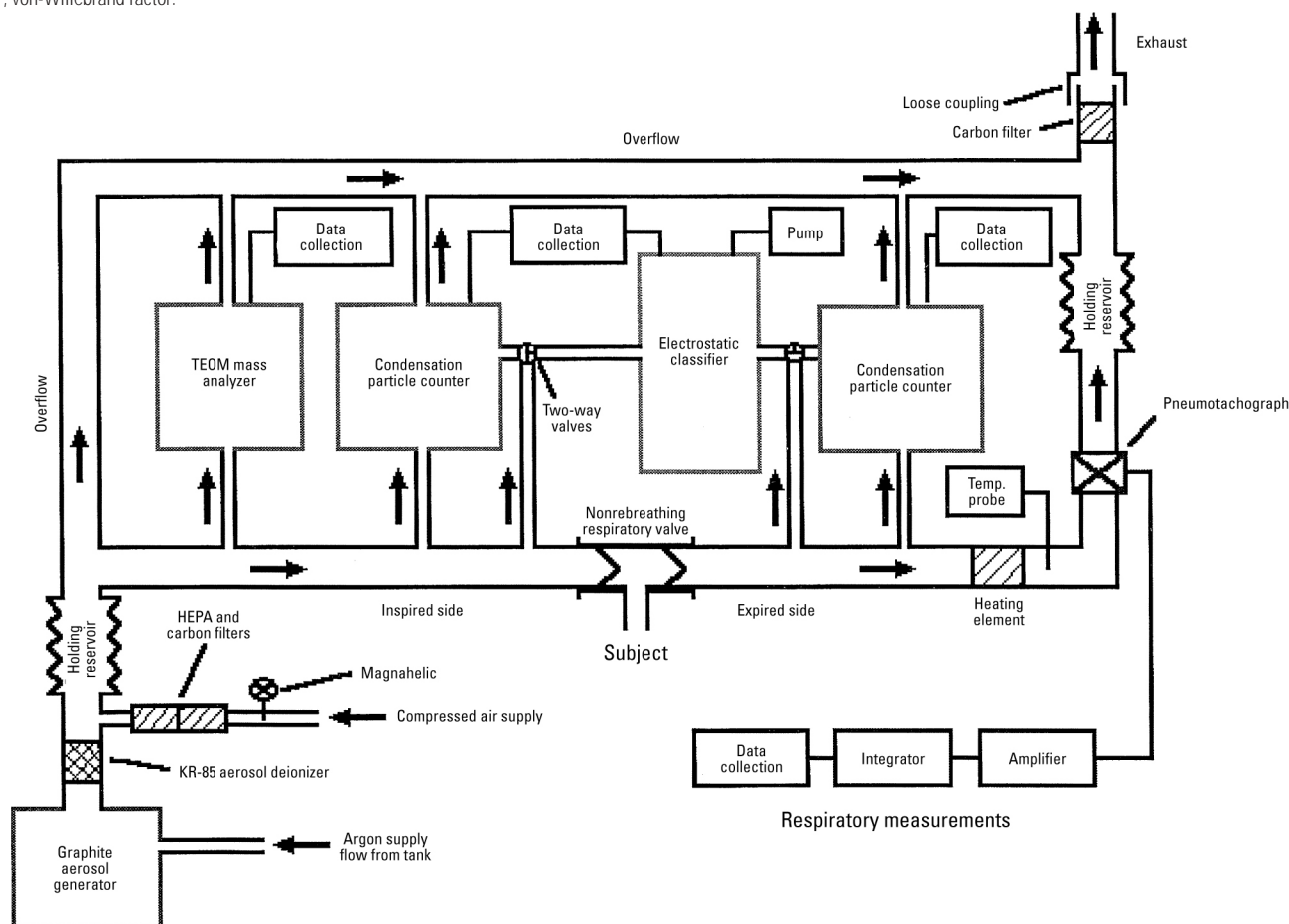
Figure 2. Design of a facility for human clinical studies of UFP exposure. TEOM, tapered element oscillating microbalance.

with no significant respiratory effects. These particles do not contain trace metals or endotoxin. For studies of UFP exposure, particles must be generated in real time so they remain in singlet form. Particle collection and resuspension results in agglomeration, with changes in particle size and surface area that influence deposition, and possible health effects.

We have developed a mouthpiece exposure system for human clinical studies of the health effects of UFPs. The system is designed to permit the quantitative determination of exposure levels, respiratory intakes, and depositions of aerosols of UFPs inhaled by human subjects. UFPs are generated in real time using a Palas generator (Karlsruhe, Germany). The carbon UFPs are generated by spark discharge in an argon atmosphere using pure graphite electrodes. Generated particles are diluted in purified air and delivered to the subject via a one-way nonrebreathing valve at a flow rate exceeding 80 L/min (Figure 2). Continuous measurements of particle mass, number, and size distribution are performed on both the inspiratory and expiratory sides of the subject. After correction for system losses, it is then possible to calculate total respiratory deposition, by particle number and mass, for each particle size fraction. Continuous measurements of minute ventilation (respiratory rate × tidal volume) also allow calculation of total inspired particle number and mass for each exposure.

For initial studies in healthy subjects, we have chosen an exposure mass concentration of 10 μg/m^3, corresponding to 2×10^6 particles/cm^3. This particle number is only slightly higher than that observed in outdoor environments. McMurry (22) measured UFP numbers exceeding 1,000,000/cm^3 in Atlanta, Georgia. We have observed peak particle numbers exceeding 1,700,000/cm^3 above a construction site outside an acute care hospital (23).

Healthy, nonsmoking subjects 18–55 years of age are exposed at rest for 2 hr to UFPs and to filtered air as a control, with a 10-min break off the mouthpiece after 1 hr. Exposures are double-blinded, randomized, and separated by at least 2 weeks. The total respiratory tract deposition fraction is determined for six different particle size fractions (midpoint diameters 7.5, 13.3, 23.7, 42.2, 75.0, and 133.4 nm) after correction for system losses, and the overall particle number and mass deposition is calculated for each subject. Respiratory symptoms, spirometry, blood pressure, pulse-oximetry, blood markers, and exhaled NO are assessed before and immediately, 3.5 hr, and 21 hr after exposure. Sputum induction is performed 21 hr after exposure to assess inflammatory cells in the conducting airways. Continuous 24-hr,

12-lead Holter monitoring is performed on the day of the exposure and analyzed for changes in heart rate variability and repolarization phenomena. Blood markers focus on parameters related to the acute-phase response, blood coagulability, and circulating leukocyte activation, including the following: complete blood leukocyte counts and differentials, IL-6, SAA, fibrinogen, and clotting factor VII. Immunofluorescence and flow cytometry are used to measure leukocyte expression of activation and adhesion molecules, including ICAM-1 and L-selectin on granulocytes and monocytes, and CD25 on lymphocytes.

Preliminary findings indicate absence of particle-associated symptoms or changes in lung function in healthy subjects (24). The overall deposition fraction was 0.66 ± 0.12 by number and 0.58 ± 0.014 by mass. The average number deposition fraction varied among subjects from 0.43 to 0.79. This relatively high overall deposition is consistent with predictions for particles in this size range (18).

Current studies are under way in healthy subjects incorporating exercise. Exercise has several effects that may enhance the effects of particle exposure. First, exercise increases respiratory minute ventilation, increasing the intake of the pollutant. Second, exercise induces adrenergic hormonal responses and changes in heart rate and heart rate variability. Finally, exercise of sufficient intensity induces a mild acute-phase response. These exercise-related effects may be enhanced by particle exposure.

Discussion

The observed associations between outdoor concentrations of fine particulate matter and health effects appear to be well established, and the remarkable consistency of the data suggests causality. However, the mechanisms by which inhaled particles result in increased morbidity and mortality have not been elucidated. We have proposed that UFPs may be important in the toxicity of the ambient fine particle fraction because of their relatively high surface area and potential for crossing the airway epithelial membrane.

The National Research Council Commission On Geosciences, Environment, and Resources has determined that data from human clinical studies are needed to identify the link between exposure and response in both healthy and susceptible populations (4). We have established a clinical facility for human exposures to laboratory-generated ultrafine carbon particles. Our preliminary studies in healthy subjects exposed at rest to ultrafine carbon particles at 10 μg/m^3 suggest no significant health effects. The relative absence of effects in these studies was expected because the least susceptible

subjects were chosen for these initial studies, the particle concentrations were low, and the particles were predicted to have low potential for toxicity.

Studies are under way with healthy subjects exposed during intermittent exercise, and planned studies will examine responses in susceptible subject groups, including patients with asthma. Future studies will use particles of varying composition, including incorporation of trace metals, and will compare the effects of UFPs with larger particles of similar composition and mass. These human clinical studies using model particles will complement ongoing studies of concentrated ambient fine particles, as well as epidemiologic, animal exposure, and *in vitro* studies, in determining the mechanisms for heath effects related to ambient particle exposure.

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