Does Inhalation of Ultrafine Particles Cause Pulmonary Vascular Effects in Humans?

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Inhalation of ambient particulate matter increases the risk of cardiovascular disease. Clinical studies play an important role in elucidating mechanisms for pollutant effects, and in establishing ambient air quality standards. Ultrafine particles (UFP; diameter <100 nm) may be important in the cardiovascular effects of ambient PM, yet there are few clinical studies of UFP health effects. Our laboratory has developed an exposure facility for clinical studies of laboratory-generated UFP. We confirmed previous predictions that UFP <50 nm in diameter deposit in the respiratory tract with a high efficiency, and have shown that exercise or the presence of asthma further increases UFP deposition. UFP exposure with exercise reduced expression of selected adhesion molecules on blood leukocytes, and also decreased the pulmonary diffusing capacity for carbon monoxide. These findings are best explained by UFP effects on pulmonary vascular function. These findings provide a possible mechanism by which inhalation of UFP may contribute to cardiopulmonary health effects in susceptible people.

Ambient air pollution particles enter the body through the lung, and deposited particles first encounter the respiratory epithelium. Particles that are inhaled and escape deposition in the upper airway can deposit in the conducting or alveolar airways. They may then be cleared from the airways via the mucociliary ladder, or they may be ingested by alveolar macrophages and either cleared via the airways or carried to regional lymph nodes. It would seem logical that adverse effects of inhaled agents would be primarily pulmonary in nature, and it is therefore not surprising that many epidemiology studies find increased respiratory morbidity and mortality associated with increases in ambient concentrations of particulate matter (PM). Cardiovascular effects would be expected only as a consequence of failure of the vital functions of the respiratory tract, for example, hypoxemia causing myocardial ischemia (Utell & Frampton, 1995).

The evidence is now quite convincing that inhalation of ambient particulate matter increases the risk for adverse cardiovascular events (Godleski, 2006; Utell et al., 2002), and there is growing evidence that long-term PM exposure may hasten the progression of atherosclerosis (Künzli et al., 2005). A review of the supporting literature is beyond the scope of this article. However, a growing number of epidemiological studies suggest that PM has both acute (days) and chronic (months to years) cardiovascular health effects (Schwartz, 2006). A recent study (Dominici et al., 2006) examined hospitalization rates of 11.5 million Medicare enrollees in 204 U.S. urban counties in relation to changes in ambient concentrations of particulate matter ≤2.5 μm in aerodynamic diameter (PM$_{2.5}$). Hospital admission rates for both pulmonary and cardiovascular diagnoses were strongly associated with daily increases in exposure to PM$_{2.5}$.

Interestingly, the strongest association was for heart failure, with a 1.28% increase in risk per 10-μg/m$^3$ increase in same-day PM$_{2.5}$.

The methods used to assess the health effects of exposure to ambient air particulate matter include epidemiological studies, panel studies of small groups of individuals, animal studies, in vitro studies, and human clinical studies. Clinical studies play an important role in elucidating mechanisms, and in establishing ambient air quality standards for particulate matter and other pollutants (Frampton et al., 2006a). However, clinical studies have limitations. Generally, they are limited to examining acute effects. The relatively small number of subjects that can be feasibly studied limits statistical power. In addition, there is a limited ability to control subjects’ prior exposures to environmental pollutants. In addition, it is not feasible to study those most susceptible to the health effects of ambient air pollution, such as people with severe chronic obstructive pulmonary disease or...
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healthy subjects inhaled 10 μm/m³ UFP (count median diameter [CMD] 26 nm, geometric standard deviation [GSD] 1.6) by mouthpiece for 2 h, with intermittent exercise. The fractional deposition by particle number at rest was 0.66 ± 0.11, a value in accordance with predictions models (ICRP, 1994). Surprisingly, the deposition fraction increased during exercise to 0.83 ± 0.04 (Figure 1). In a separate study (Chalupa et al., 2004), subjects with mild asthma showed a resting particle deposition fraction of 0.76 ± 0.05, significantly higher than for resting healthy subjects. In both healthy and asthmatic subjects, the number deposition fraction during exercise exceeded predicted values. These findings indicate that UFP deposit in the respiratory tract with much greater efficiency than particles in the fine size range, and that deposition increases further with exercise and in people with asthma. Exercise substantially increases the number of particles inhaled because of increased minute ventilation; when combined with the increase in deposition efficiency during exercise, UFP dose to the deep lung is increased many-fold during exercise.

Over the past few years, our laboratory has conducted several studies, using both healthy and asthmatic subjects, with exposures to elemental carbon UFP from 10 to 50 μg/m³. In general, these exposures did not cause symptoms, and subjects were unable to differentiate particle or air exposures. The studies have shown no effects on pulmonary function testing, airway inflammation (as measured by exhaled nitric oxide and inflammatory cells in induced sputum) or soluble markers of inflammation and coagulation (Pietropaoli et al., 2004a, 2004b).

An objective of these studies was to develop sensitive markers of both pulmonary and systemic vascular effects. One such tool is characterization of peripheral blood leukocytes using immunofluorescence staining and flow cytometry. We hypothesized that if inhalation of ultrafine particles activates pulmonary or systemic endothelial cells, by either direct or indirect mechanisms, we would find subtle changes in the surface expression of vascular adhesion molecules on blood leukocytes.

Flow cytometry was used to evaluate changes in leukocyte surface molecules in four separate exposure protocols, three in healthy subjects, and one in subjects with asthma. Each study
utilized a randomized, double-blinded, crossover design, with each subject exposed to both UFP and filtered air for 2 h on separate occasions at least 3 wk apart. UFP were freshly generated elemental carbon particles (CMD ~25 nm, GSD ~1.6). Peripheral venous blood was obtained before and at intervals after exposure.

In healthy subjects, particle exposure with exercise reduced expression of adhesion molecules, particularly CD54 (intercellular adhesion molecule-1) and CD18 (part of the B2 integrin adhesion molecule complex) on monocytes, and CD18 and CD49d (part of the B1 integrin adhesion molecule complex) on granulocytes (Frampton et al., 2006b). Figure 2 shows the change in monocyte expression of CD54 after exposure to air, and to UFP at two concentrations. Monocyte CD54 expression increased after air exposure, most likely as a consequence of the exercise performed during exposure. After UFP exposure, CD54 expression decreased relative to air at 3.5 h after UFP exposure, in a concentration-responsive fashion. In the subjects with asthma, exposure to 10 μg/m³ UFP decreased expression of CD11b (another part of the B2 integrin adhesion molecule complex) on monocytes and eosinophils, and CD54 on granulocytes. Asthmatics also showed a reduction in the percentage of CD4-positive T cells, basophils, and eosinophils in peripheral blood. We hypothesized that these reductions in leukocyte subsets and in surface expression of adhesion molecules reflected an increased retention of leukocytes in the pulmonary vascular bed following exposure to UFP. In other words, it is possible that subtle changes in pulmonary capillary blood volume or flow distribution, induced by inhaling UFP for 2 h, changed the time it takes for some blood leukocytes to get through the lungs, and this was detected as changes in peripheral blood leukocyte surface adhesion molecules.

In order to test the hypothesis that UFP exposure affected the pulmonary circulation, we measured the pulmonary diffusing capacity for carbon monoxide (DLCO) before and after exposure to 50 μg/m³ carbon UFP (Pietropaoli et al., 2004a). The DLCO is sensitive to changes in pulmonary vascular perfusion and pulmonary capillary blood volume. We postulated that pulmonary vascular changes induced by inhalation of UFP would reduce the uptake of CO from the lungs. Indeed, as shown in Figure 3, exposure to 50 μg/m³ carbon UFP for 2 h significantly decreased the DLCO 24 h after exposure (UFP: –0.76 ± 0.66 ml/min/mm Hg, versus air: –0.18 ± 0.41 ml/min/mm Hg, p = .040). There were no accompanying changes in oxygen saturation as measured by pulse oximetry.

DISCUSSION

These findings provide indirect evidence that inhalation of carbon UFP has subtle pulmonary vasoconstrictive effects, effectively prolonging transit time through the pulmonary circulation for leukocytes that are more activated, and therefore less deformable. It is well established that leukocytes must deform considerably in order to navigate the pulmonary capillary bed. At any given time, at rest, the majority of blood leukocytes in humans are in the pulmonary vascular bed, because it takes several seconds for leukocytes to traverse the pulmonary capillaries (Doerschuk, 2003). Narrowing of pulmonary capillaries by increasing intrathoracic pressure lengthens the time required for leukocytes to move through the pulmonary circulation (Markos et al., 1990, 1993). Exercise “flushes out” pulmonary vascular leukocytes as a consequence of the increased blood flow and capillary dilatation. Activation of monocytes and polymorphonuclear leukocytes (PMNs) increases expression of the CD11b/CD18 integrin complex, and decreases cell deformability through actin polymerization (Anderson et al., 2001). Exercise is known to increase expression of CD11b on peripheral blood PMNs (van Eeden et al., 1999), suggesting that cells expressing higher levels of CD11b are preferentially marginated in the pulmonary circulation, and these cells increase in the
peripheral circulation following exercise. Animal experimental data provide supportive evidence that PM has pulmonary vaso-
constrictive effects. Rats exposed to concentrated ambient fine particles showed reductions in the size of the pulmonary arteri-
oles (Batalha et al., 2002).

Our laboratory has examined the effects of ultrafine zinc ox-
ide ultrafine particles, at concentrations much higher than ambi-
ent (Beckett et al., 2005). In that study, 12 healthy adults inhaled 500 μg/m³ of ultrafine zinc oxide and, on a separate occasion, the same mass of larger, fine zinc oxide particles, compared with exposure to filtered air. Interestingly, we did not find effects on blood leukocyte subsets or adhesion molecule expression with either the ultrafine or fine particle exposures. In this study, even though the exposure mass concentration was at least 10-fold higher than for the studies with carbon particles, there were no changes in monocyte or PMN expression of surface adhesion molecules. This might reflect the fact that zinc oxide particles are soluble, and therefore would not be expected to persist as physical particles after dissolution in the alveolar lining fluid.

In summary, these studies provide evidence that inhalation of carbon UFP, with intermittent exercise, has effects on pul-
monary vascular function. These effects would not be expected to indicate adverse health consequences for healthy individu-
als. However, these findings provide a possible mechanism by which inhalation of UFP may contribute to cardiopulmonary dysfunction in patients with severely compromised respiratory or cardiovascular status, such as individuals with congestive heart failure, severe chronic obstructive pulmonary disease with cor pulmonale, or severe pulmonary hypertension.

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