OVERVIEW OF THE PMHEALTH EFECTS RESEARCH PROGRAM

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Abstract

We tested the hypothesis that exposure of healthy volunteers to concentrated ambient particles (CAPS) is associated with an influx of inflammatory cells into the lower respiratory tract. Thirty-eight volunteers were exposed to either filtered air or particles concentrated from the immediate environment of the EPA Human Studies Facility in Chapel Hill, NC. Particle concentrations in the chamber during the exposures ranged from 23.1 - 311.1 $\mu g/m^3$. Volunteers alternated between moderate exercise (15 minutes) and rest (15 minutes) for a total exposure time of 2 hours. There were no symptoms noted by volunteers after the exposure. Similarly, there were no decrements in pulmonary function. Eighteen hours after exposure, analysis of cells and fluid obtained by bronchoalveolar lavage showed an increase in neutrophils in both the bronchial and alveolar fractions in those individuals exposed to CAPS. Blood obtained after exposure to CAPS contained significantly more fibrinogen relative to samples obtained prior to exposure. Previous studies of cytokine induction by PM_{10} pollution particulates in alveolar macrophages have indicated that endotoxin is an active component in outdoor pollution. Collection of $PM_{2.5}$ and $PM_{2.5\text{-}10}$ particles of teflon filters here in Chapel Hill indicated that cytokine inducing activity was associated with a soluble component of the larger particles, but not the smaller ones. Inhibition of this activity by LPS binding protein or polymyxin B, but not a metal chelator, again implied that endotoxin was present and accounted for all actvitity. When exposing macrophages to the $PM_{2.5\text{-}10}$ particles themselves, cytokines were induced at > 50 fold higher levels than with the soluble fraction, while $PM_{2.5}$ particles increased cytokine production approx. 5 fold compared to control. Pretreating the particles with 10 ug/ml polymyxinB reduced the activity > 50%, but not completely, suggesting that other components in the course particles may contribute to cytokine production. Individual outdoor air filters collected daily over a month implied that while water extractable endotoxin levels were relatively low and ranged 0-1.2 EU/filter (cholorimetric limulus assay), bioactivities of the extracts were much more variable. Either environmental endotoxins vary in their activating potential or other components synergize with endotoxin for cytokine production. Furthermore, $PM_{2.5\text{-}10}$ inhibit phagocytosis most likely by affecting the viability of macrophages, which undergo apoptosis upon exposure. We conclude that moderate levels of ambient air particles are capable of inducing an inflammation in the lower respiratory tract as well as an increased concentration of blood fibrinogen and some portion of this effect may be associated with endotoxin content.

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Introduction

Epidemiologic studies have established an association between exposures to air pollution particles and human mortality and morbidity at concentrations currently found in major American metropolitan areas (Dockery and Pop 1994). This association has been documented in numerous investigations around the world and is remarkably consistent (UN Environment Program and WHO Report). Air pollution particles are also associated with an increased incidence of both pulmonary infections and hospitalization for respiratory disease (Bates and Sizto 1983; Dockery et. al., 1989; Pope 1989).

Despite this impressive association between particles and human mortality/morbidity, fundamental uncertainties exist as to which physical and/or chemical properties increase risk. In addition, pathophysiological mechanisms have not been determined. This uncertainty arises from the difficulty of exposing humans and animals to particles that are considered equivalent to those which populations included in the epidemiologic studies inhaled. Particles collected from specific emission sources have been demonstrated to cause an incursion of neutrophils into the lung, an injury, and changes in host defense capability in both animals and humans (Pritchard et. al., 1996; Salvi et. al., 1999). However, these specific emission sources make up a variable and rather small component of most urban airsheds making it difficult to distinguish their exact contribution to human morbidity and mortality after exposure to ambient air particles.

Particles collected from ambient air and instilled into animals can also cause a neutrophilic inflammation and lung injury (Pritchard et. al., 1996; Vincent et. al., 1997). However, the recovery of the particles on either filters or in baghouses is extremely variable. It is also uncertain whether all components can be retrieved in quantities reflecting the original particle (e.g. organics and ammonium are too volatile to be collected). In addition, these particles have been instilled at considerably higher concentrations than would be inhaled by individuals in a real world setting. Finally, there is considerable controversy as to whether this route of exposure (i.e. instillation) is equivalent to inhalation. All of these limitations make it difficult to extrapolate the results of these studies to epidemiology findings.

The recent development of ambient particle concentrators has made it possible to perform controlled exposures of animals

and humans by inhalation of "real world" particles. Concentrators allow the measurement of a range of cardiopulmonary responses and therefore testing of specific hypotheses at pertinent particle masses. Initial findings from Harvard demonstrate that particles between 0.1 and 2.5 microns are effectively concentrated, while gases and smaller particles are not (Sioutas et. al., 1997). There also does not appear to be appreciable loss of individual particle components such as metal, sulfates, nitrates, acids, elemental and organic carbon, and general classes of organics.

In the Chapel Hill area of North Carolina, ambient particulate mass is driven primarily by mobile sources such as automobiles and is similar in size distribution and chemical composition to that found in many east coast cities, albeit at lower concentrations. Concentration of particles found in Chapel Hill air 6-10 fold allows controlled exposure of humans to similar concentrations of particles as seen in many major metropolitan areas. We tested the hypothesis that concentrated particles can cause a neutrophilic inflammation in the lungs of healthy humans. In this study 38 healthy young volunteers were exposed to either filtered air (n=8) or concentrated particles ranging from 30-300 $\mu g/m^3$ (n=30). Changes in lung function, hematological parameters, and both lung inflammation and injury were measured following a two hour exposure to ambient particles.

In addition, the mechanism(s) of lung injury after exposure to air pollution particles is not known. Injury has been postulated to be mediated by ultrafine particles, endotoxin, acid aerosols, polyaromatic hydrocarbons included in the organic components of the particle, and metal-catalyzed oxidative stress. We present data suggesting a participation of endotoxin in the biological effects of particulate matter.

Materials and Methods

Ambient Aerosol Exposure System

Particles between the sizes of 0.1 and 2.5 microns present in the Chapel Hill air were concentrated using a Harvard/EPA Ambient Fine Particle Concentrator (HAPC) consisting of three-stage virtual impactors. The principles by which this concentrator works have been previously described (Sioutas et. al., 1995; Sioutas et. al., 1997). The concentrator utilizes the inertial separator technique, thus concentrating particles only, not gases. Briefly, outside air is first drawn through an Anderson high volume conventional impactor with a 2.5 μm cut-off size at a rate of 5000 L/min. The exit flow from the Anderson impactor, which contains particles mainly < 2.5 μm in diameter, is drawn into the first stage of the concentrator in which 5 virtual impactor slits (1000 L/minute per slit) are arranged in parallel. The virtual impactor consists of two parts: the upper part is in the form of a rectangular nozzle through which airflow is accelerated and the lower part is in the form of a sharp-edged slit which receives impinging particles. Each virtual impactor operates at a minor to total flow ratio of 0.2 so that 80% of airflow ejected from the rectangular nozzle is deflected to the side stream (i.e., major flow) and 20% of the flow is extracted straight down into the receiving slit (i.e., minor flow). In this design, particles > 0.1 μm achieve a sufficient momentum to cut across the deflecting major flow stream and impinge into the receiving slit, whereas particles < 0.1 μm follow the major stream and are exhausted. For this reason, particles smaller than 0.1 micron are not concentrated. Ideally, if all particles between 0.1 and 2.5 μm are condensed into the minor flow, particle concentration in the minor flow will increase by 5 times. In the present system, particles in the size range of 0.1 - 2.5 micron are concentrated about 2.5 - 3 fold in the first stage and a combined flow from five receiving slits (minor flow) is drawn into the second stage at the rate of 1000 L/min. The second stage consists of a single virtual impactor identical to those in the first stage. Here, particles are concentrated 2.5 - 3 fold again and drawn into the third stage at the rate of 200 L/min. A single virtual impactor in the third stage operates at a minor to total flow ratio of 0.4 and concentrates particles about 2 fold at a flow rate of 80 L/minute. Finally, the concentrated aerosols leaving the third stage are mixed with 120 L/min of clean and conditioned air (20° C and 50% relative humidity) and the resulting conditioned aerosols are delivered into the exposure chamber at the rate of 200 L/minute. The addition of the conditioned air dilutes the concentrated aerosols, but provides consistent temperature and humidity. Overall, particles have been concentrated 6-10 fold at the inlet of the chamber. Sham exposures are conducted by using 200 L/minute of conditioned air and no air from the HAPC.

The maximum concentration of aerosols to be delivered to the chamber varied depending on concentrations of naturally occurring aerosol in the Chapel Hill air (which usually ranges 5 to 30 $\mu g/m^3$). The exposure chamber is 4.0 x 6.7 x 7.5 feet in size and constructed with aluminum panels and heavy duty clear plexyglass for doors and windows. Because the air pumping units are located downstream of the chamber and HAPC, the chamber is operated under a slightly negative pressure (10-12 inches of water). Aerosols enter the chamber via a 6" diameter curved duct positioned on the top and middle of the chamber and exit via an exhaust duct positioned in the middle of one of the vertical walls. The subject sits between the inlet and exit duct with his/her head located less than 18 inches away from the inlet duct. A series of tests conducted in the present study have shown that the particle concentration at the subject's head position is at least 90% of that at the inlet duct.

Particle Characterization

Air was sampled just prior to entering the HAPC and again just before entering the chamber from the inlet duct. Particles were collected on pre-weighed 47 mm teflon filters (2 μm

pore, Gelman Sciences, Ann Arbor, MI) at a flow rate of 10 L/min for 2 hours during the exposure. Filters were weighed on an electrobalance (Mettler UMT2) in a temperature (20° C) and humidity (45%) controlled room. This balance has a capacity to reliably weigh masses as low as 1 micron. The end net filter weight, sampling time, and flow rate were used to calculate the particle concentration in $\mu g/m^3$. Filters with sequestered PM (both before and after concentration) were analyzed for those metals most frequently found in greatest quantity (iron and zinc) and sulfur using X-ray fluorescence (XRF) (Lawrence Livermore Labs, San Francisco, CA).

The particle size distribution was obtained using a micro-orifice uniform deposit impactor (MOUDI, MSP Corporation, Minneapolis, MN), which is an eight stage cascade impactor containing a series of micro-orifices that collect particles onto pre-weighed 37 mm Teflon filters (2 μm pore, Gelman Sciences, Ann Arbor, MI). Aerosols were sampled from the inlet duct at a flow rate of 30 L/min for 2 hours. The filter substrates from the impactor were weighed under a controlled environment following the same procedure described above for total filter samples. The weights from each stage were used to determine the mass median aerodynamic diameter (MMAD) and the geometric standard deviation (GSD). These data are reported in Table 1.

Study Population

Volunteers responding to a newspaper advertisement were pre-screened over the telephone using the following criteria: age between 18 and 40 years old; non-smokers for at least 5 years prior to study; no history of allergies or respiratory diseases (food allergy, hay fever, dust allergies, rhinitis, asthma, chronic bronchitis, chronic obstructive pulmonary disease, tuberculosis, hemoptysis or recurrent pneumonia); and not presently on any medication prescribed by a physician (except birth control pills). A urine pregnancy test was performed on all female subjects and a positive result excluded the subject from further participation in this study.

Prior to participation in the study, subjects were informed of the procedures and potential risks and each signed a statement of informed consent. The protocol and consent form were approved by the University of North Carolina School of Medicine Committee on the Protection of the Rights of Human Subjects. The screening procedures for each subject included a Minnesota Multiphasic Personality Inventory, medical history, physical examination, chest X-ray, and routine hematologic and biochemical tests.

Exposure to CAPS

Each volunteer had a single exposure to either filtered air or CAPS. Subjects were monitored continuously using telemetry and arterial saturation by oxygen. Total exposure time was two hours. Subjects entered the exposure chamber (200 cubic feet) and sat on a recumbent bicycle ergometer. Subjects exercised for thirty minutes of each hour. The schedule of exercise was 15 minutes on a cycle ergometer, 15 minutes rest, and this was repeated four times. Exercise intensity, i.e. cycle ergometer workload, was adjusted so that subjects breathed at a ventilatory rate, normalized for body surface area, of 25 $L/m^{2\cdot}$ minute. In most subjects this will be about 50 L/minute (i.e. a VO_2 of approximately 1.0 L/m). A cycle ergometer work setting of 75 to 100 watts achieved such a physiological response. During the two hour exposure, particle concentrations were monitored continuously at the inlet duct of the chamber by using Tapered Element Oscillating Microbalance (TEOM, Series 1400a, Rupprecht & Patashnick, Inc., Albany, NY). TEOM was used to monitor a consistency or short-term excursion of exposure concentration. The average exposure concentrations were determined by filter samples as described above.

Venipuncture and Pulmonary Function Testing

Venous blood was sampled from an antecubital site immediately before and 18 hours after the exposure. Measurements included a complete blood count, ferritin, blood viscosity, and fibrinogen (LabCorp, Burlington, NC).

Spirometry and plethysmographic measurement of airway resistance were similarly measured immediately before and 18 hours after the exposure. The subject inhaled completely and then exhaled rapidly and completely via a tube into a spirometer. From this maneuver, forced vital capacity (FVC), forced expiratory volume in one second (FEV_1), and peak flow (PEF) were derived. For the plethysmographic measurement of airway resistance (Raw), subjects sat in a body box and performed a brief (20 seconds) panting maneuver at 1.5 Hz through a pneumotachograph. Airflow was occluded midway through the maneuver. By measurement of unoccluded airflow, pressure at the mouth during occlusion and pressure in the box under both conditions, both thoracic gas volume and airway resistance to airflow were quantified.

Bronchoscopy with Lavage

Using a standard protocol (Ghio et. al., 1998), the volunteers underwent bronchoscopy with lavage 18 hours after exposure to either the filtered air or CAPS. The fiberoptic bronchoscope was wedged into a segmental bronchus of the lingula. Four aliquots of sterile saline were instilled and immediately aspirated. The first was 20 ml and this fraction was labeled the bronchial sample. The remaining three aliquots were 50 ml each and the return from this bronchoalveolar lavage (BAL) is considered to reflect the environment of the distal respiratory tract. These were designated the alveolar sample. The procedure was repeated on the right middle lobe again using 170 ml saline. Samples were put on ice immediately after aspiration and centrifuged

at 300 x g for 10 minutes at 4° C. Total cell counts and differentials were obtained using a hemocytometer. The supernatant was assayed for total protein, IL-8, IL-6, PGE_2, fibronectin, and alpha-1 anti-trypsin concentrations.

Acquisition of Macrophages for In Vitro Studies

Healthy, nonsmoking volunteers 18 to 35 years of age underwent fiberoptic bronchoscopy with lavage (Ghio et. al., 1998). The fiberoptic bronchoscope was wedged into a segmental bronchus of the lingula. Six aliquots of sterile saline were instilled and immediately aspirated. The first was 20 ml and this fraction was labeled the bronchial sample. The remaining five aliquots were 50 ml each and the return from these are considered to reflect the environment of the distal respiratory tract. These were designated the alveolar sample. The procedure was repeated on the right middle lobe again using 270 ml saline. Samples were put on ice immediately after aspiration and centrifuged at 300 x g for 10 minutes at 4° C. Total cell counts and differentials were obtained using a hemocytometer.

Statistics

Data are expressed as mean values ± standard error. Relationships between continuous variables were evaluated employing linear regression techniques. Differences between two groups were tested using the T-test of independent means while those between multiple groups were compared using one-way analysis of variance (Colton 1974). The post-hoc test employed was Scheffe's test. Two-tailed tests of significance were employed. Significance was assumed at $p<.05$.

Results

Study Population and Exposure

The subject population included 38 volunteers (36 males and 2 females; 26.2 ± 0.7 years old). There were eight exposures to filtered air and 30 exposures to CAPS (PM mass of 120.5 ± 14.0 $\mu g/m^3$). There was a substantial range in CAPS exposures reflecting the variation outside the facility with individual exposures ranging from 23.1 - 311.1 $\mu g/m^3$ (Table 1). The study population was divided into quartiles with the eight individuals exposed to filtered air (Quartile 1) and the remaining 30 exposures arranged into groups of ten with ascending PM mass (Quartiles 2, 3, and 4). Differences between the quartiles in PM mass were significant ($F= 41.2$; $p<.0001$). Taking into account time of exposure and ventilation rates (i.e. 50 L/minute), we estimate that individual lung exposures approximated a total dose of 1200 µg on those days with the highest PM mass (i.e. Quartile 4).

For all PM exposures, the concentration factor was 6.5 ± 0.9. Measurement of iron, zinc, and sulfur by XRF verified concentration factors which approximated the value for total mass (8.5 ± 4.4, 10.8 ± 3.9, and 6.8 ± 1.4 respectively). The size distribution of exposure aerosols was approximately log-normal with the values of MMAD (mass median aerodynamic diameter) and GSD (geometric standard deviation) being 0.65 ± 0.03 and ~2.35 respectively. There was a slight increase in MMAD from 0.54 to 0.72 µm with an increase in mass concentration from Quartile 2 to Quartile 4.

Changes in Symptoms and Lung Function

Subjects did not report any symptoms either immediately or 18 hrs after exposure to air or CAPS, nor were any abnormalities observed upon physical examination of the subjects. There were no significant differences in FEV_1, FVC, Raw, or PEF in the subjects exposed to CAPS (Table 2). Using Knudson's criteria to provide predicted values (Knudson et. al., 1983), all spirometric indices were normal.

Changes in Peripheral Blood

A recent epidemiology study has reported an association between ambient air particles and increased blood viscosity (Peters et. al., 1997). Therefore, a number of hematological parameters involved in inflammation, acute phase response, and blood viscosity or clotting were measured (Table 3). Exposure to filtered air or to CAPS had no effect on numbers of neutrophils, lymphocytes, monocytes, RBCs, or platelets in the blood 18 hours following exposure. This was true whether the data were analyzed as air versus all CAPS or broken into quartiles for analysis. Similarly there were no differences in hemoglobin, blood viscosity, or ferritin between groups exposed to air and CAPS. However, significant differences were observed in the concentration of blood fibrinogen between air and CAPS exposed subjects ($p= 0.009$). All three CAPS quartiles showed a similar change between post- and pre-exposure values (38 - 43 mg/dL), indicating there was no dependence on dose (Figure 1).

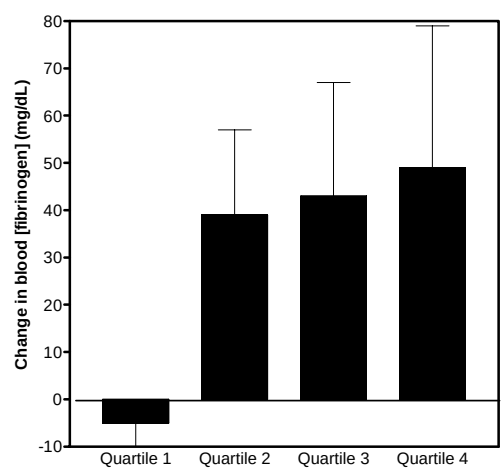

Figure 1. Changes in the concentration of blood fibrinogen after exposures to CAP and filtered air. Blood fibrinogen concentrations increased with particle inhalation relative to filtered air but this elevation did not reach significance. The

concentration of the acute phase reactant appeared to be dependent on exposure and increased with PM mass.

Changes in Bronchial Lavage Fluid and Cells

Total cells present in the bronchial lavage fraction (BL) were not effected by CAPS exposure (Table 4). There were no CAPS induced changes in percentages of macrophages, lymphocytes, and epithelial cells in the bronchial fraction. However, there was a significant CAPS induced increase in percent neutrophils in the bronchial lavage, with particle-exposed individuals having 8.1 ± 2.7% and air-exposed individuals having 2.7 ± 0.6% neutrophils (p=0.001). In addition there was a statistically significant increase in monocytes (0.76 ± 0.12% after CAPS exposures and 0.36 ± 0.13% for air exposures; p=0.010). There was also a significant increase in the number of neutrophils present in BL fluid (6.6 ± 1.7 $x10^5$ for CAPS exposures and 1.8 ± 0.05 $x10^5$ for air exposures; p= 0.011). Post-hoc testing following ANOVA revealed significant differences only between the first and third quartiles in total numbers of neutrophils (Figure 2).

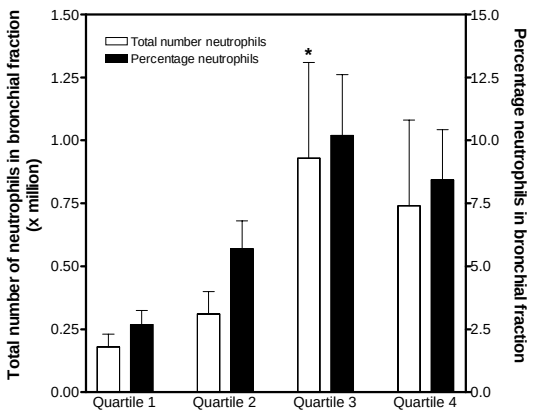

Figure 2. Neutrophil numbers in the bronchial fraction after inhalation of particles and filtered air. The total number of neutrophils in the bronchial fraction of the lavage increased with particle inhalation. Percentage neutrophils were comparably increased.

Concentrations of IL-8, IL-6, PGE_2, α1-antitrypsin, and fibronectin in the bronchial fluid were not changed after CAPS inhalation (Table 5). The concentration of protein in the bronchial fluid was decreased following exposure to CAPS; BL of air-exposed subjects contained 57.5 ± 5.7 µg/ml while BL of particle-exposed subjects contained 35.9 ± 2.5 µg/ml (p= .0006). Post-hoc tests following ANOVA revealed significant differences between the first quartile (filtered air) and the other three quartiles.

Changes in Bronchoalveolar Lavage Fluid and Cells

Total cells found in BAL fluid were increased in those individuals exposed to CAPS (Table 6). Individuals exposed to air had 15.9 ± 1.9 $x10^6$ cells while those exposed to CAPS had 21.4 ± 1.3 x 10^6 cells (p= 0.04). The percentage of macrophages, lymphocytes, monocytes, and epithelial cells were not increased after CAPS exposure. However, the percentage of neutrophils significantly increased following particle exposure (2.5 ± 0.6 for CAPS and 0.8 ± 0.3 for air; p= 0.016). In addition, absolute numbers of neutrophils were increased in BAL fluid following CAPS exposure (0.56 x 10^6) as compared with air exposure (0.09 x 10^6) (p= 0.0013). Neutrophil influx appeared to be dependent on dose with the greatest elevations occurring in the those subjects exposed to the highest concentration of particles (F= 2.9; p= .05) (Figure 3).

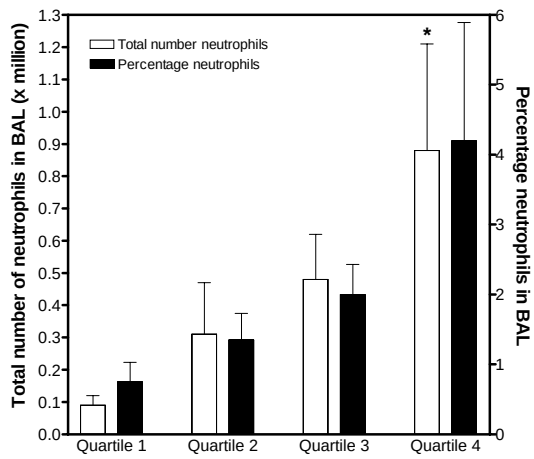

Figure 3. Neutrophil numbers in the bronchoalveolar lavage after inhalation of particles and filtered air. The total number of neutrophils in the bronchoalveolar lavage increased with particle inhalation. Percentage neutrophils were comparably increased

There was also an increase in the total number of monocytes found in the BAL of CAPS-exposed individuals (2.7 ± 0.4 $x10^5$) compared with air-exposed subjects (1.8 ± 0.3 $x10^5$), although the increase did not quite reach statistical significance (p= 0.08).

Concentrations of IL-6, IL-8, PGE_2, α1-antitrypsin, and fibronectin in BAL fluid did not change with exposure to CAPS (Table 7). Interestingly, IL-8 levels were considerably lower in the most heavily exposed individuals (66.4 ± 21.7 pg/ml) compared with those exposed to air (189.3 ± 52.1 pg/ml), although these differences did not reach statistical significance. In contrast with the bronchial fraction, protein concentrations in BAL fluid were not significantly different after inhalation of CAPS relative to air. Somewhat surprisingly, fibrinogen levels in BAL fluid were decreased in CAPS-exposed individuals (15.2 ± 1.4 mg/dL), compared with air-exposed subjects, who had 23.2 ± 2.1 mg/dL in their BAL fluid (p= 0.009).

In vitro alveolar macrophage investigation verified that endotoxin does participate in a biological effect of PM. Incubation of alveolar macrophages with the aqeuous extract of filters signficiantly increased chemiluminescence by the phagocytes (Figure 4). The greater part of the chemiluminescence was induced by the larger fraction of the PM (both the particles themselves and the aqueous extract denoted "ex").

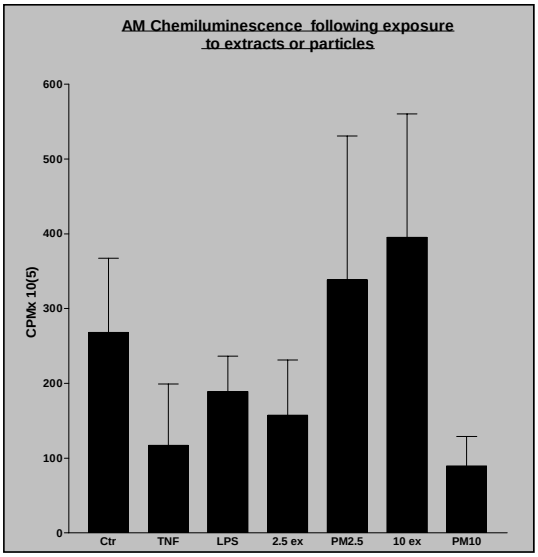

Figure 4. Chemiluminescence of alveolar macrophages (200,000/mL) after stimulation by particulate matter isolated from filters of varying size ($PM_{2.5}$vs PM2.5-10). The aqeuous soluble component denoted by "ex".

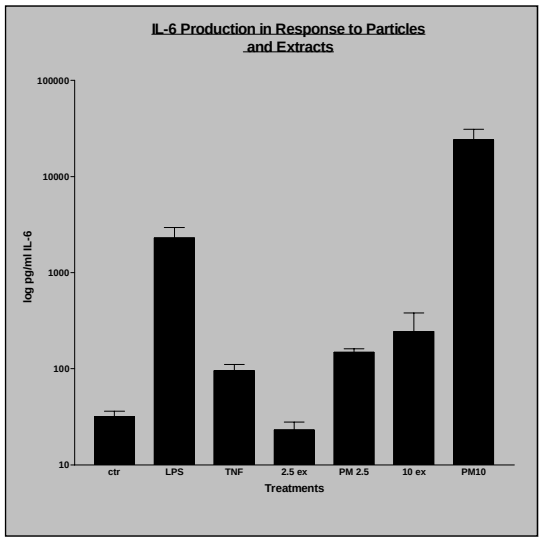

Figure 5. IL6 release measured by ELISA (R & D, Minneapolis, MN) after a 24 hour exposure of alveolar macrophages (200,000/mL) to particulate matter isolated from filters of varying size ($PM_{2.5}$vs $PM_{2.5-10}$). The aqeuous soluble component denoted by "ex".

Discussion

This study demonstrates that young, healthy volunteers exposed to realistic levels of ambient air particles develop mild inflammation in both their airways and the peripheral lung. Increased neutrophils were present in both the bronchial and bronchoalveolar fractions following CAPS exposure. The influx of neutrophils into the lung of CAPS exposed individuals was dose dependent, with those subjects exposed to the highest concentration of CAPS having the most neutrophils. The number of neutrophils present in BL or BAL lavage fluid was comparable to that found in healthy young volunteers exposed to low levels (0.10 ppm) of ozone (Devlin et. al., 1991). Neutrophils present in the BL of humans exposed to CAPS were also similar quantitatively to those found after human exposure to 300 $\mu g/m^3$ diesel exhaust for one hour (Salvi et. al., 1999). Although the latter study did not observe increased neutrophils in the alveolar fraction, this disparity may be explained by the differences in particle source, timing of bronchocoscopy (6 hours following diesel exhaust exposure versus 18 hours following CAPS exposure), or discrepancies in the total particle dose delivered to the lung in the two studies.

Rats exposed to up 350 mg/m3 of concentrated particles for 3 hours did not show evidence of lung inflammation either 3 or 24 hours after exposure (Gordon et. al., 1998). This difference between human and rat response to CAPS may be reflective of different sensitivities of the two species or reflect the higher ventilatory rate of exercising humans during exposure in this study. However, in another study rats were exposed to considerably higher levels of CAPS (5 hrs/day for 3 consecutive days to concentrated particles ranging from 206 - 733 mg/m3), and had slightly elevated BAL neutrophils (Clarke et. al., 1999). Taking into account particle concentration, exposure duration, minute ventilation, and lung surface area of humans and rats in these two studies, we estimate the rats inhaled approximately 10 times more particles than humans. Since the neutrophil levels were similar in both studies, these data lend credence to the notion that humans may be more sensitive to the effects of CAPS, at least as measured by lung inflammation.

It was somewhat surprising that CAPS did not induce increases in soluble BAL components such as IL-8, IL-6, PGE_2, protein, or fibronectin. All of these compounds were seen in BAL 24 hours after exposure of humans to low levels of ozone - a regimen which induced similar PMN levels as seen in this study. This discordance suggests the possibility that CAPS and ozone may exert their effects via different mechanisms. This possibility is further strengthened by the observation that low levels of ozone, but not CAPS, induce changes in lung function (e.g. FEV_1 and FVC).

Another noteworthy outcome of this study is that fibrinogen concentrations in the blood may be increased after inhalation of CAPS. Fibrinogen is an extracellular, dimeric glycoprotein (molecular weight of 340,000 Daltons) synthesized by the liver. It is the circulating precursor of the fibrin clot and contributes significantly to blood viscosity and coagulation, cell to cell adhesion, and platelet aggregation. Concentrations are associated with season of the year, dietary factors, geography, race, age, obesity, pregnancy, oral contraception, menopause, cigarette smoking, stress, and hypercholesterolemia (Andreotti et. al., 1999). Fibrinogen is an acute phase reactant and its levels increase during inflammation, infection, and stress. Plasma elevations in disease (e.g. hypertension, diabetes, malignancy, surgery, and inflammatory disorders) can be proportionally related to activity and tissue damage. These elevations may represent an activation of cells by an inflammatory stimulus (i.e. CAPS) with release of cytokines which induces synthesis of fibrinogen in the liver. However, the elevation of this glycoprotein did not appear to be a nonspecific elevation of an acute phase reactant as other proteins participating in this host response were not similarly increased (i.e. ferritin). Decrements of fibrinogen in the BAL of individuals exposed to CAPS supports a consumption of the protein with the activation of coagulation.

Elevations in fibrinogen after CAPS may reflect a disruption in the normal equilibrium between coagulation and fibrinolysis and present a thrombogenic risk. This disturbance in the normal equilibrium was not sufficient to be reflected by changes in prothrombin time and partial thromboplastin time (data not shown). Independent of other measures of coagulation, increases in blood fibrinogen concentrations can be associated with ischemic heart disease including myocardial infarction and sudden death (Meade et. al., 1986; Thompson et. al., 1995; Yarnell et. al., 1991). This may reflect a mechanism to explain elevated cardiovascular deaths without a significant lung injury (Sjogren 1998).

The lack of a significant finding in certain specific measures after exposure to CAPS is also remarkable. There were no changes in red blood cell counts, hematocrit, and hemoglobin which can be associated with particle inhalation (Nadziejko et. al., 1998). Similarly, and in contrast to exposure to diesel exhaust, there was no increase in either blood neutrophils or platelets (Salvi et. al., 1999). Correlations between elevations in blood viscosity and particle exposure have also been previously noted (Peters et. al., 1997) but this measure did not vary with inhalation of CAPS.

Finally, there is evidence of a relationship between lung inflammation after particle exposure and small transient decreases in pulmonary function among children and asthmatics (Pope and Dockery 1992; Romieu et. al., 1996; Vedal et. al., 1998). However, comparable to exposures to diesel exhaust (Salvi et. al., 1999), there was no evidence of a lung injury after healthy volunteers inhaled CAPS. This is a consistent result whether injury is quantified using either a biochemical index (i.e. protein) or a physiologic index (i.e. lung function). It is possible that the response of the lung to CAPS, including the neutrophilic influx, constitutes a defense of the tissue and functions to prevent such an injury.

Some portion of the *in vitro* response of the alveolar macrophages to particulate matter was mediated by endotoxin as made evident by a decrease in thechemiluminescence and IL-6 release after incubation with polymixin. The oxidative stress induced by the endotoxin could be associated with a pro-inflammatory effects through a control of transcription factors such as nF-κB and AP-1. This would result in a disruption of normal homeostasis with an inflammatory injury following as observed in the normal volunteers exposed to concentrated particles.

We conclude that exposure of healthy volunteers to CAPS can be associated with an influx of neutrophils into the lower respiratory tract. This did not appear to be associated with a lung injury in this group. *In vitro*, some portion of this response was mediated by the content of endotoxin.

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Disclaimer

This report has been reviewed by the National Health and Environmental Effects Research Laboratory, United States Environmental Protection Agency and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

References

Andreotti, R., F. Burzotta, and A Maseri. 1999. Fibrinogen as a marker of inflammation: a clinical view. *Blood Coagul. Fibrinolysis* 10(suppl. 1): S3-S4.

Bates, D.V., and R. Sizto. 1983. Relationships between air pollutant levels and hospital admissions in Southern Ontario. *Can. J. Public Health* 74:117-122.

Clarke, R.W., P.J. Catalano, P. Koutrakis, G.G. Krishna Murthy, C. Sioutas, J. Paulauskis, B. Coull, S. Ferguson, and J.J. Godleski. 1999. Urban air particulate inhalation alters

pulmonary function and induces pulmonary inflammation in a rodent model of chronic bronchitis. *Inh. Tox*. 11:637-656.

Colton, T. 1974. Statistics in Medicine. Little, Brown, & Co., Boston.

Devlin, R.B., W.F. McDonnell, R. Mann, S. Becker, D.E. House, D. Schreinemachers, and H.S. Koren. 1991. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. *Am. J. Respir. Cell & Mol. Biol.* 4:72-81.

Dockery, D.W., and C.A. Pope III. 1994. Acute respiratory effects of particulate air pollution. *Annu. Rev. Public Health* 15:107-132.

Dockery, D.W., F.E. Speizer, D.O. Stram, J.H. Ware, J.D. Spengler, and B.G. Ferris. 1989. Effects of inhalable particles on respiratory health of children. *Am. Rev. Respir. Dis.* 139:587-594.

Ghio, A.J., M. Bassett, A.N. Chall, D.G. Levin, and P.A. Bromberg. 1998. Bronchoscopy in healthy volunteers. *J. Bronchology* 5:185-194.

Gordon, T. C. Nadziejko, R. Schlesinger, and L.C. Chen. 1998. Pulmonary and cardiovascular effects of acute exposure to concentrated ambient particulate matter in rats. *Toxicol. Lett.* 96-97:285-288.

Knudson, R.J., M.D. Lebowitz, C.J. Holberg, and B. Burrows. 1983. Changes in the normal maximal expiratory flow-volume curve with growth and aging. *Am. Rev. Respir. Dis.* 127:725-734.

Meade, T.W., S. Mellows, M. Brozovic, G.J. Miller, R.R. Chadrabarti, W.R.S. North, A.P. Haines, Y. Stirling, J.D. Imeson, and S.G. Thompson. 1986. Haemostatic function and ischaemic heart disease: principle results of the Nortwick Park heart study. *Lancet* 2:533-537.

Nadziejko, C., L.C. Chen, I.T. Zelikoff, and T. Gordon. 1997. Hematological and cardiovascular effects of acute exposure to ambient particulate matter (PM) (abstract). *Am. J. Respir. Crit. Care Med.* 155:A247.

Peters, A., A. Doring, N.-E. Wichmann, and W. Koenig. 1997. Increased plasma viscosity during an air pollution episode: a link to mortality? *Lancet* 349:1582-1587.

Pope, C.A., III. 1989. Respiratory disease associated with community air pollution and a steel mill, Utah Valley. *Am. J. Public Health.* 79:623-628.

Pope, C.A., III, and D.W. Dockery. 1992. Acute health effects of PM_{10} pollution on symptomatic and asymptomatic children. *Am. Rev. Respir. Dis*. 144:1123-1128.

Pritchard, R.J., A.J. Ghio, J.R. Lehmann, D.W. Winsett, J.S. Tepper, P. Park, M.I. Gilmour, K.L. Dreher, and D.L. Costa. 1996. Oxidant generation and lung injury after particulate air pollution exposure increase with the concentrations of associated metals. *Inh. Tox.* 8:457-477.

Romieu, I., F. Meneses, S. Ruiz, J.J. Sierra, J. Huerta, M.C. White, and R.A. Etzel. 1996. Effects of air pollution on the respiratory health of asthmatic children living in Mexico City. *Am. J. Respir. Crit. Care Med.* 154:300-307.

Salvi, S., A. Blomberg, B. Rudell, F. Kelly, T. Sandstrom, S.T. Holgate, and A. Frew. 1999. Acute inflammatory responses in the airways and peripheral blood after short-term exposure to diesel exhaust in healthy human volunteers. *Am. J. Respir. Crit. Care Med*. 159:702-9.

Sioutas, C., P. Koutrakis, and S. T. Ferguson. 1995. Development and evaluation of a prototype ambient particle concentrator for inhalation exposure studies. *Inh. Tox*. 7:633-644.

Sioutas, C., P. Koutrakis, J.J. Godleski, S. T. Ferguson, C.S. Kim, and R.M. Burton. 1997. Fine particle concentrators for inhalation exposure: effect of particle size and composition. *J. Aerosol Science* 28:1057-1071.

Sjogren, B. 1998. A possible connection between furnace dust exposure, plasma fibrinogen levels and cardiovascular disease. *Sc. J. Work, Environ. & Health* 24:236-237.

Thompson, S.G., J. Kienast, S.D.M. Pyke, F. Haverkate, and J.C.W. Van de Loo. 1995. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. *N. Engl. J. Med.* 332:635-641.

U.N. Environment Program and WHO Report. 1994. Air Pollution in the world's megacities. A Report from the U.N. Environment Programme and WHO. *Environment* 36:5-37.

Vedal, S. J. Petkau, R. White, and J. Blair. 1998. Acute effects of ambient inhalable particles in asthmatic and nonasthmatic children. *Am. J. Respir. Crit. Care Med.* 157:1034-1043.

Vincent, R., P. Goegan, G. Johnson, J.R. Brook, P. Kumarathasan, L. Bouthillier, and R.T. Burnett. 1997. Regulation of promoter-CAT stress genes in HepG2 cells by suspensions of particles from ambient air. *Fundam. Appl. Toxicol.* 39:18-32.

Yarnell, J.W., I.A. Baker. P.M. Sweetnam, D. Bainton, J.R. O'Brien, P.J. Whitehead and P.C. Elwood. 1991. Fibrinogen, viscosity, and white blood cell count are major risk factors for ischemic heart disease. The Caerphilly and Speedwell collaborative heart disease studies. *Circulation* 83:836-44.

Table 1. Physical characterization of particles

	Total	Quartile 1*	Quartile 2	Quartile 3	Quartile 4
Number of Subjects	38	8	10	10	10
$PM_{2.5}$ after Concentrator ($\mu g/m^3$)	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
$PM_{2.5}$ before concentrator ($\mu g/m^3$)	26.4 ± 3.0		9.9 ± 3.0	30.3 ± 3.8	37.6 ± 4.6
Concentration factor	6.5 ± 0.9		9.4 ± 2.3	4.1 ± 0.7	6.4 ± 1.0
MMAD (microns)	0.65 ± 0.028		0.54 ± 0.06	0.67 ± 0.05	0.72 ± 0.03
Sigma G	2.35 ± 0.72		2.44 ± 0.16	2.39 ± 0.14	2.24 ± 0.084

* Filtered air

Table 2. Changes in pulmonary function following CAPS exposure (Post - Pre)

	Total CAPS	Quartile 1 (Air)	Quartile 2	Quartile 3	Quartile 4
$PM_{2.5}$	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
FEV_1 (L)	0.05 ± 0.02	0.05 ± 0.05	0.06 ± 0.04	0.04 ± 0.04	0.04 ± 0.02
FVC (L)	0.05 ± 0.02	0.09 ± 0.07	0.05 ± 0.06	0.04 ± 0.09	0.07 ± 0.09
Raw (cm H_2O/L/sec)	0.03 ± 0.04	0.12 ± 0.08	0.05 ± 0.06	0.04 ± 0.09	0.07 ± 0.09
PEF (L/sec)	0.16 ± 0.19	0.15 ± 0.23	0.43 ± 0.45	0.11 ± 0.21	0.16 ± 0.32

Table 3. Changes in blood parameters following CAPS exposure (Post - Pre)

	Total CAPS	Quartile 1 (Air)	Quartile 2	Quartile 3	Quartile 4
$PM_{2.5}$	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
RBCs/ml	0.02 ± 0.05	0.10 ± 0.09	-0.01 ± 0.11	0.1 ± 0.05	0.08 ± 0.07
Hb (g/dL)	0.12 ± 0.13	0.14 ± 0.24	0.05 ± 0.34	0.00 ± 0.12	0.28 ± 0.19
Hct (%)	0.44 ± 0.37	1.10 ± 0.83	0.09 ± 0.90	0.31 ± 0.49	0.88 ± 0.54
Neutrophils/ml	0.22 ± 0.14	-0.31 ± 0.13	-0.26 ± 0.17	-0.29 ± 0.36	-0.14 ± 0.23
Lymphocytes/ml	0.03 ± 0.05	-0.05 ± 0.04	0.06 ± 0.10	0.11 ± 0.09	-0.02 ± 0.09
Monocytes/ml	0.02 ± 0.02	0.00 ± 0.03	0.04 ± 0.03	0.01 ± 0.03	-0.02 ± 0.05
Platelets/ml	-7.47 ± 2.69	6.29 ± 16.03	-7.00 ± 4.07	-7.22 ± 6.80	-5.70 ± 3.22
Ferritin (ng/ml)	1.90 ± 2.71	3.00 ± 2.43	1.50 ± 3.57	4.33 ± 4.16	5.44 ± 3.88
Viscosity	0.01 ± 0.02	0.13 ± 0.08	0.04 ± 0.03	-0.02 ± 0.02	0.11 ± 0.03
Fibrinogen (mg/dL)	41.2 ± 12.7*	-5.3 ± 10.6	38.9 ± 17.8	43.3 ± 25.3	41.7 ± 28.8

* $p > 0.05$

Table 4. Changes in bronchial lavage cells following CAPS exposure

	Total CAPS	Quartile 1 (Air)	Quartile 2	Quartile 3	Quartile 4
$PM_{2.5}$	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
Total count ($x10^6$)	6.8 ± 0.9	6.8 ± 0.8	5.6 ± 0.9	6.7 ± 1.7	8.2 ± 1.8
Macrophages (%)	55.4 ± 2.7*	64.8 ± 1.3	58.6 ± 4.1	52.3 ± 4.2	55.3 ± 6.3
Neutrophils (%)	8.1 ± 2.7*	2.7 ± 0.6	5.7 ± 1.1	10.2 ± 2.4	8.4 ± 2.0
Lymphocyte (%)	9.0 ± 0.8	7.3 ± 1.0	9.7 ± 1.8	8.4 ± 0.9	8.9 ± 1.2
Monocytes (%)	0.8 ± 0.1*	0.4 ± 0.1	0.9 ± 0.2	0.9 ± 0.3	0.6 ± 0.1
Epithelial cells (%)	25.6 ± 2.1	26.2 ± 1.5	25.1 ± 3.5	28.3 ± 3.8	23.3 ± 4.0

* $p > 0.05$

Table 5. Changes in bronchial lavage soluble components following CAPS exposure

	Total CAPS	Quartile 1 (Air)	Quartile 2	Quartile 3	Quartile 4
$PM_{2.5}$	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
Protein (µg/ml)	35.9 ± 2.5*	57.5 ± 5.7	35.4 ± 3.7	35.5 ± 3.7	36.7 ± 5.4
IL-8 (pg/ml)	102.9 ± 22.2	54.3 ± 19.8	114.4 ± 48.2	104.1 ± 29.8	90.2 ± 40.8
IL-6 (pg/ml)	5.4 ± 0.5	4.5 ± 0.6	4.7 ± 0.6	6.7 ± 1.2	4.7 ± 0.7
PGE_2 (pg/ml)	11.2 ± 0.9	10.3 ± 1.5	9.6 ± 1.8	9.1 ± 1.4	14.1 ± 1.8
α-1 anti-trypsin (µg/ml)	20.3 ± 4.8	39.8 ± 13.4	13.0 ± 8.5	15.3 ± 4.4	31.3 ± 11.6
Fibronectin (ng/ml)	43.2 ± 7.0	66.3 ± 13.8	39.0 ± 13.2	34.9 ± 6.7	55.5 ± 15.5

* $p > 0.05$

Table 6. Changes in bronchoalveolar lavage cells following CAPS exposure

	Total CAPS	Quartile 1 (Air)	Quartile 2	Quartile 3	Quartile 4
$PM_{2.5}$	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
Total count ($x10^6$)	21.4 ± 1.3*	15.9 ± 1.9	20.3 ± 3.0	23.0 ± 2.1	20.8 ± 2.0
Macrophages (%)	80.2 ± 1.6	80.6 ± 2.9	82.4 ± 2.2	82.9 ± 1.3	75.4 ± 3.7
Neutrophils (%)	2.5 ± 0.6*	0.8 ± 0.3	1.4 ± 0.4	2.0 ± 0.4	4.2 ± 1.7
Lymphocyte (%)	15.1 ± 1.3	16.8 ± 2.4	13.1 ± 1.7	13.1 ± 1.3	19.0 ± 3.1
Monocytes (%)	1.4 ± 0.2	1.2 ± 0.3	0.9 ± 0.2	1.3 ± 0.3	2.2 ± 0.5
Epithelial cells (%)	1.1 ± 0.1	1.3 ± 0.3	0.8 ± 0.1	0.8 ± 0.1	1.4 ± 0.3

* $p > 0.05$

Table 7. Changes in bronchoalveolar lavage soluble components following CAPS exposure

	Total CAPS	Quartile 1 (Air)	Quartile 2	Quartile 3	Quartile 4
$PM_{2.5}$	120.4 ± 14.1		47.2 ± 5.3	107.4 ± 9.3	206.7 ± 19.2
Protein (µg/ml)	97.5 ± 8.3	101.7 ± 10.0	118.2 ± 16.9	86.2 ± 6.0	88.0 ± 16.8
IL-8 (pg/ml)	102.9 ± 25.2	288.8 ± 109.6	182.4 ± 67.7	59.9 ± 14.6	66.4 ± 21.7
IL-6 (pg/ml)	5.9 ± 0.9	6.6 ± 1.5	6.8 ± 2.4	5.4 ± 1.1	5.4 ± 1.1
PGE_2 (pg/ml)	7.8 ± 0.5	6.3 ± 0.9	7.7 ± 0.8	7.7 ± 1.0	8.1 ± 0.7
α-1 anti-trypsin (µg/ml)	88.1 ± 14.8	58.5 ± 13.8	77.5 ± 19.0	65.6 ± 13.5	122.7 ± 39.2
Fibrinogen (mg/dl)	15.2 ± 1.4*	23.3 ± 2.1	15.6 ± 2.9	16.1 ± 2.4	14.1 ± 2.0
Fibronectin (ng/ml)	102.6 ± 12.4	95.8 ± 16.1	103.4 ± 26.3	90.6 ± 21.7	113.7 ± 18.2

* $p > 0.05$