Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice

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THE WORLD HEALTH ORGANIZATION (WHO) recently reported that over 800,000 deaths worldwide per year can be attributed to particulate matter (PM) air pollution (65). Recent epidemiological studies have linked ambient particulate matter (PM) levels to an increased incidence of adverse cardiovascular events. Yet little is definitively known about the mechanisms accounting for the cardiovascular events associated with PM exposure. The goal of this study was to determine the effects of ultrafine (<0.1 μm) PM exposure on ischemia-reperfusion (I/R) injury. ICR mice were exposed to 100 μg of PM or vehicle by intratracheal instillation. Twenty-four hours later, mice were anesthetized with pentobarbital sodium (60 mg/kg), the left anterior descending coronary artery was ligated for 20 min, flow was restored for 2 h, and the resulting myocardial infarct (MI) size was evaluated. PM exposure doubled the relative size of the MI compared with the vehicle control. No difference was observed in the percentage of the left ventricle at risk for ischemia. PM exposure increased the level of oxidative stress in the myocardium after I/R. The density of neutrophils in the reperfused myocardium was increased by PM exposure, but differences in the number of blood leukocytes, expression of adhesion molecules on circulating neutrophils, and activation state of circulating neutrophils 24 h after PM exposure could not be correlated to the increased I/R injury observed. Additionally, aortas isolated from PM-exposed animals and studied in vitro exhibited a reduced endothelium-dependent relaxation response to acetylcholine. These results indicate that exposure to ultrafine PM increases oxidative stress in the myocardium, alters vascular reactivity, and augments injury after I/R in a murine model.

Taken together, these current studies provide compelling evidence linking PM to adverse cardiovascular effects.

The major sources of ambient PM include automobile and power plant combustion processes, mechanical processes, and environmental dust. PM produced during industrial processes is regulated by the Environmental Protection Agency (EPA) and classified into three categories based on aerodynamic diameter (41, 61). The fine (<2.5 and <0.1 μm, respectively) fractions of PM are contributed to largely by diesel exhaust (41). Coarse (2.5–10 μm) fractions of environmental PM are generated from mechanical crushing and grinding of surfaces (41). The potentially toxic components of ambient ultrafine particles include oxidant gases, organic compounds, and transition metals absorbed to the carbon core of the particles (9). Ongoing research focuses on the toxicity of these individual components, as well as the complete particulate.

Inhalation of PM is known to cause inflammation in the lungs, characterized by neutrophil and macrophage activation (49, 52, 53). The resulting pulmonary inflammation can lead to activation of the pulmonary endothelium and subsequent systemic inflammation (38, 49, 52). PM has been shown to alter the differentiation state of circulating neutrophils, enhance the release of immature neutrophils into the blood, and reduce neutrophil transit time through the bone marrow (18, 38, 49, 55). In addition, PM increases C-reactive protein, an acute-phase inflammatory marker considered to be a risk factor for cardiovascular disease (42, 51, 52, 61). Thus exposure to PM alters inflammatory parameters that are closely associated with adverse cardiovascular events.

The present study was undertaken to test the hypothesis that PM exposure alters the outcome of an adverse cardiac event. Specifically, the effect of ultrafine (<0.1 μm) PM exposure on cardiac ischemia-reperfusion (I/R) injury was examined in mice. The results provide evidence that PM exposure increased the size of the myocardial infarction induced by I/R. This increase in infarction was associated with an increased measure of oxidative stress within the myocardium and altered aortic reactivity to acetylcholine.

MATERIALS AND METHODS

Animals. Six- to ten-week-old ICR mice were obtained from Harlan (Indianapolis, IN), housed in microisolation, and cared for by the East Carolina University Comparative Medicine staff. All protocols consented to by the Institutional Animal Care and Use Committee.

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formed to the standards in the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and were approved by the East Carolina University Institutional Committee on the Care and Use of Laboratory Animals.

Particle collection and extraction. Particles were collected continuously over 7-day periods during the month of October 2002 in Chapel Hill, NC. A ChemVol High Volume Cascade Impactor (Rupprecht and Patashnick; East Greenbush, NY) with three impaction stages was used to collect different-sized fractions of ambient PM. Weekly collections were then pooled into a monthly sample. Ultrafine particles (<150 nm in diameter) were collected onto GS300 filters (Monandock Non-Wovens, Mt. Pocono, PA) previously cleaned with methanol and water, dried under sterile conditions, and stored at −80°C until extracted. Coarse particles (2.5–10 μm) and fine particles (0.15–2.5 μm) were also collected but not used in this study. Particles were extracted by prewetting the filter with small amounts of 70% ethanol, and endotoxin-free water was added to yield a total volume of 40 ml. The particles were removed by sonication for 1 h in a water bath (FS220; Fisher Scientific, Pittsburgh, PA). Particles extracted from the filters were then lyophilized and stored at −80°C. Extraction efficiency was 65–70%. Before use, particles were resuspended in sterile water at a concentration of 5 mg/ml and sonicated briefly.

Particulate exposure. Mice were anesthetized with vapors from a 1:1 mixture of isoflurane/propanol. One hundred micrograms of ultrafine PM were suspended in 100 μl of sterile PBS (composition in mM: 137 NaCl, 10 NaH2PO4, 1.47 KH2PO4, 2.7 KCl, pH adjusted to 7.4) and delivered by intratracheal instillation. Vehicle-exposed mice received 100 μl of sterile PBS.

Peripheral blood leukocyte count. Before the cardiac I/R protocol, blood was drawn from each animal via the tail vein. Total number of leukocytes in 100 μl of whole blood was determined by using a Coulter Counter (Beckman Coulter; Fullerton, CA). Differential cell counts were determined from blood smears stained with Diff-Quik (Dade Behring; Newark, DE) (20, 24). Two slides were prepared per animal, 100 cells were counted per slide, counts from each animal were averaged, and the average for all animals was reported.

I/R injury. I/R injury was performed by using an adaptation of the protocol described by Ahn et al. (1). Briefly, 24 h after exposure to PM or vehicle, animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (60 mg/kg). Supplemental injections of pentobarbital sodium (30 mg/kg) were given to maintain anesthesia throughout the surgical procedure. Body temperature was maintained at 37°C with a circulating water heating pad. A midline thoracostomy was performed, and the animals were intubated with PE-90 tubing, while mechanically ventilated with 100% oxygen at 120 strokes/min and a ventilator setting of 1.2 ml/stroke.

Control ventilation experiments, in a separate set of animals (n = 3 in each group), were performed to validate ventilator settings and determine blood oxygen saturation profiles for PM- and vehicle-exposed mice. Mice were anesthetized and mechanically ventilated as indicated above. Blood was collected from the left ventricle (LV) into a syringe containing 100 μl of heparin (1:100 dilution) after a 10-min equilibration period. Heparinized blood was run through a blood gas analyzer (Stat Profile, Critical Care Xpress; Nova Biomedical; Waltham, MA), and an average PO2 and O2 saturation were determined for each group. The average PO2 and O2 saturation measurements were not statistically different between the groups (PM: PO2 242 ± 16 mmHg, O2 saturation 99.7 ± 0.4%; vehicle: PO2 256 ± 26 mmHg, O2 saturation 99.6 ± 0.19%).

After a 10-min equilibration period, the thorax was opened with a left parasternal incision. The pericardium was gently removed from the heart, and the left anterior descending coronary artery (LAD) was identified. The LAD was ligated by using a reversible snare applied 4 mm distal to the origin between the conus arteriosus and the left atrium. Appropriate occlusion was confirmed by the appearance of myocardial cyanosis distal to the ligature. After 20 min of occlusion, the ligature was released, and the LAD was reperfused for 2 h.

Desiccation was minimized by approximating the chest walls with Parafilm.

The percentage of the LV at risk for ischemia was determined in a separate group of control animals. After the 2-h reperfusion period, the LAD was religated at the original point of occlusion. The myocardium at risk was delineated by infusing 1% Evans blue solution through the aorta as adapted from Hashimoto et al. (21). The right ventricle was removed, the entire LV was weighed, and the unstained LV region was removed and weighed to determine the percentage of the total LV tissue at risk for ischemia (14). Fifty percent of the LV was determined to be at risk for ischemia. In subsequent experiments, the percentage of the LV at risk for ischemia was calculated by multiplying the average area at risk (AAR), determined as described below, by a factor of 0.5.

After Evans blue staining, hearts from PM- and vehicle-exposed animals were excised, and 1-mm-thick serial sections were cut from the point of ligation to the apex. Sections were incubated for 20 min in a 1% solution of 2,3,5-triphenyltetrazolium chloride to demarcate the infarcted from the noninfarcted cardiac tissue (21). Both sides of all sections were photographed and analyzed by computer planimetry with the use of NIH Image software (ImageJ, version 1.34s) to determine LV tissue area, AAR, and area of infarct as described by Hazarika et al. (22). For each side of each section, the infarct was expressed as a percentage of the AAR. The values for all sections from each heart were averaged. The values reported are the means ± SE for the hearts in each group.

Histology. Hearts and lungs were fixed in 4% paraformaldehyde, embedded in paraffin blocks, cut into 5-μm sections, and stained with Gill’s hematoxylin and eosin. Sections were viewed under light microscopy. Tissue neutrophil counts were conducted on five sections per animal and three fields per section. The number of neutrophils counted in each microscopic field was then normalized to the area of the field. Lung sections were examined by a pathology-trained veterinarian blinded to each exposure group, and a semiquantitative assessment of lung inflammation was performed by assessing the following criteria: pneumonitis, edema, increased alveolar macrophages, subpleural lymphocyte aggregates, and perivascular/peribronchial cuffing. The following +/− scale was used to denote severity of each inflammatory parameter examined: − = none, + = minimal, ++ = mild, +++ = moderate, ++++ = severe. Multiple sections from each animal were analyzed, and representative scores for each group were recorded.

Myeloperoxidase assay. Blood was collected 24 h after PM or vehicle exposure in animals not subjected to I/R. Samples from four animals were pooled, and neutrophils were concentrated by sedimentation through a discontinuous Percoll gradient (1.10 g/ml, 1.095 g/ml, and 1.085 g/ml Percoll in PBS). Total and differential cell counts were determined as described above. Neutrophils were resuspended in PBS at a density of 5 × 10^6 cells/ml of PBS on the basis of total cell counts and neutrophil percentage. Cytochalasin B and N-formyl-Met-Leu-Phe (fMLP) were added to the cell suspension. After a 10-min incubation at 37°C, dimethoxybenzidine [myeloperoxidase (MPO) substrate, Sigma, D-9143; St. Louis, MO] was added to the suspension, and absorbance at 450 nm (A450) was determined by using a microplate reader (Bio-Rad; Hercules, CA). Experiments were repeated in duplicate, and absorbance readings were averaged for each mouse group and recorded.

Oxidative stress analysis. To evaluate oxidative stress in the myocardium, thiobarbituric acid reactive substance (TBARS) concentrations were determined in PM- and vehicle-exposed I/R and non-I/R injury mouse groups (TBARS assay kit; OXitex, Buffalo, NY) (27). LVs from each mouse group (5 ventricles in each group, separately analyzed in triplicate) were homogenized and analyzed for TBARS content. TBARS form a 1:2 adduct with malondialdehyde (MDA), which was detected at A532 for each sample with a spectrophotometer (SmartSpec 3000; Bio-Rad). TBARS concentrations (expressed as μmol MDA/mg LV protein) were determined on the basis of an MDA
curves were constructed for the vasoconstrictor PSS until passive force was restored. Cumulative dose-response ensure tissue viability. After stimulation, aortic rings were rinsed with beled IgG1, 553925; 1:100, FITC-labeled IgG2a; BD Biosciences). Rings were stimulated with 109 mM K+ in a condition of optimal force generation during K+ depolarization. The number of circulating neutrophils, the activation state of neutrophils was increased in the area at risk after PM exposure (Fig. 3). The percentage of the LV at risk for ischemia did not differ between the PM- and vehicle-exposed mouse hearts (33 ± 1 vs. 31 ± 1% LV at risk, P = 0.427) (Fig. 2A). In contrast, PM exposure induced a doubling of the infarct size relative to the AAR (48 ± 4 vs. 23 ± 3% AAR, P < 0.001) (Fig. 2B). Ultrasound PM exposure increased tissue neutrophil number. Serial sections through the area at risk including the infarct region were analyzed for neutrophil infiltration. The density of neutrophils was increased in the area at risk after PM exposure (Fig. 4B) compared with vehicle control hearts (Fig. 4A) (392 ± 24 vs. 270 ± 32 cells/mm2 AAR, P = 0.006; vehicle n = 12, PM n = 10). The density of neutrophils within the infarct zone exhibited a positive correlation to the size of the myocardial infarction, and the correlation was similar in data sets for both PM- and vehicle-exposed groups (R = 0.6064, P = 0.006) (Fig. 5A). The number of circulating neutrophils, the activation state of the circulating cells, and expression of chemotactic factors

Statistics. Values were reported as means ± SE. Differences between groups were compared by using Student’s t-test for unpaired observation or ANOVA with Fisher test for least significant difference. In all cases a P value of <0.05 was used to indicate statistical significance between groups.

RESULTS

Ultrasound PM exposure increased lung inflammation. At 24 h post-PM exposure, lung sections from PM-exposed animals showed evidence of pulmonary inflammation compared with the vehicle-exposed animals (Fig. 1). Increased pneumonitis was observed in all PM-exposed lungs. Alveolar edema was also associated with PM exposure but was not observed in all lung sections.

Ultrafine PM exposure reduced total circulating leukocytes. Blood was isolated from mice 24 h after PM or vehicle exposure, before I/R. PM exposure was associated with a reduction in blood total leukocyte count (2,959 ± 216 vs. 4,298 ± 473 cells/ml whole blood, P = 0.022) (Fig. 2A). Differential cell counts revealed a significant decrease in circulating neutrophil numbers in the PM- compared with the vehicle-exposed mouse groups (178 ± 46 vs. 417 ± 105 cells/ml whole blood, P = 0.041) (Fig. 2B).

Ultrafine PM exposure increased infarction after I/R injury. The percentage of the LV at risk for ischemia did not differ between the PM- and vehicle-exposed mouse hearts (33 ± 1 vs. 31 ± 1% LV at risk, P = 0.427) (Fig. 3A). In contrast, PM exposure increased the size of LV infarction. Compared with vehicle alone, PM exposure induced a doubling of the infarct size relative to the AAR (48 ± 4 vs. 23 ± 3% AAR, P < 0.001) (Fig. 2B).

Ultrafine PM exposure increased tissue neutrophil number. Serial sections through the area at risk including the infarct region were analyzed for neutrophil infiltration. The density of neutrophils was increased in the area at risk after PM exposure (Fig. 4B) compared with vehicle control hearts (Fig. 4A) (392 ± 24 vs. 270 ± 32 cells/mm2 AAR, P = 0.006; vehicle n = 12, PM n = 10). The density of neutrophils within the infarct zone exhibited a positive correlation to the size of the myocardial infarction, and the correlation was similar in data sets for both PM- and vehicle-exposed groups (R = 0.6064, P = 0.006) (Fig. 5A). The number of circulating neutrophils, the activation state of the circulating cells, and expression of chemotactic factors
were evaluated to elucidate the mechanism underlying the increased neutrophil migration into the cardiac tissue. There was no correlation between the number of circulating blood neutrophils and the number of tissue neutrophils in both mouse groups, indicating that the increased number of neutrophils seen in the myocardium was not due to a larger number of circulating neutrophils ($R = 0.2557, P = 0.358$) (Fig. 5B).

Flow cytometry was performed on blood leukocytes to examine cellular expression of L-selectin, PSGL-1, and CD11b. L-selectin is constitutively expressed on the neutrophil surface and is involved in loose cellular adherence to the vasculature through interactions with P-selectin expressed on the endothelium (25, 62). PSGL-1 interacts with P-selectin expressed on the vascular endothelial surface and mediates neutrophil rolling (25, 62). CD11b is a $\beta_2$-integrin receptor that interacts with ICAM-1 on the vascular endothelial surface and facilitates firm adherence of the neutrophil to the endothelium (25, 62). Analyses revealed no difference in mean florescence intensity for L-selectin ($P = 0.487$), PSGL-1 ($P = 0.312$), and CD11b ($P = 0.155$) between PM- and vehicle-exposed mice (Table 1).

Likewise, fMLP-stimulated neutrophil degranulation, as measured by release of MPO, did not differ between the groups. This suggests that neutrophil activity was not increased with PM exposure ($0.475 \pm 0.03$ vs. $0.475 \pm 0.05$, A$_{450}$; $n = 4$ animals in each group, experiments were repeated in duplicate).

**Ultrafine PM exposure increases oxidative stress within the myocardium.** TBARS concentrations, reported as micromoles MDA per milligram LV protein, within the myocardium were not different in PM- vs. vehicle-exposed mouse groups (Fig. 6). However, PM exposure increased oxidative status within the myocardium after I/R (196 ± 48 vs. 111 ± 28 µmol MDA/mg LV protein, $P = 0.038$) (Fig. 6).

**Ultrafine PM exposure altered vascular reactivity.** Altered vascular reactivity could affect recovery from ischemia and was evaluated as a possible explanation for the increased infarction after PM exposure. Aortic rings from PM- and vehicle-exposed mice were evaluated for their contractile and...
relaxation responses to increasing doses of PE and acetylcholine, respectively. PE-induced force generation and sensitivity were not different in aortic rings from PM- and vehicle-exposed animals (0.82 ± 0.27 mN/mm², EC₅₀ = 0.54 ± 0.08 μM vs. 0.71 ± 0.17 mN/mm², EC₅₀ = 0.53 ± 0.08 μM, respectively) (Fig. 7A).

However, the extent of vessel relaxation induced by acetylcholine at 1, 10, and 100 μM doses was significantly impaired in the aortas from PM-exposed animals. The percent relaxation ranged from 45 to 62% in the vehicle-exposed and from 29 to 39% in the PM-exposed aortas. This impairment was also associated with a slight rightward shift in sensitivity to acetylcholine (EC₅₀ = 0.32 ± 0.12 μM vs. EC₅₀ = 0.24 ± 0.07 μM) (Fig. 7B). These results indicate that PM exposure can impair endothelium-dependent relaxation.

**DISCUSSION**

Studies have linked long-term exposure to air pollution in humans with increased cardiovascular mortality due to ischemic heart disease, heart failure, arrhythmia, and cardiac arrest (5). Likewise, short-term increases in ambient air pollution have been associated with an increased incidence of hospitalizations for cardiovascular complications, including ischemic heart disease, arrhythmias, and heart failure 24 h after peak ambient pollution levels (5). Population-based studies by Liao et al. (33) have shown that short-term exposure to coarse PM is associated with plasma inflammatory and hemostatic risk factors for cardiovascular disease (33).

Chronic air pollution studies in animal models have shown that PM exposure is associated with myocardial injury. Kodavanti et al. (30) demonstrated that rats repeatedly exposed to oil combustion-derived emission PM had multifocal degeneration, chronic-active inflammation, and fibrosis within the myocardium (30). Short-term exposure studies have linked concentrated ambient particulate (CAPs) aerosol exposure to increased cardiopulmonary edema, serum lactate dehydrogenase, and oxidative stress within the lungs and hearts of rats (19).

While epidemiological and inhalation studies have provided indirect evidence that PM exposure is a risk factor for adverse

| Table 1. FACS analysis of neutrophil surface marker expression |
|-------------------|-------------------|
|                   | PM MFI            | Vehicle MFI   |
| L-selectin        | 53 ± 8            | 53 ± 11       |
| PSGL-1            | 608 ± 157         | 708 ± 162     |
| CD11b             | 205 ± 40          | 162 ± 19      |

Values represent mean fluorescence intensity (MFI) ± SE; n = 5 animals in each group, with experiments repeated 5 times. Neutrophil surface marker expression was not altered by particulate matter (PM) exposure. FACS, fluorescence-activated cell sorting; PSGL-1, P-selectin glycoprotein 1.
cardiac events, no definitive evidence has been published on the ability of particles found in ambient air to alter either the incidence or extent of cardiac injury. Thus the purpose of this study was to evaluate the effect of PM exposure on the outcome of an imposed cardiac stress. The protocol involved delivering a defined amount of PM by intratracheal instillation at a single time point. While this approach does not reproduce real-world conditions, it has been used extensively in physiological and toxicological studies as a simple, effective, and reproducible means of inducing functional responses to substances that normally enter the lungs over prolonged periods of time by respiration. The approach allows primary responses to substance exposure to be studied independent of compensatory changes that can be invoked in chronic exposures.

Exposure to ultrafine PM induced pulmonary inflammation and decreased total circulating white blood cells (WBC) 24 h after exposure (Figs. 1 and 2A). In conjunction with the inflammation, the single exposure to ultrafine PM increased oxidative stress within the myocardium, impaired endothelium-mediated relaxation, and increased the size of the myocardial infarction after I/R (Figs. 3, 6, and 7B). Our results show that ultrafine PM concentrated from ambient air and instilled intratracheally can augment the myocardial injury induced by an ischemic event.

Exposure to a single dose of ultrafine PM increased infiltration of neutrophils to the lung parenchyma and reduced total circulating WBC consistent with increased margination (Figs. 1 and 2A). It is well established that airborne particles with aerodynamic diameter <2.5 μm deposit throughout the respiratory tract and can cause pulmonary injury and activation of the pulmonary endothelium (40, 49).

Previous studies demonstrated that instillation of ultrafine particles from diverse sources into the lungs induced pulmonary neutrophilic inflammation (10, 17, 37). Using ambient particles collected from the Research Triangle Park area in North Carolina, Dick and colleagues (11) demonstrated that the ultrafine fraction induced pulmonary neutrophilic inflammation when instilled into the lungs of mice at doses of 10, 50, or 100 μg/animal. The effects of 100 μg of ultrafine PM observed in the present study are consistent with previous studies citing inflammation in lung tissue 18 h after PM exposure. Our data extend these findings by showing a continued presence of lung inflammation at 24 h postinstillation (Fig. 1).

We observed a concomitant reduction in total leukocyte count in the blood, although PM exposure did not increase either the number or activation state of the circulating neutrophils (Fig. 2A and Table 1). It should be noted that some animal studies reported increases in circulating polymorphonuclear cells with PM exposure (28, 52). The discrepancies with our results might be explained by a decreased transit time for neutrophils to reach the lung (49). At the 24-h time point we studied, a large percentage of neutrophils may have already left the blood and entered the lungs in response to the initial PM exposure.

![Graph 1](http://example.com/graph1.png)

**Fig. 6.** Oxidative stress in the myocardium before and after ischemia and reperfusion (I/R) in PM- and vehicle-exposed animals. Tissue was isolated from the LV 24 h after exposure to PM or vehicle either before or after induction of ischemia and reperfusion. Oxidative stress was estimated as the amount of thiobarbituric acid reactive substance (TBARS) represented as malondialdehyde (MDA) per milligram of total LV protein. TBARS concentrations within the myocardium are increased in the PM-exposed I/R mouse group compared with vehicle control. Analyses repeated in triplicate; n = 5 animals in each group.

![Graph 2](http://example.com/graph2.png)

**Fig. 7.** Effect of PM exposure on the contractile response of thoracic aorta to stimulation with phenylephrine (PE) or acetylcholine in vitro. Thoracic aortas were isolated 24 h after exposure to PM and vehicle, cut into rings, and suspended in muscle baths. The contractile response of the rings to cumulative additions of PE to the baths was recorded and normalized to the maximal response obtained to PE (A). Aortic rings suspended in muscle baths were stimulated with 1 μM PE. The relaxation response of the rings to subsequent cumulative additions of acetylcholine to the baths was recorded and normalized to the maximal response obtained to PE (B). Values represent means ± SE for rings from 6 PM- and 6 vehicle-exposed animals.
Our results also indicate that despite a reduced neutrophil count in the blood (Fig. 2B), tissue neutrophil density within the myocardium after I/R injury is increased with PM exposure. Myocardial neutrophil infiltration in this study could not be attributed to increased expression of cellular adhesion molecules (CD11b, PSGL-1, and L-selectin), which would enhance the ability of circulating neutrophils to adhere to the vasculature and enter the myocardium. Although not assessed in this study, PM could potentially alter vascular permeability and allow for enhanced myocardial neutrophil infiltration. A recent study by Gurgueira et al. (19) has demonstrated that 24 h after CAPs inhalation myocardial edema was increased in rats, indicating an increase in myocardium permeability. In addition, circulating inflammatory cytokines could contribute to the overall systemic inflammation seen with PM exposure and aid in the recruitment of neutrophils to the myocardium after I/R injury. Cell culture studies have shown that TNF-α, IL-1β, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor (GM-CSF) are significantly increased 24 h after incubation with coarse PM (15). Likewise, circulating levels of IL-1β, IL-6, and GM-CSF were all increased after acute exposure to PM in humans (59).

In vitro and in vivo studies have shown that various types of PM, including ultrafine PM from ambient air, induce oxidant stress (11, 19, 36, 54). The study of ambient ultrafine PM by Dick and colleagues (11) also linked pulmonary neutrophilic inflammation with elevated oxidative stress measures. Our results demonstrate that PM exposure increased cardiac oxidative stress within the myocardium after I/R, as evidenced by TBARS concentrations (Fig. 6).

Free radical formation within the myocardium leads to lipid peroxidation and cellular damage if the antioxidant capacity of the heart is overwhelmed. Lipid peroxides decompose to form carbonyl compounds, such as MDA. MDA will form a 1:2 adduct with thiobarbituric acid known as a TBARS. Thus the concentration of TBARS is an indirect indication of the oxidative state within a tissue and is widely used to assess lipid peroxidation. This technique has been used in previous studies of particulate exposure to determine the oxidative status of liver, spleen, lung, kidney, brain, testicles, and heart (34, 47).

This increase in oxidative stress may contribute to the increase in infarct size seen in the PM-exposed myocardium after I/R injury (Fig. 3B). Reports from other groups examining the effects of PM exposure in laboratory settings have found a strong correlation to the level of oxidant stress and the biological effects of PM (8, 11, 16, 19, 47).

The observed increase in oxidative stress after exposure to ultrafine particles was consistent with previous observations of lung and heart tissue after exposure to other environmental particles (29, 46). Kodavanti and colleagues (29) reported that the overall oxidant status in bronchoalveolar lavage (BAL) fluid from spontaneously hypertensive rats increased after exposure to residual oil fly ash (ROFA). Likewise, Pradhan et al. (46) found that oxidative stress was increased in BAL fluid and lung homogenates from rats exposed to mixed ambient PM collected in India, and lung antioxidant levels were reduced. Microarray studies on total RNA isolated from particulate-exposed lung and alveolar cells have shown that expression of genes involved in oxidative stress is altered with PM exposure (31, 32).

Exposure to concentrated ambient particles has also been shown to increase reactive oxygen species in the myocardium of rats. This increase in oxidative stress was accompanied by increased antioxidant enzymes, indicating that particulates could trigger adaptive responses that counterbalance the potentially damaging activity of oxygen radicals (19). In addition, a study by Van Jaarsveld et al. (60) has shown that antioxidant vitamin supplementation of rats exposed to cigarette smoke, before I/R injury, reduces mitochondrial oxidative damage (60), indicating that antioxidant therapy could potentially offer a myocardial protective effect after PM exposure. Understanding the time course of the changes in oxidants and antioxidants within the myocardium after particulate exposure may be important in understanding the epidemiological incidence of adverse cardiovascular events 24 h after periods of high ambient particulate (9, 35, 36).

In addition to increased oxidative stress within the myocardium, our results revealed an altered reactivity of the aorta after PM exposure (Fig. 7). PM exposure caused impairment in acetylcholine-induced relaxation of the aorta (Fig. 7B). We did not observe any alteration in PE-induced contraction (Fig. 7A). These observations indicate that PM affects endothelium-mediated relaxation of vessels, and such an effect could decrease reperfusion of critical vascular beds after occlusion. Although not assessed in this study, corresponding changes in the coronary vasculature could contribute to altered reperfusion in the myocardium, prolonging the recovery of the myocardium from an ischemic episode and enlarging the infarct region as seen in our study.

In the current study we found that tracheal instillation of ultrafine PM collected from ambient air was linked to increased I/R injury in association with altered vascular reactivity (Figs. 3 and 7). These results are consistent with other studies demonstrating effects of air pollution on vascular reactivity. Unfractionated ambient PM, ROFA, CAPs, titanium dioxide, and diesel exhaust particles have all been shown to alter the reactivity behavior of different vasculatures through the action of reactive oxygen species or impaired endothelial function (3, 23, 39, 57). In 2002, Brook and colleagues (6) demonstrated that inhalation of CAPs and ozone at doses representative of peak air pollution events in the United States decreased brachial artery diameter in humans. In 2004, Nurkiewicz et al. (38) reported that arterial vessels isolated from rats exposed to ROFA, which is considered an environmental PM surrogate, or titanium dioxide by intratracheal instillation exhibited less dilation than vehicle-exposed animals. This suggests an impairment of endothelial nitric oxide synthase (eNOS) (38). Other possible alterations in vascular homeostasis are implicated in the recent study by Thomson et al. (56), in which endothelin converting enzyme-1, preproET-1, and eNOS mRNA expression were found to be increased 24 h after PM exposure. Likewise, Kang et al. (26) have demonstrated that serum total endothelin concentrations were elevated in rats instilled with fine PM. These reports are in contrast to the work of Bagate et al. (2), who demonstrated an increase in acetylcholine-dependent relaxation of aortas isolated from spontaneously hypertensive rats after ambient-derived PM instillation. The apparent discrepancy of the effect of air pollution particles on vascular reactivity may be related to preexisting conditions. This could explain why relatively normal individuals do not
exhibit adverse cardiovascular outcomes when exposed to ambient PM (2, 39).

It is important to note that some studies have not revealed significant changes in inflammatory, hemostatic, and cardiac electrophysiological end points after PM exposure (4, 48). This discrepancy with the current study may reflect the fact that a low dose of an isolated constituent of PM was used, whereas the current study used complete ultrafine PM isolated from the Chapel Hill, NC, airshed. Likewise, Routledge et al. (48) did not show alterations in heart rate variability (HRV) or systemic inflammation with carbon black or sulfur dioxide exposure in healthy humans and patients with stable angina. Carbon black is considered to be representative of the carbon core of ambient PM and lacks the absorbed materials found on the environmentally derived PM used in the current study.

In addition to altered vascular reactivity, PM exposure has been linked to autonomic dysfunction, which may contribute to the cardiotoxic effects of PM. Long-term PM exposure is associated with increased mortality due to dysrhythmias (45). Studies have reported an association between ambient PM exposure and decreased HRV in humans (58). In addition, increased defibrillator charges in patients were associated with PM (58). Studies using a rat model of hypertension indicate that heart rate is increased and PQ interval prolonged after environmentally realistic PM exposure conditions (7). Wellenius et al. (64) demonstrated that exposure to CAPs increased intra-ventricular arrhythmia and decrease heart rate in a rat model of acute myocardial infarction (26).

The results of this study demonstrate the potential for ultrafine PM isolated from ambient air to exert a detrimental effect on the oxidative state of the myocardium, vascular reactivity, and recovery of the myocardium from I/R, which are important factors in determining the outcome of an adverse cardiac event. The findings warrant further study of ultrafine PM exposure on the coronary circulation and the signaling mechanisms critical for maintaining adequate myocardial perfusion.

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REFERENCES


