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Ultrafine Particulate Matter Exposure Impairs Vasorelaxant Response in Superoxide Dismutase 2 Deficient Murine Aortic Rings

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Abstract

Studies have linked exposure to ultrafine particulate matter (PM) and adverse cardiovascular events. Particulate matter-induced oxidative stress is believed to be a key mechanism underlying observed adverse vascular effects. Advanced age is one factor known to decrease anti-oxidant defenses and confer susceptibility to the detrimental vascular effects seen following PM exposure. The present study was designed to investigate the vasomotor responses following ultrafine PM exposure in wild type (WT) and superoxide dismutase 2 deficient (SOD2^{+/-}) mice which possess decreased anti-oxidant defense. Thoracic aortic rings isolated from young and aged WT and SOD2^{+/-} mice were exposed to ultrafine PM in a tissue bath system. Aortic rings were then constricted with increasing concentrations of phenylephrine, followed by relaxation with rising amounts of nitroglycerin (NTG). Data demonstrated that ultrafine PM decreased the relaxation response in both young WT and young SOD2^{+/-} mouse aortas, and relaxation was significantly reduced in young SOD2^{+/-} compared to WT mice. Ultrafine PM significantly diminished the NTG-induced relaxation response in aged compared to young mouse aortas. After ultrafine PM exposure, the relaxation response did not differ markedly between aged WT and aged SOD2^{+/-} mice. Data demonstrated that the greater vascular effect in aortic rings in aged mice *ex vivo* after ultrafine PM exposure may be attributed to ultrafine PM-induced oxidative stress and loss of anti-oxidant defenses in aged vascular tissue. Consistent with this conclusion is the attenuation of NTG-induced relaxation response in young SOD2^{+/-} mice.

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Keywords

aorta; superoxide dismutase (SOD); ultrafine PM; vasoreactivity

INTRODUCTION

Acute ambient particulate matter (PM) exposure precipitates cardiovascular events, such as vasoconstriction, increased blood pressure, arrhythmias, triggering of myocardial infarction; while chronic exposure to air pollutants promotes progression of atherosclerosis (Pope et al. 2004; 2006; 2015; Brook et al. 2010; Niemann et al. 2017; Suwa et al. 2002; Cascio 2016; Chen et al. 2015; Chiu et al. 2017). Ambient PM is a complex mixture of solid, semi-volatile and aqueous materials of various sizes and sources. PM includes coarse (2.5–10 µm in aerodynamic diameter), fine (<2.5 µm in aerodynamic diameter) and ultrafine (0.1 µm in aerodynamic diameter) particles. Increasing evidence suggests that ultrafine particles (UFP) are particularly important mediators initiating cardiovascular effects attributed to air pollution exposure (Tong et al. 2010; Cozzi et al. 2006; Lanzinger et al. 2016a; 2016b; Mills et al. 2009; Stone et al. 2017). The adverse cardiovascular effects noted following ultrafine PM exposure are thought to be due to (1) high alveolar deposition efficiency, (2) magnitudes higher particle number concentration, (3) large surface area, (4) greater concentrations of adsorbed or condensed toxic air pollutants per unit mass, and (5) potential of their associated redox-active components to reach cardiovascular target sites (Delfino et al. 2005). Ultrafine PM were found to contain polycyclic aromatic hydrocarbons (PAH), organic carbons, and elemental carbons as primary products from mobile source emissions, particularly diesel and automobile exhaust (Kim et al. 2002; Li et al. 2003), that lead to the production of cytotoxic reactive oxygen species (ROS) (Nel et al. 2001).

Oxidative stress was implicated in air pollution-induced adverse cardiovascular effects (Niemann et al. 2017; Ghio et al. 2012). Various investigators showed that ultrafine PM generated the greatest ROS activity (Cho et al. 2005; Jeng 2010; Ntziachristos et al. 2007), inducing cellular oxidative stress and mitochondrial damage in macrophages and epithelial cells (Li et al. 2003) and increasing hydrogen peroxide (H₂O₂) production from endothelial cells (Snow et al. 2014). Ultrafine particles enhance early atherosclerosis, partly due to their high content of redox cycling chemicals and ability to synergize with known pro-atherogenic mediators in the promotion of oxidative stress in tissues (Araujo and Nel 2009). In addition, advanced age is known to decrease anti-oxidant defenses and confer susceptibility of vasculature to detrimental health effects originating from PM exposure (Shumake et al. 2013).

Reactive oxygen species (ROS) levels are regulated by many pro- and anti-oxidant enzymes in cells. One of the anti-oxidant enzymes, superoxide dismutase (SOD) converts superoxide to H₂O₂, which is further degraded by either catalase (CAT) or glutathione peroxidase (GPx). Three isoforms of SOD are expressed in blood vessels: cytosolic or copper-zinc SOD (SOD1), manganese SOD (SOD2) which is present in mitochondria, and an extracellular form SOD (SOD3) (Faraci and Didion 2004). Amongst these isoforms, SOD2 is particularly responsive to and upregulated by oxidative stress (Faraci and Didion 2004) and was reported

to protect against cardiovascular disease occurrence in experimental animal models (van Deel et al. 2008; Kinouchi et al. 1991). Homozygous SOD2 deficient (SOD2^{-/-}) mice survived only up to 3 weeks of age and exhibited several pathologic phenotypes (Lebovitz et al. 1996). Heterozygous SOD2 deficient (SOD2^{+/-}) mice exhibit increased susceptibility to oxidative stress and diminished mitochondrial function (Williams et al. 1998). Mitochondrial SOD2 levels in SOD2^{+/-} mice were reduced by 50% compared to wild-type, and SOD2^{+/-} animals exhibit premature age-related decline in mitochondrial function compared to wild type (Kokoszka et al. 2001). Aging is associated with enhanced ROS production and diminished SOD2 activity (Moon et al. 2001). Previously Zhou et al. (2012) demonstrated that SOD2^{+/-} mice develop aortic stiffening over their lifespan with elevated mitochondrial superoxide generation and reduced extracellular H₂O₂ with age in aortic smooth muscle cells obtained from SOD2^{+/-} mice. The present study was designed to examine the hypothesis that vasomotor responses were impaired by ultrafine PM exposure in aged and SOD2^{+/-} mice.

METHODS and MATERIALS

Animals

Twenty-nine 4-month-old (young, BW 26.2±1.4 g) (n=7) and 16-month-old (aged, BW 30.5±1.9 g) (n=6) heterozygous SOD2 (SOD2^{+/-}) male mice and C57BL/6 wild-type (WT) young (BW 25.6±1.2 g, n=8) and aged (BW 31.1±2.0 g, n=8) littermates were used. All animal experimental procedures were performed in compliance with protocols approved by University of North Carolina at Chapel Hill IACUC according to NIH guidelines. All animals were treated humanely and with regard for alleviation of suffering. Mice were housed in ventilated cages with filtered room air and maintained at 22°C with a 12-hr light/dark cycle, and given free access to food and water.

Particle Collection, Extraction, and Exposure

Ambient pollution particles used in the present study were collected continuously over 7-day periods in August of 2002 in Chapel Hill, North Carolina outside the U.S. EPA Human Studies Facility. Particles were collected using a Chem Vol model 2400 high volume cascade impactor (Rupprecht & Patashnick Co., Albany, NY, USA) operated at flow rates of 800 L/min as previously described (Becker et al. 2005). Coarse and fine particles were collected onto polyurethane foam (PUF; McMaster-Carr, Atlanta, GA), which was previously cleaned with methanol and water and dried under sterile conditions. Ultrafine particles were collected onto G5300 filters (Monandock Non-Wovens LLC, Mt. Pocono, PA). Foam and filter were measured in an environmentally controlled room. The foam or filter was prewetted with a small amount of 70% ethanol, and endotoxin free water was added to a total volume of 40 ml. The particles were removed from foam or filter by sonication for 1 hr in a water bath (FS220; Fisher Scientific, Pittsburgh, PA). Following water extraction, the filters were further sonicated in 20 ml 0.02% Triton X-100 solution for 2 hr to release more particles. The extracted PM material was quickly frozen, dried by lyophilization and stored at -80°C. The ultrafine PM fraction was employed in this study. Particle extracts were sonicated for 15 min and vortexed for 1 min prior to each exposure experiment.

Vascular Responses in Isolated Mouse Aortic Rings

Thoracic aorta was isolated and placed in chilled Krebs-Hensleit buffer and cleaned of excessive adventitial tissue. Care was taken not to injure the endothelium during preparation of the aortic rings. Four segments of mouse aortic rings approximately 2 mm in length were suspended in individual Radnoti 4-unit organ bath chambers filled with Krebs-Hensleit buffer of the following composition (in mmol/L): 120 NaCl, 5.9 KCl, 1.2 MgSO₄, 1.8 CaCl₂, 25 NaHCO₃, and 11 glucose, pH 7.4. The solution was aerated continuously with a 95% O₂ and 5% CO₂ mixture and maintained at 37°C. Tension was recorded with a linear force transducer. All vessels were allowed to equilibrate for at least 1 hr at a resting tension of 700 mg and 1.75 V and the same baseline tension was utilized in all groups throughout the study. The vessels were then constricted with graded doses (10⁻⁷ to 10⁻⁵ mol/L) of L-phenylephrine (PE) and then relaxed with increasing concentrations (10⁻⁸ to 10⁻⁵ mol/L) of nitroglycerin (NTG). Aortic rings were then exposed to 50 µg/ml Chapel Hill (Chapel Hill, NC, USA) ultrafine PM in Krebs-Hensleit buffer for 15 min in a tissue bath system and vascular responses to PE and relaxation responses to NTG in the presence of PM were recorded. The vessel tension was continuously recorded to assess the vascular responses. The degree of vasoreactive responses was expressed as % peak tension induced by 10⁻⁵ mol/L PE as described previously (Zhou et al. 2012).

Statistical Analysis

Data are expressed as means ± SEM. Comparisons between each variable were performed by three-way ANOVA (genotype, age, and dose of vasoactivators) followed by Holm-Sidak test for multiple comparisons. T-test was used for comparisons between two groups. The statistical analysis was performed using SigmaPlot (version 13; San Jose, CA, USA). The statistical significance level was set at $p < 0.05$.

RESULTS

Vascular Responses to Phenylephrine and Ultrafine PM

Aortic rings were constricted gradually with incremental elevation in concentrations (10⁻⁷ to 10⁻⁵ mol/L) of L-PE. Peak tension was reached at a concentration of 10⁻⁵ mol/L. The tension levels were lower in the aged aortas compared to that in the young (Figure 1). Three-way ANOVA (genotype, age, and dose of PE) showed that there was a significant interaction between age and PE concentration. However, there was no marked interaction between genotype and age and between genotype and PE concentration. Ultrafine PM treatment increased the aortic ring tension by 29.7±7.3% over baseline levels in the young WT group, 32.1±11.6% in the young SOD2^{+/-} group, 22.7±3.2% in the old WT group, and 27.8±15.9% in the old SOD2^{+/-} group. There was no significant difference in responses to ultrafine PM treatment among these groups.

Age and SOD2 Deficiency Impaired Vascular Responses

As reported previously by Zhou et al. (2012), vascular reactivity was determined in thoracic aortas isolated from young and old, WT and SOD2^{+/-} mice. Compared to WT young, WT old aortas exhibited significantly less relaxant responses to NTG at concentrations of 10⁻⁷,

10^{-6} and 10^{-5} mol/L. Aortas from the SOD2^{+/-} young mice also displayed significant reduced relaxation at concentrations of 10^{-6} and 10^{-5} mol/L NTG compared to WT young (Figure 2). In addition, aged SOD2^{+/-} aortas markedly exhibited less relaxant response compared with WT young and old and young SOD2^{+/-} aortas (Figure 2). Three-way ANOVA (genotype, age, and NTG concentrations) analysis demonstrated that there was a significant interaction between genotype and age. No significant interaction between genotype and concentration and age and concentration was noted.

Ultrafine PM Worsen Vascular Relaxation in Young but not Old Mice

The effects of ultrafine PM exposure were investigated on vascular reactivity of aortic rings. As illustrated in Figure 3, ultrafine PM treatment reduced NTG-induced relaxation. Compared with untreated aortas, aortas from ultrafine-treated WT young mice displayed significantly less relaxant response to NTG at concentrations of 10^{-6} and 10^{-5} mol/L. Ultrafine PM treatment markedly reduced the vascular reactivity in SOD2^{+/-} young in response to 10^{-5} mol/L NTG compared with unexposed control. However, ultrafine PM treatment did not induce further fall in vascular responses in both aged WT and SOD2^{+/-} aorta (Figure 4). Three-way ANOVA (genotype, UF exposure, and NTG concentration) analysis showed that there was a significant interaction between genotype and UF exposure and genotype and NTG concentration in young aortas. There was no significant interaction between UF exposure and NTG concentration in the young aortas. There were no marked interactions (genotype, UF exposure, and NTG concentration) in the old aortas.

DISCUSSION

Ambient particulate matter is a risk factor for development of coronary heart disease (McGuinn et al. 2016), progression of atherosclerosis (EPA 2009; Brook et al. 2010), and triggering of myocardial infarction (Nawrot et al. 2011). This study was designed to examine the role of ultrafine PM on vascular responses of aortas and investigate the mechanism underlying PM-induced adverse vascular effects. Results demonstrated that ultrafine PM exposure increased aortic tension and significantly reduced NTG-induced relaxation response in both young WT and SOD2^{+/-} mouse aortas, with greater decrease in young SOD2^{+/-} mice.

Previously Zhou et al. (2012) noted that NTG-induced relaxation of aortas was compromised by both older age and SOD2 deficiency. The diminished relaxation was exacerbated by ultrafine PM treatment in young SOD2^{+/-} and WT mouse aortas, suggesting that ultrafine PM might impair vascular function in young as well as in oxidative stress conditions. Consistent with our observation, Cascio and colleagues (2007) showed in mice that intratracheal instillation of Chapel Hill ultrafine PM altered endothelial dependent and endothelial-independent systemic vascular tone. Further, Mills and colleagues (2011) demonstrated that PM from diesel exhaust exposure attenuated acetylcholine- and sodium nitroprusside-induced vasorelaxation in young healthy human volunteers. In their study, the average diesel particle diameter was in the ultrafine range, suggesting that combustion-derived nanoparticles produced adverse vascular effects. In addition, Mills et al. (2011) found that removal of particles from the diesel exhaust did not produce vascular dysfunction,

indicating that diesel exhaust particles themselves inhibited vascular function. To further examine the role of chemical composition of particles in producing vascular dysfunction, Mills et al. (2011) demonstrated that exposure to pure carbon nanoparticles did not initiate significant vascular dysfunction, suggesting that particle composition plays an important role in the adverse health effects attributed to air pollutants. In contrast, other investigators found that either total suspension of ambient PM or its soluble components induced relaxation of PE-precontracted rat aortic rings by affecting the smooth muscle functions (Bagate et al. 2006; Knaapen et al. 2001). Nonetheless, these observations are in agreement with the postulation that particle composition determines adverse health effects (Mills et al. 2011) as control particles, TiO₂, did not elicit any apparent vasodilatory effects (Knaapen et al. 2001).

Brook et al. (2010) suggested that oxidative stress and inflammatory pathways mediate the adverse health effects due to air pollution exposure. Particle size, surface area, and surface chemistry are thought to serve as important determinants of the adverse health effects attributed to air pollutants. Air pollutant surfaces are coated with highly reactive oxidative transition metals (Gurgueira et al. 2002) and ultrafine PM has the highest ROS activity (Cho et al. 2005; Jeng 2010; Ntziachristos et al. 2007). Therefore, the cytotoxicity mediated by ultrafine PM might stem from ROS-induced pathways. Oxidative stress has been implicated in the adverse cardiovascular effects induced by air pollution (Ghio et al. 2012). Subchronic inhalation of ultrafine PM for 5 hr per day, 3 days per week, for a total of 75 hr induced significantly larger early atherosclerotic lesions and systemic oxidative stress in mice (Araujo et al. 2008). The mechanistic studies underlying ROS-mediated adverse health effects related to air pollution exposure (1) utilize pharmacological inhibitors of ROS release, (2) directly measure ROS production, or (3) indirectly measure pro- and antioxidant enzyme activities regulating ROS levels (Sun et al. 2008; Gurgueira et al. 2002; Araujo and Nel 2009; Araujo et al. 2008). No apparent study has been conducted to investigate the impact of deleting anti-oxidant genes on air pollution-induced health effects, such as deletion of the SOD gene. In this study, aortas from SOD2^{+/-} mice treated with ultrafine PM were employed and data demonstrated that ultrafine PM significantly reduced NTG-induced relaxation of aortic rings, suggesting that the vascular effects attributed to ultrafine PM exposure may be due to ultrafine PM-induced oxidative stress and loss of anti-oxidant defenses in vascular tissue.

Another interesting finding of this study is that ultrafine PM treatment did not further reduce the compromised NTG-induced vascular relaxation observed in aged WT and SOD2^{+/-} mice. Moon et al. (2001) reported that SOD2 activity is significantly lowered in vascular smooth muscle cells from aged mice and in human arteries in older individuals (D'Armiento et al. 2001). Further, SOD2 deficiency accelerated atherosclerosis (Ballinger et al. 2002) and endothelial dysfunction (Ohashi et al. 2006) in mice. In addition, Zhou et al. (2012) previously reported that aging initiated aortic stiffness in WT and SOD2^{+/-} mice, providing evidence that anti-oxidant activity falls over a lifetime. Thus, ultrafine PM treatment showed no further effect on decreased NTG-induced vascular reactivity seen in older mice that already exhibited reduced anti-oxidant defense due to aging processes.

There are limitations to this study. To eliminate the impact of estrogens on vascular tone, only male mice were employed in this study as vascular responses differ in gender. Estrogen receptors are located on vascular endothelial and smooth muscle cells. Estrogens affect vascular tone indirectly by modulating release of endothelium-derived vasoactive factors and directly by modulating intracellular calcium in vascular smooth muscle cells (Miller 1999; Orshal and Khalil 2004). The ultrafine PM concentration may not be relevant for systemic exposure. Yet the high concentration of PM was used to investigate the mechanism underlying PM-induced vascular effects.

CONCLUSIONS

Data demonstrated that ultrafine PM significantly reduced NTG-induced relaxation responses in young WT and SOD2^{+/-} mouse aortas, with greater attenuation in young SOD2^{+/-} mice. The relaxation response after ultrafine PM exposure did not markedly differ between aged WT and aged SOD2^{+/-} mice. Data suggest that the greatest compromise in vascular response of aortic rings in aged mice after ultrafine PM exposure may be due to ultrafine PM-induced oxidative stress and loss of anti-oxidant defenses in SOD2^{+/-} vascular tissue.

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List of Abbreviations

H₂O₂	hydrogen peroxide
NTG	nitroglycerin
PAH	polycyclic aromatic hydrocarbons
PE	L-phenylephrine
PM	particulate matter
ROS	reactive oxygen species
SOD2	superoxide dismutase 2 deficient
WT	wild type

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Figure 1. Phenylephrine (PE) increased aortic tension in the WT and SOD2^{+/-} mouse aortic rings. N=6–8 mice each group. *p<0.05 aged WT (WT-O) vs. young WT (WT-Y) aortic rings.

Figure 2.

Vascular response is impaired with age and SOD2 deficiency in isolated young (4 months old) and aged (16 months old) mouse aortic rings. Nitroglycerine (NTG) induced relaxation (% of peak PE response) of aortic rings pre-constricted with 1 μ mol/L phenylephrine (PE) treatment. N=6–8 mice each group. *p<0.05 vs. young WT (WT-Y) aortic rings; #p<0.05 aged SOD2 ^{+/-} (SOD-O) vs. aged WT (WT-O) aortic rings.

Figure 3.

Ultrafine PM treatment decreased the vascular relaxation response in isolated young SOD^{2+/-} and WT mouse aortic rings. Nitroglycerine (NTG) induced relaxation (% of peak PE response) of aortic rings pre-constricted with 1 μ mol/L phenylephrine (PE). N=6–8 mice each group. *p<0.05 ultrafine PM treated young WT (UF-WT-Y) vs. untreated young WT aortic rings (WT-Y), and #p<0.05 ultrafine PM treated young SOD (UF-SOD-Y) vs. untreated young SOD (SOD-Y) aortic rings.

Figure 4.

Ultrafine PM treatment had no further effect on the vascular relaxant response in isolated aged SOD2^{+/-} and WT mouse aortic rings. Nitroglycerine (NTG) induced relaxation (% of peak PE response) of aortic rings pre-constricted with 1 μ mol/L phenylephrine (PE). N=6–8 mice each group.