Air pollution and cardiometabolic remodeling: a role for RhoA/Rho-kinase

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AMBIENT AIR POLLUTION mediated by fine particulate matter (PM) of <2.5 μm in aerodynamic diameter (PM2.5) has been associated with adverse cardiovascular outcomes (8, 31, 39, 49). One important mechanism is the elevation in blood pressure (BP) that may occur within hours to days after exposure to high concentrations of PM2.5 (6, 16, 21, 52). Recent studies (21, 58) have shown that commonly encountered levels of airborne pollutants can result in a prohypertensive response in humans that may be exaggerated in predisposed individuals. This potentiating effect of inhaled particulates has been noted with other chronic conditions or risk factors such as atherosclerosis, diabetes, and postmenopausal status (31, 40, 49). Although the precise mechanisms remain elusive, there is increasing evidence that PM2.5 exposure results in rapid changes in the vasculature (7, 32). We (51) have previously shown that PM2.5 exposure results in the activation of RhoA/Rho-kinase (ROCK) through ROS pathways and hypothesized that this important signaling cascade may mediate at least some of the prohypertensive effects of PM2.5. Given the important role of RhoA/ROCK in a multitude of processes, including the regulation of smooth muscle tone, cellular migration, and hypertrophy (36, 47), in the present study we examined the effect of PM2.5 exposure on vascular function and cardiac remodeling and tested the effects of ROCK antagonism.

MATERIALS AND METHODS

All experimental procedures were approved by the Committees on Use and Care of Animals of New York University and The Ohio State University.

Animals and PM2.5 Exposure Protocol

Male C57BL/6 mice (8 wk old) were purchased from the Jackson Laboratory (Bar Harbor, ME). Animal exposure and the monitoring of exposure atmosphere and ambient aerosol were performed as previously described (49, 51) using a versatile aerosol concentration enrichment system. Briefly, 52 mice were exposed to concentrated PM2.5 or filtered air (FA) in a mobile trailer at The Ohio State University campus of Columbus, OH (“Ohio’s air pollution exposure system for the interrogation of systemic effects”). FA-exposed mice received an identical protocol with the exception of a high-efficiency particulate air filter positioned in the inlet valve position to remove PM2.5 in the filtered air stream. The exposure protocol was composed of 10 × ambient concentration for 6 h/day, 5 days/wk, for a total of 12 wk from May to August 2008. The exposure was followed by an osmotic minipump implantation for the infusion of angiotensin II (ANG II) or vehicle. This was followed by treatment with fasudil or placebo. Blood pressure was monitored, followed by analysis of vascular function and ventricular remodeling indexes. PM2.5 exposure potentiated ANG II-induced hypertension, and this effect was abolished by fasudil treatment. Cardiac and vascular RhoA activation was enhanced by PM2.5 exposure along with increased expression of the guanine exchange factors (GEFs) PDZ-RhoGEF and p115 RhoGEF in PM2.5-exposed mice. Parallel with increased RhoA activation, PM2.5 exposure increased ANG II-induced cardiac hypertrophy and collagen deposition, with these increases being normalized by fasudil treatment. In conclusion, PM2.5 potentiates cardiac remodeling in response to ANG II through RhoA/Rho kinase-dependent mechanisms. These findings have implications for the chronic cardiovascular health effects of air pollution.
ANG II Infusion and Fasudil Treatment

At the end of the 12-wk exposure, mice received an osmotic pump (Alza, Mountain View, CA) containing ANG II (0.75 mg/kg/day ANG II in 0.15 mol/l NaCl and 0.01 N acetic acid) or vehicle for a duration of 14 days. This dose of ANG II provides a plasma concentration similar to that reported in patients with renovascular hypertension and was based on prior publications (19, 41). Fasudil (1 mg·kg·day⁻¹) or placebo (0.9% NaCl) was started the day after ANG II and was administered intraperitoneally for 13 days. A previous study (47) has shown that fasudil is a inhibitor of ROCK.

BP Monitoring

Systolic BP (SBP) was measured using a computerized noninvasive tail-cuff manometry system (Visitech IITC model 129 System, Visitech Systems, Apex, NC). To avoid procedure-induced anxiety, mice were trained for 5 consecutive days before the experimental procedure, and fasudil was administered 1 h earlier. The first 10 of 20 BP values recorded with each measurement were disregarded, and the remaining 10 values were collected and averaged for analysis of each mouse. BP was recorded daily after ANG II or placebo infusion for duration of 14 days.

Myograph Experiments

Myograph experiments were performed as previously described (49). Briefly, at the end of the study period, the aorta was removed, and 2-mm thoracic aortic rings were mounted in individual organ chambers. Rings were subjected to graded doses of the vasoconstrictor phenylephrine (PE; 10⁻⁶ mol/l). To examine functional RhoA/ROCK activity in the aorta, aortic rings were precontracted by PE (10⁻⁶.5 mol/l) with fasudil added in an cumulative manner. The degree of relaxation was taken as an indication of Rho/ROCK activity.

RhoA Activity Assays

We measured RhoA activity using two approaches. In the first approach, we measured the translocation of RhoA from the cytosol to membrane; in the second approach, we measured RhoA activity based on the binding of “active” Rho-GTP.

RhoA translocation assay. Cardiac tissue was homogenized in cold homogenization buffer [containing (in mM) 0.0 Tris HCl (pH 7.4), 1 EGTA, 1 EDTA, 1 PMSF, and 1 Na₃VO₄]. The homogenate was resuspended in homogenization buffer containing 1% Triton X-100. Protein concentrations were determined with a bicinchoninic acid kit (Pierce). RhoA migration was determined by Western blot analysis. Briefly, equal amounts of proteins were separated by SDS-PAGE and subsequently transferred to a nitrocellulose membrane. The membrane was then incubated with monoclonal Rho antibody. Finally, membranes were incubated with a horseradish peroxidase-linked secondary antibody and visualized with an enhanced chemiluminescence kit (Amersham).

RhoA activity. RhoA-GTP levels in the aorta were determined with the G-LISA RhoA activation assay kit (Cytoskeleton, Denver, CO) according to the manufacturer’s instructions. The RhoA G-LISA kit contains a Rho-GTP-binding protein linked to the wells of a 96-well plate. Active GTP-bound RhoA is removed during washing steps.

Quantitative Real-Time PCR

Total RNA was prepared from heart tissue with a RNeasy Mini kit (Qiagen, Valencia, CA). cDNA was synthesized by a Transcriptor First-Strand cDNA Synthesis Kit (Roche, Indianapolis, IN). Quantitative real-time PCR was performed using the iQ5 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA). Sequences of primers used for real-time PCR in this study are shown in Table 1.

Collagen Staining

Masson’s trichrome staining and picrosirius red staining were used for the histochemical determination of collagen expression in heart tissues. Four successive sections were collected on the same slide, and at least 10 sections from 3 consecutive slides per area per mouse were examined. Each image was digitized with a digital camera and analyzed under a research microscope (Zeiss Axioskop with Spot I digital camera, Jena, Germany) with NIH Image software (version 1.61, http://rsb.info.nih.gov/nih-image). Results are expressed as percentages of the total selected area. All analyses were performed blindly without knowledge of the origin of the samples.

Picrosirius red staining. Frozen heart cross sections were fixed with ice-cold methanol for 5 min and washed twice in PBS. Sections were stained with picrosirius red (0.1% Sirius red in a saturated aqueous solution of picric acid, Sigma Chemical, St. Louis, MO) for 1 h and washed in acidified water (0.5% acetic acid). Sections were then dehydrated and mounted in a resinous medium. Type I/III collagen appears red on light microscopy, and collagen I appears yellow, whereas collagen III appears red on polarizing microscopy.

Table 1. Primers used for real-time quantitative RT-PCR in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Forward Oligonucleotides</th>
<th>Reverse Oligonucleotides</th>
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<tbody>
<tr>
<td>GEF-H1</td>
<td>5'-GAAGGCGAAAGAAGCTTGGAG-3'</td>
<td>5'-GCATCGGCTTTTCTGTC-3'</td>
</tr>
<tr>
<td>p115RhoGEF</td>
<td>5'-GAAGCCTGAGAGGACTG-3'</td>
<td>5'-AGGAAGTTGAGCGAGCA-3'</td>
</tr>
<tr>
<td>PDZ-RhoGEF</td>
<td>5'-GGGTGCGGCTGACATGGG-3'</td>
<td>5'-TAGGTGACGAGTTAGAC-3'</td>
</tr>
<tr>
<td>t-Argrin</td>
<td>5'-GAAGCTGCGGCTGACATGGG-3'</td>
<td>5'-TAGGTGACGAGTTAGAC-3'</td>
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<tr>
<td>ROCK-I</td>
<td>5'-TGGGACGCAATGATGAGTATAAT-3'</td>
<td>5'-CCGACTAGAATGATG-3'</td>
</tr>
<tr>
<td>ROCK-II</td>
<td>5'-CCGACTAGAATGATG-3'</td>
<td>5'-TCCATATGGAAGCAGACC-3'</td>
</tr>
<tr>
<td>Guanine dissociation inhibitor</td>
<td>5'-ATTGACAGAGTGGACCAAG-3'</td>
<td>5'-TGGACAGAGTGGACCAAG-3'</td>
</tr>
<tr>
<td>Atrial natriuretic peptide</td>
<td>5'-TTTGTGTCCTACATGCTGCTC-3'</td>
<td>5'-AGGAAGAATGAGCTC-3'</td>
</tr>
<tr>
<td>α-Tubulin</td>
<td>5'-TGGGCGGCTGACATGGG-3'</td>
<td>5'-TAGGTGACGAGTTAGAC-3'</td>
</tr>
<tr>
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<td>5'-TGGTGTGTCCTACATGCTGCTC-3'</td>
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<tr>
<td>Collagen type III</td>
<td>5'-AGGAGTTGACGAGTTAGAC-3'</td>
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<tr>
<td>Osteopontin</td>
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<tr>
<td>GAPDH</td>
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<td>5'-AGGAAGAATGAGCTC-3'</td>
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GEF, guanine nucleotide exchange factor; ROCK, Rho-kinase; MMP, matrix metalloproteinase.
Masson’s trichrome staining. Frozen heart cross sections were fixed with acetone. Sections were then stained with Masson’s trichrome stain. Collagen appears blue on light microscopy.

Gelatin Zymography for Matrix Metalloproteinase Activity

Gelatin zymography was performed to determine gelatinolytic activities of matrix metalloproteinase (MMP)-2 and MMP-9 as previously described (42). Myocardial protein (40 μg) was treated with sampling buffer (0.5 mol/l Tris-HCl, glycerol, 10% SDS, and 0.1% bromphenol blue) in a final solution of 20 μl. SDS-PAGE was performed using a 10% polyacrylamid gel containing 0.1% gelatin at 125 V for 60 min. SDS was removed with Triton X-100 for 60 min, and the gel was incubated in a developing buffer (Tris base, Tris-HCl, NaCl, CaCl₂, Brij-35, and ZnCl₂) overnight. Gels were stained for 3 h with 0.5% Coomassie G250 and destained for 60 min in 7% acetic acid and 35% methanol. Gelatinolytic activities were detected as a clear band against a black background (in arbitrary units/cm) and analyzed as relative optical densities.

Morphometric Analyses of Cardiac Hypertrophy

Frozen heart cross sections were stained with hematoxylin and eosin. Four successive sections were collected on the same slide, and at least 10 sections from 3 consecutive slides per area mouse were examined. Each image was digitized with a digital camera and analyzed under a research microscope (Zeiss Axioskop with Spot I digital camera, Jena, Germany) with NIH Image software (version 1.61). Nuclear and cytosolic areas were quantified, respectively, and the results are expressed as ratios of nuclear to cytosolic areas. All analyses were performed blindly without knowledge of the origin of the samples.

Data Analyses

All data are expressed as means ± SD unless otherwise specified. Differences among groups were tested by one-way ANOVA and Bonferroni’s post hoc test. In addition, the interaction between FA and PM was analyzed by two-way ANOVA using Graphpad Prism. In addition, the interaction between FA and PM was analyzed by two-way ANOVA using Graphpad Prizm software (version 4). P values of <0.05 were considered statistically significant.

RESULTS

PM₂.₅ Concentrations during the Study Period

Whole body exposure to PM₂.₅ was made using a versatile aerosol concentration enrichment system as previously described (11, 30). The mean daily ambient PM₂.₅ concentration at the study site was 6.5 ± 5.5 μg/m³, whereas the mean concentration inside the PM₂.₅ chamber was 74.3 μg/m³. During the exposure time period, the outdoor mean temperature was 27.4 ± 6.3°C (median 28.5°C), and the mean outdoor humidity was 65.8 ± 22.2% (median 61.5%). Because mice were exposed 6 h/day, 5 days/wk, the equivalent PM₂.₅ concentration to which mice were exposed “normalized” over the 12-wk period was 11.4 μg/m³ after taking into account nonexposed time and weekends, which is well within the annual average PM₂.₅ National Ambient Air Quality Standard of 15.0 μg/m³ [United States Environmental Protection Agency (51a)]. XRF elemental analysis revealed higher concentrations of a range of metals. Of note, in the transition metal category, levels of both Fe and Zn in the transition metals were disproportionately high (Supplemental Figs. 1 and 2).

BP Changes

There were no significant differences in mean SBP after 12 wk of PM₂.₅ exposure (101.4 ± 11.8 vs. 108.8 ± 7.4 mmHg for FA and PM, respectively, n = 12, P = 0.079). At the end of this exposure period, mice were randomized to receive vehicle, ANG II, or ANG II plus fasudil. No significant differences were noted between vehicle-treated PM₂.₅ and FA mice during the 14 days (Fig. 1, A and B). However, when ANG II was infused in conjunction with PM₂.₅, differences in SBP emerged after 1 wk of ANG II infusion (Fig. 1, A and B). Fasudil treatment decreased SBP to a greater degree in PM₂.₅ compared with FA mice (Fig. 1, A and B).

Vascular Reactivity

Figure 2, A and B, shows the responses of thoracic aortic rings to the α-adrenergic agonist PE. PM₂.₅ exposure potenti-
ated maximal aortic contractile responses to PE in control animals, but, importantly, the increase in maximal constriction was highest in the group that received ANG II and was exposed to PM2.5. Fasudil treatment reduced maximal constriction in both FA and PM groups but to a higher level in PM animals. There were no changes in EC50 values in any of the groups. PM2.5 exposure increased relaxation to the ROCK inhibitor fasudil compared with FA/H11001 vehicle and FA/H11001 ANG II-treated mice, respectively (Fig. 2, C and D). Notably, fasudil treatment decreased the aortic sensitivity to fasudil.

**Cardiac Remodeling With PM2.5**

Figure 3 shows that compared with vehicle-treated FA mice, cardiac mass in vehicle-treated PM2.5 mice increased but did not attain statistical significance. Cardiac mass in the PM2.5 + ANG II-treated mice, respectively (Fig. 2, C and D). Notably, fasudil treatment decreased the aortic sensitivity to fasudil.

**Fig. 2. PM2.5 exposure enhances aortic response to phenylephrine (PE) through activation of RhoA/Rho-kinase (ROCK).** A and B: aortic rings were mounted in myograph chambers. After equilibration, responses to PE were analyzed. n = 6 animals/group. *P < 0.05 vs. the same exposure infused with vehicle; #P < 0.05 vs. FA infused with the same drug; $P < 0.05 vs. the same exposure infused with ANG II. C and D: aortic rings were precontracted by PE (10^{-7.5} M), and fasudil was then added in an accumulative manner. n = 6 animals/group. *P < 0.05 vs. the same exposure infused with vehicle; #P < 0.05 vs. FA infused with the same drug; $P < 0.05 vs. the same exposure infused with ANG II.

**Fig. 3. Effect of PM2.5 exposure on cardiac mass.** n = 6 animals/group. *P < 0.05 vs. the same exposure in animals infused with vehicle; #P < 0.05 vs. FA exposure in animals infused with the same drug; $P < 0.05 vs. the same exposure in animals infused with ANG II.
ent significantly attenuated the increase in cardiac mass in PM$_{2.5}$ + ANG II mice and eliminated the difference between the two groups (Fig. 3). We assessed hypertrophy at the cellular level by measuring the nuclear-to-cytosol ratio of cardiomyocytes and quantified the expression of cardiac hypertrophy markers atrial natriuretic peptide (ANP) and α-tubulin by real-time PCR. Figure 4, A and B, shows that PM$_{2.5}$ exposure did not significantly affect the nuclear-to-cytosol ratio of cardiomyocytes in vehicle-treated mice but significantly decreased the nuclear-to-cytosol ratio of cardiomyocytes in ANG II-treated mice. This effect of PM$_{2.5}$ exposure was abolished by fasudil. Consistent with the morphometric analyses, PM$_{2.5}$ exposure increased the mRNA expression of ANP and α-tubulin in ANG II-treated mice, with this effect also being reversed by fasudil treatment (Fig. 4, C and D).

**Cardiac and Vascular RhoA Activity**

Figure 5 shows that PM$_{2.5}$ exposure increased RhoA translocation to the membrane in vehicle-treated mice compared with FA. ANG II alone had a significant effect on RhoA activation. However, in conjunction with PM$_{2.5}$, there was a marked increase in RhoA translocation in PM$_{2.5}$ + ANG II mice. Fasudil treatment decreased these increases in RhoA translocation and to a higher level in PM$_{2.5}$-exposed mice. In separate experiments, we also evaluated Rho activity in the aorta to confirm that these effects of PM$_{2.5}$ extended to the vasculature, as previously demonstrated by us (50). PM$_{2.5}$ in conjunction with ANG II increased Rho activity 139.3 ± 9.4% compared with the FA + ANG II group (100 ± 18.6%).

**Fig. 4.** PM$_{2.5}$ exposure enhances ANG II-induced cardiomyocyte hypertrophy. A: slides of heart tissue were stained by hematoxylin and eosin. B: results are expressed as the ratio of nuclear to cytosolic size. C and D: mRNA expression of the cardiomyocyte hypertrophy markers atrial natriuretic peptide (ANP; C) and α-tubulin (D) were analyzed by real-time RT-PCR. n = 6 animals/group. *P < 0.05 vs. the same exposure in animals infused with vehicle; #P < 0.05 vs. FA exposure in animals infused with the same drug; $P < 0.05$ vs. the same exposure in animals infused with ANG II.
Expression of Guanine Nucleotide Exchange Factors With PM2.5 Exposure

Since RhoA activity was increased by PM2.5 exposure, we next analyzed the expression of genes that may play a role in RhoA activation. PM2.5 alone did not significantly increase mRNA expression of any of the guanine nucleotide exchange factors (GEFs). In contrast, mRNA for L-arginine, PDZ-RhoGEF, and p115-RhoGEF (Fig. 6), but not GEF-H1 (data not shown), was increased in the FA/H11001 ANG II group compared with the FA/H11001 vehicle group. mRNA expression of PDZ-RhoGEF and p115-RhoGEF but not L-arginine was increased in PM2.5/H11001 ANG II-treated mice (Fig. 6). These increased expression levels of Rho-GEFs in the FA + ANG II and PM + ANG II groups were markedly inhibited by fasudil treatment (Fig. 6). Neither ANG II nor PM2.5 exposure affected the mRNA expression of guanine dissociation inhibitor or the expression of ROCK-I and ROCK-II (data not shown). Protein expression of RhoA was also analyzed by Western blot analysis. No significant difference between groups were observed (data not shown). Results in the aorta mirrored the responses seen in the myocardium (data not shown).

Cardiac Collagen Deposition

To investigate the role of PM2.5 exposure in cardiac collagen deposition, Masson’s trichrome staining was performed. Figure 7, A and B, shows that PM2.5 exposure significantly enhanced cardiac collagen deposition in ANG II-treated mice, with fasudil treatment reducing the increase in collagen deposition. These results were confirmed by picrosirius red staining (Fig. 7, C and D). Some markers of cardiac fibrosis were also analyzed by real-time PCR. Figure 7, E–H, shows the effects on collagen I/III, transforming growth factor (TGF)-β, and osteopontin. Fasudil treatment abolished these additive effects of PM2.5 exposure (Fig. 7, E–H).

Cardiac Expression and Activity of MMPs

Studies (5, 9, 13, 29, 46) have shown that ANG II induces cardiac remodeling through an upregulation of MMP activity. We examined the expression of MMPs in cardiac tissue by real-time PCR and evaluated MMP activity by zymography. Figure 8, A–D, shows that ANG II increases the mRNA expression level of MMP-2, with PM2.5 exposure further enhancing this increased expression of MMP-2. In addition to its effect on enhancement of mRNA expression of MMP-2, PM2.5 exposure increased activated lower-molecular-weight forms of MMP-2, as shown by zymography (Fig. 8, E and F).

DISCUSSION

In this study, we demonstrated an important effect of PM2.5 preexposure on BP and cardiovascular remodeling in a model of experimental hypertension. PM2.5 activated RhoA in the aorta and myocardium with resultant cardiac fibrosis and the activation of matrix-degrading enzymes. These changes were prevented by the ROCK antagonist fasudil. RhoA activation may represent an important pathway through which PM2.5 mediates its effects.

In a prior publication (50), we demonstrated an important effect of PM2.5 preexposure on BP and cardiovascular remodeling in a model of experimental hypertension. PM2.5 activated RhoA in the aorta and myocardium with resultant cardiac fibrosis and the activation of matrix-degrading enzymes. These changes were prevented by the ROCK antagonist fasudil. RhoA activation may represent an important pathway through which PM2.5 mediates its effects.
regulation of systemic, pulmonary, and mesenteric vascular tone. Abnormal activation of the RhoA/ROCK pathway has been implicated in the pathogenesis of hypertension by Ca\(^{2+}\)/H\(_{11001}\) sensitization of the contractile apparatus (28, 35, 43). Our results strongly implicate RhoA/ROCK as playing a pathophysiological role in PM2.5-mediated BP effects and cardiovascular remodeling (21, 52, 58).

An important finding in this study is that short-term PM2.5 exposure enhances ANG II-induced cardiac remodeling, as evidenced by the increase in cardiac mass, cardiomyocyte hypertrophy, and collagen deposition. While left ventricular remodeling may represent an adaptive response to pressure or volume overload to preserve cardiac function, an exuberant remodeling response predisposes to heart failure, arrhythmia, and sudden death. PM2.5 exposure increased cardiac RhoA activation, with reversal of these effects by fasudil, strongly indicating that RhoA/ROCK plays a significant role in mediating the effects of inhaled particulates on cardiac remodeling and BP. RhoA/ROCK has been demonstrated to be rapidly activated in response to various stimuli (3, 18, 23, 44). Overexpression of constitutively active RhoA or dominant negative mutants in cardiomyocytes modulates the expression of various genes and pathways involved in cardiac hypertrophy (3, 10, 24), whereas ROCK inhibition attenuates cardiac hypertrophy in hypertensive strains and in response to exogenous ANG II (44, 54). Recent studies (18, 23, 44, 54) have also suggested an important role for RhoA/ROCK in myocardial fibrosis. A characteristic consequence of inhibition of ROCK is an amelioration of left ventricular fibrosis, as has been previously demonstrated in these studies. A heterozygous deletional mutant of ROCK-I (predominantly expressed in the vasculature and heart) is characterized by reduced perivascular fibrosis (43). Consistent with a potential role for this pathway in cardiovascular disease, the same injurious stimuli such as ANG II and smoking, which are well known to trigger fibrosis, can trigger the activation of fibroblasts to a synthetic phenotype resulting in reparative fibrosis (38, 56). This process is initiated by TGF-\(\beta\), which is typically produced in response to cellular damage and hormones such as ANg II (22, 38). TGF-\(\beta\), in turn, stimulates the expression of genes that are characteristic of myofibroblasts, including smooth muscle actin, fibronectin, and osteopontin (25). We demonstrated an important effect of PM2.5 on both TGF-\(\beta\) and osteopontin, molecules that have been implicated as being obligatory in the activation of the myofibroblast and the transformation to a cell type actively involved in extracellular matrix synthesis (25, 57). Indeed, prior studies (22, 57) have suggested that both TGF-\(\beta\) and osteopontin are correlated with the transition to heart failure. While PM2.5 has been associated with a robust profibrotic response, these changes were not accompanied by parallel increases in collagenolytic enzymes such as MMP-1 and MMP-9. However, MMP-2 activity and expression were increased. The reasons for the preferential activation of MMP-2 in our work may be related to the influence of ROS on the activation of this enzyme, as previously demonstrated by us (42). However, we cannot exclude an effect of PM2.5 on MMP-1 activity, as has been demonstrated in pulmonary alveolar epithelial cells (1).

Our findings thus may have strong implications for the association between air pollution and congestive heart failure admissions and suggest a direct effect of PM2.5 on myocardial structure (6, 12, 53, 55). Such alterations could theoretically then increase myocardial stiffness, compliance, and diastolic filling properties of the left ventricle, resulting in a predisposition to diastolic heart failure.

How may PM2.5 mediate Rho activation? In our previous work (51), we demonstrated an important effect of PM2.5 in the activation of the NAD(P)H oxidase system and demonstrated that the ROS generated through this pathway in response to PM activates RhoA/ROCK. PM2.5 has previously been shown to regulate ROS generation through a NADPH oxidase-dependent...
mechanism in a variety of cell types and may represent an important upstream pathway by which PM2.5 may potentiate the effects of ANG II (4, 17, 27). Since ANG II is well known to regulate NADPH oxidases, this may well represent an important upstream pathway by which PM2.5 exerts additive effects on ANG II-mediated BP and cardiovascular remodeling (14, 41). In addition, PM2.5 may have direct effects on ANG II receptor subtype 1 signaling that could partly explain our findings. In a study with isolated pulmonary arterial rings and pulmonary arterial endothelial cells, Li et al. (26) demonstrated that ultrafine PM (SRM1648) at concentrations from 1 to 100 μg/ml could induce constriction in pulmonary artery rings and that this effect was blocked by losartan, an ANG II receptor subtype 1 antago-

Fig. 7. PM2.5 exposure enhances ANG II-induced cardiac collagen deposition. A and B: representative micrographs (A) and summary of data (B) analyzed by Masson's trichrome staining. C and D: representative micrographs (C) and summary of data (D) analyzed by picrosirius red staining. E–H: mRNA expression of cardiac collagen deposition-related genes analyzed by real-time RT-PCR and expressed as fold increases over GAPDH. TGF, transforming growth factor. n = 6 animals/group. *P < 0.05 vs. the same exposure in animals infused with vehicle; #P < 0.05 vs. FA exposure in animals infused with the same drug; $P < 0.05 vs. the same exposure in animals infused with ANG II.
In their study (26), Zn, a significant component of the PM sample, could replicate these effects. We believe that our data provides a mechanistic basis for the link between PM2.5 exposure and increases in BP and strongly implicates RhoA/Rho-kinase as playing a pathophysiologic role in the acute tonal responses and in the maladaptive remodeling response that sustains hypertension. The presence of high concentrations of transition metals in the inhaled particulates may argue for a role for reactive metals in potentiating free radical reactions. The locus of these interactions at the present time is unclear but there is intriguing information that inhaled particulates particularly those in the ultrafine range, may directly transgress into the circulation where it may mediate effects. (33, 34, 45) There is also interesting evidence of heightened oxidant stress with inhaled particulates in the myocardium mediated through the autonomic nervous system. (15) Regardless of the mechanism, the fact that inhaled particles induce these effects in the myocardium in hypertension has important implications for the cardiovascular effects of air pollution.

There are several limitations to our study, including the inability to distinguish the BP-dependent and -independent effects of RhoA/ROCK activation induced by PM2.5 exposure and whether the left ventricular changes are a cause of consequence of changes in systemic vascular resistance. These issues may be difficult to sort out in the context of an in vivo study as these ventricular-vascular adaptive responses are sometimes inseparable. Additionally, almost any drug that alters Rho/ROCK will also simultaneously affect BP. An additional limitation pertains to the use of pharmacological inhibitors of the ROCK pathway as these inhibitors may have nonspecific effects through targeting of other kinases, including PKC (48). Thus, some of our effects may relate to non-ROCK effects of fasudil. A final limitation pertains to the use of noninvasive BP monitoring used in this study. We exerted considerable care in the measurements with careful training of the mice.

In conclusion, our results suggest that environmentally relevant, low concentrations of ambient PM2.5 exposure may have detrimental effects on the cardiovascular system, especially in...
conjunction with other predisposing conditions or risk factors. PM2.5 exposure alone has weak effects on measurable outcomes, but in conjunction with these additional factors may have a substantive impact in modulating outcomes such as BP and myocardial remodeling. Our findings have important implications for the association of PM2.5 with hypertension and heart failure and provide one putative mechanism by which PM2.5 may mediate its effects.

GRANTS

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REFERENCES


FASUDIL PREVENTS AIR POLLUTION-INDUCED VENTRICULAR REMODELING


