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Diesel exhaust induced pulmonary and cardiovascular impairment: The role of hypertension intervention

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ABSTRACT

Exposure to diesel exhaust (DE) and associated gases is linked to cardiovascular impairments; however, the susceptibility of hypertensive individuals is poorly understood. The objectives of this study were (1) to determine cardiopulmonary effects of gas-phase versus whole-DE and (2) to examine the contribution of systemic hypertension in pulmonary and cardiovascular effects. Male Wistar Kyoto (WKY) rats were treated with hydralazine to reduce blood pressure (BP) or L-NAME to increase BP. Spontaneously hypertensive (SH) rats were treated with hydralazine to reduce BP. Control and drug-pretreated rats were exposed to air, particle-filtered exhaust (gas), or whole DE (1500 μg/m^3), 4 h/day for 2 days or 5 days/week for 4 weeks. Acute and 4-week gas and DE exposures increased neutrophils and γ-glutamyl transferase (γ-GT) activity in lavage fluid of WKY and SH rats. DE (4 weeks) caused pulmonary albumin leakage and inflammation in SH rats. Two-day DE increased serum fatty acid binding protein-3 (FABP-3) in WKY. Marked increases occurred in aortic mRNA after 4-week DE in SH (eNOS, TF, tPA, TNF-α, MMP-2, RAGE, and HMGB-1). Hydralazine decreased BP in SH while L-NAME tended to increase BP in WKY; however, neither changed inflammation nor BALF γ-GT. DE-induced and baseline BALF albumin leakage was reduced by hydralazine in SH rats and increased by L-NAME in WKY rats. Hydralazine pretreatment reversed DE-induced TF, tPA, TNF-α, and MMP-2 expression but not eNOS, RAGE, and HMGB-1. ET-1 was decreased by HYD. In conclusion, antihypertensive drug treatment reduces gas and DE-induced pulmonary protein leakage and expression of vascular atherogenic markers.

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Introduction

Epidemiological and experimental evidence suggests that exposures to gas and ambient particulate matter (PM) are associated with increased vasoconstriction, increased blood pressure (BP), myocardial infarction, stroke, ischemia, and even arrhythmias in healthy individuals and those with preexisting cardiovascular disease (Bell and HEI Health Review Committee, 2012; Brook et al., 2010). Those with highly compromised cardiovascular health are likely to cross the threshold leading to increased mortality (Colais et al., 2012); however, how air pollution impacts those with relatively mild impairments, such as hypertension, is not well understood. More than 30% of adults worldwide suffer from systemic hypertension (WHO, 2012), and many take antihypertensive therapy. Thus, the vulnerability of those with hypertension to air pollution effects becomes a significant public health concern. And therefore, it is important to determine how hypertensive individuals under antihypertensive therapy (and individuals taking medication that may increase BP) respond to air pollution.

A number of studies have employed genetically predisposed rat model of human hypertension in examining susceptibility difference to PM and also diesel exhaust (DE) (Hazari et al., 2012; Kodavanti et al., 2000). We recently have shown that, although myocardial tissue of healthy Wistar Kyoto (WKY) rats shows many gene expression changes after DE exposure, the spontaneously hypertensive (SH) rat myocardium does not respond to DE (Gottipolu et al., 2009). Even though the DE-exposed WKY rats' myocardial expression pattern seems to mimic expression in hypertensive rats without DE, these changes are associated with decreased BP and contractile response in WKY rats (Gordon et al., 2012). The systolic BP changes might result from peripheral vasculature alterations; however, thus far, no studies have compared the systemic vascular and myocardial effects of DE in SH and WKY rats. It is not known if genetic factors or physiological presence of hypertension might be altering responses to air pollution.

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Numerous studies have employed DE as a prototypic near-road air pollution source in human clinical and animal toxicological studies (Adar and Kaufman, 2007; Barath et al., 2010). However, limited information is available on how gas-phase components versus PM contribute to adverse cardiopulmonary and vascular health outcomes (Gordon et al., 2012). Because carbon monoxide (CO), one of the gas-phase components, is a vasodilator (Leffler et al., 2011), DE-associated CO might influence the vasculature differently than does particulate DE.

The purpose of the present study was twofold. First, we sought to determine the nature of pulmonary and cardiovascular effects of gas-phase components versus whole DE in normotensive WKY and SH rats. Second, we wanted to understand the contribution of the physiological state of hypertension in modifying pulmonary and cardiovascular response to gas or DE. We used pharmacological agents to manipulate the hypertension status of WKY and SH rats and examined pulmonary, systemic, aortic, and cardiac effects using tissue analysis approaches used in our previous study (Kodavanti et al., 2011).

Materials and methods

Animals and drug treatments. Healthy male WKY and SH rats (11 weeks old) were purchased from Charles River Laboratories, Inc., Raleigh, NC. The rats were acclimatized for 1 week (21 ± 1 °C, 50 ± 5% relative humidity, and 12-h light–dark cycle) and were single-housed in polycarbonate, individually ventilated cages with beta chip bedding. All animals received standard Purina rat chow (Brentwood, MO) and water ad libitum. Ten days prior to exposure, two groups of WKY rats received hydralazine, a direct-acting, vascular smooth-muscle relaxant shown to decrease BP (Vidrio et al., 2003), or L-nitro-arginine methyl ester (L-NAME), a nonselective inhibitor of nitric oxide synthase shown experimentally to induce hypertension (Kameyama et al., 2005), each at 150 mg/L in drinking water. This regimen was continued until necropsy (Fig. 1). Use of animals in this study was approved by the U.S. EPA NHEERL Animal Care and Use Committee.

DE generation and animal exposures. DE generation and exposure methodology for gas and whole-DE recently was described (Gordon et al., 2012). Briefly, a 4.8 kW (6.4 hp) Yanmar L70 V engine and low sulfur diesel fuel (32 ppm) were used. Diluted whole exhaust was directed to one Hazelton 1000 (984 L) exposure chamber, and a second chamber received gas-phase components following filtration of PM through a high-efficiency particulate air (HEPA) filter. A third chamber received HEPA-filtered room air. The target DE particulate concentration was 2 mg/m^3, and the actual concentrations achieved are listed in Table 1. Continuous emission monitors (CEMs) were used to measure chamber concentrations of PM and gas components as described in Gordon et al. (2012). The organic carbon/elemental carbon ratio of ~0.6 for particulate fraction, using similar fuel and combustion conditions, has been reported (Sharkhuu et al., 2010). Rats were exposed 4 h/day for 2 consecutive days or 4 h/day, 5 days/week for 4 consecutive weeks.

BP. Systolic BP was monitored using tail-cuff methodology at day −10 (before the drug treatment began), 0 day (prior to beginning of exposure to gas and DE), 2 days after exposure, and 2 days prior to a 4-week necropsy. We used the IITC Life Sciences (Woodland Hills, CA) model 179 BP Analyzer, model 20NW Cuff pump, B60-7/16″ tail cuff, and restraining tubes. Rats were acclimatized twice prior to the beginning of the −10-day determination.

Necropsy, sample collection, histology, and bronchoalveolar lavage fluid (BALF) markers. One day after final exposure, rats were weighed and anesthetized with intraperitoneal sodium pentobarbital (50–100 mg/kg). Blood was collected from the abdominal aorta directly into blood collection tubes containing ethylenediaminetetraacetic acid (EDTA; for complete blood counts), citrate (for platelet aggregation), or in serum separator tubes without an anticoagulant (for cytokine and biomarker assays). The heart was removed, blotted dry, weighed, and cut into two mid-longitudinal halves. One half was fixed in 10% neutral formalin for histological evaluation. From the second half, the right ventricle was discarded, and the left ventricle plus septum was snap-frozen in liquid nitrogen and retained for ribonucleic acid (RNA) isolation. Segments of aorta were snap-frozen for RNA isolation and aortic arches were fixed in 10% formalin. Heart, lung, and aorta tissues were processed for histology, and slides were stained with hematoxylin and eosin for examination at light microscopy level. The right lung was lavaged as described in Gordon et al. (2012). BALF analysis for lung inflammation and injury markers was performed as described in a previous publication (Kodavanti et al., 2011).

Complete blood count, platelet aggregation, and serum biomarker analysis. Aliquots of serum from EDTA-collected blood were analyzed for complete blood counts by a Beckman–Coulter AcT blood analyzer (Beckman-Coulter, Inc., Fullerton, CA). Adenosine diphosphate-induced aggregation was measured using the BioData Corporation Platelet Aggregation Profiler (PAP-8E) and protocol (Horsham, PA). Serum samples were processed for troponin, von Willebrand Factor (vWf), soluble E-selectin (sE-selectin), soluble ICAM-1, and adiponectin

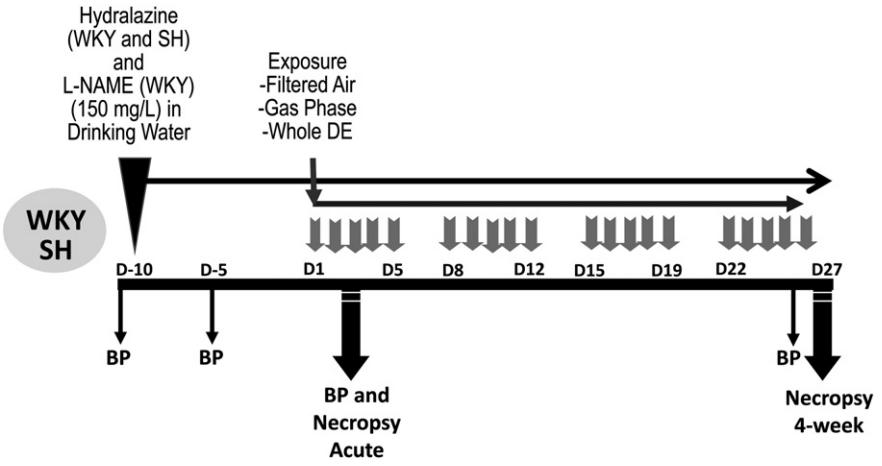

Fig. 1. Schematic of the experimental protocol. WKY = Wistar Kyoto rats; SH = spontaneously hypertensive rats, DE = diesel exhaust, BP = systolic blood pressure. The solid black horizontal line and the text just above represent days. Drug treatments began 10 days prior to exposure (D-10). Exposure began at day 1 (D1).

Table 1
Summary of concentrations and characteristics of the gas-phase and whole DE within the animal exposure chambers.[a]

		2-Day exposure			4-Week exposure	
Constituent	Air	Gas phase	Whole DE	Air	Gas phase	Whole DE
Particle mass (TEOM), mg/m3	NA	NA	1220 ± 480	NA	NA	1494 ± 541
Particle mass (filter), mg/m3	4 ± 10	19 ± 19	1762 ± 193	14 ± 44	24 ± 24	1786 ± 275
Oxygen (O_2), %	NA	19.8 ± 0.2	19.8 ± 0.2	20.7 ± 0.2	19.7 ± 0.2	19.9 ± 0.3
Carbon monoxide (CO), ppm	NA	37.3 ± 4.4	39.4 ± 3.6	3.7 ± 2.7	28.4 ± 12.3	24 1 ± 2.6
Nitric oxide (NO), ppm	NA	26.8 ± 1.7	26.2 ± 1.9	<0.5	25.5 ± 3.0	21.7 ± 5.8
Nitrogen dioxide (NO_2), ppm	NA	1.0 ± 0.1	0.9 ± 0.1	<0.5	0.8 ± 0.2	0.7 ± 0.2
Sulfur dioxide (SO_2), ppm	NA	<1.0	<1.0	<1.0	<1.0	<1.0
Temperature, °C	72.1 ± 0.8	72.5 ± 0.9	71.7 ± 0.8	73.6 ± 1.3	74.6 ± 1.6	74.6 ± 1.0
Relative humidity, %	52.4 ± 1.4	85.1 ± 4.1	78.4 ± 4.1	49.2 ± 3.5	79.5 ± 5.8	69.7 ± 7.0

[a] Particle mass by tapered element oscillating microbalance (TEOM), O_2, CO, NO, NO_2, SO_2, temperature, and relative humidity data represent mean values ± SE from continuous measurements taken over all exposure days over a six weeks period. Filter data represent mean values ± SE from one measurement per day taken over all exposure days. NA = not analyzed. Rats were exposed 4 h/day for 2 consecutive days or 4 h/day, 5 days/week for 4 consecutive weeks.

according to kits and protocol using the Luminex 100 system (Luminex Corporation, Austin, TX). Sample values were normalized based on standard curves, and data were calculated using Luminex software. Serum fatty acid binding protein-3 (FABP-3), myosin, and brain-type natriuretic peptide (BNP) were analyzed using kits and protocols from Mesoscale Discovery, Inc. (Gaithersburg, MA).

RNA isolation and gene expression. Thoracic aorta and left ventricular total RNA were isolated using the Qiagen RNeasy Fibrous Tissue Kit (Valencia, CA). Left ventricular markers of contractile response and aorta markers for inflammation, thrombosis, vasoconstriction, and receptors that recognize oxidized lipids and proteins were analyzed at messenger RNA (mRNA) level using real-time reverse transcription polymerase chain reaction (RT-PCR). Applied Biosystems, Inc., model ABI 7900 HT Sequence Detection System was used as described earlier (Kodavanti et al., 2011). Gene-specific primers for control and targets were purchased from Applied Biosystems, Inc. (Carlsbad, CA). Super-Script III Platinum One-Step Quantitative RT-PCR kits were purchased from Invitrogen (Grand Island, NY).

Statistical analysis. BP data were analyzed using two-way analysis of variance (ANOVA), considering strain and treatment as two variables, followed by Holm–Sidak post hoc comparison. Baseline PCR data were analyzed using one-way ANOVA followed by Holm–Sidak post hoc comparison. All other data were analyzed using strain and exposure within air group or treatment and exposure within a given strain as two factors. Two-way ANOVA, followed by Duncan's method for all pair-wise comparisons, was used.

Results

Exposure conditions

Average PM mass concentrations achieved in the DE chamber for 2 days were (mean ± SE) 1220 ± 480 μg/m^3 and, for 4 weeks, were 1494 ± 541 μg/m^3 (Table 1). Gas concentrations were elevated in whole DE and gas-phase-only exposures to comparable degrees.

BP

Tail cuff methodology provided measures of BP difference between WKY and SH rats and the effectiveness of drug therapy (Fig. 2). SH rats, as anticipated, had increased systolic BP when compared with WKY rats. Hydralazine treatment significantly reduced BP in SH rats without affecting BP in WKY rats. L-NAME treatment of WKY rats indicated a trend of an increase in BP. BP could not be measured for more than 50% rats treated with L-NAME. This could have occurred due to change in tail blood flow by L-NAME. Changes in peripheral vascular resistance have been presumed to interfere with blood pressure measurement using tail cuff methodology in rats (Swali et al., 2010). Thus, the data in Fig. 2 likely underestimate the effect of L-NAME. No effects of gas or DE on BP could be discerned using tail cuff methodology.

Pulmonary BALF effects of gas and DE exposures

Although total lavagable cells were not changed by drug treatment or exposures (data not shown), BALF neutrophils were increased significantly by both gas and DE in WKY rats and by gas in SH rats after 2 days of exposure (Fig. 3). Hydralazine or L-NAME did not affect baseline or acute gas- or DE-induced increase in neutrophils in WKY rats but significantly reduced gas-induced neutrophil increase in SH rats. Small increases in neutrophils were also apparent in WKY rats exposed to gas and SH rats exposed to DE for 4 weeks; neither hydralazine nor L-NAME had significant effects. BALF gamma-glutamyl transpeptidase (γ-GT) activity was increased in WKY rats exposed to gas and DE for 2 days and also for 4 weeks. This effect in SH rats was only evident with gas at 4 weeks; the treatment with hydralazine or L-NAME did not influence this effect of DE or gas in WKY rats at either time point. The increases in BALF γ-GT activity were significant in hydralazine-treated SH rats exposed to DE for 2 days and gas as well as DE for 4 weeks. In contrast, pulmonary albumin leakage was

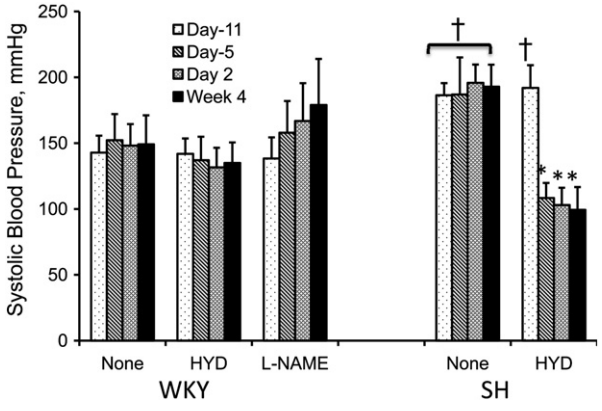

Fig. 2. Systolic BP in WKY and SH rats prior to and during drug treatment as determined using tail cuff methodology. The BP in rats was measured using tail cuff methodology prior to drug treatment (D-11), 5 days after drug treatment (D-5), 2 days following gas and DE exposure (D2), and 4 weeks after exposure (week 4) in all rats exposed for 4-weeks. Because no exposure-related changes could be discernible using this methodology, the data for all exposure groups were combined for a given treatment. Note that the BP signal could not be detected for more than 50% of the WKY rats treated with L-NAME at 4 weeks ($n = 18$; 7 rats could be measured), likely because of the blood flow changes in the tail. The data in the graph represent only the animals for which BP could be detected using tail cuff methodology. Hyd. = hydralazine. * = significant ($p \leq 0.05$) treatment effect. † = significant ($p \leq 0.05$) strain difference.

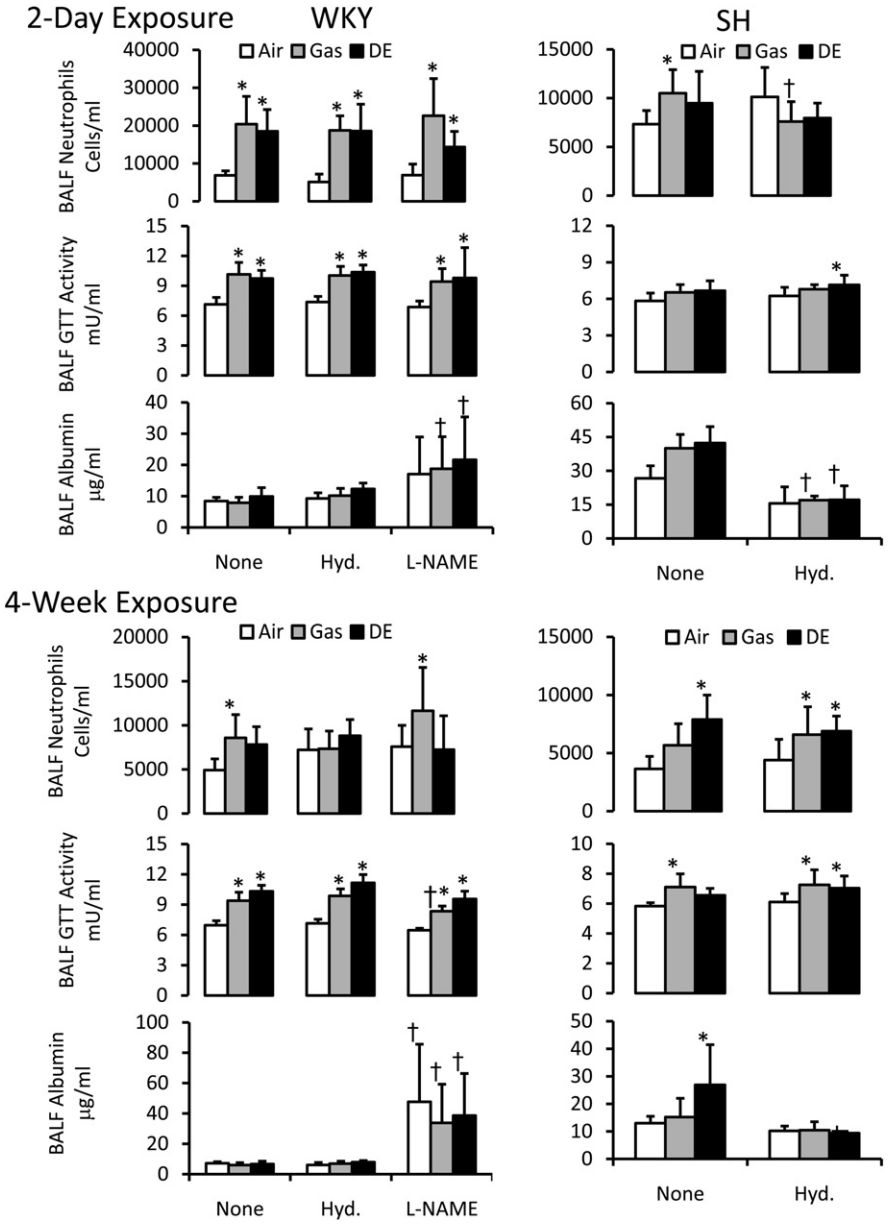

Fig. 3. The contribution of systemic hypertension in pulmonary injury and inflammation in WKY and SH rats exposed to gas-phase (gas) and whole diesel exhaust (DE) for 2 days or 4 weeks. WKY rats were treated with no drug, hydralazine (Hyd.; 150 mg/L), or L-NAME (150 mg/L), and SH rats with no drug or hydralazine (Hyd.; 150 mg/L) in drinking water. Treatment began 10 days prior to air, gas-phase components (gas) or whole diesel exhaust (DE) exposure and continued until necropsy. Values represent mean ± SD, $n = 6$/group. * = significant ($p \leq 0.05$) exposure effect within a given treatment group. † = significant ($p \leq 0.05$) treatment effect within a given exposure group.

affected by exposure in a strain-specific manner and with drug treatments. BALF albumin did not increase in WKY rats either with gas or DE following 2-day or 4-week exposures, and hydralazine did not change albumin in WKY rats, but treatment with L-NAME increased BALF albumin in all WKY rats, regardless of exposure. BALF albumin was increased significantly in SH rats exposed to DE for 4 weeks. More importantly, DE-induced increase in albumin were reversed by hydralazine in SH rats, suggesting that hydralazine decreases pulmonary vascular leakage without markedly affecting inflammatory potential.

Complete blood count, platelet aggregation, and serum biomarkers

Strain differences in hematological and platelet aggregation parameters were consistent with known differences between WKY and SH rats (Kodavanti et al., 2002). Small effects of drug treatments were seen in some hematological (supplemental materials, Tables 1 and 2) and platelet aggregation parameters (supplemental materials, Tables 3 and 4); however, few effects occurred with any exposure conditions (Supplementary materials, Results), except for small increases in platelet number and aggregation by DE in SH rats (Supplementary materials, Tables 1–4).

Circulating biomarkers of inflammation and cardiac injury were analyzed in serum samples. Serum levels of von Willebrand Factor (vWf), soluble E-selectin (sE-selectin), soluble intercellular adhesion molecule-1 (sICAM-1), and adiponectin were significantly different at baseline in both strains (Supplementary materials, Table 5). However, few gas or DE effects were noted, except for a slight adiponectin decrease in DE-exposed WKY rats and in sE-selectin, as well as sICAM-1, in DE-exposed, nondrug-treated SH rats (Supplementary materials, Results). Troponin levels were not affected by strain, drug treatment, or exposure. Cardiac injury biomarkers, such as FABP-3, myosin, and BNP were measured only in 2-day exposure groups (Supplementary materials, Table 6). Gas and DE increased serum

FABP-3 in WKY rats. Hydralazine (but not L-NAME) also tended to increase FABP-3 in WKY rats. No consistent changes occurred with any other exposure conditions. L-NAME increased serum BNP levels in WKY. Gas exposure was also associated with increases in serum BNP in non drug treated WKY; however high variability was apparent within individual treatment groups.

Myocardial contractile response and aortic atherogenic and vasoconstriction markers

Left ventricular markers of contractile activity (voltage-dependent calcium channel, L-type, alpha 1C subunit; ATPase2a2; phospholamban; sarcolipin; epoxide hydrolase, fibromodulin and tumor necrosis factor -α) were analyzed at mRNA level in control and hydralazine-treated WKY and SH rats exposed to DE, but no effects of exposure or drug treatment were noted, except for DE-induced decrease in mRNA expression of calcium channel protein (voltage-dependent, L-type, alpha 1C subunit) (Supplementary materials, Table 7). The lack of effect of hydralazine treatment on left ventricular expression of genes involved in myocardial function but reduction in BP in SH rats suggests that these markers might not be transcriptionally regulated and that the decrease in BP, resulting from pharmacological intervention, might be reflective of peripheral vasculature tone.

Both drug treatments and exhaust exposures produced a number of effects on aorta gene expression. Baseline aorta gene expression differences between SH and WKY rats are shown in the Supplementary materials, Fig. 1. The effects of gas and DE exposures, in general, were more pronounced in SH rats when compared with WKY rats exposed for 4 weeks. Nondrug-treated WKY rats exposed for 2 days did not show any exposure effects of gas or DE (Supplementary materials, Figs. 2 and 3). Small effects in gas- and DE-exposed nondrug-treated SH rats were noted in tissue factor (TF), tissue plasminogen activator (tPA), matrix metalloproteinase-1 (MMP-1), high-mobility group box 1 (HMGB-1), heme oxygenase-1, and receptor for advanced glycation endproducts (RAGE) (Supplementary materials, Fig. 3). Drug treatments also caused small changes in selected markers (Supplementary materials, Fig. 3).

Four-week gas and DE exposures produced several effects, especially in SH rats. The effects in WKY rats were minimal. DE exposure increased SH rat aorta endothelial nitric oxide synthase (eNOS), TF, tPA, tumor necrosis factor-alpha (TNF-α), MMP-2, RAGE, and HMGB-1 (Figs. 4 and 5). SH rats exposed to gas showed trends of increases that were significant only for TNF-α, tPA, and TF. Hydralazine treatment in SH rats reversed the DE effects on TF, tPA, TNF-α, and MMP-2 but not the effects on RAGE and HMGB-1. Moreover, DE increased eNOS expression in SH rats. The effect of gas on eNOS expression was increased in hydralazine-treated SH rats. Hydralazine markedly reduced plasminogen activator inhibitor-1 (Fig. 4) and lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1; Fig. 5) in SH rats with high baseline expression for these markers. The effects of hydralazine in all WKY rats were inconsistent. L-NAME tended to increase endothelin-1 (ET-1), TNF-α, and LOX-1 but decreased RAGE, HMGB-1, and eNOS expression in 4 week WKY rats.

Histology

Pathologist examined lung, heart and aorta tissues for exposure related changes. No effects were seen in aorta and myocardial tissues at light microscopy level; however, pigmented macrophages were readily apparent in the lungs of DE but not gas exposed SH and WKY rats (supplemental materials, Fig. 4).

Discussion

Underlying cardiovascular diseases have been associated with exacerbated PM effects and systolic BP. However, the role of preexistent hypertension in modifying systemic and pulmonary response to air pollution is not clearly understood. Furthermore, it is not understood whether genetic factors or phenotypic presence of hypertension might play a differential role in susceptibility to air pollution. Because most PM experimental studies are done with consideration of a single variable or an animal model and a limited number of biomarkers to address one specific question, a more comprehensive picture often is not understood, especially relating lung effects to cardiovascular and systemic effects. In this study, we wanted to understand the role of pharmacological hypertension intervention in normotensive WKY and genetically predisposed SH rats in modifying gas or DE-induced lung and cardiovascular effects. Thus, a number of related hypotheses were examined to understand interactions of several biological responses and exposure scenarios in a comprehensive manner.

Hydralazine and L-NAME treatment effects

We selected hydralazine, a direct acting and nonspecific muscle relaxant that has been widely used experimentally and therapeutically to reduce BP in rats. The precise mechanism of how hydralazine reduces BP is not known; however, it is thought to produce vasorelaxation through impairing calcium homeostasis in smooth muscle cells (Vidrio et al., 2003). With hydralazine treatment, we were able to achieve expected BP reduction in SH rats without affecting the BP of normotensive WKY rats. Because hydralazine tended to reduce pulmonary albumin leakage in all SH rats (air control and gas or DE exposed), one may speculate that smooth muscle contraction within pulmonary vasculature might contribute to increased protein leakage at baseline and following acute injury in these rats. Surprisingly hydralazine did not have remarkable effects on hematological parameters or platelet activity but markedly reduced LOX-1 mRNA expression in SH, which already have elevated levels at baseline when compared with WKY rats (Supplementary materials, Fig. 1). Because LOX-1 is involved in pathogenic signaling on binding to circulating oxidatively modified lipid ligands (Sawamura et al., 2012), its downregulation by hydralazine might reduce vascular pathologies during hypertension.

We presumed that, by elevating the BP of normotensive WKY rats, we will be able to mimic some of the baseline alterations of SH rats (relative to WKY) and also examine potential exacerbation of DE and gas-induced injury response. L-NAME, an inhibitor of nitric oxide (NO) synthase, is known to increase BP through inhibition of NO-mediated vasorelaxation (Kameyama et al., 2005). L-NAME treatment regimen has been shown to increase BP in WKY rats (Mishra et al., 2010). Although our BP data could not ascertain significant increase with L-NAME in WKY rats, the trend was readily apparent, and eNOS-expression in aorta was inhibited significantly by LNAME at the 4-week time point in WKY rats, suggesting that, at the level of blood vessel, L-NAME effect was apparent. L-NAME treatment has been shown to increase platelet aggregation *in vivo* and in response to thrombin (Tymvios et al., 2009). Authors concluded that the presence of NO originating from the environment external to the platelet was needed for regulation of platelet aggregation. They also concluded that raised levels of endogenous eNOS inhibitors were not sufficient to enhance platelet activity, and eNOS was not essential for normal platelet function. The lack of effect of L-NAME on platelet numbers and a modest decrease of *in vitro* adenosine diphosphate-induced platelet aggregation at the 2-day but not at 4 weeks, suggests that platelet aggregation is not impacted markedly by L-NAME in our study.

Exacerbation of pulmonary albumin leakage by L-NAME, regardless of exposure status in WKY rats, indicates the involvement of pulmonary vascular contractility (Heerdt and Pleimann, 1996) in albumin leak and corroborates with our previous and present studies showing an increase in albumin leakage in SH rats at baseline relative to normotensive WKY rats (Kodavanti et al., 2000). The effects of L-NAME on aorta mRNA markers provide further insight into the relationship

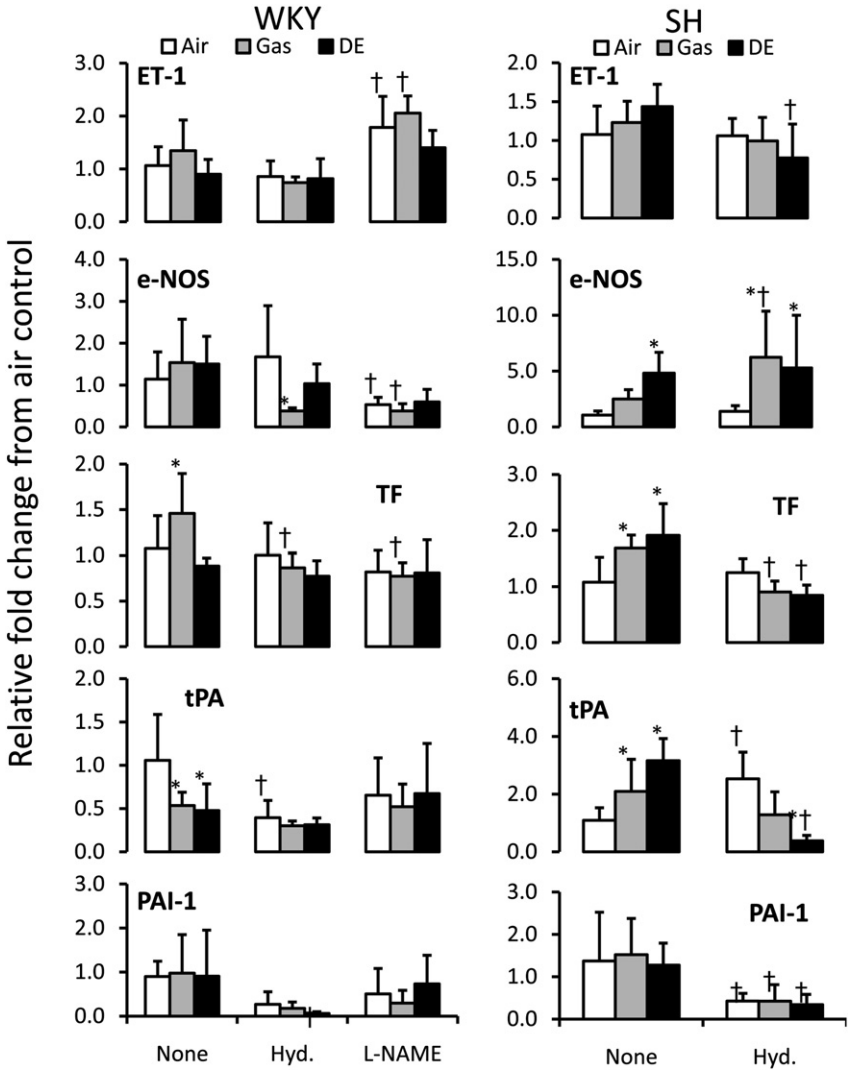

Fig. 4. The contribution of systemic hypertension in aortic mRNA expression for vasoconstriction and thrombosis markers in WKY and SH rats exposed to gas-phase (gas) and whole diesel exhaust (DE) for a 4-week period. WKY rats (left panels) were treated with no drug, hydralazine (Hyd.; 150 mg/L), or L-NAME (150 mg/L), and SH rats (right panels) with no drug or hydralazine (Hyd.; 150 mg/L) in drinking water 10 days prior to air, gas-phase components (gas), or whole diesel exhaust (DE) exposure until necropsy. Values represent mean ± SD, $n = 5$–6/group analyzed in duplicate. The data for WKY and SH rats were analyzed separately using different plates. Therefore, all WKY rat data are normalized to nondrug-treated WKY rats exposed to air, and all SH rat data are normalized to nondrug-treated SH rats exposed to air. * = significant ($p \leq 0.05$) exposure effect within a given treatment group. † = significant ($p \leq 0.05$) treatment effect within a given exposure group.

between vascular impairment and hypertension. Interestingly L-NAME treatment-mediated increases in expression of ET-1, TNF-α, and LOX-1 and decreases in eNOS, and RAGE at baseline in WKY parallel baseline differences between SH and WKY rats and contradict effects of hydralazine in SH rats. Thus, some vascular abnormalities that are associated with systemic hypertension in SH rats can be mimicked by L-NAME in WKY rats.

Gas and DE exposure effects

A variety of systemic and cardiovascular ailments has been variably linked to vehicular emissions, especially DE. Because gas and particulate components of these exhausts are chemically distinct, we wanted to determine how whole exhaust versus gas-phase components induces toxicity to the lung and the vasculature. Our data support the following interpretation. (1) Whole DE and gas, in general, produced similar acute pulmonary injury and inflammation responses that were more readily apparent in normotensive WKY rats relative to SH rats. (2) At 4 weeks, pulmonary neutrophilic inflammation and albumin leakage were significantly increased with DE but not gas in SH rats. (3) Four-week but not 2-day exposure to gas and DE (gas < DE) caused marked upregulation of many markers of vasoconstriction and atherogenesis in the aorta of SH rats; the effects in WKY rats were modest. The predominance of aortic changes in SH rats might reflect their greater vulnerability to acquire atherogenic vascular effects of DE. SH rats have been shown to be more vulnerable than WKY rats to cardiac physiological effects of PM, including DE (Lamb et al., 2012) but not gene expression changes (Gottipolu et al., 2009). The effects of both particle-free and whole exhaust exposures were specific to cardiac physiological endpoints examined (Lamb et al., 2012). Our data with vascular and pulmonary toxicity endpoints support the notion that gas and whole DE effects were endpoint specific. In general, the gas effects were noted primarily in the lung and with 2-day exposure in both strains. However, vascular effects predominated after 4 weeks DE exposure in SH rats. Our recent paper with WKY rats exposed to gas and whole DE shows that the BP effect was more pronounced at the 4-week time point and evident only with whole DE (Gordon et al., 2012).

Whole DE contains respirable soot particles, aldehydes, and organic components, as well as combustion-related gases (carbon monoxide [CO] and nitrogen oxides [NO_x]), and all have shown biological effects (Gordon et al., 2012; McDonald et al., 2011). Gas phase also contains

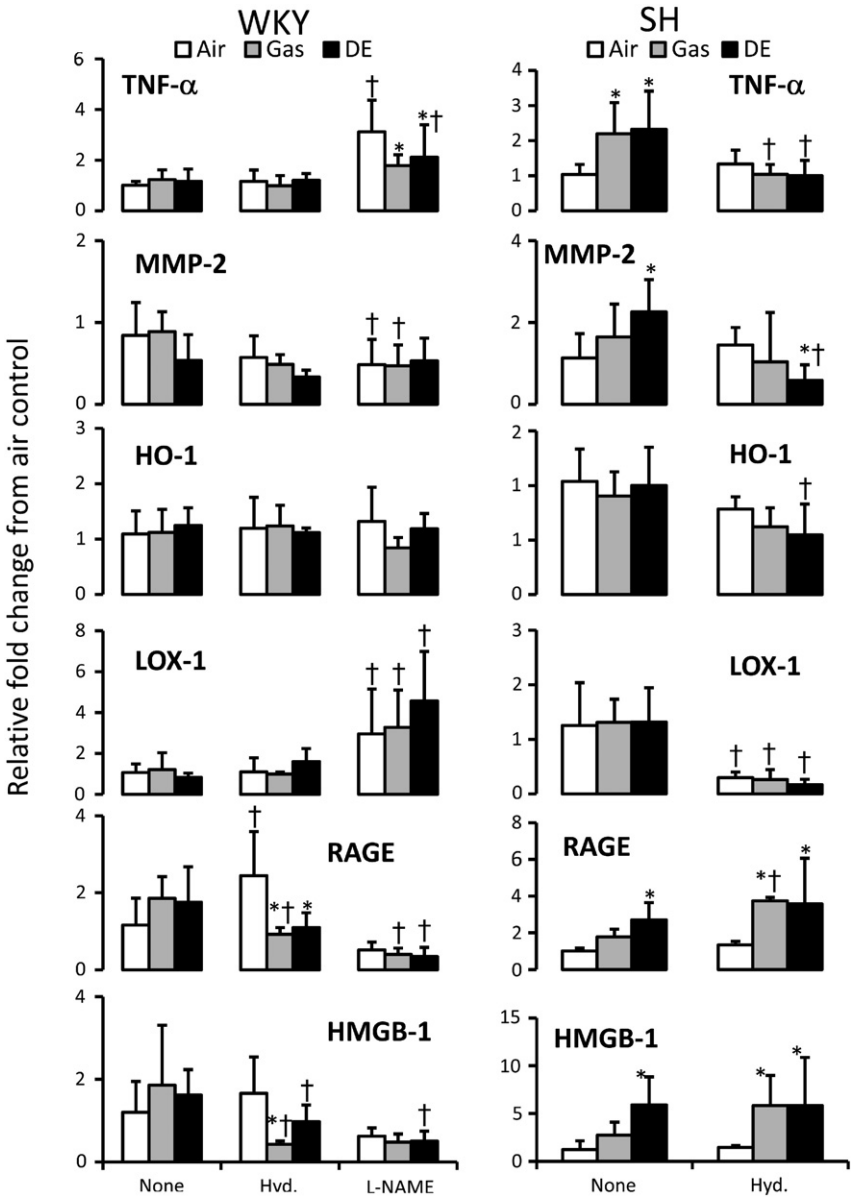

Fig. 5. The contribution of systemic hypertension in aortic mRNA expression for inflammation and atherogenic signaling markers in WKY and SH rats exposed to gas-phase (gas) and whole diesel exhaust (DE) for a 4-week period. WKY rats (left panels) were treated with no drug, hydralazine (Hyd.; 150 mg/L), or L-NAME (150 mg/L), and SH rats (right panels) with no drug or hydralazine (Hyd.; 150 mg/L) in drinking water 10 days prior to air, gas-phase components (gas), or whole diesel exhaust (DE) exposure until necropsy. Values represent mean ± SD, $n = 5–6$/group analyzed in duplicate. The data for WKY and SH rats were analyzed separately using different plates. Therefore, all WKY data are normalized to nondrug-treated WKY rats exposed to air, and all SH data are normalized to nondrug-treated SH rats exposed to air. * = significant ($p \leq 0.05$) exposure effect within a given treatment group. † = significant ($p \leq 0.05$) treatment effect within a given exposure group.

polycyclic aromatic hydrocarbons (Portet-Koltalo et al., 2011). NO being an endogenous vasodilator has been shown to be therapeutic in acute respiratory distress syndrome while also known to cause injury depending on the concentration and the host condition (Allen et al., 2009). Similarly CO also has vasodilatory effects in the lung (Leffler et al., 2011). It is not known how these two gases will cause biological response when inhaled as a mix. Exposures to gas components of DE have been shown to cause acute electrocardiographic changes in ApoE −/− mice (Campen et al., 2005) and rats (Gordon et al., 2012). Particulate fraction (often studied using bulk collected particles) also has been shown to elicit cardiovascular effects in animal studies (Kodavanti et al., 2011; Yokota et al., 2008). A more recent study shows that, in humans, particulate but not gas-phase components are responsible for the thrombogenic effect of DE (Lucking et al., 2011). In corroboration with the study done by Lucking et al. (2011), our data show that, although pulmonary injury and inflammation are noted with both gas and DE, generally, the long-term aorta thrombogenic and vascular effects in SH rats are more readily apparent with DE.

Intravascular thrombosis and platelet aggregation are enhanced following exposure to DE and other respirable PM (Nemmar et al., 2006, 2011); however, the roles of endothelial and circulating mediators in DE-induced platelet aggregation remain unclear. A modest increase in platelet number and aggregation by acute DE in SH rats exposed for 2 days and increased expression of aorta markers of thrombosis at 4 weeks supports our previous study showing increased expression of thrombogenic markers in the aortas of WKY rats after bulk DE exposure (Kodavanti et al., 2011). Procoagulant effects have been associated with decrease in BP after DE exposure in a mouse model of hypertension (Nemmar et al., 2011).

Systemic biomarker changes have been reported extensively by many DE studies of a wide range of concentrations involving humans and animals (Carlsten et al., 2008; Gerlofs-Nijland et al., 2010; Peretz

et al., 2008; Zuurbier et al., 2011); however, there is no consistent pattern of change in any biomarker that could be attributable to DE exposure across all studies. We examined markers of cardiac injury, thrombosis, and endothelial activation. Strain-related differences in serum biomarkers were consistent in all exposure scenarios, suggesting that the measurements actually reflect the chronic disease condition. Increases in circulating FABP3 levels by gas and DE exposures in WKY rats and gas in SH rats suggest that fatty acid regulation might be impacted. This is a multifunctional small molecular weight protein detected in the circulation that modulates the cellular uptake of fatty acids (Imamura, 2011). Circulating brain-type natriuretic peptide (n-terminal proBNP) has been implicated in cardiac injury (de Antonio et al., 2012) and right ventricular alterations from exposure to biomass fuels (Emiroglu et al., 2010). The levels of BNP in circulation tended to increase in WKY rats exposed to gas and DE suggesting that BNP might be involved in gas and DE induced acute cardiovascular response.

Modification of DE and gas effects by drug pretreatment

Although a number of studies have examined the exacerbation of PM effects in hypertensive rats (Kodavanti et al., 2000, 2002; Gottipolu et al., 2009; Lamb et al., 2012), the contribution of physiological presence of hypertension is not well understood. We show that DE-induced pulmonary protein leakage, but not neutrophilic inflammation or lung cell injury (γ-GT increase in BALF) was reversed by normalizing the BP of SH rats with hydralazine treatment. Concomitantly L-NAME treatment in WKY rats was associated with increased protein leakage (regardless of exposure status), without affecting neutrophil influx in the lung or BALF γ-GT activity. In addition, hydralazine reversed DE- and gas-induced increases of mRNA markers for thrombosis and inflammation in SH rats but did not change the effects on eNOS, RAGE, and HMGB-1. Thus, physiological presence of hypertension likely drives lung vascular leakage and pathogenic effects that occur in the SH rat aorta after DE exposure but not lung inflammation and cell injury. The increased expression of eNOS in SH rat aorta by DE and a slight exacerbation of gas-induced eNOS expression by hydralazine supports the hypothesis that DE in rats actually might produce a vasodilatory effect; however, DE increasing albumin leakage in SH rats argues against this assumption suggesting that gas and particulate component of whole exhaust might have differential effects. Although we have recently shown that long-term DE reduces BP in WKY rats (Gordon et al., 2012), the effects on SH rats will need to be examined to affirm the role of BP reduction in this model.

We have reported that, when SH and WKY rats are exposed to DE in a similar exposure protocol, the gene expression pattern of DE-exposed WKY rat myocardium is changed to appear like that of SH rats without DE (Gottipolu et al., 2009). This suggests that hypertensive SH rat hearts actually might have diminished contractile ability at baseline that could not be further exacerbated by DE, and that WKY rat myocardial gene expression changes, rather, reflect diminished contractile response. The lack of hydralazine or DE effect on selected myocardial mRNA markers, despite decrease in BP of SH rats, suggests that other markers might be regulating cardiac contractility at a transcriptional level.

The protective effects of hydralazine on DE-induced vascular effects in hypertensive rats have implications for how air pollution can affect pulmonary and cardiovascular systems of those hypertensive individuals who are on antihypertensive therapy. Because hypertension in SH rats originates from genetic predisposition (Morrissey et al., 2011), the exacerbated DE aortic effects in SH rats relative to WKY rats suggest contribution of hypertension and associated genetic differences. However, the reversal of DE-induced vascular effects by hydralazine emphasizes the contribution of the physiologic presence of hypertension and the potential option of therapeutic intervention.

Acute exposure to DE is associated with impairment of endothelium-dependent acetylcholine-mediated vasorelaxation (Mills et al., 2011). Oxidation of NO and consequent inhibition of its vasodilatory function have been postulated to mediate vasoconstrictive effect of DE (Cherng et al., 2011); however, physiologically, this is not necessarily associated with increased BP in animals (Gordon et al., 2012; Nemmar et al., 2011). We did not see increases in aorta transcripts of eNOS following 2-day exposure; however, 4-week DE exposure led to increases in eNOS expression in SH rats, and this effect was exacerbated slightly by hydralazine, which produces smooth-muscle-dependent vasorelaxation suggesting a potential role of eNOS in DE- and hydralazine-induced vasorelaxation. The lack of increase in expression of a vasoconstrictor ET-1 and inhibition of its basal expression by hydralazine in SH rats exposed to DE for 4 weeks supports the conclusion that, in SH rats, DE might not contribute to vascular effect through ET-1 (Knuckles et al., 2011).

In conclusion, we show that pulmonary protein leakage and atherogenic vascular effects of long-term DE exposures can be effectively reversed by normalizing BP in hypertensive rats. However, the effects of DE on aortic vasoactivity markers are more similar to the effect of BP-lowering hydralazine, emphasizing the contribution of peripheral vasculature in regulation of hypertension.

Conflict of interest

The authors declare that there are no conflicts of interest.

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Disclosures

The authors have nothing to disclose.

Disclaimer

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Appendix A. Supplementary data

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