RESEARCH ARTICLE

Particulate matter inhalation exacerbates cardiopulmonary injury in a rat model of isoproterenol-induced cardiomyopathy

Alex P. Carll1, Najwa Haykal-Coates2, Darrell W. Winsett2, William H. Rowan III3, Mehdi S. Hazari2, Allen D. Ledbetter2, Abraham Nyska4, Wayne E. Cascio5, William P. Watkinson2,†, Daniel L. Costa6, and Aimen K. Farraj2

1Environmental Sciences and Engineering, UNC Gillings School of Global Public Health, Chapel Hill, North Carolina, USA, 2Experimental Toxicology Division, US EPA, Research Triangle Park, North Carolina, USA, 3ITT Corporation, Advanced Engineering and Sciences, Alexandria, Virginia, USA, 4Sackler School of Medicine, Tel-Aviv University, Timrat, Israel, 5Department of Medicine, Brody School of Medicine, East Carolina University, Greenville, North Carolina, USA, and 6Office of Research and Development, US EPA, Research Triangle Park, North Carolina, USA

Abstract

Ambient particulate matter (PM) exposure is linked to cardiovascular events and death, especially among individuals with heart disease. A model of toxic cardiomyopathy was developed in Spontaneously Hypertensive Heart Failure (SHHF) rats to explore potential mechanisms. Rats were infused with isoproterenol (ISO; 2.5 mg/kg/day subcutaneous [sc]), a β-adrenergic agonist, for 28 days and subsequently exposed to PM by inhalation. ISO induced tachycardia and hypotension throughout treatment followed by postinfusion decrements in heart rate, contractility, and blood pressures (systolic, diastolic, pulse), and fibrotic cardiomyopathy. Changes in heart rate and heart rate variability (HRV) 17 days after ISO cessation indicated parasympathetic dominance with concomitantly altered ventilation. Rats were subsequently exposed to filtered air or Harvard Particle 12 (HP12) (12 mg/m3)—a metal-rich oil combustion-derived PM—at 18 and 19 days (4 h/day) after ISO infusion via nose-only inhalation to determine if cardio-impaired rats were more responsive to the effects of PM exposure. Inhalation of PM among ISO-pretreated rats significantly increased pulmonary lactate dehydrogenase, serum high-density lipoprotein (HDL) cholesterol, and heart-to-body mass ratio. PM exposure increased the number of ISO-pretreated rats that experienced bradyarrhythmic events, which occurred concomitantly with acute alterations of HRV. PM, however, did not significantly affect mean HRV in the ISO- or saline-pretreated groups. In summary, subchronic ISO treatment elicited some pathophysiologic and histopathological features of heart failure, including cardiomyopathy. The enhanced sensitivity to PM exposure in SHHF rats with ISO-accelerated cardiomyopathy suggests that this model may be useful for elucidating the mechanisms by which PM exposure exacerbates heart disease.

Keywords: Air pollution; arrhythmia; cardiomyopathy; heart failure; heart rate variability; isoproterenol

Abbreviations: AVB: atrioventricular block; BALF: bronchoalveolar lavage fluid; BP: blood pressure; DBP: diastolic blood pressure; dP/dt max: peak derivative of aortic pressure; HF: high frequency power; HF: heart failure; HP12: Harvard particle 12; HR: heart rate; HRV: heart rate variability; ISO: d,l-isoproterenol-hydrochloride; LDH: lactate dehydrogenase; LF: low frequency power; LV: left ventricular; MAP: mean arterial pressure; MMAD: mass median aerodynamic diameter; Penh: enhanced respiratory pause; PM: particulate matter; pNN15: percent of pairs of normal adjacent R-R intervals that differ by >15 ms; pNN50: percent of pairs of normal adjacent R-R intervals that differ by >50ms; QA interval: time between ventricular depolarization and ejection; RR interval: time between two normal beats; RMSSD: square root of the mean of squared differences between successive RR intervals; RV: right ventricular; SBP: systolic blood pressure; SDNN: standard deviation of the RR interval; SHHF: Spontaneously Hypertensive Heart Failure; SHR: Spontaneously Hypertensive rat; SVPB: supraventricular premature beat; Tco: core temperature; VPB: ventricular premature beat

†Deceased.

Address for Correspondence: Aimen K. Farraj, PhD DABT, US Environmental Protection Agency, Experimental Toxicology Division, Mail Code: B1430-01, Research Triangle Park, NC 27711, USA. Phone: (919) 541-5027; Fax: (919) 541-0034; E-mail: farraj.aimen@epa.gov

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Introduction

Day to day patterns in airborne particulate matter (PM) associated with combustion-derived air pollution have been linked to increases in cardiovascular morbidity and mortality (Pope et al., 1999). Epidemiological studies show that individuals with heart failure (HF) are particularly vulnerable to the adverse health effects of PM. For instance, elevations in daily fine particulate matter (PM$_{2.5}$) concentrations have been associated with increased HF hospitalizations (Dominici et al., 2006; Pope et al., 2008) and mortality (Goldberg et al., 2000); and increases in PM$_{10}$ levels propagate new HF diagnoses and deaths in survivors of myocardial infarction (Zanobetti and Schwartz, 2007). Several mechanisms of PM-induced cardiac dysfunction in humans have been postulated, including autonomic imbalance, direct effects on cardiac ion channels, myocardial ischemia, and vascular effects related to systemic inflammation (Brook et al., 2003; Schulz et al., 2005). Cardiomyopathy resulting from disease or toxicity may predispose individuals to one or more of these responses to PM.

The study of the effects of PM exposure on heart failure has been limited by the absence of practical experimental animal models that mimic the pathogenesis and characteristics of human HF. Many models have been developed by means ranging from physical occlusion of key blood vessels to pharmacological induction, but these fall short in integrating multiple conditions that often combine to elicit HF: The Spontaneously Hypertensive Heart Failure (SHHF) rat strain, a breed bred for its genetic predisposition to HF, replicates important human cardiovascular risk factors and cardiac deficits. Lean male SHHF/MccCrL-Lepr$^{+/-}$ rats progress from compensated hypertrophic cardiomyopathy to dilated cardiomyopathy and decompensated heart failure at 18 months of age primarily as a consequence of hypertension (Heyen et al., 2002; Anderson et al., 1999; Tamura et al., 1999). Although the etiology of cardiac disease in the lean SHHF rat mirrors some of the characteristics of human HF, the protracted time required to progress to decompensated HF detracts from its utility as an experimental model with which to study mechanisms. Recently, however, investigators have shown that continuous infusion of isoproterenol (ISO), an exogenous catecholamine and nonselective $\beta$-adrenoreceptor agonist, promotes progression from cardiac hypertrophy to dilatation and pump dysfunction in the Spontaneously Hypertensive rat (SHR), a genetic precursor to the SHHF strain with similar pathology (Badenhorst et al., 1995) and 3.99 mg/m$^3$ for PM$_{2.5}$ (Cavallari et al., 2008) have been reported. We hypothesized that exposure to Harvard Particle 12 (HP12)—a ROFA particle with lower metal content that is known to impair cardiac function in hypertensive rats (Wichers et al., 2004a)—would exacerbate cardiopulmonary injury in SHHF rats with ISO-induced cardiomyopathy. Thus, we examined physiologic (blood pressure, electrocardiogram [ECG], heart rate, HRV, and body temperature), biochemical, and histologic indicators of cardiopulmonary impairment in ISO-treated SHHF rats at baseline and after inhalation-exposure to HP12.

Methods

Animals and radiotelemetry implantation

Lean male SHHF rats (MccCrL-Lepr$^{+/-}$; $n = 19$, 60 days old; Charles River Laboratories, Kingston, NY) were implanted with radiotransmitters capable of measuring ECG, heart rate (HR), blood pressure (BP), and core body temperature ($T_c$) (model TL11M2-C50-PXT; Data Sciences International, St. Paul, MN). The total number of rats was limited by animal availability from the supplier and blood pressure monitoring capacity. Telemetry implantation adhered to previously described preoperative, anesthetic, and surgical procedures. The telemeter was placed in the abdominal cavity, the blood pressure catheter tip was inserted into the descending abdominal aorta, and the electrode leads approximated the Lead II orientation as previously described (Wichers et al., 2004a). “Lean” and “obese” SHHF rats differ in disease severity and progression because of their differing responsiveness to leptin, a neurohormone responsible for satiety; obese rats are leptin resistant while lean rats are not (Radin et al., 2003). Rats were shipped after a 2-week recovery period to an Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-approved animal facility, housed in a room (22°C $\pm 1^\circ$C, 50% $\pm 5$% relative humidity, 12-h light:dark cycle 0600:1800 h), and provided standard Purina rat chow (5001; Brentwood, MO) and water ad libitum. All studies conformed with the guidelines of the US Environmental Protection Agency (EPA) Institutional Animal Care and Use Committee (IACUC).

Isoproterenol infusion

After random assignment to four groups (Saline-Air, Saline-HP12, ISO-Air, ISO-HP12), rats (110 days) were injected intraperitoneally with 1.0–2.0 mg/kg ketamine-xylazine (Sigma; St. Louis, MO) and implanted subcutaneously with osmotic minipumps (Alzet model 2004; Durect, Cupertino, CA) through a 1.0-cm interscapular incision to continuously infuse 0.9% saline or 2.5 mg/kg/day $dl$-isoproterenol hydrochloride subcutaneous (sc) (ISO; Sigma-Aldrich, to fine particulate air pollution and is rich in transition metals (Dreher et al., 1997), which play a key role in the adverse health effects of air pollution (Ostro et al., 2007). Occupational exposures of boiler overhaul workers to concentrations as high as 6.69 mg/m$^3$ for PM$_{10}$ (Hauser et al., 1995) and 3.99 mg/m$^3$ for PM$_{2.5}$ (Cavallari et al., 2008) have been reported. We hypothesized that exposure to Harvard Particle 12 (HP12)—a ROFA particle with lower metal content that is known to impair cardiac function in hypertensive rats (Wichers et al., 2004a)—would exacerbate cardiopulmonary injury in SHHF rats with ISO-induced cardiomyopathy. Thus, we examined physiologic (blood pressure, electrocardiogram [ECG], heart rate, HRV, and body temperature), biochemical, and histologic indicators of cardiopulmonary impairment in ISO-treated SHHF rats at baseline and after inhalation-exposure to HP12.
St. Louis, MO) dissolved in saline. Pumps were removed 28 days post implantation through a similar incision during anesthesia with 3% isoflurane (Isoflo; Abbott Laboratories, North Chicago, IL).

Exposure to particulate matter
Due to concern over the rats’ ability to withstand nose-only restraint soon after ISO treatment, rats were acclimated for 1 h to the restraint 17 days after pump removal (45 days after pump implantation). Rats were exposed 24 and 48 h later to either filtered air or PM (HP12 aerosol; 12 mg/m³) for 4 h each day (0900–1300 h; Figure 1). Previous studies by Kodavanti et al. (1998) and this laboratory (Farraj et al., 2009) found that exposure to similar concentrations of a more metal-rich ROFA (Florida Power & Light) in different strains elicited electrocardiographic lesions and cardiac arrhythmias. We were interested in whether or not similar exposures to a different ROFA with significantly less transition metal content would elicit changes in heart rate variability, blood pressure, and arrhythmias. Animals were only exposed for 2 days to reduce stress on the animals as the nose-only restraint appeared particularly stressful to the SHHF strain (relative to previous studies using other strains), despite acclimation. HP12 was derived from accumulated particulate emission deposits scraped from the inside stack wall of a Boston-area power plant burning no. 6 residual oil, and the bulk sample was ground, sieved, and analyzed as previously described (Wichers et al., 2004a; Gilmour et al., 2004). Water-soluble metals accounted for 3.3% of total HP12 particle mass, with Zn as the predominant water-leachable metal (1.1% particle mass) and soluble Ni, V, and Fe (0.7%, 0.1%, and 0.002% particle mass, respectively) at lower, more environmentally relevant levels than those of more commonly studied ROFA particles (Wichers et al., 2004a; Gilmour et al., 2004). HP12 particles in the current study had a mass median aerodynamic diameter (MMAD) of 1.1 μm and a geometric standard deviation of 2.85 μm. HP12 was administered with a dry dust aerosol generator combined with a flow-by, nose-only exposure chamber as described elsewhere (Ledbetter et al., 1998). Each exposed animal was restrained in a separate, conical, plastic tube (Lab Products, Seaford, DE). Chamber aerosol concentration was determined gravimetrically every hour. Aerosol-size distribution was determined once per exposure using a seven-stage cascade impactor (Intox Products, Albuquerque, NM). After each inhalation exposure, animals were returned to their cages for radio-telemetry monitoring.

Radiotelemetry data acquisition and analysis
Radiotelemetry was used to track changes in cardiovascular and thermoregulatory function by continuously monitoring ECG, HR, mean arterial BP (MAP), systolic BP (SBP), diastolic BP (DBP), pulse pressure (the difference between diastolic and systolic pressures), QA interval (the time from initiation of ventricular depolarization to initiation of ventricular ejection), T_{co}, and activity in awake, unrestrained rats as described in previous studies (Wichers et al., 2004a). Telemetry data parameters were acquired at 5-min intervals starting at 4 weeks before osmotic pump implantation until euthanasia. Ten-second averages were collected for each animal every 5 min and averaged over 1 h for each treatment group. ECG and BP waveform segments were acquired for 1 min every hour at a sample rate of 1000 Hz. MAP, SBP, and DBP were calculated from the BP waveform. QA interval was calculated from ECG and BP waveforms with specialized computer software (DataART 3.01; Data Sciences International) and used as an index of cardiac contractility (Cambridge and Whiting, 1986). To corroborate QA interval measurements, the same software was used to calculate the peak derivative of aortic pressure (dP/dt_max)—another indirect index of left ventricular contractility as well as a predictor of death and heart transplantation in HF patients (Germano et al, 1998; Tartiere et al., 2008)—over 12 h at four select time-matched sampling periods: 3 days after osmotic pump removal (day 31), 1 day before the initial inhalation exposure (day 45), and immediately after both inhalation exposures (days 46 and 47; 1500–0200 h).

ECG waveforms were analyzed for arrhythmias, mean RR intervals, and HRV using HRV analysis software (ECG-auto 1.5.12.48; EMKA Technologies, Falls Church, VA) that identified R-wave peaks and measured RR intervals. Continuous 1-min ECG waveforms were analyzed every hour for 12-h intervals (1500–0200 h) on the day before infusion pump implantation (day –2), the day before inhalation exposure (day 45), and the days of inhalation exposure (days 46 and 47, post exposure).

For HRV analysis, thorough visual inspection was conducted to identify and exclude arrhythmias, artifacts, and 1-min ECG waveforms lacking distinguishable R-waves for more than 30 s. HRV analysis generated heart rate (HR) and time-domain measures, including mean time between adjacent QRS-complex peaks (RR interval), standard deviation of the RR interval (SDNN), SDNN normalized for the effects of heart rate (SDNN/(RR interval × 100)), square root of the mean of squared differences of adjacent RR intervals (RMSSD), and percent of adjacent normal RR

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**Figure 1.** Study regimen. Four groups of lean male SHHF rats were continuously infused sc starting at 110 days of age with either saline or isoproterenol (ISO: 2.5 mg/kg/day) by osmotic pump for 28 days. Nose-only exposure occurred at 18 and 19 days following pump removal for 4 h each day to either filtered air or PM (HP12) at a concentration of 12 mg/m³ and a mass median aerodynamic diameter (MMAD) of 1.1 μm.
intervals differing by ≥15 ms (pNN15). pNN15 is a measure of parasympathetic tone comparable to pNN50 in humans (Massin, 1999). SDNN represents overall HRV, whereas RMSSD represents parasympathetic influence over heart rate (Rowan et al., 2007). HRV analysis also calculated frequency domain parameters, particularly low frequency (Lf) and high frequency (Hf), and the ratio of these two frequency domains (Lf/Hf). Lf is generally believed to represent a combination of sympathetic and parasympathetic tone, whereas Hf indicates cardiac vagal (parasympathetic) tone, and Lf/Hf serves as an index of sympathovagal balance (Notarius and Floras, 2001).

Arrhythmias were verified from time-matched BP, and identified as ventricular premature beats (VPBs), supraventricular premature beats (SVPBs), atrial premature beats (APBs), sinoatrial blocks, or atrioventricular blocks (AVBs). Any shortening of a PP interval to 40 ms < the mean of the three preceding normal PP intervals was indicative of an APB and was deemed 'nonconducted' if unaccompanied by a QRS complex. VPBs and SVPBs were distinguished from normally conducted beats. VPBs were distinguished from SVPBs using previously reported guidelines (O’Grady and O’Sullivan, 2007).

Bradyarrhythmias were characterized according to previously published criteria (Chung, 1983; Barold, 2001; Watkinson et al., 1998). Identification of a second-degree AVB required a P-wave lacking a subsequent QRS complex and comprising a normal PP interval. Second-degree AVBs were differentiated as either Mobitz type I or Mobitz type II. A Mobitz I event was indicated by an RR interval increase greater than 100 ms but less than double the preceding PP interval, a prolongation in PR interval immediately prior to the event, and a noticeably decreased PR interval in the first QRS complex thereafter. Alternatively, identification of a Mobitz II event required an RR interval twice to 3-fold the adjacent normal PP intervals, a prolongation in PR interval immediately prior to the event, and a noticeably decreased PR interval in the first QRS complex thereafter. Advanced AVBs were identified as any skipped beats resembling the Mobitz type II arrhythmias but more than 3-fold the adjacent normal RR intervals. Advanced AVBs were differentiated as either Mobitz type I or Mobitz type II. A Mobitz I event was indicated by an RR interval increase greater than 100 ms but less than double the preceding PP interval, a prolongation in PR interval immediately prior to the event, and a noticeably decreased PR interval in the first QRS complex thereafter. Alternatively, identification of a Mobitz II event required an RR interval twice to 3-fold the preceding PP interval and a nonconducted P wave accompanied by roughly constant PR intervals in the preceding two normal QRS complexes. Sinoatrial blocks resembled second-degree AVB Mobitz type II arrhythmias but with the skipped beat lacking a P-wave. Advanced AVBs were identified as any skipped beats resembling the Mobitz II but more than 3-fold the adjacent normal RR intervals. To facilitate statistical analysis of each arrhythmia type and allow the data to converge under the Poisson distribution, the absence of an event within every 1-min ECG waveform was counted arbitrarily as 0.01.

Whole-body plethysmograph data acquisition and analysis

To examine the pulmonary effects of ISO pretreatment, data were collected on ventilatory parameters by a barometric whole-body plethysmography system (Buxco Electronics, Sharon, CT). The plethysmography methodology permitted continuous monitoring of tidal volume, breathing frequency, minute volume, and enhanced respiratory pause (Penh). Measurements occurred at 1 day before osmotic pump implantation, 2, 7, 14, 21, and 27 days after pump implantation, and 7 and 14 days following the removal of pumps (35 and 42 days after pump implantation, respectively). Daily calibration of plethysmography chambers (model PLY3213; Buxco Electronics) preceded every animal loading. A bias flow regulator delivered fresh air (1.8 L/min) to each cylindrical chamber, preventing CO2 buildup within the chamber. Unrestrained animals were placed in individual cylindrical dyspnea boxes containing a built-in reference chamber for measuring respiration-induced pressure fluctuations. Data were channeled to computer software (BioSystem XA; Buxco Electronics) that calculated respiratory parameters. After animals were placed in plethysmography chambers and allowed 1 min to stabilize from handling, their ventilation was measured for 5 min. Data were collected for each parameter every 10 s and averaged over 1-min intervals; automated breath-by-breath analyses were performed using a rejection algorithm to eliminate breaths that were outside a given range. After 5-min of data acquisition, rats were removed and returned to home-cages.

BALF and serum analysis

At 24 (n=10) and 48 (n=9) h after the second HP12 exposure, rats were anesthetized with sodium pentobarbital (50–100 mg/kg intraperitoneal [ip]), euthanized by exsanguination from the descending abdominal aorta, and subjected to intratracheal cannulation. Whole blood was collected from the descending abdominal aorta in serum separator tubes and centrifuged. Serum aliquots were frozen at −80°C. Hearts were excised and trimmed free of arterial tissue and fat, washed free of excess blood, and blotted dry. The atria as well as the right ventricular free wall were dissected away from the left ventricle, which remained attached to the intraventricular septum. All heart sections were weighed, transferred to 10% formalin, and refrigerated (4°C).

The whole lung was lavaged with a total of 35 ml/kg phosphate-buffered saline injected into and withdrawn from the trachea twice. Bronchoalveolar lavage fluid (BALF) was centrifuged at 1500 rpm for 10 min, and the supernatant was collected for biochemical analyses. The remaining pellet was resuspended to count total cells (Coulter counter model Z1; Beckman Coulter, Miami, FL), and a separate aliquot was centrifuged (Shandon 3 Cytospin; Shandon, Pittsburgh, PA) to prepare cell differential slides that were later dried at room temperature and stained with Wright-Giemsa using an automated slide stainer (Hematek 2000; Miles, Elkhart, IN). Macrophages, neutrophils, and eosinophils were enumerated using light microscopy (≥200 cells per sample).

BALF and serum samples were analyzed with a Konelab 30 clinical chemistry analyzer (Thermo Clinical Labsystems, Espoo, Finland). BALF was analyzed for albumin (Diasorin, Stillwater, MN), lactate dehydrogenase
Isoproterenol cardiomyopathy and PM susceptibility

(LDH) activity (Thermo DMA, Melbourne, Australia), N-acetyl glucosaminidase activity (Roche Diagnostics, Mannheim, Germany), and total protein (Coomassie Plus Protein Reagent; Pierce, Rockford, IL; Protein Standards, Sigma-Aldrich). Serum was analyzed for albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase, lactate dehydrogenase-1, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, total protein, and triglycerides (Thermo Electron, Melbourne, Australia); α-hydroxybutyrate dehydrogenase and lipoprotein(a) (Randox Laboratories, UK); total bilirubin (Diagnostic Chemicals, Oxford, CT); C-reactive protein (Diasorin); myoglobin (Roche); sorbitol dehydrogenase (Diagnostic Chemicals); and total antioxidant status (Randox Laboratories). Enzyme-linked immunosorbent assay (ELISA) kits were used to analyze serum for rat cardiac troponin-I (Life Diagnostics, West Chester, PA) and rat interleukin (IL)-6 (R&D Systems, Minneapolis, MN). Optical density was read at 450 nm in a spectrophotometer (Spectramax 340 PC; Molecular Devices, Sunnyvale, CA), and results were analyzed using computer software (Softmax Pro 3.1.2; Molecular Devices).

Histopathology
A 2-mm-thick cross-section of the intact left ventricle (LV) of each rat was made. A longitudinal section was then made of the right ventricle (RV) and the apical half of the LV. These three sections were imbedded in paraffin, sectioned with a thickness of 5 µm, mounted onto slides, and then stained with hematoxylin and eosin (H&E) or Barbeito-Lopez Trichrome. The Barbeito-Lopez Trichrome staining method was applied for routine diagnosis of myocardial degeneration or necrosis (Milei and Bolomo, 1983).

Cardiac histopathological changes were described and scored by a certified pathologist using semiquantitative grading (grades 0–4) for the severity of changes according to the number of cardiomyopathic foci randomly distributed in the myocardium: 0 = No Lesion; 1 = Minimal Change—up to 3 foci of cardiomyopathy; 2 = Mild Change—up to 6 foci; 3 = Moderate Change—up to 12 foci; 4 = Marked Change—more than 12 foci. Cardiomyopathy is marked by a spectrum of randomly distributed focal to multifocal lesions containing necrotic myofibers and mononuclear cells (mainly macrophages) that are eventually replaced with fibrotic collagen deposits (Jokinen et al., 2005).

Statistics
Software (Fishing License Method) designed specifically for time-series data analysis was used to evaluate telemetry parameters (HR, MAP, SBP, DBP, pulse pressure, \( T_{co} \), QA interval) for treatment effects (\( p < .05 \)) of specific durations occurring at unspecified moments (Nadziejko, 2004). Different protocols were used to detect infusion- or inhalation-related group differences. The preinhalation analysis of data spanned from 7 days before pump implantation until 17 days after pump removal (1 h before the first nose-only exposure). To analyze these data for infusion-related differences, groups were consolidated according to infusion agent (e.g., saline or ISO) and compared for significant differences lasting approximately 3, 6, 12, 24, 48, 96, or 192 h. To detect inhalation exposure-related differences, data from all four groups (Saline-Air, Saline-HP12, ISO-Air, ISO-HP12) spanning 96 h before inhalation exposure to 44 h after the second inhalation exposure were compared for differences lasting 1, 4, and 16 h. Of note, data were not collected during nose-only treatment, but acquisition recommenced 1–2 h after animals were removed from the nose-only chambers and returned to home cages.

The statistical analyses for HRV, ventilatory parameters, body mass, cardiomyopathy scores, cardiac mass ratios, serum, BALF, lung cellularity measures, and bradyarrhythmia counts in this study were performed using SAS.
version 9.1.3 software (SAS Institute, Cary, NC) using PROC MIXED and PROC GLIMMIX procedures. A linear mixed model with restricted maximum-likelihood estimation analysis (SAS) and least squares means post hoc test were used to determine statistical differences for all data. In addition, preinhalation HRV data collected 17 days after pump removal (day 45) were arranged into two cohorts according to infusion treatment, and a two-tailed unpaired t test was used to detect significant differences between these two cohorts prior to nose-only exposure (p < .05).

Results

Effects of ISO infusion on cardiopulmonary physiology

Relative to saline-treated rats, ISO infusion caused various ventilatory alterations during the infusion regimen including tachypnea (ventilatory rate +53%, days 7–28; p < .05), elevated Penh (+95%, days 2–28; p < .05), and decreased tidal volume (−30%, days 2–28; p < .05). Upon infusion of ISO, immediate changes in HR, body temperature, and blood pressure were noted with some of these changes lasting the entire infusion period (Figure 2). Over the entire 28-day infusion, ISO treatment caused a significant increase in HR (+39%; p < .05) concomitant with peak HR. Relative to saline treatment, systolic and diastolic pressures decreased with ISO treatment (−12% and −10%, respectively; p < .05) during the first 12 days of infusion but normalized for the remainder of the infusion (Figure 2). Likewise, ISO decreased pulse pressure (Figure 2) by 11% and QA interval (the time between ventricular depolarization and ejection) by 16% (QA,Saline = 39.3 m, QA,ISO = 33.1 ms) during infusion. Changes in the ECG were apparent including inverted T-waves (48–65 h into infusion), downward sloping ST depression with T-wave flattening (4–28 days into infusion), and deep Q waves (>25% of R-amplitude)—the latter occurring simultaneously with T-wave inversion and ST-depression. Notably, ECG abnormalities on day 2 were accompanied by the most dramatic changes in minute volume (−24%; Saline = 587.5, ISO = 444.75 ml/min; p < .05) and Penh (+119%; Saline = 0.71, ISO = 1.56; p < .05) that were observed during the entire study.

Over the 17-day period that followed pump removal and preceded inhalation-exposure, Penh levels in ISO-treated rats remained significantly higher (+32%; p < .05) than those of saline-treated rats. ISO-infused rats also had a significantly lower HR (−9%; p < .05) and pulse pressure (−11%; p < .05), but there were no differences in Tco. Some physiological differences emerged after pump removal but subsided prior to inhalation exposure, including a 9-day hypotensive period (−9% in SBP and DBP, days 29–38; p < .05), a 3-day increase in QA interval (+7%, days 29–32; p < .05), and a decrease in aortic dP/dt max (−7% on day 3; ISO = 2552 ± 197 mm Hg, Saline = 2750 ± 152 mm Hg; p < .05) relative to saline-treated rats. More than half of the ISO-treated rats (5 of 8) had Q-waves 6 days after pump removal. One day prior to nose-only treatment (17 days after pump removal), ISO-treated rats had significantly greater RMSSD (Table 1; +35%; p = .007), pNN15 (+323%; p = .001), Lf (+137%; p = .0139), and Hf (+159%; p = .019) and a 6% lower heart rate (p < .008) than saline-treated rats. There were no group differences in body mass immediately after the infusion regimen and just before inhalation exposure.

Effects of particulate inhalation

Cardiac function and hemodynamics

HP12 exposure had no effect on HR, pulse pressure, QA interval, Tco, MAP, SBP, and DBP in either ISO- or saline-treated rats.

Autonomic function

No statistically significant differences were observed between the pre- (day 45) and postinhalation HRV values of any single group (Figure 3). Notably, the ISO–HP12 group had a trend toward increased pNN15 (134% increase; p = .192) after the second inhalation exposure (day 47) relative to preinhalation, whereas no similar trends were observed in any of the other groups (ISO–Air: day 47 versus day 45, p = .603; all other intragroup comparisons of postinhalation values to day 45, p = 1.0).

Table 1. Effects of ISO infusion on heart rate variability parameters prior to inhalation exposure.

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Isoproterenol</th>
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<tbody>
<tr>
<td>Heart rate (bpm)</td>
<td>293 ± 4</td>
<td>278 ± 3*</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>9.0 ± 0.4</td>
<td>9.6 ± 0.7</td>
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<tr>
<td>RMSSD (ms)</td>
<td>4.3 ± 0.3</td>
<td>5.8 ± 0.4*</td>
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<tr>
<td>pNN15 (%)</td>
<td>0.65 ± 0.18</td>
<td>2.75 ± 0.47*</td>
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<tr>
<td>Lf (ms²)</td>
<td>0.9 ± 0.1</td>
<td>2.0 ± 0.4*</td>
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<tr>
<td>Hf (ms²)</td>
<td>1.0 ± 0.2</td>
<td>2.6 ± 0.6*</td>
</tr>
<tr>
<td>Lf/Hf</td>
<td>1.1 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>

Note. Values are means ± SE, n = 8/group, of ECG data acquired 17 days after infusion cessation, corresponding with the day prior to initiation of nose-only inhalation exposure. Groups are presented according to infusion treatment. *Different from Saline (p < .05).

Table 2. Cardiac histopathology scores and bradyarrhythmia counts.

<table>
<thead>
<tr>
<th></th>
<th>Saline–Air</th>
<th>Saline–HP12</th>
<th>ISO–Air</th>
<th>ISO–HP12</th>
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</thead>
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<tr>
<td>Heart histopathology</td>
<td>3.6 ± 0.2</td>
<td>3.4 ± 0.2*</td>
<td>3.6 ± 0.2*</td>
<td>3.6 ± 0.2*</td>
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<tr>
<td>Cardiomyopathy score</td>
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<td>0.8 ± 0.3</td>
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<td>Bradyarrhythmias</td>
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<td>Preinhalation (day 45)</td>
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<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Postinhalation (day 46)</td>
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</tr>
<tr>
<td>Postinhalation (day 47)</td>
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Note. Histopathology values are means ± SE (n = 4–5/group). Bradyarrhythmia counts are total frequency of events from ECG data (n = 4/group) collected daily from a 1-min ECG trace per rat sampled once every hour over 12 h. *Significantly different from Saline–Air (p < .05). †Significantly different from Saline–HP12 (p < .05).
Figure 3. Effects of inhalation exposure on heart rate and HRV parameters. Values are presented as group means acquired over 12 h (1500–0200 h) on specific days relative to osmotic pump implantation (day 0). Vertical bars indicate standard error. From left, upper horizontal bars indicate the period of infusion (28 days), and the two nose-only inhalation exposures (4 h/day). Asterisks mark significant differences within groups relative to their own preinfusion values (day −2; \( p < .05 \)), whereas no significant differences were detected between day 45, 46, and 47 values of the same group (\( p > .05 \)). Differences from the Saline-Air and Saline-HP12 groups on corresponding days were also noted by ‘a’ and ‘b’, respectively (\( p < .05 \)).
Cardiac arrhythmia
VPBs were not influenced by treatment or exposure. Bradyarrhythmias were rare prior to inhalation exposure (baseline); only a single second-degree AVB was observed, which occurred in a rat pretreated with ISO and was the more benign type I Mobitz arrhythmia. There were no statistically significant effects of inhalation exposure on incidence of bradyarrhythmias; nevertheless, 10 of the 11 bradyarrhythmias that occurred after nose-only exposure (91%) were in the ISO-HP12 group (Table 2). HP12 inhalation increased the proportion of ISO-pretreated rats with bradyarrhythmias. Three out of four ISO-pretreated rats had bradyarrhythmias after HP12 exposure, which collectively included seven second-degree AVB Mobitz type II events and three sinoatrial block events (Figure 4).

In contrast, no bradyarrhythmias were observed among ISO-pretreated rats in the HP12 group prior to inhalation exposure, and only a single bradyarrhythmia was observed among all other groups after nose-only treatment. Time-matched comparisons of acute (1-min) HRV measures prior to and following inhalation exposure revealed that the AVB Mobitz II arrhythmias in ISO-HP12 rats were accompanied by elevations in \( L_f \) and \( L_f/H_f \) relative to the HRV values at the corresponding hour 1 day before PM inhalation.

In one of the four ISO-HP12 rats, six second-degree AVB Mobitz type II arrhythmia events were observed 7 h after cessation of the second HP12 inhalation. Each of these arrhythmias was accompanied by progressive sinus slowing (increasing PP intervals) and the entire 1-min

Figure 4. Sample bradyarrhythmias observed after PM inhalation in ISO-pretreated rats. ECG and blood pressure waveforms of a second-degree atrioventricular nodal block Mobitz type II arrhythmia (A), and a sinoatrial block arrhythmia (B). Arrow indicates nonconducted P-wave. See Methods for arrhythmia identification criteria.
sampling period was characterized by a 150-fold increase in \( L_f \) and a 170-fold increase in \( L_f/H_f \) (14% decrease in \( H_f \)) relative to the same hour on the day preceding inhalation exposure. Two hours after the second HP12 inhalation period, another ISO-HP12 rat had a second-degree AVB Mobitz II arrhythmia that was accompanied by a 71-fold increase in \( L_f \) and 48-fold increase in \( H_f \), resulting in a 40% increase in \( L_f/H_f \).

### Indicators of injury and inflammation in BALF
ISO infusion alone did not increase macrophage, neutrophil, protein, or albumin levels in lavage fluid (Figure 5). In contrast, ISO infusion followed by HP12 exposure increased lavage LDH relative to the ISO-Air group (73% greater; \( p < .05 \)). The ISO-HP12 group also had a trend toward a greater LDH than the Saline-Air group (63% greater; \( p = .096 \)).

### Serum biomarkers
The ISO-HP12 regimen doubled serum HDL relative to ISO-Air (Figure 6; \( p < .05 \)) and increased HDL by 74% relative to the Saline-Air regimen (\( p = .0597 \)), whereas the Saline-HP12 treatment had no effect on HDL. The ISO-HP12 group had a 10% increase in serum total protein relative to the Saline-HP12 group \( (p < .05) \) and a 5% increase above the ISO-Air group \( (p = .058) \). Treatment with ISO and/or HP12 did not significantly alter serum total cholesterol, LDL, lipoprotein(a), triglycerides, total protein, \( \alpha \)-hydroxybutyrate dehydrogenase, cardiac troponin-I, creatine kinase, myoglobin, lactate dehydrogenase-1, IL-6, C-reactive protein, sorbitol dehydrogenase, ALT, AST, total antioxidant status, or bilirubin, relative to corresponding control groups.

### Histopathology
ISO infused rats exposed to air had a cardiomyopathy that was characterized by multifocal lesions (Figure 7) containing necrotic myofibers, macrophages, collagen deposits, and proliferating fibroblasts; the histology score of the lesions in this group was significantly greater than that in saline-infused rats \( (p < .05) \). HP12 exposure did not discernibly worsen the histopathologic features relative to air-exposure regardless of infusion pretreatment (Table 2).

### Heart and body mass
Heart mass increased with the combination of ISO pretreatment and HP12 inhalation relative to either ISO or HP12 treatment alone. In heart-to-body mass ratio, the ISO-HP12 group significantly exceeded both saline groups by 8.3% and exceeded the ISO-Air group by 2% (Figure 8; \( p < .05 \)). ISO-HP12 rats also surpassed Saline-Air and ISO-Air rats in atrial mass by 25% and 19%, respectively \( (p = .036 \) and \( p = .0524 \), respectively), but these differences were no longer significant when normalized to heart or body mass. Additionally the ISO-HP12 group had trends towards a significantly increased ratio of RV-to-heart mass relative to the ISO-Air and Saline-Air groups \( (+13\%, \ p = .098, \) and \( +12\%, \ p = .058 \), respectively).
Both the air- and the HP12-exposed ISO groups significantly surpassed the HP12-exposed saline group in LV-to-body mass ratio by 10.8% and 8.4%, respectively (p < .05), but were not significantly greater than the Saline-Air group. Although the ISO-Air group’s mean heart-to-body mass ratio was 5.7% higher than both saline groups, this difference failed to reach significance (p = .61, both comparisons). There was a minor trend toward decreased LV mass in the Saline-HP12 group versus its corresponding air-control (p = .20), and this trend was less apparent in the ISO-HP12 group relative to ISO-Air (p = .68). These ratios were not driven by body mass changes as all groups experienced similarly minor declines in body mass after 2 days of nose-only exposure to air or HP12 (data not shown) that likely resulted from restraint.

**Discussion**

We hypothesized that PM exposure would exacerbate cardiopulmonary injury in a rat model of ISO-induced cardiomyopathy. In the present study, ISO alone elicited hypotension, tachycardia, ECG abnormalities, and hypoventilation during infusion, as well as severe fibrotic cardiomyopathy, bradycardia, hypotension, and increased heart rate variability after infusion. It is likely that ISO treatment also depressed cardiac output after infusion as suggested by decreased heart rate, pulse pressure, and aortic dP/dt_max and by increased QA interval, but these effects would have to be confirmed with direct measurement of cardiac function in future studies. The mechanisms by which ISO altered chronotropy and hemodynamics post
infusion are unclear, but they may include remodeling associated with cardiomyopathy or decreased cardiac β₁-adrenergic receptor expression, the latter of which has been shown to reduce HR and increase HRV (Ecker et al., 2006), follow long-term administration of ISO (Murray et al., 2000; Nishikawa et al., 1993; Zhang et al., 2005), and contribute to human HF (Rockman et al., 2002; Diwan and Dorn, 2006). HP12 exposure in ISO-treated rats increased lung LDH, serum protein, serum HDL, heart weight, and prevalence of bradyarrhythmias. Collectively, these findings suggest that this rat model of ISO-induced cardiomyopathy is sensitive to the effects of air pollution inhalation.

Pulmonary LDH and serum total protein increased solely in PM-exposed ISO-infused rats, suggesting that ISO-induced cardiomyopathy enhanced sensitivity to PM-induced injury. This was associated with trends of increased lung protein and albumin. There was no evidence of significant pulmonary injury in saline-treated PM-exposed rats, thus paralleling the observations of Wichers et al. (2004b), who saw a similar lack of effect in SH rats exposed to 0.83 mg/kg HP12 via intratracheal instillation (IT). In this past study, the IT dose was more than twice the equivalent dose from a 6-h inhalation of fine ROFA particles at the same concentration as our study (12 mg/m³) (Costa et al., 2006). The absence of significant lung injury with HP12 exposure at these levels likely relates to transition metal content. Investigators in previous studies have shown that the inhalation of a more metal-rich ROFA, or its constituent metals, including Ni and V, causes airway inflammation and lung injury in rats (Campen et al., 2001; Kodavanti et al., 1998; Gardner et al., 2004). Gardner and colleagues (2004) found significant lung injury (alveolitis, bronchiolar hyperplasia, and inflammation) after 3

![Figure 8](image-url)

**Figure 8.** Heart mass. Values are presented as group means with standard error bars. Horizontal bars with stars indicate significantly different values \((p < .05)\).
consecutive days of 6-h 15 mg/m³ exposures. Although in our study HP12 exposure significantly increased lung LDH and serum protein levels only in ISO-treated rats, HP12 had no significant effect on pulmonary neutrophil infiltration in either the ISO- or saline-pretreated groups, which may be explained by the fact that HP12 has 2% the soluble vanadium and 20% the soluble nickel of the most extensively studied ROFA (Florida Power & Light; Wichers et al, 2004a; Dreher et al., 1997). Meanwhile, PM-induced pulmonary injury may have been enhanced by ISO pretreatment due to β₂-adrenergic receptor down-regulation, which impairs pulmonary clearance and has been observed among heart failure patients (Snyder et al., 2006) as well as ISO-infused rodents (Nerme et al., 1990). Further work on the different factors that may influence pulmonary injury after PM exposure should be investigated.

PM inhalation increased heart-to-body weight ratio in ISO-pretreated rats, indicating that PM exposure worsened overall cardiac health. Changes in cardiac mass are associated with heart failure progression (Diwan and Dorn, 2006). Although there was no significant change in left ventricular (LV) mass, multiple studies have shown that increased LV mass is associated with disease severity in individuals with heart failure (Van Hee et al., 2009; Diwan and Dorn, 2006). Interestingly, in a recent publication describing results from the Multi-Ethnic Study of Atherosclerosis, Van Hee et al. (2009) demonstrated a strong inverse relationship between LV mass and annual PM₁₀ levels. In contrast, Rivero and coworkers (2005) found that a single intratracheal instillation of fine PM in the healthy Wistar rat, a genetic precursor of the SHHF rat, increased cardiac mass via edema. The increased heart-to-body mass ratio in the ISO-HP12 group may have stemmed from a trend towards increased right ventricular mass. The change was apparent despite a mild trend towards decreased LV and septal mass with HP12 exposure in these rats. The exact mechanisms accounting for these varied impacts on cardiac chamber wall mass are unclear and merit further investigation. Notably, cardiac hypertrophy has been associated with slower conduction across cardiomyocyte junctions (Cooklin et al., 1997), which, by impeding both sinoatrial and atrioventricular conduction, may account for the increased prevalence of bradyarrhythmias in the ISO-HP12 group.

This study is the first to demonstrate an increase in serum HDL cholesterol after PM exposure in rats and is in parallel with a study that showed analogous increases with PM exposure in humans (Tomao et al., 2002). Although PM exposure elevated HDL, there was no correlative evidence of liver dysfunction or toxicity (as indicated by AST, ALT, sorbitol dehydrogenase, bilirubin), and thus the significance of this finding is unclear. Interestingly, HP12 induced a 2-fold increase in serum HDL cholesterol only in the ISO-infused group. Although HDL cholesterol is popularly considered protective of cardiovascular health, this issue continues to be debated within the scientific community (Taubes, 2008). A recent trial of an HDL-increasing pharmaceutical therapy that also decreases LDL levels was associated with significant increases in major cardiovascular events and cardiovascular deaths (Rader, 2007). Notably, serum HDL cholesterol in our study correlated significantly with pulmonary injury (lavage LDH; \( P = .002; r^2 = .47 \)), indicating that the increase in HDL may correspond with increasing pulmonary injury. Thus, the increase in HDL cholesterol among HP12-exposed ISO-treated rats may indicate increased cardiovascular risk; further work on the impact of HDL on cardiovascular health and potential links with PM exposure is warranted.

In the present study, HRV measures only discretely alluded to PM-induced increases in cardiac vagal tone as seen in a trend towards increased pNN15 in the ISO-HP12 rats. Nevertheless, rapid changes in HRV measures in individual rats that were paired with the occurrence of bradyarrhythmias suggest an enhanced acute phase sympathetic response to PM exposure in animals with underlying ISO-induced cardiomyopathy—a condition common to heart failure. The increased number of ISO-pretreated rats with bradyarrhythmias after PM exposure—especially atrioventricular block resembling the human Mobitz type II—parallels our recent findings in ROFA-exposed SHR (Farraj et al., 2009) and those in second-hand smoke-exposed mice (Chen et al., 2008), and may have been linked to acute sympatho-excitation. Arrhythmogenic sympatho-excitation was implicated by arrhythmias occurring concomitantly with acute (1-min) upward spikes in LF and LF/HF parameters, changes that have been previously shown in conjunction with PM-induced cardiac oxidative stress (Rhoden et al., 2005). ISO treatment caused elevations in RMSSD and hence vagal dominance before PM exposure. This was followed by extreme and brief sympathoexcitation concomitant with the bradyarrhythmias that followed PM exposure, which may have reflected an acutely arrhythmogenic shift in sympathovagal balance. These changes support the value of short-term measurements of HRV for providing insight into the mechanistic relationship between acute-phase responses to PM and cardiac events, especially in the presence of underlying cardiac disease. Additionally, PM-induced pulmonary inflammation can activate pulmonary irritant receptors and lead to enhanced cardiac vagal tone that may predispose individuals to fatal bradyarrhythmias (Pope et al., 1999; Stone and Godleski, 1999; Brook et al., 2003). Pope et al. (1999) noted significant increases in RMSSD with increasing ambient PM₁₀ levels among patients with HF; and other epidemiological studies have noted analogous responses not only in RMSSD but also in pNN50 (Yeatts et al., 2007; Routledge et al., 2006; Riediker et al., 2004). This scenario may also exist in animal models as shown by Tankersley et al. (2004), who reported similar findings with carbon PM exposure in terminally senescent mice. Because lung injury (increased lavage LDH) and bradyarrhythmias were evident only in HP12-exposed ISO-pretreated rats, pharmacologically induced
cardiomyopathy may have enhanced sensitivity to the pulmonary toxicity of PM. Furthermore, it is possible that PM-induced lung injury in this group played a role in the induction of bradyarrhythmias and increased heart mass. Lower concentrations of HP12 would help reveal whether pulmonary injury is necessary to elicit these changes to cardiac conduction and structure. Furthermore, we hope to examine the contribution of inflammatory, autonomic, and direct effects of PM components (e.g., cardiac oxidative stress, direct ion channel interference) in air pollution-induced cardiac dysfunction.

In conclusion, although PM inhalation did not affect chronotropic, hemodynamic, or thermoregulatory parameters in control or ISO-pretreated rats, HP12 exposure did exacerbate some cardiopulmonary responses relative to control rats. The subtlety of the effects of PM inhalation in this study may be due to the prolonged delay (17 days) between pump removal and PM exposure; this lapse in time may have allowed for some recovery from ISO-induced cardiac injury. In a subsequent study (Carll et al., 2009), inhalation of a similar PM at a much lower concentration (580 µg/m³) 5 days after pump removal elicited significant pulmonary inflammation, hypotension, and ECG alterations (e.g., prolonged PR interval, decreased Q wave amplitude) in ISO-infused rats (367).

References


Declaration of interest

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