Abstract

Rachel McIntosh-Kastrinsky
Multi-pollutant mixtures and their cardiac health effects in mice
(Under the direction of Ilona Jaspers)

A growing body of evidence from epidemiological and toxicological studies indicates a strong link between air pollution and cardiovascular morbidity and mortality. Many studies have focused on the role of single pollutants, but this report along with others indicates the importance of ambient air pollutant mixtures. This report utilizes two different air pollution mixtures to underline the importance of moving from a single-pollutant approach to a multi-pollutant approach: (1) photochemically-altered volatile organic compounds (VOCs), nitric oxide and nitrogen dioxide (NO_x) and ozone (O_3) and (2) concentrated ambient particles [CAPs, fine ($PM_{2.5}$) and ultrafine (UFP)] and O_3. In both studies the Langendorff cardiac perfusion model was used to access cardiac function. In these studies, mice demonstrated decreased cardiac function after multi-pollutant exposure, as indicated by decreased ventricular pressure and contractility. These two studies together help demonstrate the complexity of gaseous and particulate multi-pollutants and their adverse cardiovascular health effects.

Acknowledgements and Dedication

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I would like to dedicate this work to my husband, Josh Kastrinsky, for his continuous and unfaltering support throughout my career.

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Chapter 1: Introduction

According the Centers for Disease Control and Prevention (CDC) heart disease is the leading cause of death in the United States (Hoyert & Xu, 2012). Therefore it is important to understand the many factors involved in heart disease. Though not commonly addressed by the general population, air pollution has been linked to heart disease in numerous epidemiological, human and animal studies (D. Dockery et al., 1993; Kodavanti et al., 2011; Riediker et al., 2004). The American Heart Association has stated that cardiovascular health consequences of air pollution in general equal or exceed those due to pulmonary disease (R. Brook et al., 2002). Furthermore, studies have highlighted the key role particulate matter (PM), multi-pollutant mixtures and photochemical reactions play in cardiovascular disease related to air pollution (R. D. Brook et al., 2010). Since cardiovascular disease is the leading cause of death in the United States and the World Health Organization estimates that 28,000 premature deaths per year in North American are from air pollution, it is important to further investigate the link between air pollution and cardiovascular disease (Cohen et al., 2004; Hoyert & Xu, 2012).

Many studies have indicated a link between adverse cardiovascular health effects and particulate matter, but far fewer studies have considered the role of multi-pollutant mixtures (R. D. Brook et al., 2010). Though it is important to understand individual pollutants, air pollutants are not found separately in the ambient atmosphere, but instead

are found as complex mixtures (Dominici, Peng, & Bell, 2010). Therefore it is important to shift from a single-pollutant to a multi-pollutant approach to clarify the role of adverse health effects from air pollution (Dominici et al., 2010). This technical reports focuses on the adverse cardiovascular health effects from exposure to the complex mixtures of (1) photochemically altered volatile organic compounds (VOCs), nitric oxide and nitrogen dioxide (NO_x), and ozone (O_3) and (2) particulate matter (PM) and O_3.

Particulate Matter (PM)

PM is classified under the National Ambient Air Quality Standards (NAAQS) under the Clean Air Act of 1971, deeming it as harmful to the public health and the environment (USEPA, 2012). PM is a heterogeneous mixture of solid and liquid particles suspended in the air, continually varying in size and chemical composition in space and time (R. D. Brook et al., 2004). There are three categories of PM according to the particle aerodynamic diameter: coarse (PM_{10}), fine ($PM_{2.5}$) and ultrafine PM (UFP) (U S Environmental Protection Agency, 2010). PM_{10}, $PM_{2.5}$ and UFP have a nominal mean aerodynamic diameters of less than 10 μm, 2.5 μm and 0.1 μm, respectively (U S Environmental Protection Agency, 2010). The United States Environmental Protection Agency (USEPA) regulates PM_{10} and $PM_{2.5}$ with standards set at 150 $\mu g/m^3$ and 35 $\mu g/m^3$ per a day, respectively (USEPA, 2012). The origin, deposition and health effects differ for each fractionation.

PM_{10} originates through mechanical processes and typically consist of human-made and natural dust particles (John H Seinfeld & Pandis, 2006). PM_{10} deposits in the extrathoracic region and upper airways of the respiratory tract (U S Environmental Protection Agency, 2010). PM_{10} more commonly affects the respiratory system than the

cardiovascular system though studies have shown detrimental effects in both physiological systems with long and short-term exposures (U S Environmental Protection Agency, 2010).

$PM_{2.5}$ originates from coagulation of UFP particles and condensation of vapors onto existing particles, causing them to grow into their size range (John H Seinfeld & Pandis, 2006). $PM_{2.5}$ deposits in the lower airways of the respiratory system, and depending on the breathing pattern it can even reach the bronchioles (U S Environmental Protection Agency, 2010). $PM_{2.5}$ has been demonstrated to cause detrimental cardiovascular health effects with both short-term and long-term exposure, while the respiratory system appears to be more affected by long-term exposure to $PM_{2.5}$ (R. D. Brook et al., 2010; U S Environmental Protection Agency, 2010).

UFP originate from condensation of hot vapors during combustion processes and from the nucleation of atmospheric species to form fresh particles (John H Seinfeld & Pandis, 2006). UFP deposits in the most distal airways, reaching the alveoli and has been shown to translocate into the blood stream through the capillaries (Oberdorster et al., 2002; U S Environmental Protection Agency, 2010). UFP typically do not affect the respiratory system due to their small size, but have been shown to affect the cardiovascular system possibly due to their ability to translocate into the bloodstream (R. D. Brook et al., 2010; Oberdorster et al., 2002).

Many studies have indicated that there are three potential mechanisms in which PM can cause adverse cardiovascular health effects. The first mechanism is through systemic oxidative stress and inflammation, wherein an inhaled pollutant causes oxidative stress and inflammation starting in the lungs and then entering the blood stream

to become systemic (R. D. Brook et al., 2010). The second mechanism is through imbalance of the autonomic nervous system, wherein the inhalant reacts in the respiratory tract and causes an imbalance between the sympathetic and parasympathetic nervous system (R. D. Brook et al., 2010). The third mechanism is through translocation of particulate matter into the bloodstream, allowing direct damage to be incurred on the cardiovascular system (R. D. Brook et al., 2010). Though all these mechanisms are possible it is likely that a combination of them results in the adverse cardiovascular health effects attributed to air pollution.

Ozone (O_3)

Ozone is a criteria pollutant (NAAQS) under the Clean Air Act with the current standard set at 75 ppb per 8-hour time period (USEPA, 2012). Ozone formation in the urban and regional atmosphere is driven by short-lived volatile organic compounds emitted from anthropogenic and biogenic sources, including alkenes, aromatic, and oxygenated organic species (J. H. Seinfeld & Pandis, 2006). Many studies have demonstrated the adverse respiratory health effects of ozone and more recent studies have shown the cardiovascular implications of ozone (Bell, McDermott, Zeger, Samet, & Dominici, 2004; M. J. Hazucha, 1987). Because ozone is a highly reactive atmospheric gas it is important not only to consider ozone's effect individually, but its effect on other pollutants and those resulting health effects.

Volatile Organic Compounds (VOCs)

There are many sources of volatile organic compounds (VOCs) including combustion processes, production, treatment storage and distribution of fossil fuels, application of volatile organic solvents, industrial production processes, and biological processes (Friedrich & Obermeier, 1998). The major classes of VOCs are alkanes, alkenes, aromatic hydrocarbons and oxygenated compounds (J. H. Seinfeld & Pandis, 2006). In typical urban environments the non-methane VOCs are composed of 40-45% alkanes, 10% alkenes, 20% aromatic hydrocarbons, 10-15% oxygenates and the rest are unidentified VOCs (Atkinson & Arey, 2003). While in the atmosphere VOCs react with hydroxyl radicals and O_3 during the daylight and NO_x during the nighttime (Atkinson & Arey, 2003). Most reactive products of VOCs include an alkyl or substituted alkyl radical (for example, hydroalkyl, nitroalkyl or oxoalkyl) with key intermediates being oxygen radicals (Atkinson & Arey, 2003). Few studies have looked at the adverse health effects from atmospheric VOCs, but some studies indicates they may play a role in the adverse respiratory and cardiovascular health effects from air pollution (Doyle, Sexton, Jeffries, Bridge, & Jaspers, 2004; McDonald et al., 2010; Smith, 1965).

Nitric Oxide and Nitrogen Dioxide (NO_x)

Nitric oxide (NO) is emitted into the atmosphere by both natural and anthropogenic sources, while nitrogen dioxide (NO_2) is emitted in small quantities from combustion along with NO and is usually formed in the atmosphere by oxidation of NO (J. H. Seinfeld & Pandis, 2006). The atmospheric sum of NO and NO_2 is usually designated as NO_x (J. H. Seinfeld & Pandis, 2006). The U. S. Environmental Protection

Agency (EPA) regulates NO_2 as a criteria pollutant (NAAQS) as surrogate for NO_x, because it is the most prevalent anthropogenic form of NO_x in the atmosphere (U.S. Environmental Protection Agency, 1999). The current standard for NO_2 is 53 ppb averaged over a year (USEPA, 2012). NO_2 reacts with VOCs in the atmosphere to form ozone and other intermediates (Friedrich & Obermeier, 1998). Increases in NO_2 levels have been correlated with increased levels of particulate matter (R. D. Brook et al., 2004). Though NO_x does have adverse respiratory and cardiac health effects alone, it's reactivity with VOCs, ability to produce ozone and correlation with PM levels may be more important with regard to public health (Hoppenbrouwers, Calub, Arakawa, & Hodgeman, 1981; U.S. Environmental Protection Agency, 1999).

Irradiation Chamber

In the ambient environment pollutants are exposed to sunlight, which causes photochemical reactions, further altering pollutants. An irradiation chamber, also known as a smog chamber, can be used to create complex photochemical environments that simulate the ambient environment (Jeffries, Fox, & Kamens, 1976). Outdoor smog chambers allow for the natural diurnal variations in solar intensity that may not be considered in indoor chambers (Jeffries et al., 1976). Modeling has shown that the natural diurnal variations in solar intensity may cause strong fluctuations in hydroxyl and hydroperoxy radical concentrations (Jeffries et al., 1976). Because outdoor smog chambers are exposed to these diurnal variations in solar intensity, they can appropriately simulate the ambient environment. Furthermore cell and animal models can be exposed to these complex mixtures to assess adverse health effects.

Concentrated Ambient Particles (CAPs)

The ambient particle concentrator is a recently developed technology employed to conduct studies in which humans and animals can to exposed to elevated levels of ambient PM (Ghio & Huang, 2004). There is no modification of the physico-chemical characteristics of the particles, which is important since the aerosols on the surface of PM may play a key role in their adverse health effects (Campen et al., 2010; Ghio & Huang, 2004). This method also allows isolation of the particles without atmospheric gases, therefore enabling control of the gaseous exposure mixture (Ghio & Huang, 2004). Though the chemical composition of particles varies depending on location of collection, this method allows for exposure and control of particles from the ambient environment (R. D. Brook et al., 2010).

Cardiac Perfusion Model

The Langendorff cardiac perfusion is a standard *ex vivo* technique used to investigate cardiac contractile strength and the clinical relevance of myocardial infarction from ischemia-reperfusion injury (Tong et al., 2009). The simplicity of the isolated mammalian preparation, the broad spectrum of measurements which can be done using this method, its high reproducibility and relatively low cost make it a very useful tool in cardiovascular research (Skrzypiec-Spring, Grotthus, Szelag, & Schulz, 2007). Therefore, the Langendorff cardiac perfusion is a useful technique to assess cardiac function after air pollution exposure.

Technical Report Aim

The focus of this technical report is to investigate the cardiac health effects of multi-pollutant mixtures. Both studies aimed to look at adverse cardiac health effects from multi-pollutant mixtures in chamber inhalation systems followed by cardiac perfusion. The first study looked at the inhalation of photochemically-altered gaseous air pollutants (VOCs, NO_x and O_3), while the second study addressed the effects of inhaling ambient particulate matter and ozone as co-pollutants. It was hypothesized that both photochemically-altered gaseous air pollutants (smog) and PM with O_3 would decrease cardiac function.

Chapter 2: Photochemically alter air pollution depresses cardiac function

Introduction

Epidemiological studies indicate a relationship between acute and chronic ambient air pollution exposure and cardiovascular disease (Peters et al., 2004). Specifically, ambient air pollution exposure has been shown to increase the risk of ischemic heart disease, heart failure, and arrhythmias (R. D. Brook et al., 2010; Peters et al., 2004). Numerous studies have investigated the effect of individual pollutants or simple mixtures, such as particulate matter (R. Brook et al., 2002), far fewer have considered the complex mixtures encountered in the environment (Sexton et al., 2004). Though individual pollutants can cause serious health effects, multi-pollutant mixtures are encountered daily in the ambient environment. Shifting from a single-pollutant to a multi-pollutant approach could help enhance scientific knowledge of adverse health effects and provide greater public health protection (Dominici et al., 2010). It is important to consider photochemically-altered mixtures, which are encountered in the ambient environment, since ambient pollutants react in the environment forming secondary pollutants (Ruiz et al., 2007).

Environmental irradiation chambers, or "smog chambers," simulate the complex mixtures found in the environment by using sunlight to initiate photochemical reactions,

which naturally occur in an ambient urban environment (Jeffries et al., 1976). Outdoor smog chambers allow for the natural diurnal variations in solar intensity that may not be considered in indoor chambers (Jeffries et al., 1976). Modeling has shown that the natural diurnal variations in solar intensity may cause strong fluctuations in hydroxyl and hydroperoxy radical concentrations (Jeffries et al., 1976). Smog chambers allow the study of photochemically altered air pollutants as present in the ambient environment.

Studies have shown that exposure to photochemically-altered air pollutants have led to adverse health effects. Smith noted decreased respiratory function during exertion when subjects were exposed to a photochemically altered component of Los Angeles, CA smog, perooxyacetyl nitrate (PAN) (1965). Human epithelial lung cells exposed in a smog chamber showed an increase in inflammatory marker IL-8 and in cell death marker LDH (Sexton et al., 2004). After exposing human alveolar cells to photochemically-altered 1,3-butadiene, isoprene and NO_x cells showed increased cell death and inflammation (Doyle et al., 2004; Doyle, Sexton, Jeffries, & Jaspers, 2007). In a toxicogenetic study, human epithelial cells showed increased cell death when exposed to a photochemically-altered gaseous mixture of hydrocarbons, NO_x and O_3 (Rager et al., 2011). Furthermore the study's toxicogenetics suggest a link between cardiovascular disease and the photochemically-altered gaseous mixture exposure (Rager et al., 2011). These results help elucidate the respiratory health effects and mechanisms from complex ambient air pollution, but indicate a need for further investigation into their adverse cardiovascular health effects.

In this study a Langendorff model was used to assess the cardiovascular effects from exposure to a complex air pollution mixture. The Langendorff model has been used

previously to assess the cardiovascular effects and mechanisms from air pollution exposure in animals (Simkhovich, Marjoram, Kleinman, & Kloner, 2007; Tong et al., 2009; Tong, Cheng, Samet, Gilmour, & Devlin, 2010). This study was designed to investigate the cardiovascular effects in mice exposed to photochemically altered products of volatile organic hydrocarbons urban mixtures (smog). The cardiovascular health effects of the urban air pollution were assessed through the mechanical function and hemodynamics of the heart. In this study, we hypothesized that photochemically altered urban mixtures of volatile organic compounds (VOCs), NO_x and O_3 (smog) would decrease cardiac function in mice.

Materials and Methods

Experimental Animals

All studies were conducted with the approval of the Institutional Animal Care and Use Committee for the University of North Carolina at Chapel Hill and in accordance with the National Institutes of Health Guides for the Care and Use of Laboratory Animals. This study used C57BL/6 mice (18 to 21 weeks of age, average body weight 23.9±0.39g). Animals were given access to both food and water ad libitum.

Generation of Photochemical Urban Mixtures

The University of North Carolina-Chapel Hill's outdoor environmental irradiation chamber was used to generate multi-pollutant exposure conditions. Synthetic Urban

Mixture (Scott Specialty Gases, Plumsteadvillle, PA), a VOC mixture, and NO_x (nitric oxide and nitrogen dioxide) were used as the starting materials for the test atmosphere. The Synthetic Urban Mixture contains 55 different hydrocarbons at specific ratios that represent chemicals present in urban atmospheres (Sexton, Jeffries et al. 2004). On the morning of the exposure, the chamber was humidified naturally by pre-flushing with HEPA-filtered ambient air. At 0700 hours, the volatile organics of Synthetic Urban Mixture were drawn from a gas cylinder into the photochemical reaction chamber while a liquid mixture containing less-volatile organics was injected into the chamber. NO_x was drawn from a gas cylinder (AirGas, National Welders, Morrisville, NC) into the chamber to establish a test atmosphere containing 0.2 ppm NO_x

Five-month old mice were exposed for 4 hours (2000-2400 hours) in a whole body inhalation system to photochemically aged Urban Mixture (smog), 0.3 ppm of ozone (O_3), or filtered air (FA) generated in an outdoor photochemical irradiation chamber.

Cardiac Perfusion

The procedure for cardiac perfusion has been previously described (Tong et al. 2010). Briefly, 8 hours after exposure, mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.). Heparin (100 units) was injected intravenously through the vena cava before removal of heart. The hearts were rapidly removed and placed in ice-cold Krebs-Henseleit buffer, after which the aortas were cannulated. Retrograde perfusion via the aorta was performed under constant pressure (100 cmH_2O) above the heart. The non-recirculating perfusate was a Krebs-Henseleit buffer containing (in mmol/L) 120 NaCl,

5.9 KCl, 1.2 $MgSO_4$, 1.75 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose. The buffer was aerated with 95% O_2—5% CO_2 and maintained at pH 7.4 and a temperature of 37°C.

For assessment of contractile function, a latex balloon on the tip of a polyethylene catheter was inserted through the left atrium into the left ventricle. The catheter was connected to a pressure transducer (Argon Medical Devices, Athens, TX) at the same height as the heart. A PowerLab system was used to collect and process the left ventricular pressure data (AD Instruments, Milford, MA). All hearts were perfused for 25 min prior to the start of a 20 min-no-flow ischemia, followed by 2 h of reperfusion. Heart rate, cardiac contractile function indexes (left ventricular systolic and diastolic pressures), and their derivatives (dP/dt), which are the rates of rise/fall in left ventricular pressure, were measured continuously. Left ventricular developed pressure (LVDP) was measured as the difference in systolic and diastolic pressure in the left ventricle and ischemic time to contracture was measured as the time from the start of ischemia until initial contracture (at least 5 cmH_2O increase in left ventricular developed pressure). Recovery of LVDP, expressed as a percentage of the initial pre-ischemic LVDP, was measured at 20, 40 and 60 min of reperfusion following 20 min of ischemia.

Cardiac Necrosis Evaluation

At the end of 2 h reperfusion, hearts were perfused with 15mL of 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) dissolved in Krebs-Henseleit buffer, then incubated in 1% TTC at 37°C for 10 min, and then fixed in formalin. The area of necrosis was measured by taking cross-sectional slices through the ventricles, which were then photographed using a digital camera mounted on a stereo-microscope. The resulting

images were quantified by measuring the areas of stained (viable tissue) versus unstained tissue (infarct) with the use of Abode Photoshop. Infarct size was expressed as a percentage of the total ventricular section and averaged from the four images.

Isolated Aortic Ring Constriction

Thoracic aorta was isolated and placed in chilled, modified Krebs buffer (all in mM 120 NaCl, 5.9 KCl, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, adjusted to pH 7.40 with 1N HCl and stored at 4°C), cleaned of excessive adventitial tissue, and cut into 2-mm ring segments. Each ring segment was suspended in individual organ chambers filled with Krebs buffer (25 ml) of the following composition (mM): 120 NaCl, 5.9 KCl, 1.2 $MgSO_4$, 1.8 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose, pH 7.40 as described previously (Rajagopalan et al., 1996). The solution was aerated continuously with a 95% O_2—5% CO_2 mixture and maintained at 37°C. Care was taken not to injure the endothelium during preparation of the rings. Tension was recorded with a linear force transducer. Vessels were allowed to equilibrate for at least 1 h at a resting tension of 700 mg before being exposed to graded doses of L-phenylephrine (PE), starting with 0.025μM and increasing ten-fold every 2 min until a final concentration of 250μM was reached. The vessels were then relaxed with acetylcholine (Ach), starting at 0.001μM and increasing at a ten fold every 45 sec until a final concentration of 1μM was reached. The vessels' buffer was then flushed four times with fresh buffer, refilled and allowed to rest for 15 min before a second run was conducted. The vessels buffer was changed every 30 min to ensure adequate glucose. The vessel tension was continuously recorded to assess the vessel constriction. Subtracting the baseline tension from the highest

constriction tension and dividing that by each relaxation dose tension minus the baseline constriction tension calculated the percent constriction. Percent constriction from the two runs was averaged for the final percent constriction.

Measurement of Lung Function and BAL

Mice were anesthetized with pentobarbital (5mg/mL), given the paralytic pancuronium bromide, underwent tracheostomies and were mechanically ventilated (Scireq, Montreal, Canada) 12-24 hours (smog 12h, other 24h) after exposure, as previously described (Allen et al., 2012). They were exposed to aerosol challenges of increasing concentrations methacholine (MCh, Sigma-Aldrich) for 30 sec, using an ultrasonic nebulizer (Scireq, Montreal, Canada). Airway reactivity was determined by assessing forced oscillatory mechanics every 10 sec for 3 min following MCh challenge. Like other studies using forced oscillary mechanics to evaluate airway function, we focused our analysis on total airway resistance (R), minor airway resistance (Raw), and tissue damping (G).

After respiratory challenge measurements were completed, mice were euthanized and blood was gathered by cardiac puncture, spun down for 15 min at 10,000 rpm to separate and collect serum. The lungs were lavaged five times with 1 mL saline, and the resultant bronchoalveolar lavage fluid (BAL) was centrifuged to separate the cellular components from the supernatants. Total BAL cellularity was determined using hemoacytometer, and the BAL composition was evaluated morphologically following differential staining (Diff-Quik; Dade Behring) as described previously (Allen et al., 2012).

Statistical Analysis

Data are expressed as means ± SEM. Comparisons between each variable (smog1, smog2, and O_3) group and filtered air (FA) control group were performed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons. The statistical significance levels was set at $p<0.05$.

Results

Composition of Smog Exposure

Table 2-1 details the compositions of the exposure groups. Composition (50+ hydrocarbons, Table 2-1) is based on EPA analyses of 40+ cities and was used to create two test mixtures of different concentrations but of the same composition of the primary organics. The smog groups contained about 0.3 ppm of ozone and secondary carbonyls.

As seen in a previous study (Rager, Lichtveld et al. 2011), average NO_2 and NO levels decreased across the day, and levels of secondary chemical products, such as ozone, formaldehyde, and peroxyacetyl nitrate increased. No particulate matter or secondary organic aerosol was formed within the chamber.

Cardiac Effects

Hearts from the two smog-exposed groups had significantly lower baseline LVDP (69.2±16.0 cmH_2O for smog-group-1 and 75.1±23.0 cmH_2O for smog-group-2) compared to FA-group (146.4±14.8 cmH_2O; $p<0.05$), though there was no significant

difference between FA-group and O_3-group (Figure 2-1). Baseline left ventricular contractility was also significantly depressed by the smog and ozone only inhalation. The baseline maximum d*P*/d*t* was significantly lower in both smog groups (2764±558.8 cmH_2O/sec for smog-group-1 and 2945±765.7 cmH_2O/sec for smog-group-2) and O_3-group (3447±847.8 cmH_2O/sec) compared to FA group (5405±400.2 cmH_2O/sec; $p<0.05$) (Figure 2-2). In addition, the baseline minimum d*P*/d*t* was depressed by the smog inhalation (-1822±335.5 cmH_2O/sec) for smog-group-1, (-2070±625.3 cmH_2O/sec) for smog-group-2 and O_3-group (-2104±499.7 cmH_2O/sec) compared to the FA group (-3675±242.0 cmH_2O/sec $p<0.05$) (Figure 2-2). In addition, time to ischemic contracture was also significantly prolonged in smog-group-1 (17.3±0.5 min) compared to FA (13.2±1.4 min; $p<0.05$), but not in smog-group-2 or O_3-group (Figure 2-3). There was no statistically significant difference of baseline heart rate, baseline coronary artery flow rate (Table 2-2) and post-ischemia hemodynamics and infract size (data not shown) in the smog1, smog2, and O_3 groups compared to FA control, although there was a tendency to have lower baseline heart rate in the smog groups (data not shown).

Vascular Effects

Although there was no significant difference between the four groups, there was a trend for the smog1 and smog2 groups to be less constricted compared to the FA group (Figure 2-4). On the contrary the O_3 group trended toward an increase in aortic constriction compared to the FA group (Figure 2-4). The results presented here were not significant, but their trends indicate further investigation of aortic constriction is needed.

Lung Function and BAL

Lung function was assessed through MCh challenges, which indicated no significant statistical difference between the groups (data not shown). BAL samples were analyzed to assess pulmonary inflammation. BAL fluid showed that there was no significant difference between the four groups for cell count (data not shown).

Discussion

In the present study, results demonstrate that photochemical reactions of hydrocarbon, O_3 and NO_x (smog) can significantly depress ventricular pressure and contractility without altering lung function and inflammation. Though many studies have linked adverse cardiovascular health effects to particulate matter, our study indicates that it may be important to consider the adverse effects from gaseous mixtures as well. Photochemical reactions of hydrocarbons and NO_x generate many products (including many volatile and semi-volatile organic hydrocarbons), which may exert toxic effects on the cardiovascular system (Rager et al., 2011). Mice exposed to the gaseous portion of gasoline engine emissions and not the particulate matter fraction have demonstrated vascular remodeling (Lund et al., 2007). Monoxide gases and volatile hydrocarbon species appear to be drivers of vascular remodeling (McDonald et al., 2010). Campen et al. noted that combustion related gases, possibly alkanes and aldehydes, may have a greater role in driving adverse effects on coronaries than do particles (2006). These experimental studies along with our results suggest gaseous components of urban smog may hinder cardiac function.

No seed particles were injected into the irradiation chamber, indicating the cardiac depression was solely from photochemically altered-products of hydrocarbons, O_3 and NO_x. This is unique compared to many other findings which indicate particulate matter as the principle cause of cardiac dysfunction (R. D. Brook et al., 2010; Cho et al., 2009; Kyotani & Iwatsuki, 2002; Maté, Guaita, Pichiule, Linares, & Díaz, 2010; MohanKumar, Campbell, Block, & Veronesi, 2008; Peters, 2005; Peters et al., 2004; Polichetti, Cocco, Spinali, Trimarco, & Nunziata, 2009; Tong et al., 2009, 2010). Campen et al. (2005, 2006), Heyder and Takenaka (1996), and Lund et al. (2007, 2009) have all shown results, demonstrating a distinct role for the gaseous components of air pollution in cardiac suppression. Results presented here further demonstrate this cardiovascular effect and bolster the evidence for the role of gaseous components of air pollution in cardiac depression. These results illustrate the cardiac effects of multi-gaseous pollutants that have been photochemically reacted and formed secondary products (smog), which resemble events in the ambient environment.

Volatile organic compounds (VOCs), O_3 and NO_x are highly reactive and therefore can form new and potentially more toxic compounds when reacted, particularly when photochemical reactions from sunlight are considered (Kesselmeier & Staudt, 1999). Findings indicate a substantial biological role for the gaseous components of whole emissions (Campen et al., 2010). It has been documented that ozonation of VOCs can results in products that are more potent than the parent compound (Kozumbo & Agarwal, 1990; Pitts et al., 1980). In a recent study it was demonstrated that monoxide gases and VOCs appear to be likely drivers of some vascular remodeling during atherosclerosis (Campen et al., 2010). Furthermore the higher solubility of volatile gases

may indicate that they more readily access the circulation particles, demonstrating potential biological plausibility (Campen et al., 2005). Though particles have demonstrated an apparent toxic link to cardiovascular disease, environmental exposure to PM never occurs without concomitant exposure to numerous gaseous co-pollutants (Campen et al., 2010). Unfortunately, limited information about the role of gaseous co-pollutants is available (McDonald et al., 2010). The results presented here add to the growing information about the role of reactive gases and indicate a role for photochemically altered VOCs, NO_x and O_3 in depressing cardiac function through decreased ventricular pressure and contractility.

A reduction of contractility, but not heart rate could indicate mechanistic implications of cardiac suppression from gaseous air pollutants. Reduced contractility could indicate a dysfunction of the heart's calcium channels (Giordano, 2005). Some studies have found an association between air pollution, heart rate variability (HRV), and calcium channels (Chahine et al., 2007; Park, O'Neill, Vokonas, Sparrow, & Schwartz, 2004). Unfortunately, limited information on how air pollution affects the heart's calcium channels is available and additional studies are needed to clarify the potential role of calcium channels in reduced cardiac contractility from air pollution.

Remarkably, no change in respiratory function or lung inflammation was detected even though there was significant reduction in cardiac function. Many studies have focused on respiratory inflammation as a pathway for cardiovascular damage (R. D. Brook et al., 2010; Cho et al., 2009; Rager et al., 2011; Sexton et al., 2004). Other studies have demonstrated that respiratory inflammation is not necessarily a precursor to

cardiac injury, which the results presented from this study confirm (Campen et al., 2005, 2006; Lund et al., 2007; Tong et al., 2009, 2010).

Conclusion

Short-term inhalation of photochemical altered urban mixtures (VOCs, NO_x and O_3) without particles can acutely depress cardiac function through reduced ventricular pressure and contractility, without eliciting a respiratory response. This study indicates distinct roles of gaseous air pollutants in cardiovascular injury, possibly through alteration of the calcium channels. Additional studies are needed to clarify the underlying mechanism and extent in which complex photochemical mixtures can lead to cardiac injury.

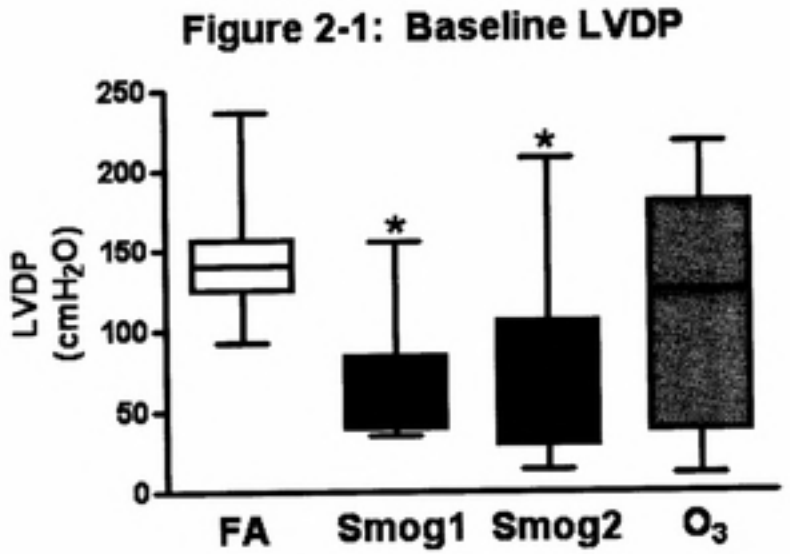


Figure 2-1: Baseline LVDP

The left ventricular developed pressure (LVDP) at baseline prior to ischemia 8 h after inhalation exposure to filtered air (FA), smog-1, smog-2, or ozone (O_3). Values are means ± SEM (n = 6-8 in each group). * $p < 0.05$ compared with FA control group.

Figure 2-2: Assessment of Contractility

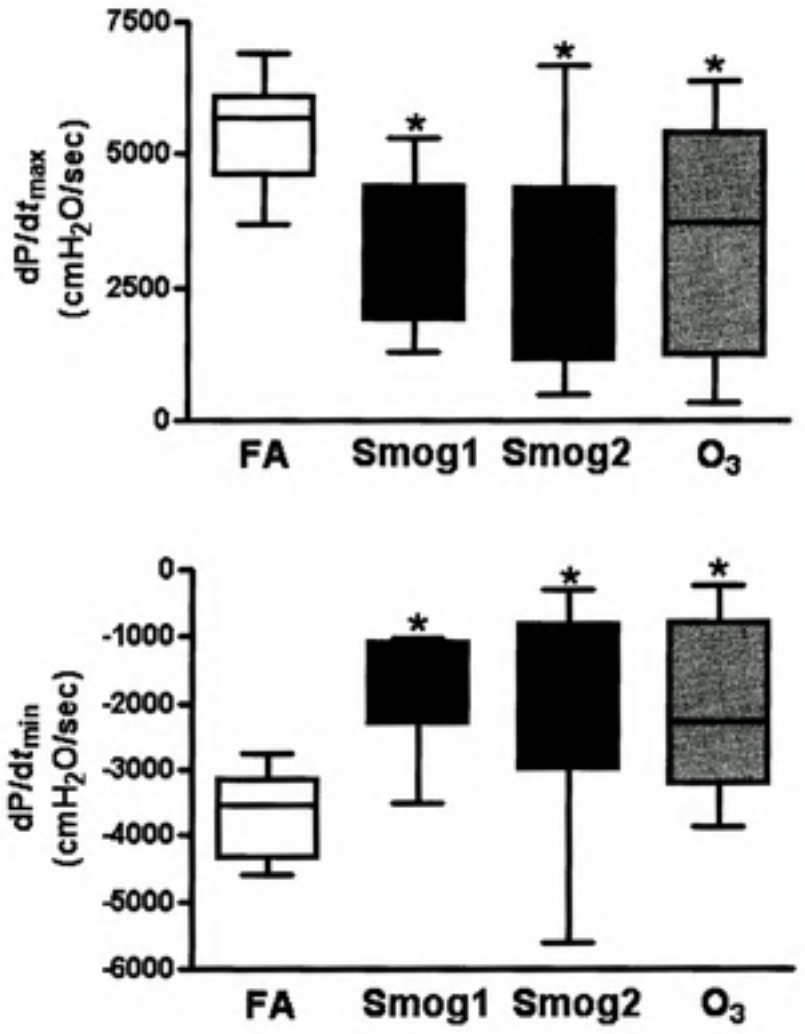


Figure 2-2: Assessment of Contractility

Assessment of contractility (dP/dt_{max}), upper panel, and lusitripsy (dP/dt_{min}) lower panel, baseline prior at baseline prior to ischemia 8 h after inhalation exposure to filtered air (FA), smog-1, smog-2, or ozone (O_3). Values are means ± SEM (n = 6-8 in each group).

* $p < 0.05$ compared with FA control group.

Figure 2-3: Time to Contracture

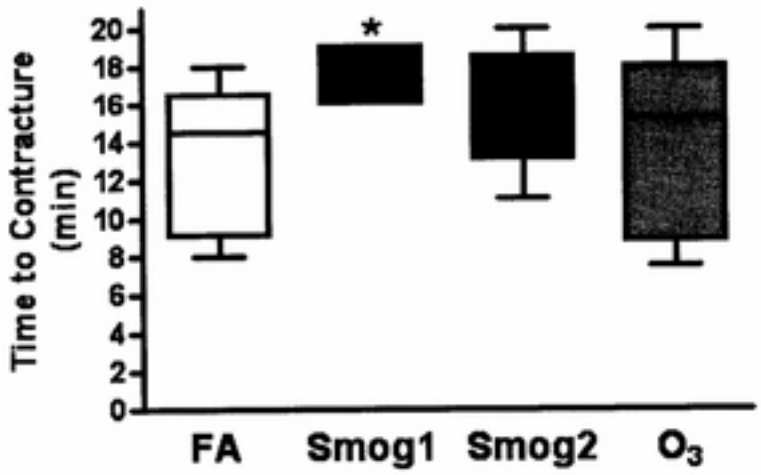


Figure 2-3: Time to Contracture

The time to contracture after 20 min of no-flow ischemia 8 h after inhalation exposure to filtered air (FA), smog-1, smog-2, or ozone (O_3). Values are means ± SEM (n = 6-8 in each group). * $p < 0.05$ compared with FA control group.

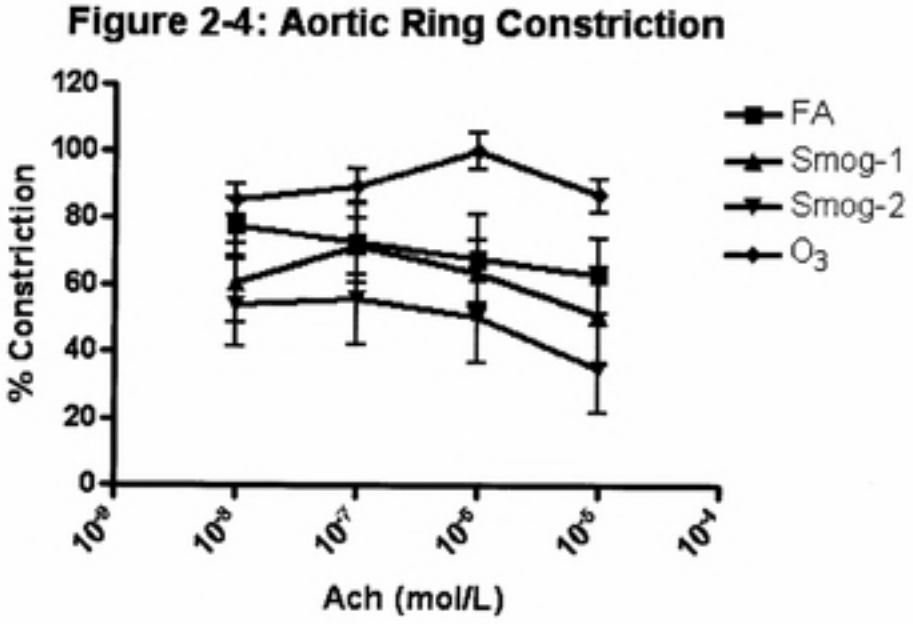


Figure 2-4: Aortic Ring Constriction

Aortic ring constriction 8 h after inhalation exposure to filtered air (FA), smog-1, smog-2, or ozone (O_3). Values are means ± SEM (n = 6-8 in each group). * $p < 0.05$ compared with FA control group.

Table 2-1: Chamber Experiments Exposure Composition

Species	Smog 1 (9/13/1201)	Smog 2 (9/29/2011)	Ozone (11/8/2011)	Filtered Air (9/22/2011)
Ozone (ppb)	243	257	245	10
NO (ppb)	0	0	0	1
NO_2 (ppb)	18	10	4	7
CO (ppm)	0.5	0.4	0.3	0.3
PAN (ppb)	1.3	7.4	<0.2	<0.2
PM1 ($\mu g/m^3$)	<5	<5	<5	<5
Formaldehyde (ppb)	64.2	38.8	12.2	12.2
Acetaldehyde (ppb)	22.1	20.2	0.0	0
Acetone (pppb)	11.1	11.4	0.0	0
Unknown cluster (ppb)	74.9	34.5	9.5	9.5
2-hexanone (ppb)	17.1	5.5	0.0	0
Glyoxal (ppb)	11.7	1.9	0.6	0.6
Methylglyoxal (ppb)	15.7	3.2	0.8	0.8
Estimated Total Carbonyls (ppb)	286.0	141.0	23.1	23.1
Organic Precursors (ppbC)				
Total, ppbC	2000	3000	<20	<20
Alkanes, 23 total	1133	1699		
Isopentane	173	259		
n-butane	147	220		
Propane	92	138		
Ethane	77	115		
Alkenes, 16 total	262	393		
Ethene	53	80		
2,3,3-trimethyl-1-butene	32	48		
c-2-pentene	27	40		
t-2-butene	23	35		
Aromatics, 13 total	605	908		
Toluene	138	207		
1,2,4-trimethylbenzene	112	168		
m-xylene	74	111		
Benzene	44	66		

Table 2-1: Chamber Experiments Exposure Composition

Chemical composition of smog chamber during exposure time point for Smog1, Smog2, Ozone and filtered air groups.

Table 2-2: Baseline hemodynamic properties and the onset time to ischemia contracture

Group	*n*	LVDP (mmHg)	HR (bpm)	Flow rate (mL/min)	dP/dt_{max} (cmH_2O/sec)	dP/dt_{min} (cmH_2O/sec)	Time to contracture (min)
FA	8	146.4±14.87	331.6±29.66	2.750±0.7783	5405±400.2	-3675±242	13.25±1.436
Smog1	7	69.22±16.03*	286.7±79.15	2.429±0.6209	2764±558.8*	-1822±335.5*	17.29±0.5216*
Smog 2	8	75.10±23.05*	271.2±97.85	3.800±1.653	2945±765.7*	-2070±625.3*	15.25±1.191
O_2	6	113.7±29.16	254.4±66.79*	1.533±0.2404	3447±847.8*	-2104±499.7*	14.00±1.839

Table 2-2: Baseline hemodynamic properties and the onset time to ischemia contracture

Values are means ± SE. LVDP=left ventricular developed pressure; HR=heart rate; flow rate=coronary flow rate; dP/dt_{max}=maximum 1st derivative of the change in left ventricular pressure/time; dP/dt_{min}=minimum 1st derivative of the change in left ventricular pressure/time; time to contracture=onset time to ischemic contracture. * $p < 0.05$ compared with FA control group.

Chapter 3: Ozone co-exposure with fine and ultrafine particulate matter alters cardiac response

Introduction

Epidemiological studies demonstrate that exposure to particulate matter (PM) increases risk for adverse clinical and vascular events (R. D. Brook et al., 2010). A wide range of both cardiac and vascular responses manifest in controlled human and animal PM exposure studies have provided biological plausibility to these PM-induce health effects (R. Brook et al. 2002; D. Dockery et al. 1993; Kodavanti et al. 2011; Peters et al. 2005). Some of these responses have been shown to be similar in humans and animals. For instance, some human subjects exposed to PM have decreased heart rate variability (HRV), a predictor of increased risk (Gold et al. 2000; Gong et al. 2004; Tsuji et al. 1994, 1996) and enhanced arrhythmogenesis (Whitsel et al., 2009). Experiments in animals not only demonstrate a similar PM-induced decrease in HRV and increased incidence of arrhythmia, but also more functional decrements in the heart such as diminished left ventricular developed pressure (LVDP), and decreased contractility (Chen & Hwang 2005; Gurgueira et al. 2002).

Epidemiological studies indicate that fine ($PM_{2.5}$) and ultrafine PM (UFP) are the principle instigators of adverse clinical cardiovascular events (Brook et al. 2010). However, today's air pollution is a mixture of not only PM, but also gaseous irritants,

vapors, and biological substances. Thus when examining the effects of any given pollutant, the influence of other components muses decreased HRV, systolic blood pressure and heart rate (HR) in rats (Wang, Jiang, Zhao, & Song, 2012), few studies have addressed the health effects of simultaneous exposures with distinct pollutants. As such, studies need to determine whether pollutants act synergistically or antagonistically with one another in a multi-pollutant mixture; this is particularly true of PM and ubiquitous gaseous co-pollutants like O_3.

Studies have investigated simultaneous PM and O_3 exposures but the combined effects are still not fully understood. For instance, Brook et al. demonstrated acute arterial vasoconstriction in healthy subjects co-exposed to $PM_{2.5}$ and O_3 (2002), whereas Urch et al. (2005) found no significant changes in mean arterial pressure, systolic blood pressure or HR in a similar study population; although constriction was observed with $PM_{2.5}$ alone (Urch et al., 2005). Animal studies also indicate that the effect of combining pollutants does not necessarily yield the expected synergistic response. For instance, Kodavanti et al. demonstrated an increase in thrombotic factors and hemeoxygenase-1, an indicator of oxidative stress, in the aortas of rats exposed to diesel exhaust particles (DEP) or O_3 alone, but not as co-pollutants (Kodavanti et al. 2011). The respiratory effects of O_3 and PM co-exposure are equally conflicting. For example, rats instilled with ozonized DEP had increased inflammatory cells and protein in the lungs (Madden et al. 2000). In contrast, Farraj et al. (Farraj, Boykin, Ledbetter, Andrews, & Gavett, 2010) showed that sensitized mice co-exposed to O_3 and DEP did not have increased cytotoxicity or inflammation; however they did demonstrate an increase in

bronchoconstriction, a functional measure of the lungs. Similar investigations into the effects of simultaneous exposure on cardiac function have not been conducted.

Thus, in this study, the Langendorff perfusion apparatus was used to further clarify the effects of simultaneous PM and O_3 exposure, and more specifically assess its impact on cardiac function. Only a handful of studies have assessed how air pollution alters the mechanical function (e.g. contractility) of the heart; however, given the Langendorff cardiac perfusion is a standard *ex vivo* technique used to investigate cardiac contractile strength and the clinical relevance of myocardial infarction from ischemia/reperfusion injury, it is a suitable approach to assess these acute effects of exposure. We hypothesized that (1) a single inhalation exposure to UFP alone would cause decrements in cardiac function after exposure and decrease recovery after ischemia-reperfusion injury; (2) exposure to fine PM or O_3 alone would cause similar decrements, however, the response would not be as great; and (3) co-exposure to O_3 would potentiate the PM-induced cardiac dysfunction.

Methods and Materials

Experimental Animals

Twelve to fourteen-week old female C57BL/6 mice (body weight = 20.2 ± 0.2 g) were used in the study. Upon arrival, animals were housed five per cage with food and water available ad libitum in an AAALC-approved facility; all animals were treated humanely and with regard to alleviation of suffering. All experimental protocols were conducted with the approval of the Environmental Protection Agency Institutional

Animal Care and Use Committee and in accordance with the National Institutes of Health Guides for the Care and Use of Laboratory Animals.

Experimental Groups

Mice were assigned to one of the following groups: (1) fine concentrated ambient particles (FCAPs); (2) ultrafine CAPs (UFCAPs); (3) ozone (O_3); (4) FCAPs and O_3 co-exposure (FCAPs + O_3); (5) UFCAPs and O_3 co-exposure (UFCAPs + O_3); and (6) filtered air (FA). Each group had n = 5-8.

Generation of Concentrated Ambient Particles

Particles between 2.5 μm and 0.1 μm in diameter were used for FCAPs and particles less than 0.1 μm in diameter were used for UFCAPs. Mice were placed into the chamber and allowed at least 30 minutes to acclimate before being exposed for four hours. Mice were then removed and placed in their cages for 20-24 hours before cardiac perfusion was preformed.

Cardiac Perfusion

The procedure for cardiac perfusion has been previously described (Tong et al. 2010). Briefly, 24 hours after exposure, mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.). Heparin (100 units) was injected intravenously through the vena cava before removal of heart. The hearts were rapidly removed and placed in ice-cold Krebs-Henseleit buffer, after which the aortas were cannulated. Retrograde

perfusion via the aorta was performed under constant pressure (100 cmH_2O) above the heart. The non-recirculating perfusate was a Krebs-Henseleit buffer containing (in mmol/L) 120 NaCl, 5.9 KCl, 1.2 $MgSO_4$, 1.75 $CaCl_2$, 25 $NaHCO_3$, and 11 glucose. The buffer was aerated with 95% O_2—5% CO_2 and maintained at pH 7.4 and a temperature of 37°C.

For assessment of contractile function, a latex balloon on the tip of a polyethylene catheter was inserted through the left atrium into the left ventricle. The catheter was connected to a pressure transducer (Argon Medical Devices, Athens, TX) at the same height as the heart. A PowerLab system was used to collect and process the left ventricular pressure data (AD Instruments, Milford, MA). All hearts were perfused for 25 min prior to the start of a 20 min-no-flow ischemia, followed by 1 h of reperfusion. Heart rate, cardiac contractile function indexes (left ventricular systolic and diastolic pressures), and their derivatives (dP/dt), which are the rates of rise/fall in left ventricular pressure, were measured continuously. Left ventricular developed pressure (LVDP) was measured as the difference in systolic and diastolic pressure in the left ventricle and ischemic time to contracture was measured as the time from the start of ischemia until initial contracture (at least 5 cmH_2O increase in left ventricle developed pressure). Recovery of LVDP, expressed as a percentage of the initial pre-ischemic LVDP, was measured at 20, 40 and 60 min of reperfusion after 20 min of ischemia.

Statistical Analysis

Data are expressed as means ± SEM. Comparisons between each group (FCAPs, UFCAPs, O_3, FCAPs + O_3 and UFCAPs + O_3) and the filtered air (FA) control group

were performed by one-way ANOVA followed by Tukey's post hoc test for multiple comparisons using Prism GraphPad (La Jolla, CA). The statistical significance levels were set at $p < 0.05$.

Results

Composition of Exposure Groups

Table 3-1 shows the concentration of O_3 and PM in each exposure group. The FA groups had an average concentration of 4.0 ppb of O_3 and 4.9 $\mu g/m^3$ of total PM. The O_3 groups had an average concentration of 299.0 ppb of O_3 and 3.4 $\mu g/m^3$ of total PM. The FCAPs groups had an average concentration of 33.1 ppb of O_3 and 190.9 $\mu g/m^3$ of FCAPs. The UFCAPs groups had an average concentration of 25.7 ppb of O_3 and 138.8 $\mu g/m^3$ of UFCAPs. The FCAPs+O_3 groups had an average concentration of 300.0 ppb of O_3 and 211.5 $\mu g/m^3$ of FCAPs. The UFCAPs+O_3 groups had an average concentration of 298.3 ppb of O_3 and 85.7 $\mu g/m^3$ of UFCAPs.

Cardiac Effects at Baseline

Post-exposure (baseline) hemodynamics and the onset time to ischemic contracture for each of the exposure groups are listed in Table 3-2. As shown in Figure 3-1, there was a significant decrease in LVDP in the FCAPs (31.9 ± 6.7 cmH_2O), O_3 (54.7 ± 12.6 cmH_2O) and UFCAPs + O_3 (45.0 ± 9.2 cmH_2O) groups compared to FA (96.7 ± 9.6 cmH_2O) 24 hours after exposure and before ischemia. Left ventricular contractility was also significantly depressed in the UFCAPs, FCAPs, O_3 and UFCAPs + O_3 groups

compared to the FA control group the maximum d*P*/d*t* was significantly lower in FCAPs (1397 ± 296 cmH_2O/sec), O_3 (2483 ± 480 cmH_2O/sec) and UFCAPs + O_3 (1975 ± 306 cmH_2O/sec) when compared to FA (3880 ± 208 cmH_2O/sec) (Figure 3-2). Figure 3-3 shows the minimum d*P*/d*t* before ischemia was also significantly lower in the UFCAPs (-1452 ± 395 cmH_2O/sec), FCAPs (-982 ± 259 cmH_2O/sec), O_3 (-1520 ± 318 cmH_2O/sec) and UFCAPs + O_3 (-1323 ± 286 cmH_2O/sec) groups when compared to FA (-2744 ± 317 cmH_2O/sec). There was no difference in HR, coronary flow rate or ischemic time to contracture between any exposure groups at before ischemia (Table 3-2).

Discussion

This study examined the effects of O_3 co-exposure on PM-induced cardiac mechanical dysfunction in mice. Our data demonstrate that a single inhalation exposure to FCAPs decreases ventricular mechanical performance in mice. A similar, but lesser, response was observed in mice exposed to O_3 alone. Contrary to our original hypothesis UFCAPs alone did not cause a significant decrement in ventricular function. However, introduction of O_3 as a co-pollutant with UFCAPs caused a decrease in cardiac contractility 24 hours after exposure, which was similar to the response observed in the FCAPs and O_3 alone groups. Surprisingly, O_3 significantly blunted the effects of FCAPs on cardiac contractility. These results were not entirely as hypothesized and clearly reflect the complexity of interactions between individual pollutants and the resultant short-term cardiac response.

Based on previous findings (Tong et al., 2010), UFCAPs was hypothesized to cause the greatest degree of cardiac dysfunction. Ultrafine black carbon particles have

been shown to translocate into the blood circulation and have the potential to cause direct effects on the cardiovascular system (Oberdorster et al., 2002). UFP cause heterogeneity of cardiac repolarization as well as decreased HRV in humans (Samet et al., 2009), whereas more functional assessments in animals reveal decreased LVDP, contractility and coronary flow (Bagate et al., 2006; Hwang et al. 2008; Simkhovich et al. 2007; Tong et al. 2010). One reason we did not observe any effects with UFCAPs may be related to the method of exposure. In this study, animals were exposed via whole-body inhalation as opposed to instillation (Bagate et al., 2006; Tong et al., 2010), direct perfusion (Hwang et al., 2008; Simkhovich et al., 2007), or nasal inhalation (Amatullah et al., 2012) of UFP as in other studies; this could have resulted in a lower effective dose (Osier and Oberdorster 1997; Costa et al. 2006). Other studies exposed animals to higher concentrations (10 mg/kg i.t. - Bagate et al., 2006; 100 μg particles i.t. - Cozzi et al. 2006; 27.8 μg particles infused directly into the heart - Simkhovich et al., 2007; 100 μg particles i.t. - Tong et al., 2010). As such, there was likely differential deposition of UFCAPs and FCAPs, which would have resulted in variable sensory neural activation, epithelial injury, inflammation and clearance (Johnson, 2004). This is particularly important to consider when addressing the timeline of response, its latency and transience from a single exposure. Even though the results were not significant, there was a tendency for LVDP, dP/dt_{max} and dP/dt_{min} to be lower in the mice exposed to UFCAPs compared to the FA control, suggesting a higher concentration of UFCAPs might have produced a more robust response.

Much of the current research is focused on PM as a cardiotoxicant, and even though O_3 has been traditionally viewed as a pulmonary toxicant, various studies have

also noted the adverse cardiovascular effects of O_3 inhalation; including decreased HR, alteration of cardiac repolarization, and increased inflammation (Farraj et al., 2012; Kodavanti et al., 2011; Watkinson et al.2001). In addition, Tankersley et al. (2012) showed that an increase in total peripheral resistance (hypertension) and right ventricular pressure after O_3 contributed to reduced cardiac output, which the authors suggested occurred by reduced end-systolic and end–diastolic left ventricular volumes. This was in contrast to the effects of carbon black particles, which caused similar losses of cardiac function but with an increased end-diastolic left ventricular volume. Thus, the type of functional cardiac responses following air pollution may be dependent on the type of pollutant. We speculate that both pollutant-types, particulates and gases, produce similar cardiac decrement. Yet, the mechanisms mediating the response may not be the same. For instance, recent studies have demonstrated that O_3 activates transient receptor potential A1 (TRPA1) channels (Taylor-Clark & Undem, 2010) on airway sensory nerves; hence O_3 might cause airway irritation and thereby altered autonomic balance, which could acutely change cardiac function. Fine PM has been shown to cause similar shifts in autonomic imbalance but through elicitation of cardiac oxidative stress, although this response is probably not exclusive to PM (Ghelfi, Wellenius, Lawrence, Millet, & Gonzalez-Flecha, 2010; Rhoden, Wellenius, Ghelfi, Lawrence, & González-Flecha, 2005). In fact, Wang et al. (2012) recently showed that exposure to PM caused a significant decrease in superoxide dismutase (SOD) activity and an increase in malondialdehyde (MDA), both of which indicate cardiac oxidative stress, whereas O_3 alone did not elicit any change.

The combined effects of PM and O_3 are less easily explained. PM and O_3 together can cause acute arterial vasoconstriction in healthy humans subjects (Brook et al. 2002 and 2009), and although flow-mediated dilatation (FMD) was decreased after PM and O_3 co-exposure (Brook 2009), FMD was also decreased with PM alone suggesting it may be the main driver of the response. Because certain responses were observed in one city (Ann Arbor) but not in another (Toronto) the type of PM and the different sources contributing to the chemical physical characteristics of the PM might account for such differences in response. In this study, we noted a disparity in the response of mice to PM of different sizes, particularly when O_3 was included in the exposure. Depressed cardiac function occurred in mice exposed to UFCAPs + O_3, but not FCAPs + O_3; suggesting co-exposure resulted in increased toxicity for one mixture and reduced toxicity for the other. The disparate effect may be due to a number of factors, although differences in the chemical composition of the PM and chemical interactions between the PM and O_3 might be the most relevant.

The responses to FCAPs and UFCAPs combined with O_3 can be partially explained by the chemical changes occurring in PM upon ozonization. Ozone is highly reactive and therefore it has the potential to react with certain components of PM such as the aromatic compounds (Madden et al., 2000). It has been documented that ozonization of aromatic substances can result in the formation of carbonyls, carboxylic acids, quinones, and epoxides, which can be more toxic than the parent compound (Kozumbo & Agarwal 1990; Pitts et al. 1980). Therefore, the ozonization of UFCAPs in this study could have increased the biological potency of UFCAPs, which may have led to decreased cardiac function. In contrast, the "over-ozonization" of aromatic substances has

been shown to ultimately decrease the biological potency of the reaction products relative to the products formed by less O_3 exposure (Madden et al., 1993). It also appears that the organic content may determine whether O_3 potentiates or reduces the bioactivity of the PM (Madden et al. 2000). Whether this is the driving mechanism or not remains to be determined, however, it may partially explain why the FCAPs + O_3 group had no effect. Additionally, O_3 may cause epithelial injury facilitating PM effects (Elder, Gelein, Finkelstein, Cox, & Oberdorster, 2000). Adamson et al. (1999) showed that O_3 and urban particulate co-exposure resulted in greater epithelial injury and interstitial inflammation than for either component alone; not to mention UFP did not have a large biological effect without O_3. As such, co-exposures may produce differential responses due to toxicological interactions within the host. For instance, Thomson et al. (2005) showed that on their own, PM and O_3 increased expression of the potent vasoconstrictor endothelin-1 (ET-1) in the lungs and its circulating levels in the plasma, however, together they only caused an upregulation (i.e. without plasma "spill-over"). Thomson et al. (2005) suggest increased oxidative stress due to co-exposure may have overwhelmed translation of ET-1; this is probably not the case for our study given we used significantly lower pollutant concentrations.

Though we saw a decrease in cardiac function in the FCAPs, O_3 and UFCAPs + O_3 groups, we did not see any change in lung function or lung inflammation markers for any of the exposure groups (data not shown). Similar lung function results have been seen in other studies (Cho et al. 2009; Kodavanti et al. 2011; Tong et al. 2009, 2010). These results indicate that cardiac decrements can occur without any apparent lung injury. Previous studies have noted that lung function decrements tend to happen prior to

the 24h post-exposure mark, indicating a delayed response in cardiac function compared to respiratory function and not a lack of respiratory response (D. W. Dockery & Pope, 1994; Easton & Murphy, 1967; M. J. Hazucha, 1987; M. Hazucha & Sant' Ambrogio, 1993; Pope, 2000). Another explanation is that cardiac dysfunction attendant to toxicant inhalation occurs due to subtle underlying changes which do not manifest as overt toxicity (e.g. autonomic dysfunction, impaired stress responsiveness).

Conclusion

The findings of this study indicate that inhalation of PM, even at relatively low concentrations, causes acute decrements in cardiac function as measured by decreases in LVDP and contractility. These decrements indicate that the heart undergoes mechanical "deficiencies" post-exposure, which might impair its function as a pump. Although these acute responses are likely transient and quickly reversible, the results presented here are demonstrative of the potentially deleterious effects of even a single exposure to air pollution. These effects may be of particular significance in those individuals who might already have underlying cardiovascular disease. Although many of these responses are elicited by PM, these results indicate that the effect of co-exposure to a second air pollutant is not simply additive or synergistic, nor is it generalizable. When assessed based on the findings of our results and those of other researchers, the data suggest that chemical reactivity might play a major role in determining not only the degree of toxicity, but also the target organ. In these experiments, the mechanism causing the depressed cardiac function is unclear and warrants further investigation, but the suppressed

contractility would lead one to consider the function of the calcium channels (Giordano, 2005); clearly further investigation is required.

Figures and Tables

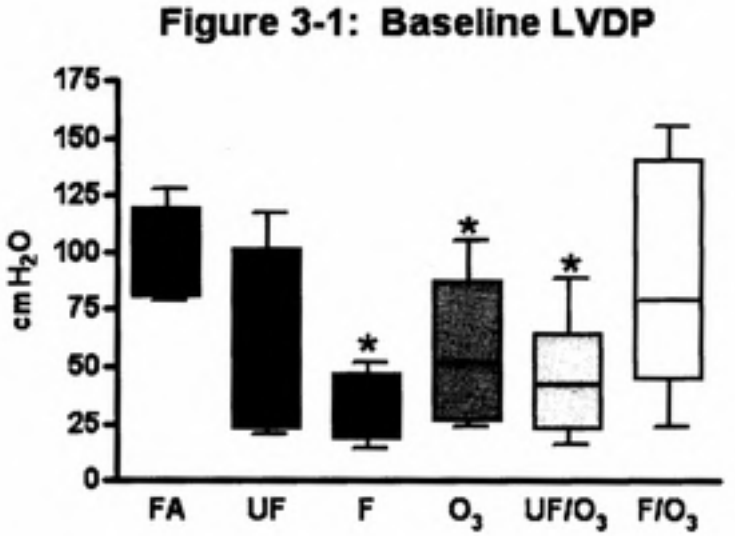


Figure 3-1: Baseline LVDP

The left ventricular developed pressure (LVDP) at baseline prior to ischemia 20-24 h after inhalation exposure to filtered air (FA, Ultrafine CAPs (UF), Fine CAPs (F), ozone (O_3), Ultrafine CAPs+O_3 (UF/O_3), or Fine CAPs+O_3 (F/O_3). Values are means ± SEM (n = 5-8 in each group). * $p < 0.05$ compared with FA control group.

Figure 3-2: Assessment of Contractility

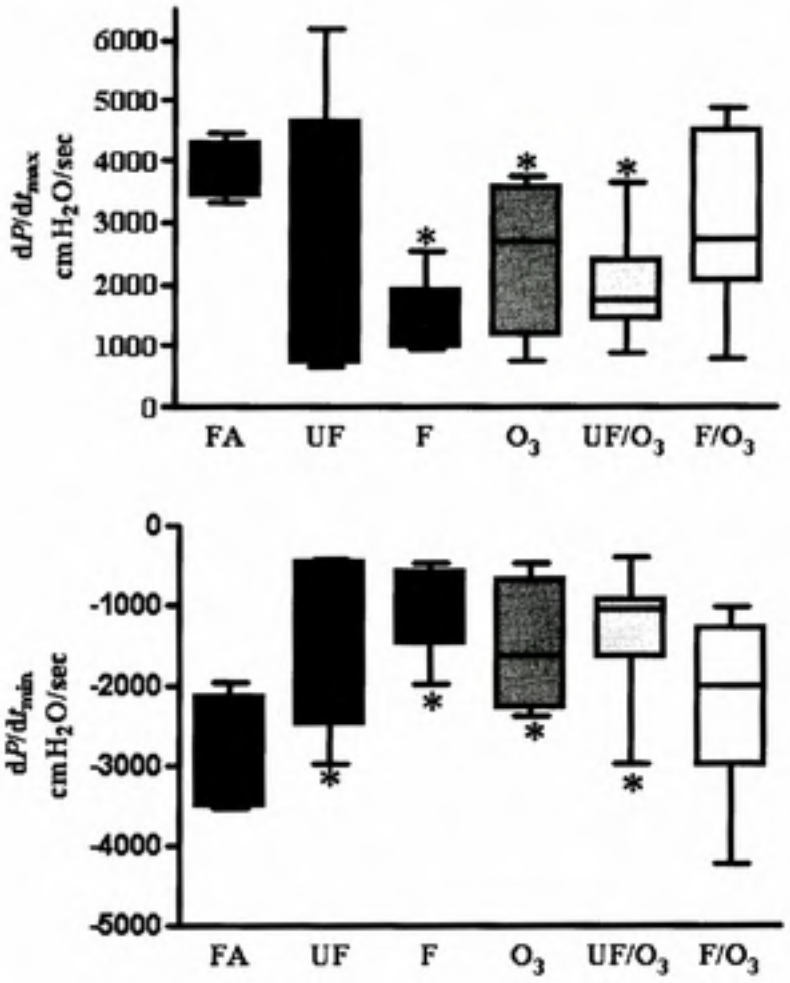


Figure 3-2: Assessment of Contractility

Assessment of contractility (dP/dt_{max}), upper panel, and lusitripsy (dP/dt_{min}) lower panel, baseline prior at baseline prior to ischemia 20-24 h after inhalation exposure to filtered air (FA), Ultrafine CAPs (UF), Fine CAPs (F), ozone (O_3), Ultrafine CAPs+O_3 (UF/O_3), or Fine CAPs+O_3 (F/O_3). Values are means ± SEM (n = 5-8 in each group). * $p < 0.05$ compared with FA control group.

Table 3-1: Composition of Exposure Groups

	Filtered Air	O_3	FCAPs	FCAPs+O_3	UFCAPs	UFCAPs+O_3
Duration (hrs)	4	4	4	4	4	4
O_3 Concentration (ppb)	4.0	299.0	33.1	300.0	25.7	298.3
PM Mass Concentration ($\mu g/m^3$)	4.9	3.4	190.9	211.5	138.8	85.7
PM Total # Concentration (pt/cc)	24.2	20.2	10100.0	11200.0	213000.0	161000.0
PM Median (nm)	122.0	136.0	246.0	235.0	76.0	72.0
PM Mean (nm)	142.0	163.0	242.0	239.0	88.0	83.0
Geometric Mean (nm)	-	125.0	202.0	197.0	77.0	73.0
Mode (nm)	120.0	104.0	305.0	305.0	72.0	67.0
Geometric Standard Deviation	-	2.2	2.0	2.0	1.7	1.7
Chamber Temperature	72.2	72.5	71.6	71.9	73.4	72.7
Chamber Relative Humidity	50.2	52.4	59.8	59.0	70.5	56.0

Table 3-1: Composition of Exposure Groups

Reported statistics are an average of each group's exposure condition for all exposures. Each exposure group consists of multiple animals with exposures performed over multiple days.

Table 3-2: Baseline hemodynamic properties and the onset time to ischemia contracture

Group	*n*	LVDP (cmH_2O)	HR (bpm)	Flow rate (mL/min)	dP/dt_{max} (cmH_2O/sec)	dP/dt_{min} (cmH_2O/sec)	Time to contracture (min)
FA	5	96.7 ± 9.6	306.6 ± 17.5	1.7 ± 0.3	3880 ± 208	-2744 ± 317	14.3 ± 1.9
UFCAPs	6	59.7 ± 15.4	270.3 ± 19.6	1.7 ± 0.5	2564 ± 825	-1452 ± 395*	14.6 ± 1.8
FCAPs	5	31.9 ± 6.7*	301.3 ± 27.6	5.7 ± 2.9	1397 ± 296*	-981 ± 259*	11.1 ± 0.5
O_3	6	54.7 ± 12.6*	246.3 ± 36.4	2.5 ± 0.6	2483 ± 480*	-1520 ± 318*	15.3 ± 1.6
UFCAPs+O_3	8	45.0 ± 9.3*	295.1 ± 18.5	2.0 ± 0.5	1975 ± 306*	-1323 ± 286*	15.1 ± 1.6
FCAPs+O_3	8	88.0 ± 18.4	277.6 ± 15.7	1.7 ± 0.2	3034 ± 528	-2219 ± 394	12.9 ± 1.3

Table 3-2: Baseline hemodynamic properties and the onset time to ischemia contracture

Values are means ± SEM. Flow rate = coronary flow rate; dP/dt_{max} = maximum 1st derivative of the change in left ventricular pressure/time; dP/dt_{min} = minimum 1st derivative of the change in left ventricular pressure/time; time to contracture = onset time to ischemic contracture. * $p < 0.05$ compared with FA control group.

Chapter 4: Discussion and Conclusion

Discussion

The aim of these studies was to examine the cardiac health effects of multi-pollutant mixtures at concentrations observed in the ambient environment. As hypothesized both multi-pollutant studies decreased cardiac function through ventricular pressure and contractility. Photochemically-altered smog decreased LVDP and contractility compared to the filtered air control. Fine PM and ozone as co-pollutants decreased LVDP and contractility, but contrary to the hypothesis ultrafine PM and ozone as co-pollutants did not alter cardiac function compared to the filtered air control. In addition, ultrafine and ozone as individual pollutants both caused decrements in ventricular function. These results indicate the relationship of multi-pollutants is a complex interaction, which cannot be simply defined as antagonistic or synergistic.

Photochemically-altered VOCs, NO_x and O_3 are highly reactive in the environment and have been shown to cause adverse cardiovascular effects. Human respiratory cells exposed to photochemically-altered VOCs, NO_x and O_3 showed increased inflammation and cell death (Sexton et al., 2004). Furthermore a toxicogenetic analysis of human respiratory cells exposed to photochemically-altered smog (VOCs, NO_x and O_3) also indicated photochemically-altered products may induce toxic effects on the cardiovascular system (Rager et al., 2011). Campen et al. demonstrated that

monoxide gases and VOCs may be a key driver of some vascular remodeling during atherosclerosis (Campen et al., 2010). The high solubility of these volatile gases may indicate that they can more readily access the circulation, demonstrating a potential biological plausibility (Campen et al., 2005). The results presented in this report and in corroboration with previous studies indicate that photochemically-altered gaseous components in the atmosphere may play a role in the adverse cardiovascular health effects from air pollution through decreasing ventricular pressure and contractility.

Particulate matter has been clearly linked to cardiovascular disease through epidemiological, clinical and animal studies, however PM is not found alone in the atmosphere (R. D. Brook et al., 2010). Therefore it is important to consider the role of other pollutants along with PM. Ozone is a highly reactive gaseous component of the atmosphere, whose adverse cardiopulmonary health effects have been fairly well categorized (Farraj et al., 2012; M. J. Hazucha, 1987). There is not a clear consensus from previous studies regarding the cardiovascular health effects of PM and O_3 exposure. For instance, Brook et al. demonstrated acute arterial vasoconstriction in healthy subjects co-exposed to $PM_{2.5}$ and O_3 (2002), whereas Urch et al. (2005) found no significant changes in mean arterial pressure, systolic blood pressure or HR in a similar study population; although constriction was observed with $PM_{2.5}$ alone (Urch et al., 2005). In this report $PM_{2.5}$ and O_3 as co-pollutants did not alter cardiac function even though as individual pollutants they each depressed cardiac function. Furthermore UFP, which studies have indicated as the fractionation most closely linked with cardiovascular morbidity and mortality, did not cause changes in cardiac function when exposed alone, but did when co-exposed with O_3 (R. D. Brook et al., 2010). The different responses

from $PM_{2.5}$. UFP with and without O_3 could be due to the differing chemical composition, deposition and mechanistic pathway. These results indicate there is a complex relationship between PM and O_3 during exposure that cannot simply be defined as antagonistic or synergistic, and warrant further investigation.

In future studies, it would be useful to investigate the cardiovascular health effects of the VOCs, NO_x and O_3 mixture with PM. This would give a more accurate picture of ambient pollutant mixtures and may give key insights to the health effects seen in epidemiological studies. It would also be useful to further investigate the different pathways leading to the observed adverse cardiovascular health effects. Since both study present here showed decrease ventricular contractility it would be valuable to examine the role calcium channels play in these adverse effects. Finally since epidemiological studies indicate individuals with cardiovascular disease may be more susceptible to air pollution, one may consider utilizing more susceptible animal models with these mixture to assess the role of susceptibility.

Limitations

Though this report provides good insights for the field it does have its limitations. The use of the Langendorff cardiac perfusion system is valuable in defining the mechanical function of the heart, but because it is an isolated organ system it does not consider the body system as a whole. The irradiation chamber though very useful has its limitations. Foremost it is dependent on weather cloud patterns, which replicates the ambient environment, but also can be difficult to repeat. The concentrated ambient particle generator does produce intact particles, but it can be difficult to obtain a specified

concentration. Though these limitations should be considered, they do not negate the usefulness of these systems.

Conclusion

In conclusion, the findings of this report indicate that the inhalation of complex mixtures of air pollutants at ambient levels causes acute decrements in cardiac function as measured by decreases in LVDP and contractility. These decrements indicate the heart may undergo mechanical "deficiencies" after exposure, which might impair its function as a pump. Although these acute responses are likely transient and quickly reversible, these results demonstrate the potentially deleterious effects of a single exposure to air pollution. This is of particular interest when one considers a more susceptible population. The two complex mixture presented in this report indicate that adverse cardiovascular health effects observed in epidemiology studies are due to complex mixtures and not a single pollutant, clearly demonstrating a need to move from a single-pollutant to a multi-pollutant approach.

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