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$PM_{2.5}$ exposure in utero contributes to neonatal cardiac dysfunction in mice☆

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ABSTRACT

Objective: Exposure of fine particulate matter ($PM_{2.5}$) to pregnant dams has been shown to be strongly associated with adverse cardiovascular outcomes in offspring at adulthood, however, effects evident during neonatal periods are unclear. We designed this study to examine cardiac function of neonatal mice (14 days old) exposed to in utero $PM_{2.5}$.

Methods: Pregnant FVB female mice were exposed either to filtered air (FA) or $PM_{2.5}$ at an average concentration of 91.78 $\mu g/m^3$ for 6 h/day, 5 days/wk (similar to exposure in a large industrial area) throughout the gestation period (21 days). After birth, animals were analyzed at day 14 of life.

Results: Fourteen day old mice exposed to $PM_{2.5}$ during the in utero period demonstrated decreased fractional shortening (%FS, 41.1 ± 1.2% FA, 33.7 ± 1.2% $PM_{2.5}$, $p < 0.01$) and LVEDd (2.87 ± 0.08 mm FA, 2.58 ± 0.07 mm $PM_{2.5}$, $p < 0.05$) compared to FA exposed mice. Contractile kinetics and calcium transients in isolated cardiomyocytes from $PM_{2.5}$ exposed mice illustrated reduced peak shortening (%PS, 16.7 ± 0.5% FA, 14.7 ± 0.4% $PM_{2.5}$, $p < 0.01$), negative contractile velocity (-dL/dT, −6.91 ± 0.3 μm/s FA, −5.46 ± 0.2 μm/s $PM_{2.5}$, $p < 0.001$), increased time to relaxation 90% (TR90, 0.07 ± 0.003 s FA, 0.08 ± 0.004 s $PM_{2.5}$, $p < 0.05$), decreased calcium transient amplitude (Δ340/380, 33.8 ± 3.4 FA, 29.5 ± 2.8 p.m.$_{2.5}$) and slower fluorescence decay rate (τ, 0.72 ± 0.1 s FA, 1.16 ± 0.15 s $PM_{2.5}$, $p < 0.05$). Immunoblotting studies demonstrated alterations in expression of Ca^{2+} handling proteins- SERCA-2A, p-PLN, NCX and $Ca_V1.2$ in hearts of 14 day old in utero $PM_{2.5}$ exposed mice compared to FA exposed hearts.

Conclusion: $PM_{2.5}$ exposure during the critical in utero period adversely affects the developing mouse fetus leading to functional cardiac changes that were evident during the very early (14 days) stages of adolescence. These data demonstrated that exposure to $PM_{2.5}$ during the gestation period significantly impacts cardiovascular outcomes early in life.

1. Introduction

It has been estimated that environmental factors account for significant risks of developing cardiovascular diseases (CVDs) (O'Toole et al., 2008). Ambient particulate matter (PM) pollution has gained significant attention as a global health concern in recent years after being ranked as the 9th cause of overall disease burden (Lim et al., 2012). In particular, $PM_{2.5}$ (ambient particles with diameters of <2.5 μm, $PM_{2.5}$) exposure contributes to significant CVD burden (Brook et al., 2010). $PM_{2.5}$ exposure is associated with CVDs, including arrhythmias, hypertension, myocardial infarction and remodeling (Rich et al., 2005; Wold et al., 2012).

Since $PM_{2.5}$ inhalation adversely impacts cardiovascular function, it is becoming increasingly crucial to gain understanding of the effects of $PM_{2.5}$ on the developing fetus. Significant evidence has accumulated concerning the adverse pregnancy outcomes and infant health following air pollution exposure (Fleischer et al., 2014; Fleisch et al., 2015). An understanding of developmental plasticity

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provides a conceptual basis for these observations (Bateson et al., 2004). There is also substantial evidence suggesting a positive correlation between particulate air pollution exposure and adverse cardiovascular outcomes during the postnatal periods (Gorr et al., 2014; Breton et al., 2016). In another study, cardiomyocytes cultured in media from PM-treated lung epithelial cells demonstrated contractile dysfunction (Gorr et al., 2015). The multitude of $PM_{2.5}$ effects (both direct and indirect) on the cardiovascular system are also well reviewed by others (Du et al., 2016; Nelin et al., 2012) These studies are suggestive that $PM_{2.5}$ exposure is associated with critical biological and cardio-developmental mechanisms leading to harmful effects later in life. However, this is the first study demonstrating the potential association between in utero exposure to ambient air pollution and neonatal cardiac function.

In our previous work, we demonstrated the role of $PM_{2.5}$ exposure during developmental periods in influencing an adult disease phenotype (Tanwar et al., 2017), however, it did not determine whether $PM_{2.5}$ exposure during the in utero period is equally detrimental to neonatal cardiac function. Here, we hypothesized that $PM_{2.5}$ in utero exposure can influence cardiac function in neonatal mice (14 day old). The purpose of the present study was to examine the impact of in utero $PM_{2.5}$ exposure on cardiac function in neonatal mice and to further study potential cellular and molecular mechanisms mediating these harmful cardiac effects.

2. Materials and methods

2.1. Animals and $PM_{2.5}$ exposure

All animal experiments were approved and performed according to NIH guidelines under an Institutional Animal Care and Use Committee (IACUC) protocol (IACUC#: 2013A00000074) at The Ohio State University, Columbus, Ohio. Male and female mice of the FVB strain were housed for 1 week in our facility before breeding and the presence of a vaginal plug was considered as confirmation of pregnancy. Once pregnancy was confirmed, we exposed pregnant dams to either $PM_{2.5}$ or FA for 6 h/day, 5 days/week throughout pregnancy (21 days). The aerosol concentration system located at the Ohio State University (Wold et al., 2012) was used for the concentrated $PM_{2.5}$ exposure from the Columbus, OH region. The average $PM_{2.5}$ concentration that the dams were exposed to was 91.78 $\mu g/m^3$. Since the exposures were performed for 6 h/day, 5 days/week, the 24-h average $PM_{2.5}$ concentration was 22.94 $\mu g/m^3$, which was below the daily national ambient air quality standard (NAQS) of 35 $\mu g/m^3$ set by the current U.S. Environmental Protection Agency (U.S. EPA, 2012). An identical system was used for the FA exposure except that a HEPA filter at the inlet to the system was used to remove all ambient particles. After birth, both groups of pups were placed in room air until day 14. At 2 weeks of age, all experiments were conducted on male mice as described below.

2.2. Echocardiographic assessment

At 14 days of age, in vivo cardiac function was determined (40 MHz transducer, Vevo 2100; Visualsonics *In Vivo* Imaging System (Toronto, ON, Canada)). Internal body temperature was maintained at 37 °C under 1.5–2% isoflurane (delivered in 100% O_2) anesthesia supplied through a nose cone. Following induction of anesthesia, the chest fur was removed using a hair removal cream. Ultrasound gel (prewarmed) was placed on the chest and a 15 MHz (optimized for mice) probe placed in the parasternal, short axis orientation. Three cine loops of M mode data were captured per mouse and analyzed for LV end-systolic and end-diastolic internal dimensions (LVESd and LVEDd), as well as systolic and diastolic posterior wall thickness (PWTs and PWTd). Percent fractional shortening (%FS) was calculated using the equation: %FS = [(LVED*d* - LVES*d*)/LVED*d* * 100]. LV end diastolic volume (LVEDV) was calculated from LVEDd (7/(2.4 + LVEDd)*LVEDd (Brook et al., 2010)). LV end systolic volume (LVESV) was calculated from LVESd (7/(2.4 + LVESd)*LVESd (Brook et al., 2010)) and ejection fraction (EF= (LVEDV-LVESV)/LVEDV*100). Stroke volume was calculated by subtracting LVESV from LVEDV (LVEDV-LVESV). Cardiac output was calculated from stroke volume multiplied by heart rate (SV*HR). An average of three cardiac cycles per mouse was used for data interpretation. Analyses of all the parameters were performed according to the AHA technique by an investigator blinded to group assignment.

2.3. In vitro assessment of cardiomyocyte function

At 14 days of age, mice were euthanized and hearts were harvested for myocyte isolation using enzymatic digestion (Liberase; Roche, Indianapolis, IN) through coronary retrograde perfusion via the aorta using a standard protocol and contractile kinetics were assessed using a Myopacer Field-Stimulator system (IonOptix, Milton, MA) as described previously (Wold et al., 2012). The following functional indices were determined: basal sarcomere length, % peak shortening normalized to sarcomere length (%PS; in vitro equivalent of %FS), time-to-90% PS (TPS) and time-to-90% relengthening (TR90), maximal velocities of shortening (+dL/dt) and relengthening (-dL/dt).

2.4. Measurement of intracellular Ca^{2+} transients

Ca^{2+} transients were measured to identify alterations in Ca^{2+} cycling using the Ionoptix system as listed in our previous publication (Wold et al., 2012). Fura-2 fluorescence was used to monitor basal Ca^{2+}, the amplitude of the Ca^{2+} transient (Δ340/380), time to peak transient, and the maximum rate of rise and decay of the Ca^{2+} transient. Fluorescence decay rate (tau) was used as an indication of the intracellular Ca^{2+} clearing rate.

2.5. Quantitative real-time PCR

Total RNA was isolated from snap frozen heart tissue of 14 day old neonates using the RNeasy kit (Qiagen, Hilden, Germany) and concentration was determined using a NanoDrop 2000c (ThermoScientific, Wilmington, DE). 1 ng of RNA was reverse transcribed to generate cDNA using the iScript Supermix kit (Bio-Rad, Hercules, CA) on a CFX96 Thermocycler (BioRad, Hercules, CA). Primers were used at a final concentration of 0.25–0.5 μM for target genes and normalized to *GAPDH* expression. Relative gene expression levels were quantified using the formula $2^{-\Delta\Delta Ct}$ (Livak and Schmittgen, 2001). Gene-specific primer sequences are presented in Table 1. The following three step amplification protocol was used for amplification: denaturation step at 95 °C for 10 min followed by 45

Table 1
Primer sequences used for PCR amplification.

Gene	Forward primer	Reverse primer
Gapdh	CTCACTCAAGATTGTCAGCAATG	GAGGGAGATGCTCAGTGTTGG
IL-1β	CCAAGAGGTGAGTGCTTCCC	CTGTTGTTCAGACTCTCTCCCT
IL-6	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG
CD62P	CATCTGGTTCAGTGCTTTGATCT	ACCCGTGAGTTATTCCATGAGT
CD62E	ATGAAGCCAGTGCATACTGTC	CGGTGAATGTTTCAGATTGGAGT
COL-1	GCTAACGTGGTTCGTGACCGTG	GGTCAGCTGGATAGCGACATC
MMP9	CAGGAGTCTGGATAAGTTGGGTC	GGTACTGGAAGATGTCGTGTGAG
MMP13	GACAGATTCTTCTGGCGCCT	CATAACTCCACACGTGGTTCTCAG