Environmental Pollution 231 (2017) 812–820

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Alterations in cardiovascular function by particulate matter in rats using a crossover design☆

Hsiao-Chi Chuang [a, b, c], Yin-Jyun Lin [d], Charles C.K. Chou [e], Jing-Shiang Hwang [f], Chu-Chih Chen [g], Yuan-Horng Yan [d, h], Hui-I. Hsieh [i], Kai-Jen Chuang [j, k], Tsun-Jen Cheng [d, l, *]

[a] School of Respiratory Therapy, College of Medicine, Taipei Medical University, Taipei, Taiwan
[b] Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Division of Pulmonary Medicine, Department of Internal Medicine, Shuang Ho Hospital, Taipei Medical University, New Taipei City, Taiwan
[d] Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, Taipei, Taiwan
[e] Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan
[f] Institute of Statistical Science, Academia Sinica, Taipei, Taiwan
[g] Division of Biostatistics and Bioinformatics, Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan
[h] Department of Medical Research, Kuang Tien General Hospital, Taichung, Taiwan
[i] Department of Family Medicine, Cathay General Hospital, New Taipei City, Taiwan
[j] Department of Public Health, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[k] School of Public Health, College of Public Health, Taipei Medical University, Taipei, Taiwan
[l] Department of Public Health, College of Public Health, National Taiwan University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 5 April 2017
Received in revised form
21 August 2017
Accepted 21 August 2017
Available online 31 August 2017

Keywords:
Air pollution
Black carbon
Blood pressure
Heart rate
Heart rate viability
$PM_{2.5}$

ABSTRACT

The objective of this study was to investigate associations between cardiovascular effects and urban ambient particle constituents using an *in vivo* crossover experimental design. Ambient particles were introduced to an exposure chamber for whole-body exposure of WKY rats, where the particulate matter with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$) mass concentration, particle number concentration, and black carbon (BC) were monitored. Organic carbon (OC), elemental carbon (EC), and soluble ions of $PM_{2.5}$ were determined. In a crossover design, rats were exposed to ambient particles or high-efficiency particle arrestance (HEPA)-filtered control air for 7 days following a 7-day washout interval. The crossover exposure between particles and HEPA-filtered air was repeated 4 times. Radiotelemetric data on blood pressure (BP) [systolic BP (SBP), diastolic BP (DBP), pulse pressure (PP), and mean arterial pressure (MAP)], heart rate (HR), and heart rate viability (HRV) were subsequently obtained during the entire study. Exposure to the $PM_{2.5}$ mass concentration was associated with decreases in the SBP, DBP, MAP, and HR ($p < 0.05$), whereas no significant changes in the BP or HR occurred with the particle number or black carbon. For HRV, the ln 5-min standard deviation of the normal-to-normal (NN) interval (LnSDNN) and the ln root mean square of successive differences in adjacent NN intervals (LnRMSSD) were positively associated with the $PM_{2.5}$ mass concentration ($p < 0.05$). There were no significant effects of the particle number concentration or BC on HRV. Alterations in the HR were associated with OC, EC, Na^+, Cl^-, and NO_3^-. Cl^- was associated with the DBP, MAP, HR, SDNN, and RMSSD. NO_3^- was correlated with the SBP, MAP, HR, SDNN, and RMSSD. In conclusion, we observed cardiovascular responses to ambient particles *in vivo* using a crossover design which can reduce animal use in future environmental studies.

☆ This paper has been recommended for acceptance by David Carpenter.

* Corresponding author. Institute of Occupational Medicine and Industrial Hygiene, College of Public Health, National Taiwan University, 17 Xu-Zhou Road, Taipei 100, Taiwan.

E-mail addresses: r92841005@ntu.edu.tw (H.-C. Chuang), yinjyun@gmail.com (Y.-J. Lin), ckchou@rcec.sinica.edu.tw (C.C.K. Chou), jshwang@stat.sinica.edu.tw (J.-S. Hwang), ccchen@nhri.org.tw (C.-C. Chen), d97841006@ntu.edu.tw (Y.-H. Yan), shirleyhsieh.hhi@gmail.com (H.-I. Hsieh), kjc@tmu.edu.tw (K.-J. Chuang), tcheng@ntu.edu.tw (T.-J. Cheng).

http://dx.doi.org/10.1016/j.envpol.2017.08.082
0269-7491/

1. Introduction

Atmospheric variations in air pollution are considered to be risk factors for cardiovascular morbidity and mortality (Brook et al., 2010). Individuals who have preexisting cardiopulmonary disease were reported to be susceptible to changes in air pollution levels (Chuang et al., 2005). Furthermore, exposure to particulate matter with an aerodynamic diameter of <2.5 μm ($PM_{2.5}$) was suggested to be able to exacerbate cardiac and vascular pathophysiology, leading to the development of cardiopulmonary disease (Pope et al., 2004; Rajagopalan et al., 2005). Mechanisms underlying particle-driven cardiac dysfunction were postulated (Brook et al., 2010), but little is known regarding interactions between particle physicochemistry and cardiovascular regulation.

Epidemiological evidence showed seasonal effects of particulate air pollution on cardiovascular disease (Yi et al., 2010); nonetheless, different environmental conditions may have distinct effects on human health. Combustion-derived organic vapors cool in the atmospheric environment and subsequently sublimate into primary particles, an essential mechanism of formatting primary particles (Lipsky and Robinson, 2006). Incomplete combustion produces more vapor, allowing the formation of more primary particles (Chuang et al., 2013). Carbonaceous particles provide a platform for the intermixing or intercalation of inorganic and organic substances (Murr, 2008). However, the specific components responsible for the effects on cardiovascular function remain unclear. Based on previous results, we postulated that the effects of pulmonary exposure to particles on cardiovascular disorders would be associated with the physicochemical properties of the particles. Changes in cardiovascular function and heart rate variability (HRV) may denote human pathophysiological responses to particle exposure as reported by the American Heart Association (Brook et al., 2010). Blood pressure (BP), heart rate (HR), and indices of HRV are commonly used in epidemiological and toxicological settings to evaluate the cardiovascular toxicities of particles (Chuang et al., 2007; Delfino et al., 2005; Schneider et al., 2008). Nonetheless, associations between cardiovascular function and the physicochemical properties of particles remain unclear.

Experimentally, models with large numbers of diseased animals are commonly used to investigate the circadian cycle of hemodynamic parameters for delineating the true effects of particles. The current study set out to reduce the number of animals used for the experiment and to understand the cardiovascular effects of pulmonary exposure to particles using an *in vivo* crossover design. In addition, component-specific cardiovascular responses by particles were investigated.

2. Materials and methods

2.1. Animals

Every experimental procedure was carried out in accordance with institutional guidelines. Eight-week-old male Wistar Kyoto (WKY) rats were obtained from the National Laboratory Animal Breeding and Research Center (Taipei, Taiwan). Rats were acclimated in a temporary animal room with high-efficiency particle arrestance (HEPA)-filtered clean air, accredited by the Animal Research Committee of National Taiwan University (Taipei, Taiwan), during the post-surgical period and washout period. All experimental protocols were approved by the animal and ethics review committee of the Animal Research Committee of National Taiwan University.

2.2. Experimental design

Rats were randomized into 2 exposure conditions in a crossover study, with an interval of 7 days for washout between exposures. Before exposure, PhysioTel radiotelemetric transmitters (Data Sciences International, USA) were implanted in WKY rats at 9 weeks of age (Fig. 1). Details of transmitter implantation were previously reported (Carll et al., 2010; Cesarovic et al., 2011). After 10 days of acclimatization in HEPA-filtered clean air, rats were randomly assigned to group 1 ($n = 4$) or group 2 ($n = 3$) for 7 days of exposure to particle or HEPA-filtered air, respectively. The particle-exposed group 1 was then exposed to 7 days of HEPA-filtered air after a washout, whereas the HEPA-filtered control group 2 was then exposed to 7 days of particles after the washout. During the washout period, rats were maintained in HEPA-filtered clean air for 7 days. The rationale for a 7-day washout period was that these cardiac parameters (BP, HR, and HRV) are temporary physiological responses to particle exposure, and these are able to recover to normal background levels in healthy rats after 7 days. The crossover exposure between particles and HEPA-filtered air was repeated 4 times during the experiment as shown in Fig. 1. Real-time radiotelemetric data were monitored during the exposure period (particles and HEPA-filtered control).

2.3. Exposure system and monitoring

A continuous whole-body exposure system to ambient particles was applied in this study, which was previously described (Yan et al., 2014). Briefly, ambient particles were homogeneously distributed within each cage in this system. Ambient air was introduced into the system, and then the air stream was divided into two exposure conditions: particles and the HEPA-filtered control. Rats were exposed to traffic-dominated ambient air of Taipei City (Taiwan) 24 h/day, 7 days/week for a total of 4 weeks in January and February 2012. The HEPA filter was located in the inlet valve position to remove most of the particles. The temperature and relative humidity (RH) were recorded using a Thermo Recorder TR-27Ui (T&D, Matsumoto, Japan). The $PM_{2.5}$ mass concentration was determined using a dust monitor (DUST-check, model 1.108; Grimm Labortechnik, Ainring, Germany). A scanning mobility particle spectrometer (SMPS, TSI 3936, USA) was used to measure particle numbers in the size range of 13.6–736.5 nm. Black carbon (BC) mass concentrations were monitored with an Aethalometer (Magee AE31, USA). Ozone (O_3) was also monitored throughout the experimental period (Thermo Recorder TR-72Ui, T&D Corporation). The resolution of data collected from the instruments mentioned above was every minute for 1–73-h moving averages. Simultaneously, during the study period, 24-h integrated $PM_{2.5}$ samples were collected using a pair of ChemComb™ 3500 Cartridge Samplers equipped with a $PM_{2.5}$ size-selective inlet (Thermo Scientific, USA). Both samplers were operated at air flow rate of 10.0 L/min. One of the cartridges was loaded with a Teflon filter, whereas the other was equipped with a quartz fiber filter. The quartz filters were baked at 900 °C for 3 h before sampling to eliminate organic contamination.

2.4. Chemical analyses

Details of the chemical analysis were previously reported (Salvador and Chou, 2014). Briefly, Teflon filter samples were