Environmental Research 150 (2016) 306–319

Contents lists available at ScienceDirect

Environmental Research

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

Associations of oxidative stress and inflammatory biomarkers with chemically-characterized air pollutant exposures in an elderly cohort

Xian Zhang [a], Norbert Staimer [a], Daniel L. Gillen [b], Tomas Tjoa [a], James J. Schauer [c], Martin M. Shafer [c], Sina Hasheminassab [d], Payam Pakbin [d], Nosratola D. Vaziri [e], Constantinos Sioutas [d], Ralph J. Delfino [a,*]

[a] Department of Epidemiology, School of Medicine, University of California, Irvine, CA, USA
[b] Department of Statistics, School of Information and Computer Sciences, University of California, Irvine, CA, USA
[c] University of Wisconsin-Madison, Environmental Chemistry and Technology Program, Madison, WI, USA
[d] Department of Civil and Environmental Engineering, Viterbi School of Engineering, University of Southern California, Los Angeles, CA, USA
[e] Division of Nephrology and Hypertension, Department of Medicine, School of Medicine, University of California, Irvine, CA, USA

ARTICLE INFO

Article history:
Received 14 March 2016
Received in revised form
18 May 2016
Accepted 10 June 2016

Keywords:
Air pollution
Inflammation
Oxidative stress
Particulate matter components
Biomarkers

ABSTRACT

Background: Exposure to air pollution has been associated with cardiorespiratory morbidity and mortality. However, the chemical constituents and pollution sources underlying these associations remain unclear.

Method: We conducted a cohort panel study involving 97 elderly subjects living in the Los Angeles metropolitan area. Airway and circulating biomarkers of oxidative stress and inflammation were measured weekly over 12 weeks and included, exhaled breath condensate malondialdehyde (EBC MDA), fractional exhaled nitric oxide (FeNO), plasma oxidized low-density lipoprotein (oxLDL), and plasma interleukin-6 (IL-6). Exposures included 7-day personal nitrogen oxides (NO_x), daily criteria-pollutant data, five-day average particulate matter (PM) measured in three size-fractions and characterized by chemical components including transition metals, and *in vitro* PM oxidative potential (dithiothreitol and macrophage reactive oxygen species). Associations between biomarkers and pollutants were assessed using linear mixed effects regression models.

Results: We found significant positive associations of airway oxidative stress and inflammation with traffic-related air pollutants, ultrafine particles and transition metals. Positive but nonsignificant associations were observed with PM oxidative potential. The strongest associations were observed among PM variables in the ultrafine range (PM < 0.18 μm). It was estimated that an interquartile increase in 5-day average ultrafine polycyclic aromatic hydrocarbons was associated with a 6.3% (95% CI: 1.1%, 11.6%) increase in EBC MDA and 6.7% (95% CI: 3.4%, 10.2%) increase in FeNO. In addition, positive but nonsignificant associations were observed between oxLDL and traffic-related pollutants, ultrafine particles and transition metals while plasma IL-6 was positively associated with 1-day average traffic-related pollutants.

Conclusion: Our results suggest that exposure to pollutants with high oxidative potential (traffic-related pollutants, ultrafine particles, and transition metals) may lead to increased airway oxidative stress and inflammation in elderly adults. This observation was less clear with circulating biomarkers.

1. Introduction

Epidemiological studies have shown positive associations between short-term exposures to air pollutants and cardiopulmonary morbidity and mortality as reviewed by Franklin et al. (2015). The particular mechanisms linking air pollution to acute respiratory and cardiovascular events are not completely understood. Particulate matter (PM) air pollution consists of discrete particles that range from coarse-sized particles 2.5–10 μm ($PM_{2.5-10}$), to accumulation mode particles 0.1–2.5 μm ($PM_{0.1-2.5}$), and finally to ultrafine particles < 0.1 μm in diameter ($PM_{0.1}$). Particle size is an important determinant of deposition in the respiratory tract, and an indicator of chemical composition and source (Delfino et al., 2005; Sioutas et al., 2005). Smaller particles have a higher pulmonary deposition fraction and penetrate deeper in the lung (Lippmann, 1977). The large surface area of ultrafine

* Corresponding author.
E-mail address: rdelfino@uci.edu (R.J. Delfino).

http://dx.doi.org/10.1016/j.envres.2016.06.019
0013-9351/

particles also carries high concentrations of pro-oxidant chemical components, such as polycyclic aromatic hydrocarbons (PAHs) and transition metals, each of which has been shown to induce oxidative stress responses (Li et al., 2003) and can translocate from pulmonary sites to the circulatory system (Elder et al., 2006). Experimental studies have provided evidence that ultrafine particles can induce the greatest amount of oxidative stress and inflammation per unit of PM mass (Li et al., 2002; Cho et al., 2005). This may in-turn impact physiologic responses that ultimately increase the risk of acute cardiorespiratory morbidity (Weichenthal, 2012). In epidemiological studies, some have shown that ultrafine particulate matter air pollution is more strongly associated with adverse health effects when compared to larger PM diameters (Delfino et al., 2009; Franck et al., 2011) while other studies have shown $PM_{2.5}$ to have stronger or as strong associations as ultrafine PM (Ruckerl et al., 2014; Lanzinger et al., 2016). The inconsistent epidemiological evidence may be due to the fact that the sources and components of pollutants vary from study to study. In urban cities, such as Los Angeles, traffic-related pollutants are the main source for PM mass concentrations (Pant et al., 2013). Those traffic-related pollutants have been shown to contain redox active chemicals that are able to generate reactive oxygen species (ROS) responsible for increases in oxidative stress (Ayres et al., 2008). By quantifying the inherent capacity of PM to oxidize target molecules, oxidative potential is proposed to be a more attractive and biologically more relevant exposure metric than PM mass (Borm et al., 2007). Therefore, the direct measurement of PM oxidative potential may show stronger or more precise associations than either ultrafine PM or traffic-related air pollutants.

Most previous epidemiologic studies have used ambient air monitoring data from central air monitoring stations, and focused primarily on the U.S. Environmental Protection Agency (EPA)'s criteria air pollutants, namely, $PM_{2.5}$, PM_{10}, ozone (O_3), nitrogen dioxide (NO_2) and carbon monoxide (CO). Several research groups including ours have been investigating the associations between biomarkers of effect and size-fractionated and/or chemically-characterized particulate air pollutants (Delfino et al., 2008, 2009, 2010a; Chen et al., 2015; Wu et al., 2015). However, there is still a need to systematically explore oxidative stress and inflammatory biomarkers in both pulmonary and circulatory systems in relation to chemically-characterized PM.

To better understand the underlying mechanism of the association between cardiorespiratory morbidity and short-term exposure to air pollution, we conducted a panel study to investigate the potential roles of air pollutant components and pollution source tracers on both airway and systemic biomarkers of oxidative stress and inflammation in an elderly cohort. The focus on an elderly cohort is motivated by findings that reveal that older subjects tend to be more susceptible to air pollution-induced health effects, and hence represent a high risk population (Brook et al., 2010). We hypothesized that chemicals from fossil fuel combustion, related ultrafine particles, and PM oxidative potential would have stronger adverse health effects than other air pollutant variables. To our knowledge, this is the first study to relate biomarkers of airway and systemic oxidative stress responses to exposure markers of PM oxidative potential in an elderly population.

2. Methods

2.1. Study design

To investigate short-term health effects of exposures to air pollution, we conducted a cohort panel study of repeated measures of outcomes and exposures for 97 elderly non-smoking adults (age ≥ 65) living in two Los Angeles California metropolitan areas (downtown Los Angeles and Anaheim, CA) between 2012 and 2014. The repeated measures design can effectively allow for the control of between and within-subject variability in regression models. A study design flowchart can be found in Appendix, Fig. A.1. Briefly, two groups of subjects in each area were followed alternatively for up to 12 weeks in two discrete 6-week periods in order to incorporate seasonal differences in air pollution levels in the Los Angeles metropolitan area (Daher et al., 2013; Hasheminassab et al., 2014b). Specifically, one 6-week period took place during the warm season (July–October) and the other took place the cool season (November–February). Weekly clinical visits for study participants were scheduled on the same day of week and at the same time of day in order to minimize potential biases induced by weekly and circadian variation (94% of the subjects arrived to clinic with a variability of ± 2 h).

Subjects were excluded from study participation if they lived or were employed outside of the monitored community (18 km radius), smoked within the last 12 months, abused drugs or alcohol, or reported exposure to environmental tobacco smoke at home or on a regular basis at other locations. Additional health criteria for exclusion included the presence of psychiatric disorders or dementia that would prevent the subject from full participation, dialysis treatment or renal failure, daily oral corticosteroids, and active cancer. Observations following the previous 7 days when subjects reported any acute infection (7.62%) were excluded *a priori* given the known major impact of infections on systemic and respiratory inflammation.

2.2. Baseline questionnaires

Background questionnaires collected at the beginning of the study included information on socioeconomic status, medical history, current medication use, history of smoking, and environmental exposure profile. In addition, a baseline fasting blood sample was taken to obtain plasma lipid profiles and glucose levels.

2.3. Markers of airway oxidative stress and inflammation

2.3.1. Exhaled breath condensate (EBC) sampling and malondialdehyde (MDA) analysis

We collected EBC samples during normal breathing with the RTube™ Collection System (Respiratory Research, Inc., Austin, TX) using standard procedures recommended by the American Thoracic Society and European Respiratory Society (Holvoet et al., 2003). Room air was inhaled through a one-way valve and the exhaled air was directed into a collection chamber (solid aluminum tube pre-chilled on dry ice) where vapors, aerosols and moisture in the breath condense. EBC was then collected from the walls of the condenser. The samples were transported to our laboratory on dry ice and then frozen at -80 °C until analysis. We analyzed EBC samples for MDA using HPLC analysis by modifying the Larstad et al. (2002) protocol (details are given in the online supplement). The estimated limit of quantification (LOQ) for MDA in EBC samples is 3 nM. All values $<$ LOQ were set to 1.5 nmol. We excluded MDA results if the concentration was greater than the upper limit of quantification (12.5 nM) and coefficient of variation (CV) $>$ 25% (occurring in 1.16% of samples).

2.3.2. Fractional concentration of exhaled nitric oxide (FeNO) measurement

We used the NIOX MINO (Aerocrine Inc, New Providence, NJ) to noninvasively measure FeNO. Based on previous research (ATS/ERS, 2005), a questionnaire was administered to ascertain the following information prior to the FeNO measurement: (1) did the subject have a meal (breakfast if in the morning, lunch if in the