International Journal of Hygiene and Environmental Health 220 (2017) 387–394

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

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

Associations between urinary biomarkers of oxidative stress and air pollutants observed in a randomized crossover exposure to steel mill emissions

Guillaume Pelletier[a,*], Marc Rigden[a], Lisa Marie Kauri[a], Robin Shutt[a], Mamun Mahmud[a], Sabit Cakmak[a], Premkumari Kumarathasan[a], Errol M. Thomson[a], Renaud Vincent[a], Gayle Broad[b], Ling Liu[a], Robert Dales[a,c,d]

[a] Environmental Health Science and Research Bureau, Health Canada, Ottawa, Ontario, Canada
[b] The NORDIK Institute, Algoma University, Sault Ste. Marie, Ontario, Canada
[c] University of Ottawa, Ottawa, Ontario, Canada
[d] Ottawa Hospital Research Institute, Ottawa, Ontario, Canada

ARTICLE INFO

Article history:
Received 29 June 2016
Received in revised form
15 November 2016
Accepted 24 November 2016

Keywords:
Air pollution
Steel industry
Human exposure
Urinary biomarkers
Oxidative stress

ABSTRACT

The effects of industrial air pollution on human health have not been as thoroughly investigated as those of urban air pollution which originates mostly from automotive transport. To better assess the health impacts of point sources of industrial air pollution, a randomized crossover exposure study was conducted. Sixty one young and healthy volunteers were randomly assigned to spend five consecutive eight-hour days near a steel mill or at a location five kilometres away. After a nine or sixteen-day washout period, volunteers spent another five consecutive days at the second site. Meteorological conditions and air pollutants were monitored at both exposure sites. On each exposure day, the first morning urine was collected along with a second urine sample obtained immediately before leaving the exposure site at the end of the day. Urinary levels of biomarkers of oxidative stress 8-hydroxy-2′-deoxyguanosine (8-OHdG, a biomarker of oxidative DNA damage), malondialdehyde (MDA, a biomarker of lipid peroxidation), 8-isoprostane (8-IsoP, a bioactive metabolite resulting from the peroxidation of arachidonic acid) and Vascular Endothelial Growth Factor (VEGF, involved in response to oxidative stress) were measured. According to mixed-effects linear regression models, intra-individual variations in 8-OHdG urinary levels were significantly associated with exposure site, but surprisingly, lower levels were observed at the steel mill site. Delayed, temporally-defined associations with specific air pollutants were observed for 8-OHdG, 8-IsoP and VEGF. However, these associations were subtle, presented complex patterns and their biological consequences remain unclear.

1. Introduction

Associations between air pollution and adverse human health effects have been firmly established in numerous epidemiological studies (Adam et al., 2015; Atkinson et al., 2015; Brook et al., 2010; Shah et al., 2013). However, the health effects of point sources of industrial air pollution are not as well studied as those of diffuse urban air pollution resulting mostly from automotive transport. Steel is an important Canadian industrial sector and epidemiological studies already suggest association between respiratory symptoms and steel mill emissions (Pope, 1989; Pope and Dockery, 1992; Pouliou et al., 2008). The molecular mechanisms underlying the effects of air pollution on pulmonary and cardiovascular functions are not yet fully elucidated. However, there are several lines of evidence pointing toward the involvement of reactive oxygen species (ROS) and oxidative stress in target tissues (Brook et al., 2010; Yang and Omaye, 2009), while systemic inflammation and oxidative stress biomarkers are not as consistently observed in human exposure studies (Brook et al., 2010).

The intra-individual variations of pulmonary and cardiovascular functions of young and healthy volunteers exposed to steel mill air pollutant emissions or to ambient air at a site located five kilometres away from the steel mill were previously assessed using a

* Corresponding author at: Environmental Health Centre, 50 Colombine Driveway, P.L. 0803B, Tunney's Pasture, Ottawa, Ontario, K1A 0K9, Canada.
E-mail address: guillaume.pelletier@canada.ca (G. Pelletier).

http://dx.doi.org/10.1016/j.ijheh.2016.11.010
1438-4639/

randomized crossover exposure study design (Dales et al., 2013; Liu et al., 2014). Briefly, after controlling for confounding factors, exposure to air pollutants near a steel mill resulted in slightly (but significantly) lower forced expiratory volume in 1-s to forced vital capacity (FEV_1/FVC), forced expiratory flow between 25% and 75% of the FVC and total lung capacity, functional residual capacity and residual volume values (Dales et al., 2013). Although increased heart rate was the only cardiovascular endpoint significantly associated with proximity to the steel mill, alterations of diastolic blood pressure, pulse pressure, pulse rate and flow-mediated vasodilation in the same volunteers presented significant associations with specific air pollutants (Liu et al., 2014). Furthermore, metal levels measured in particulate matter were significantly higher at the steel mill site and significantly associated with observed changes in cardiovascular and respiratory physiology (Cakmak et al., 2014).

Volunteers from this randomized crossover exposure study (Dales et al., 2013; Liu et al., 2014) also provided urine samples. Biomarkers of oxidative stress such as 8-hydroxy-2′-deoxyguanosine (8-OHdG, a biomarker of oxidative DNA damage), malondialdehyde (MDA, a biomarker of lipid peroxidation), 8-isoprostane (8-IsoP, a biomarker of oxidative stress and mediator of inflammation generated from the peroxidation of arachidonic acid) and Vascular Endothelial Growth Factor (VEGF, a growth factor involved in response to oxidative stress) have been previously used to assess the effects of air pollution on human health (de Oliveira et al., 2014). In the current investigation, we measured the concentrations of these biomarkers of oxidative stress in urine samples and explored their associations with exposure sites and air pollutant levels using mixed-effects linear regression models.

2. Materials and methods

2.1. Study design

The experimental design of this human exposure study has been previously described (Dales et al., 2013; Liu et al., 2014) and approved by both Health Canada and Algoma University Research Ethics Boards. Briefly, 61 volunteers were randomly assigned to one of the two exposure sites. The Bayview site was located on the edge of a residential neighbourhood, within 100 m of a steel mill. The College site was located on a college campus located approximately five kilometres away from the steel mill. Outdoor exposures were carried out in the Spring–Summer of 2010 (May–August) in Sault Ste. Marie, Canada. Awnings were installed at both exposure sites to protect the participants from direct sunlight and precipitations. After spending five consecutive eight-hour days at the first exposure site (from Monday to Friday), participants went through a 9-day washout period and were then crossed over to the second site, where they also spent 5 consecutive eight-hour days. A 16-day washout period was also used over Canada Day (as exposures were suspended on the week of this national holiday), and to occasionally accommodate volunteers. First morning urine was collected by the participants at home and provided to the study team upon arrival at the site. Another urine sample was collected in the afternoon, near the end of the exposure day. Participants completed 30 min of moderate exercise on an elliptical trainer at 60% of predicted maximum heart rate in the middle of the exposure day, but otherwise they spent the day resting and undergoing pulmonary and cardiovascular function measurements.

2.2. Subject recruitment and characteristics

Sixty-six potential volunteers for this study answered the advertisement posted at a local college and university. They provided informed written consent and information on their age, sex, weight, height, residence, tobacco exposure, medical history and current medications was obtained through a questionnaire. Pregnant or breast-feeding women, smokers or those exposed to cigarette smoke at home and residents of the Bayview neighbourhood were excluded. Volunteers that suffered from diabetes mellitus, chronic cardiovascular and respiratory disease, who took medications that might interfere with the endpoints assessed or presented conditions that may have hindered daily on-site exercise were also excluded. Five volunteers were excluded from this study based on these criteria. The remaining 28 males and 33 females were young (median age: 22 years) and healthy volunteers whose detailed characteristics were previously presented (Dales et al., 2013; Liu et al., 2014).

2.3. Air pollution measurement

Temperature, relative humidity and air pollutants were monitored hourly at each study site for every exposure day. Sulphur dioxide (SO_2) was measured by ultraviolet fluorescence, ozone (O_3) by ultraviolet photometry, carbon monoxide (CO) by infrared photometry and nitrogen oxides (NO and NO_2) by chemiluminescence using an Air Pointer® air quality monitor (Recordum Messtechnik GmbH, Mödling, Austria). Air particles presenting a diameter of 2.5 μm or less ($PM_{2.5}$) were quantified by nephelometry using an Air Pointer®, while ultrafine particles ranging from 0.01 to 0.1 μm diameter (UFP) were measured by condensation using a Model 3007 Ultrafine Particle Counter (TSI, Shoreview, MN, USA).

2.4. Urinary biomarker measurement

Urinary creatinine was determined using a modified Jaffé method with reagents purchased from Roche Diagnostics (Laval, QC, Canada, Cat# 11489291). Urine samples were diluted 1:10 with 0.85% saline and assays were performed using a Cobas Fara II clinical chemistry analyzer. Urinary malondialdehyde (MDA) levels were measured by HPLC following the method of Larstad et al. (2002) with the following modifications: Urine samples were diluted 1:5 with 0.85% saline. Duplicate injections on an Agilent 1200 series HPLC with a G1311A quaternary pump and G1321B fluorescence detector were done for each sample. Peak integration was performed using Chemstation (V C.01.02) and MDA concentration was determined using linear standard curves generated daily. Levels of 8-Isoprostane (8-IsoP) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) were determined in duplicate using ELISA kits from Cayman (Ann Arbor, MI, USA, Cat# 516351) and Japan Institute for the Control of Aging (JAICA, Fukuroi, Shizuoka, Japan, Cat# KOGHS-10E), respectively, according to the manufacturers' instructions. Optical density values were obtained using a Spectramax Plus 384 (Molecular devices) and biomarker concentrations determined using Softmax Pro (V 5.0.1) software. Vascular Endothelial Growth Factor (VEGF) levels were measured in duplicate by a luminescence-based ELISA from R&D Systems (Minneapolis, MN, USA, Cat# QVE00B) using a Fluostar Optima (BMG) plate reader and Optima software (V 2.20R2). Duplicate values were averaged. Samples whose duplicates presented a variance greater than 15% were reassessed again in duplicate. All urinary MDA, 8-IsoP and VEGF values were above their respective limit of quantification (LOQ), while 3.5% of samples presented 8-OHdG values below LOQ. These missing values were replaced by 1/2 LOQ for statistical analyses.

2.5. Data analysis

Data were log-transformed to ensure normal distribution. Comparisons of environmental factors between sites were evaluated by non-parametric Wilcoxon Rank-Sums test. Correlation between