Science of the Total Environment 463–464 (2013) 884–893

Contents lists available at ScienceDirect

Science of the Total Environment

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

Biomarkers of occupational exposure to air pollution, inflammation and oxidative damage in taxi drivers

Natália Brucker [a,b], Angela M. Moro [a,b], Mariele F. Charão [a,b], Juliano Durgante [b], Fernando Freitas [b], Marília Baierle [a,b], Sabrina Nascimento [a,b], Bruna Gauer [b], Rachel P. Bulcão [a,b], Guilherme B. Bubols [a,b], Pedro D. Ferrari [c], Flávia V. Thiesen [c], Adriana Gioda [d], Marta M.M.F. Duarte [e], Iran de Castro [f], Paulo H. Saldiva [g], Solange C. Garcia [a,b,*]

[a] Post-graduate Program in Pharmaceutical Sciences, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[b] Laboratory of Toxicology (LATOX), Department of Clinical Analysis, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[c] Toxicology Institute, Pontifical Catholic University of Rio Grande do Sul, Porto Alegre, RS, Brazil
[d] Department of Chemistry, Pontifical Catholic University of Rio de Janeiro (PUC-Rio), Rio de Janeiro, RJ, Brazil
[e] Department of Health Sciences, Lutheran University of Brazil, Santa Maria, RS, Brazil
[f] Institute of Cardiology, University Cardiology Foundation, Porto Alegre, RS, Brazil
[g] Department of Pathology, College of Medicine, University of São Paulo, São Paulo, SP, Brazil

HIGHLIGHTS

- Chronic exposure to air pollution was linked to increase of atherogenic predictors.
- Higher homocysteine levels were found in taxi drivers.
- 1-OHP levels showed important influence on increase of IL-1β, IL-6 and TNF-α levels.
- Air pollution was related to depletion of antioxidants leading to oxidative damage.

ARTICLE INFO

Article history:
Received 21 March 2013
Received in revised form 27 May 2013
Accepted 24 June 2013
Available online 19 July 2013

Editor: Adrian Covaci

Keywords:
Occupational exposure
1-hydroxypyrene
Inflammation
Oxidative stress
Taxi drivers

ABSTRACT

Exposure to environmental pollutants has been recognised as a risk factor for cardiovascular events. 1-hydroxypyrene (1-OHP) is a biomarker of exposure to polycyclic aromatic hydrocarbons (PAHs) from traffic-related air pollution. Experimental studies indicate that PAH exposure could be associated with inflammation and atherogenesis. Thus, the purpose of this study was to evaluate whether the biomarker of PAH exposure is associated with biomarkers of inflammation and oxidative stress and if these effects modulate the risk of developing cardiovascular diseases in workers exposed to air pollution. This study included 60 subjects, comprising 39 taxi drivers and 21 non-occupationally exposed persons. Environmental $PM_{2.5}$ and benzo[a]pyrene (BaP) levels, in addition to biomarkers of exposure and oxidative damage, were determined. Inflammatory cytokines (IL-1β, IL-6, IL-10, TNF-α, IFN-γ and hs-CRP) and serum levels of oxidised LDL (ox-LDL), auto-antibodies (ox-LDL-Ab) and homocysteine (Hcy) were also evaluated. $PM_{2.5}$ and BaP exhibited averages of 12.4 ± 6.9 μg m^{-3} and 1.0 ± 0.6 ng m^{-3}, respectively. Urinary 1-OHP levels were increased in taxi drivers compared to the non-occupationally exposed subjects ($p < 0.05$) and were positively correlated with pro-inflammatory cytokines and negatively correlated with antioxidants. Furthermore, taxi drivers had elevated pro-inflammatory cytokines, biomarkers of oxidative damage, and ox-LDL, ox-LDL-Ab and Hcy levels, although antioxidant enzymes were decreased compared to the non-occupationally exposed subjects ($p < 0.05$). In summary, our findings indicate that taxi drivers showed major exposure to pollutants, such as PAHs, in relation to non-occupationally exposed subjects. This finding was associated with higher inflammatory biomarkers and Hcy, which represent important predictors for cardiovascular events. These data suggest a contribution of PAHs to cardiovascular diseases upon occupational exposure.

1. Introduction

Epidemiological studies have provided strong evidence that exposure to air pollution represents a risk factor in the morbidity and mortality of

* Corresponding author at: Avenida Ipiranga 2752, Santa Cecília, Porto Alegre, RS, CEP.: 90610-000, Brazil. Tel.: +55 3308 5297; fax: +55 51 3308 5437.
E-mail address: solange.garcia@ufrgs.br (S.C. Garcia).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.06.098

cardiovascular diseases (Brook, 2008; Pope et al., 2004). Air pollution caused by vehicular exhaust is one of the most important problems in urban environments, especially in developing countries (Yoshida et al., 2010).

Emissions from vehicles contain a heterogeneous mixture of hazardous substances. An important fraction of air pollution contains particulate matter of less than 2.5 μm ($PM_{2.5}$), which contains various chemicals adsorbed to its surface that act as efficient carriers of other pollutants (Delfino et al., 2009). Pope et al. (2002) showed that each 10 $\mu g/m^3$ increment of $PM_{2.5}$ levels was associated with a 6% increase in the risk of developing cardiovascular damage. Indeed, the smallest particles in urban environments, such as $PM_{2.5}$, are the most dangerous, as they can lead to the induction of inflammation and oxidative damage (Araujo and Nel, 2009; Kunzli et al., 2005).

Toxicological evidence indicates that $PM_{2.5}$ contains large amounts of polycyclic aromatic hydrocarbons (PAHs), such as benzo[a]pyrene. These are ubiquitous and persistent contaminants that are mainly derived from incomplete combustion of organic materials in occupational settings or in the environment (Hong et al., 2009; Rossner et al., 2008; Sorensen et al., 2003). The toxicity of PAHs also appears to be related to their biotransformation into reactive metabolites, resulting in the generation of reactive oxygen species (ROS) that are capable of inducing lipid and protein oxidation and the depletion of endogenous antioxidants within an organism (Lodovici and Bigagli, 2011; Rossner et al., 2008; Sorensen et al., 2003).

Humans are commonly exposed to PAH mixtures through the inhalation of polluted air, dietary intake and cigarette smoke (Burgaz et al., 2002; Fan et al., 2012). Furthermore, many studies have indicated that exposure to these chemicals in cell culture and animal models is associated with the induction of pro-inflammatory cytokines, the generation of ROS and atherosclerosis (Curfs et al., 2004; Knaapen et al., 2007; Podechard et al., 2008; Umannova et al., 2011; Vogel et al., 2005). Several studies have utilised urinary 1-hydroxypyrene (1-OHP) levels to biologically monitor the uptake of individual PAHs from occupational exposures (Liu et al., 2010; Sellappa et al., 2011). Likewise, there are reports in the literature regarding the use of this biomarker to assess traffic-related air pollution exposure (Burgaz et al., 2002; Demetriou et al., 2012; Hansen et al., 2004, 2008).

Studies indicate that the inhalation of small environmental particles may directly or indirectly result in ROS formation and the activation of inflammatory mediators (IL-6, TNF-α, hs-CRP). However, the mechanisms responsible for vascular damage remain unknown (Bhatnagar, 2006; Brook, 2008; Huang et al., 2012; Sorensen et al., 2003). One hypothesis is that ROS contribute to endothelial dysfunction and the progression of atherosclerosis, consequently increasing the risk of cardiovascular events (Brook et al., 2009; Pope et al., 2004; Steinvil et al., 2008).

The constant increase in the number of circulating vehicles raises concern about the negative impact of traffic emissions on human health. There is a lack of experimental data regarding the consequences of occupational exposure to pollution (Lewne et al., 2006; Manini et al., 2006; Piccardo et al., 2004). Measuring biomarkers of effect, susceptibility and exposure is helpful in assessing the hazards of occupational exposure (Manini et al., 2006; Manno et al., 2010; Rossner et al., 2008). Biomonitoring of taxi drivers may help to determine the risk of developing work-related pathologies as a result of spending several hours per day in traffic (Burgaz et al., 2002; Petchpoung et al., 2011).

The present study aimed to evaluate whether the biomarker of exposure to PAHs was associated with biomarkers of inflammation and oxidative stress. Furthermore, this study also investigated whether inflammation and oxidative stress caused by exposure to PAHs modulate the risk of developing cardiovascular disease during occupational exposure to air pollution.

2. Materials and methods

2.1. Subjects

One hundred and seventeen male participants were recruited. Subjects older than 60 years old, smokers, those with a history of cardiovascular disease, those diagnosed with diabetes mellitus and chronic diseases, those taking vitamin supplementation, those who had failed to collect samples or participate in any stage of the study were excluded. Smokers were excluded to avoid the confounding factor of smoking on PAH levels. Based on these exclusion criteria, 60 men were enrolled. The exposed group consisted of 39 taxi drivers potentially exposed to vehicle emissions from the traffic of Porto Alegre, Brazil. All the professional drivers were self-employed and worked during the daytime, and the working hours ranged from approximately 8 h to more than 12 h. The control group consisted of 21 men that were non-occupationally exposed to traffic exhaust.

The taxi drivers were recruited through invitations and advertisements in radio stations and in taxi driver syndicates. For recruitment of the control group, invitations and announcements were made at the Federal University of Rio Grande do Sul (administrative activities) for men living in the same city without indication of any occupational exposure to air pollution. The groups simultaneously underwent equivalent examinations and procedures.

Each subject was personally interviewed utilising a questionnaire regarding individual characteristics, health status, history and lifestyle (smoking, alcohol drinking habits, diet, medication, exercise habits) and other general information regarding the work shift (years of service and time spent inside the car). This study was approved by the Ethics Committee for Research of the Federal University of Rio Grande do Sul/RS (Nr. 20322/11). All the participants were informed about the study and signed a consent form according to the guidelines of the local committee.

2.2. Sample collection

All recruitment and sample collections were performed during the winter of 2011 because this season is characterised by high levels of air pollutants. Evaluations of the subjects were performed during the same period of $PM_{2.5}$ sampling. All the subjects were monitored once during pre-work shifts between 7:00 am and 9:00 am. Pre-work shift urine and blood samples were collected from participants after a 12-hour fasting period. Fifty millilitres of fresh urine was collected for the determination of 1-hydroxypyrene and creatinine levels. The urine samples were frozen at −80 °C until further analysis. The blood venous samples from all the subjects were collected by venipuncture using vacutainer tubes. The first EDTA blood tube (2 mL) was used for a haemogram and to measure carboxyhaemoglobin (COHb) levels. The second EDTA blood tube (2 mL) was immediately centrifuged at 1500 *g* for 10 min at 4 °C, and the plasma was used to quantify malondialdehyde (MDA) and protein carbonyl (PCO) levels, while the erythrocytes were used to analyse non-protein thiol groups. A blood tube with heparin (4 mL) was collected and stored at −80 °C until analysis to determine catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) enzymatic activities. Plasma was collected from a citrate vacutainer (2 mL) for fibrinogen analysis. Another blood collection tube without anticoagulants (2 mL) was centrifuged at 1500 *g* for 10 min at room temperature. The serum was removed and was immediately used to determine glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), and triglyceride (TG) levels. The remaining serum was aliquoted and stored at −80 °C for determination of high sensitivity C reactive protein (hs-CRP), oxidised-LDL (ox-LDL), autoantibodies against ox-LDL (ox-LDL-Ab), homocysteine (Hcy), cytokines and vitamin C levels.