Environmental Pollution 208 (2016) 261–270

Contents lists available at ScienceDirect

Environmental Pollution

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

Acute exposure to Buenos Aires air particles (UAP-BA) induces local and systemic inflammatory response in middle-aged mice: A time course study

Nadia S. Orona [a, b, *], Sebastián A. Ferraro [a, b], Francisco Astort [a], Celina Morales [c], Fernando Brites [d], Laura Boero [d], Gisela Tiscornia [a], Guillermo A. Maglione [a], Paulo H.N. Saldiva [e], Sebastian Yakisich [f], Deborah R. Tasat [a, g]

[a] Center for The Studies in Health and Environment, School of Science and Technology, National University of General San Martín, San Martín, Buenos Aires, Argentina
[b] Committee for Scientific Research, La Plata, Buenos Aires, Argentina
[c] Institute of Cardiovascular Physiopathology, Department of Pathology, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
[d] Laboratory of Lipids and Lipoproteins, Department of Clinical Biochemistry, School of Pharmacy and Biochemistry, University of Buenos Aires, Buenos Aires, Argentina
[e] Experimental Atmospheric Pollution Laboratory, School of Medicine, Sao Paulo University, Sao Paulo, Brazil
[f] Department of Pharmaceutical Sciences, Hampton University, Hampton, VA, USA
[g] Department of Histology and Embryology, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina

ARTICLE INFO

Article history:
Received 17 April 2015
Received in revised form
14 July 2015
Accepted 16 July 2015

Keywords:
Urban air pollution
Cardiorespiratory system
Aging
Inflammation
Oxidative stress

ABSTRACT

Exposure to air particulate matter (PM) is associated with increased cardiovascular morbimortality. However, PM doesn't affect equally to all people, being the old cohort the most susceptible and studied. We hypothesized that another specific life phase, the middle-aged subpopulation, may be negatively affected. Therefore, the aim of this study was to analyze *in vivo* the acute biological impact of two environmental particles, Urban Air Particles from Buenos Aires and Residual Oil Fly Ash, on the cardiorespiratory system of middle-aged mice, evaluating oxidative metabolism and inflammation. Both PM provoked a local and systemic inflammatory response, leading to a reduced alveolar area in the lung, an epicard inflammation in the heart, an increment of IL-6, and a reduction on PON 1 activity in serum of middle-aged animals. The positive correlation of local parameters with systemic markers of oxidative stress and inflammation could be responsible for associations of cardiovascular morbimortality in this subpopulation.

1. Introduction

There is increasing evidence that air pollution has a negative impact on health on urban populations, particularly due to the burning of fossil fuels from industries and vehicles. The World Health Organization (WHO) has ranked air pollution as one of the top ten causes of disability, and the leading cause of death in the industrialized world (Delfino et al., 2011; Brook, 2008). Cities are by nature concentrations of human, industrial and vehicular activities and therefore could be considered as a source of pollution (PM and gases) affecting the health of its inhabitants. Epidemiological studies showed that exposure to air particulate matter (PM) in urban zones, even when particle concentrations are well below established air quality standard levels, is associated with an increase in cardiovascular morbidity and mortality rates (Analitis et al., 2006; Brunekreef and Forsberg, 2005; Dockery et al., 1993; Pope, 2000; Schwartz et al., 1991). Hence, a greater understanding of the underlying molecular and cellular mechanisms by which pollutants exacerbate and/or promote cardiorespiratory injury is imperative.

Lungs are the primary target of PM exposure, where particles can translocate to the blood system, heart and brain exerting not only pulmonary but, systemic adverse effects (Brook et al., 2004; Brook, 2008). Buenos Aires is a Latin American megacity with a population of more than 2.8 million people (National census, 2010), where vehicular emissions are the main problem. Officially, around 1.2 million vehicles circulate daily in the city; growing steadily in

* Corresponding author. School of Science and Technology, National University of San Martín, Martín de Irigoyen 3100, 1653 San Martín, Buenos Aires, Argentina.
E-mail address: naorona@gmail.com (N.S. Orona).

http://dx.doi.org/10.1016/j.envpol.2015.07.020
0269-7491/

the metropolitan area (5.2% in 2011 and 5.7% in 2012). Our group has previously determined in downtown Buenos Aires a 72 ug/m^3/day particle average concentration of PM 2.5 during wintertime, being public transport (cars, taxis, trucks and vans), the responsible for 94.7% of the total annual PM emission.

In demographic terms, Buenos Aires slightly differs from the whole country. An important fact for public health is given by the age composition of its population being the percentage of the aged inhabitants significantly higher at the city level than at the national level. In so, the impact of environmental factors, as air pollution, on the health of the aged population grow in importance (Abrutzky et al., 2012).

PM does not equally affect everybody, identifying young less than 2 years old and adults over 65 years old as susceptible subpopulations (Pope, 2000; Saldiva et al., 1995; Samet et al., 2000). It is well known that throughout the normal aging process, physical and biochemical changes occur both in the respiratory and cardiovascular systems that, in turn, may affect the response of the lung to inhaled xenobiotics and, consequently, the cardiac function. Although aging is a continuous process, usually it is treated as a discrete variable, where the old cohort is the most studied. Regarding the cardiovascular system, together with age, the principal causes of cardiac failure are the decrease in elasticity and the loss of ability of the arterial system to respond to changes in pressure (compliance). In addition, it has been shown that air pollution may adversely affect cardiovascular physiology such as heart rate and blood pressure (Rich et al., 2012). Furthermore, in the respiratory system it is well known that acute PM exposure induces local oxidative stress, subsequent inflammation and ultimately irreversible lung damage (Riva et al., 2011).

Herein we hypothesized that another specific life phase, the middle-aged subpopulation, may be negatively affected by airborne particles. Therefore, the aim of the present study was to perform an *in vivo* comparative analysis through time on the lung and heart histology, on oxidative metabolism, on inflammatory parameters and on serum biomarkers of cardiovascular disease from middle aged mice acutely exposed to Urban Air particles from downtown Buenos Aires (UAP-BA) and Residual Oil Fly Ash (ROFA), two PM of different morphochemical composition.

2. Materials and methods

2.1. Animals

BALB/c mice were purchased from the breeding facility of the School of Exact and Natural Sciences, University of Buenos Aires, and were acclimated to our research facility for at least one week prior to any experimental manipulation. Animals were kept on a 12:12 h light:dark schedule and given food and water *ad libitum*. According to Jackson Laboratory's definition, in our study we employed 10–12 months old mice defined as middle aged. Animal treatment was carried out following the local ethical guidelines from the National University of San Martín (UNSAM) and the 6344/96 regulations of the Argentinean National Drug, Food and Medical Technology Administration (ANMAT) guidelines.

2.2. Particulate matter (UAP-BA and ROFA) collection and characterization

UAP-BA (Urban Air Particles from downtown Buenos Aires, Argentina) was collected as previously described by our group (Martin et al., 2007). Briefly, A MiniVol™ Portable Air Sampler (Airmetrics, OR) with 2.5 μm cut-point impactor was employed (Baldauf et al., 2001). The MiniVol's pump draws air at 5 L/minute through a particle size separator (impactor) and then through a Teflon 47 mm (filter Sartorious, 0.2 μm pore size) and each filter was placed in a clean plastic cassette during transport and storage. The filters were weighed (after moisture equilibration) before and after sampling to determine the net particulate mass gain with a microbalance (Mettler M3, weighing accuracy of 1 μg), using an α source to remove the electrostatic charge.

Residual Oil Fly Ash (ROFA) was collected from Boston Edison Co., Mystic Power Plant, and Mystic, CT, USA, and was kindly provided by Dr. J. Godleski (Harvard School of Public Health, MA, USA).

Both particulate matter, UAP-BA and ROFA, were morphologically and chemically characterized employing scanning electron microscopy (SEM) and energy dispersive X-ray spectroscopy (EDX), respectively. For SEM observations, collected particles were coated with gold by direct current sputtering. Stub preparations were examined in a quanta SEM FEG-S50 (SEI, Oregon, USA). Chemical composition was analyzed with a Phillips SEM 505 SEM (Philips Electron Optics, NL, USA) coupled to a EDX dispersion detection unit (EDAX Inc., NJ, USA).

2.3. UAP-BA and ROFA animal exposure – experimental model

BALB/c mice (12 pg/experiment) were anesthetized intraperitoneally with 1 ml/kg body weight) of ketamine (50 mg/ml) and xylazine (2%) and exposed to PM by intranasal instillation in a single dose (UAP-BA or ROFA) (1.0 mg/kg body weight) or Phosphate Buffer Salt (PBS) (control group).

Briefly, animals were immobilized in an inclined supine position while 50 μL of UAP-BA or ROFA suspension was delivered dropwise to the nares (Leong et al., 1998; Southam et al., 2002). Due to the presence of fluid in the mouse nasal cavity, a respiratory reflex is triggered which ensures that the maximum delivered volume reaches the lung. After 3, 24 or 48 h of exposure, animals were euthanized and lung and heart samples were collected. For all time points, control mice were instilled with 50 μL of PBS. The selected dose falls within the range of concentrations consistently used in several animal studies (Dye et al., 2001; Nurkiewicz et al., 2006).

For clarity the experimental model and all endpoints evaluated in lung and/or heart from control and exposed PM animals are shown in Fig. 1.

2.4. Lung and heart tissue samples for histology

After exposure to either PM or PBS, BALB/c lungs and hearts were excised and kept in PBS (pH 7.4) at 4 °C. Samples were employed for histological (1) or biochemical (2) purposes.

1) Organs were placed in 10% buffered formalin for no more than 48 h, dehydrated in alcohol and embedded in paraffin. Histological sections of 5–7 μm thicknesses were cut with a Reichert-Jung micrometer (Nossloch, Germany) for light microscopy observation. For all post instillation time points (3- 24–48 h), two lung longitudinal sections and two heart cross sections from both control and PM exposed mice were obtained and stained with Haematoxylin and Eosin. Regarding the heart, histological examinations of the left ventricle, right ventricle, septum and mesothelial cells in epicardium were conducted. The lesion severity was assessed based on semiquantitative criteria previously outlined by Herman et al. (2000). This grading system is based on the percentage of myocytes showing myofibrillar loss, cytoplasmic vacuolization and inflammatory infiltrate: 0 = no damage; 1 = <5%; 1.5 = 5–15%; 2 = 16–25% 2.5 = 26–35% y 3 = >35%.
2) Organs were washed in PBS, weighted and homogenized in the same buffer for superoxide dismutase (SOD) and catalase (CAT) determinations.

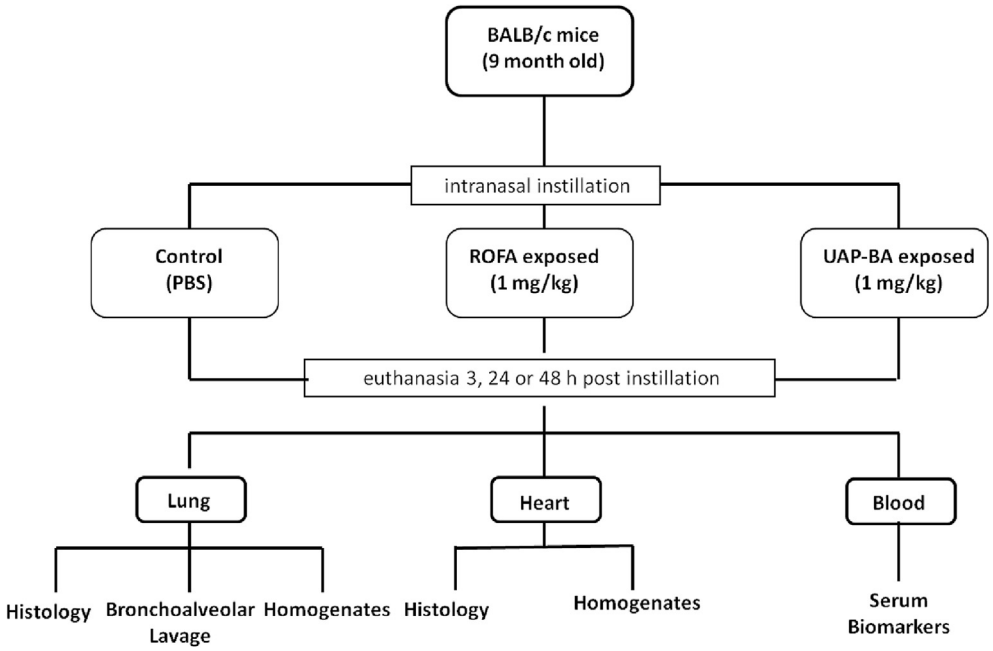

Fig. 1. Experimental animal model. Middle-aged BALB/c mice were randomized in three groups and acutely exposed to UAP-BA (1 mg/kg BW), ROFA (1 mg/kg BW) or PBS. Cellular and biochemical parameters from control and exposed PM animals were evaluated in lung, heart and serum after 3, 24 or 48 h post-exposure.

2.5. Lung bronchoalveolar lavage (BAL)

After treatment, lung bronchoalveolar lavage (BAL) was performed as previously described by Tasat and de Rey (1987). Briefly, the thoracic cavity was partly dissected and the trachea was cannulated with an 18-gauge needle. The excised lung was then gently massaged and lavaged twelve times with 1 ml of cold sterile PBS (Ca++Mg free, pH 7.2–7.4). The BAL was immediately centrifuged at 800× g at 4 °C for 10 min and resuspended in PBS. Total cell number (TCN) was determined with a Neubauer chamber. Based on morphological criteria, control animals showed >95% of alveolar macrophages (AM).

2.6. Total cell number (TCN) and cell differential (CD) in the bronchoalveolar lavage (BAL)

TCN was determined under a Neubauer chamber. To easily identify different cell types on the BAL, smears were prepared, fixed with methanol and stained with hematoxilin and eosin (CD). At least 200 cells in each sample were counted by light microscopy.

2.7. Superoxide anion generation in the bronchoalveolar lavage (BAL)

Superoxide anion (O_{2^-}), a main reactive oxygen specie (ROS) generated during the respiratory burst, was evaluated using the Nitro Blue Tetrazolium (NBT) reduction test (Segal, 1974).

The intracellular release of this ROS was evidenced by the amount of a blue formazan precipitate in the cells after NBT reduction as previously described by Tasat and de Rey (1987). BAL cells were treated with NBT in the presence or absence of TPA, a known inductor of O_2^- generation. Immediately after isolation, samples were incubated with NBT for 60 min at 37° C. In positive controls, TPA was added at a concentration of 0.5 mg/ml for the last 15 min. Cells showing a blue formazan precipitate were considered reactive, whereas those without precipitate were scored as non reactive. The percentage of reactive and non reactive cells was evaluated by light microscopy.

2.8. Superoxide dismutase (SOD) and catalase (CAT) activities in lung and heart homogenates

Tissue samples were homogenized in PBS (pH 7.4) (1:5). The suspension was centrifuged at 600x g for 10 min at 0–4° C to remove nuclei and cell debris. The pellet was discarded and the supernatant was used as "homogenate" (Evelson et al., 2001).

Superoxide dismutase (SOD) activity was determined spectrophotometrically by following the inhibition of the rate of adenochrome formation at 480 nm, in a reaction medium containing 1 mM epinephrine and 50 mM glycine/NaOH (pH 10.5). Enzymatic activity was expressed as SOD units/mg protein. One unit was defined as the amount of sample able to inhibit the rate of adenochrome formation by 50% (Misra and Fridovich, 1972).

Catalase (CAT) activity was evaluated by following the decrease in absorbance at 240 nm in a reaction medium consisting of 100 mM PBS (pH 7.4) and 20 mM hydrogen peroxide (70). Results were expressed as pmol catalase/mg protein.

2.9. General biochemical determinations

Serum levels of glucose, total bilirubin, triglycerides and total cholesterol were quantified by standardized methods (Roche Diagnostics, Mannheim, Germany) in a COBAS C501 autoanalyser (Roche Diagnostics, Mannheim, Germany). HDL-C concentration was determined by selective precipitation methods employing phosphotungstic acid in the presence of magnesium ions (Warnick et al., 1982).

2.10. Serum biomarkers

2.10.1. Proinflammatory cytokines production

To determine the effect of PM on proinflammatory cytokine production, tumor necrosis factor alpha (TNFα) and interleukin (IL-6) levels were assayed in the BAL fluid or blood serum, respectively. After

mice PM exposure (3, 24 and 48 h), blood serum and BAL supernatants were collected and frozen at −80 °C until use. Both cytokines were detected using a commercially available specific enzyme linked immunosorbent assay (ELISA) kit, following the manufacturer's instructions. ELISA plates (Corning, Newark, CA) were coated with 1:125 cytokine-specific capture antibodies diluted in coating buffer (0.1 M Na_2CO_3, pH 9.5) at 4° C overnight. Wells were blocked with PBS containing 10% FCS for 1 h at RT. Cytokine standards and BAL supernatants or blood sera were added to wells and incubated for 2 h. Following three washes, biotinylated cytokine-specific detection antibody 1:250 was added for 1 h. After washing, streptavidin-peroxidase/TMB detection system was employed for 30 min. Absorbance was measured on a microplate reader (Bio-Rad, bench mark) at 655 nm. All samples were run in triplicates.

2.10.2. Paraoxonase 1 (PON 1) activity

The enzyme PON 1 was evaluated employing two different substrates: paraoxon (Sigma Chemical Co.; PON 1 activity) and phenylacetate (Sigma Chemical Co.; ARE activity). Both activities were measured in serum samples following the method of Furlong et al. (Furlong et al., 1989). PON 1 activity was assessed by adding serum samples (20 μl) to 2 ml Tris/HCl 10 buffer (100 mmol/l, pH = 8.0) containing 2 mmol/l CaCl2, 2.6 mmol/l paraoxon (O,O-diethyl-O-p-nitrophenylphosphate) and 1.0 mol/l NaCl. The rate of generation of p-nitrophenol was determined at 405 nm and 25 °C, in a Hitachi U- 1100 spectrophotometer. Increases in the absorbance were recorded at 45- second intervals during 5 min, after 30 s of initial pre-incubation. Enzymatic activity was calculated from the molar extinction coefficient (17,000 l mol^{-1} cm^{-1}) and results were expressed as nmol/ml.min. Measurements were all carried out within the same assay. Within-run precision (CV) was 5.5%.

2.10.3. Protein content

Protein concentration was measured by the method of Lowry et al. (Lowry et al., 1951) using bovine serum albumin as standard.

2.11. Statistical analysis

Results were expressed as mean values ± standard deviation of the mean and representing the mean of 3 independent experiments. To analyze differences between control and PM exposed groups, ANOVA followed by Newman–Keuls post-test was used. Statistical significance was set up at $p < 0.05$.

3. Results

3.1. Particulate matter morphological and chemical characterization

As we have previously shown, no metal traces were found in UAP-BA particles, while both HAPs and PCBs were found adsorbed to the particle-carbon core (Ferraro et al., 2011; Martin et al., 2007; Orona et al., 2014). Particle size was homogeneous depicting a mean aerodynamic diameter <0.2 μm (data not shown). ROFA

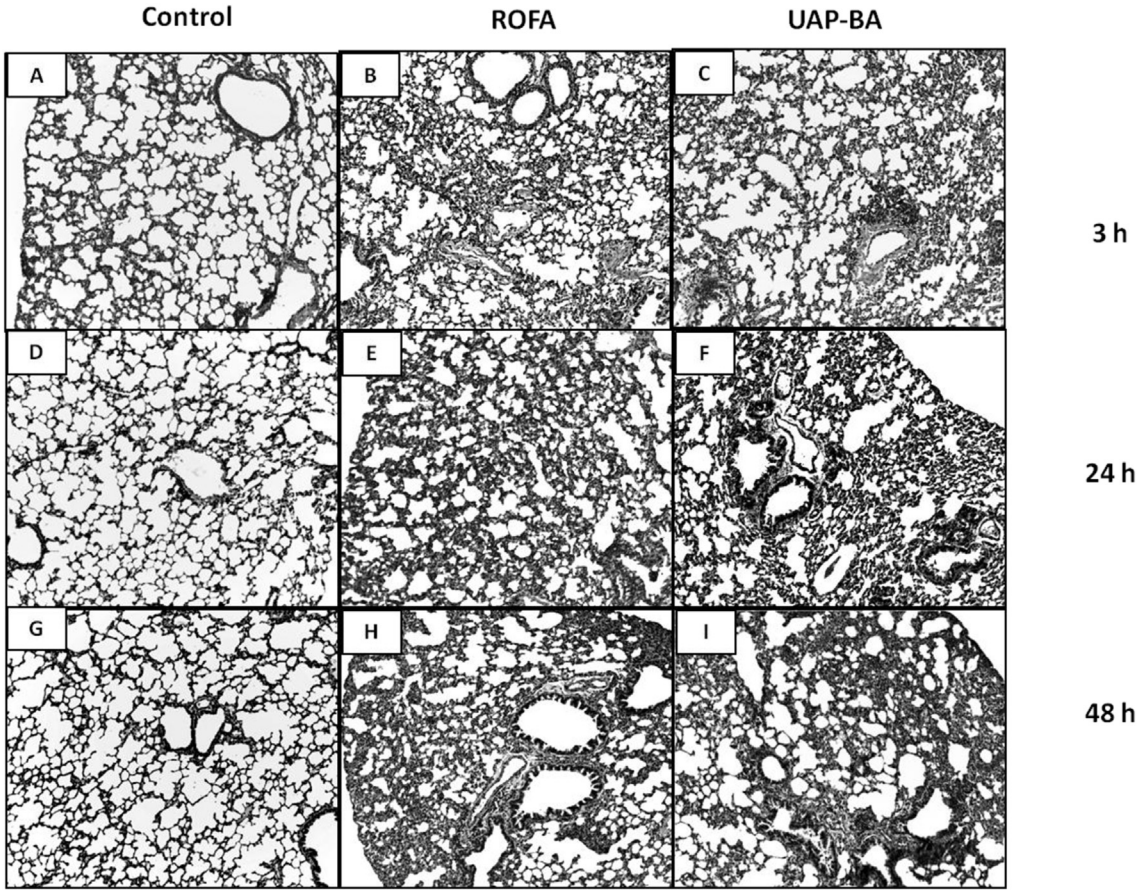

Fig. 2. Lung histology. Microphotographs showing the lower respiratory tract of PBS (A-D-G), ROFA (B-E-H) or UAP-BA (C–F–I) exposed animals. A marked reduction in lung airspace and inflammatory infiltrates are observed both in ROFA and UAP-BA exposed mice through time with respect to controls. Figs. A-B-C; D-E-F and G-H-I represent lung sections at 3, 24 and 48 h post-exposure respectively. Ori. Mag. 100X.

sample, in accordance with Killingsworth et al. (1997) and previous work from our laboratory (Ferraro et al. 2011; Martin et al., 2007; Orona et al., 2014), proved to be heterogeneous depicting in its composition vanadium, nickel and iron metals traces with a particle mean aerodynamic diameter of 2.06 ± 1.57 μm (data not shown).

3.2. Lung and heart histological evaluation

Particle intranasal instillation in mice was found to induce measurable inflammatory response after exposure both in lung and heart. Lung histology corresponding to the lower respiratory tract from PM exposed mice clearly shows differences in the alveolar area (Fig. 2A–I). Both PMs provoked through time a marked reduction in the alveolar airspace due to cell infiltration mainly composed of lymphocytes and polymorphonuclear cells, through the whole parenchyma occurring preferentially around bronchiole and blood vessels. Lungs from ROFA (B-E-H) or UAP-BA (C-F-I) exposed mice showed a time dependent interstitial inflammation. Three hours post-intranasal instillation; focal intracellular infiltration was observed increasing through time, depicting at 24 and 48 h post-instillation a moderate diffuse cell infiltration when compared to non-exposed control mice.

In all PMs exposed groups heart associated histopathological findings were observed only in the right ventricle (RV) (Fig. 3). Control animals hearts showed normal myocardium (grade = 0). On the contrary, after 3 h ROFA exposure, RV epicardium focally showed the presence of reactive mesothelial cells (grade = 0) (Fig. 3B), while the RV of the group exposed to UAP-BA, depicted vascular dilation, inflammatory infiltrate in the epicardium, and interstitial inflammation in subepicardial myocardium composed mainly of lymphocytes (grade = 1) (Fig. 3C). In both exposed groups, either to ROFA or UAP-BA, alterations in the epicardium and subepicardial myocardium were similarly observed at 24 and 48 h (Fig. 3E-F-H-I). Notably, the heart response to UAP-BA was always observed earlier in time than to ROFA.

3.3. Lung BAL total cell number (TCN) and cell differential (CD) after acute PM exposure

Fig. 4A and B shows the temporal kinesis of the lung cellular influx in the bronchoalveolar lavage (BAL) from control and ROFA or UAP-BA acutely exposed middle-aged mice. A significant increase in TCN was found only in the BAL from ROFA exposed mice after 3 h post-exposure. Twenty-four hours later, this increment decreased to control values and was sustained onwards. On the contrary, UAP-BA did not modify TCN at any time point assayed (Fig. 4A). Regarding cell differential (CD), exposed mice either to ROFA or UAP-BA elicited from the earliest time point tested (3 h), different cellular subpopulation distribution in the BAL when compared to controls as shown in Fig. 4B. A rise in the percentage of polymorphonuclear cells (PMN) was seen in all particle-exposed animals. UAP-BA led to a sharp increase at 3 h, which was maintained over time (24 h and 48 h) while ROFA particles induced a increment in the PMN fraction at 3 h reaching a maximum value 24 h post-exposure. To characterize the degree of inflammation at a molecular level, we analyzed TNFα secretion in the BAL fluid. Exposure to UAP-BA or ROFA did not affect TNF-α level in middle-aged mice through time (data not shown).

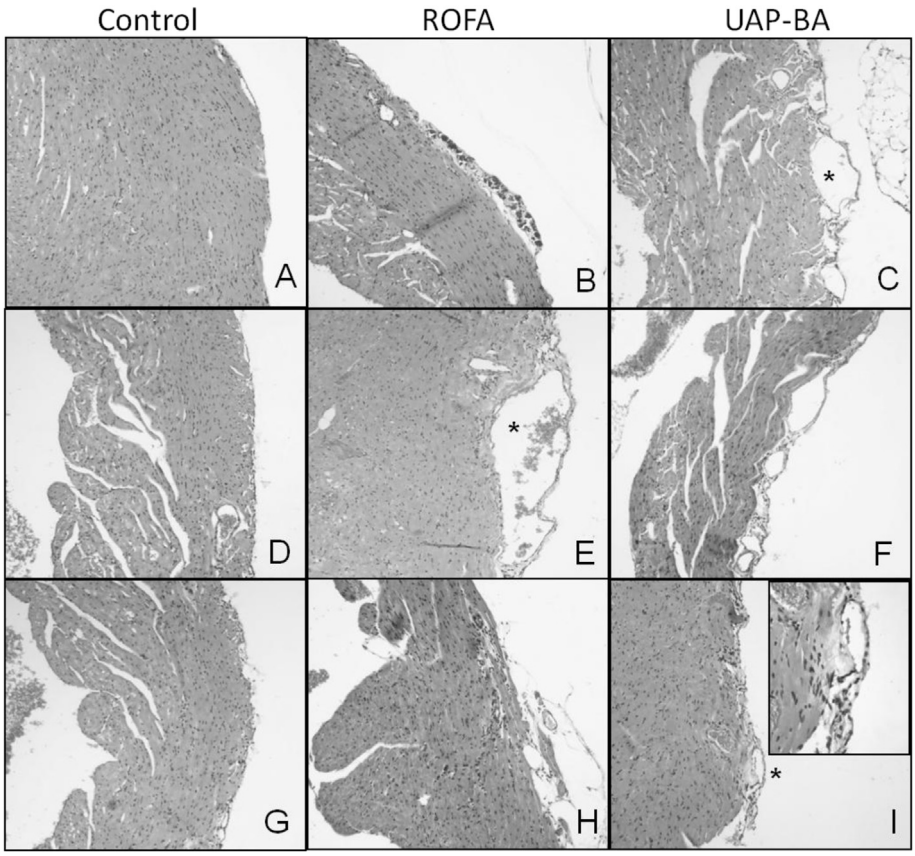

Fig. 3. Heart histology. Microphotographs showing heart histology of PBS (A-D-G), ROFA (B-E-H) or UAP-BA (C–F–I) exposed mice. PM histopathological findings were observed only in the right ventricle (RV). Figures A-B-C; D-E-F and G-H-I represent heart sections at 3, 24 and 48 h post-exposure respectively. Asterisks depict vascular dilation and/or inflammatory infiltration Orig. Mag. 100X.

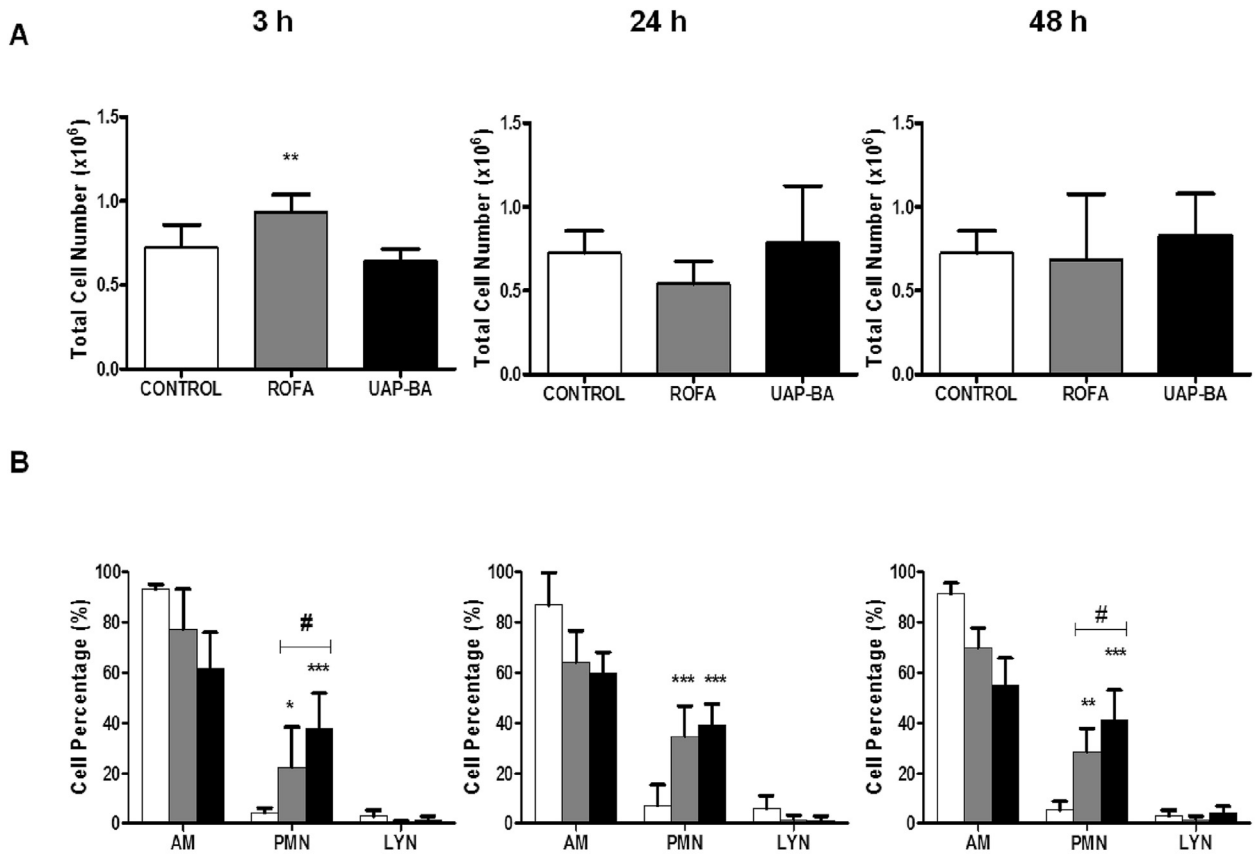

Fig. 4. Total cell number and cell differential in bronchoalveolar lavage. Total Cell Number (A) and Cell Differential (B) in the bronchoalveolar lavage of PBS (white bars), ROFA (grey bars) or UAP-BA (black bars) exposed mice. Results are expressed as media ±SD, *p < 0.05, **P < 0.01, ***p < 0.001 compared with controls and #P < 0.05 between ROFA and UAP-BA.

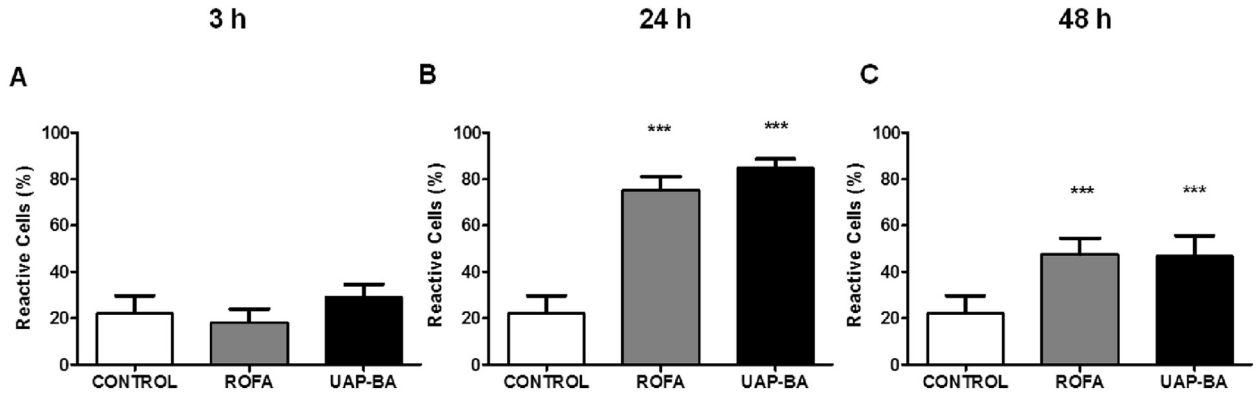

Fig. 5. Superoxide anion generation in PM-exposed BAL cells from aged animals. Superoxide anion generation evaluated as percentage of reactive dark-blue cells, measured by the NBT test in BAL cells from PBS (white bars), ROFA (gray bars) or UAP-BA (black bars) exposed mice. Results are expressed as media ±SD, ***p < 0.001 compared with controls.

3.4. Lung superoxide anion generation after acute PM exposure

Superoxide anion (O_{2^-}) generation in control and PM exposed middle-aged mice through time is shown in Fig. 5. Irrespective of the time point assayed, most cells (80%) from controls were colorless and non-reactive. Nevertheless, a time dependent response for ROFA or UAP-BA exposed animals was observed. At the earlier time studied (3 h), a similar percentage of BAL reactive cells from controls and PM-exposed animals was observed (Fig. 5A). Nevertheless, at 24 and 48 h post-instillation, both PM were able to stimulate a marked and significant ($p < 0.05$) increase in the production of cells with an intense (dark blue, violet) reaction as a result of formazan precipitation (Fig. 5B and C). It is worth to note that the generation of O_{2^-} in the BAL of middle-aged animals exposed to ROFA or UAP-BA showed a similar pattern.

3.5. Lung and heart antioxidant enzyme activity after acute PM exposure

SOD and CAT activities in the heart and lung homogenates from middle-aged animals exposed to ROFA or UAP-BA were evaluated through time at 3, 24 and 48 h post-instillation. The effect of PM

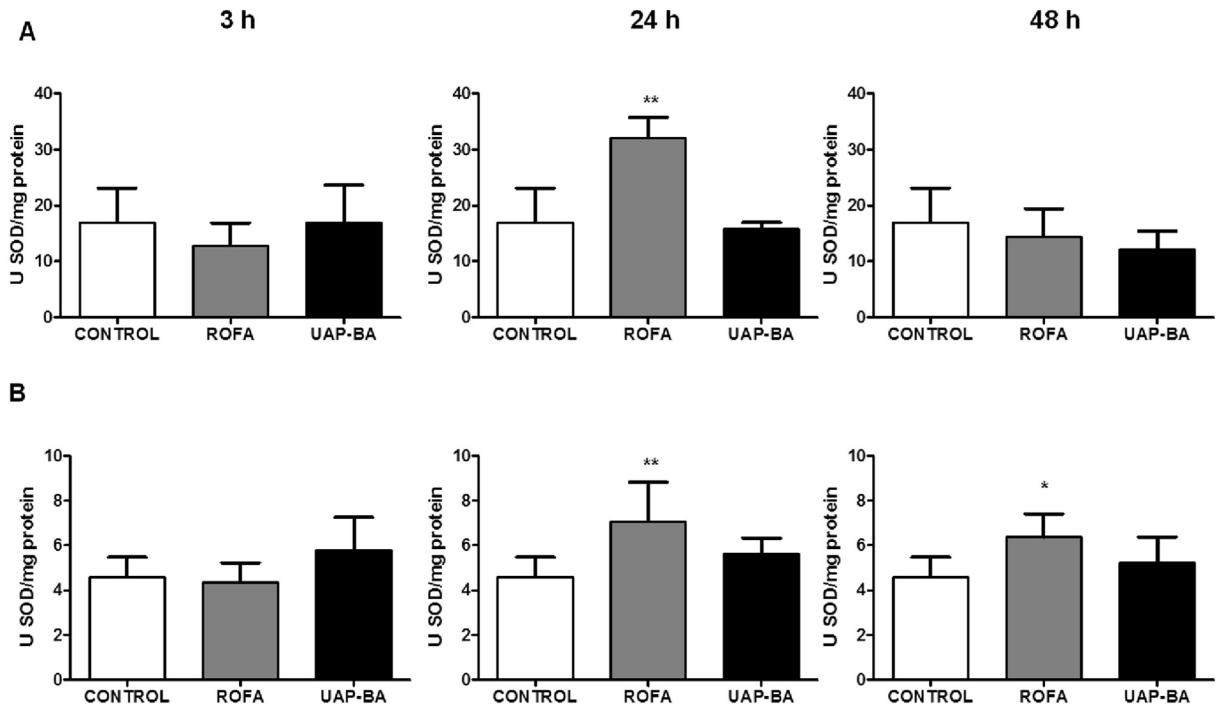

Fig. 6. Antioxidant activities in mice lung and heart homogenates. SOD activity in lung (A) or heart (B) homogenates from middle-aged mice exposed to PBS (white bars), ROFA (gray bars), or UAP-BA (black bars). Results are expressed as mean ± SD. $^{**}p < 0.01$ or $^{*}p < 0.05$ compared with controls.

intranasal instillation on SOD activity is shown in Fig. 6. A significant increase in SOD activity was seen on ROFA-exposed lung homogenates at 24 h compared to the corresponding controls (Fig. 6A, middle panel). On the contrary, UAP-BA exposed mice showed no changes on the antioxidant activity of this enzyme through time (Fig. 6A). SOD activity in heart homogenates significantly increased only in ROFA exposed mice at 24 h and 48 h post-instillation. Similarly to what was observed in the lung, UAP-BA was not able to cause variations in the SOD activity through time (Fig. 6B).

CAT activity remained unchanged in both lung and heart homogenates from either ROFA or UAP-BA exposed middle-aged mice with respect to controls for the range of time point assayed (data not shown).

3.6. Systemic response after acute PM exposure

Serum level of glucose, total bilirubin, triglycerides and total cholesterol in neither ROFA nor UAP-BA exposed mice showed differences with respect to controls (data not show).

The occurrence of systemic inflammation was confirmed by the significant increase in IL-6 in sera at 3 h after intranasal exposure of ROFA ($p < 0.001$) or UAP-BA ($p < 0.01$) compared to control mice (Fig. 7). No increase in IL-6 levels was observed at later time points in UAP-BA compared to their respective controls, while ROFA was able to sustain IL-6 rise up to 24 h ($p < 0.01$).

As seen in Fig. 8, albumin did not change through time neither for ROFA nor UAP-BA exposed mice. Interestingly, at the earlier time-point assayed (3 h) both PM-exposed mice showed a significant increase in total protein content in comparison with PBS-exposed mice. On the contrary, total protein significantly decreased in ROFA-exposed as well as UAP-BA mice at 48 h with respect to controls.

PON activity, an antioxidant enzyme exclusively transported by HDL particles, was found to be significantly reduced 3, 24 and 48 h after intranasal exposure of UAP-BA ($p < 0.05$) in comparison to control mice and to animals exposed to ROFA (Fig. 9A). Interestingly, this decrease cannot be attributed to a reduced level of its carrier given HDL-C levels did not show any statistically significant change (Fig. 9B).

4. Discussion

It is well known that epidemiological associations of air pollution as a risk factor for acute cardiopulmonary events (eg, stroke, myocardial infarction) and chronic morbidity (eg, deep vein thrombosis, atherosclerotic cardiovascular disease) are largely driven by aging and those individuals with cardiorespiratory diseases (Mateen and Brook, 2011). In this context, in Buenos Aires, where the percentage of the aged population is high, UAP-BA adverse impact on health might induce or aggravate physiologic

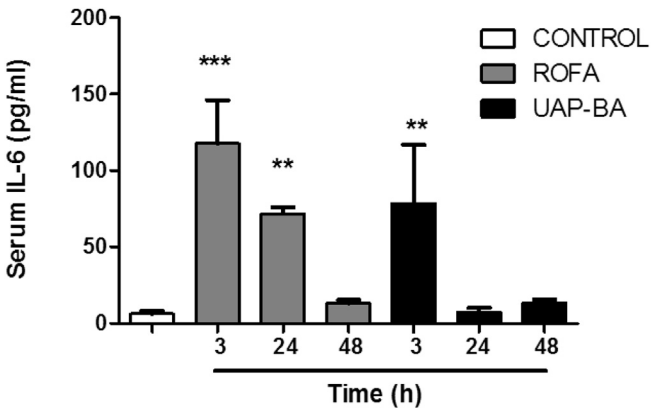

Fig. 7. Proinflamatory IL-6 secretion. Interlukin 6 (IL-6) level in serum from middle-aged mice exposed to PBS (control), ROFA, or UAP-BA at 3, 24 and 48 h post-instillation. Results are expressed as mean ± SD. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared with controls.

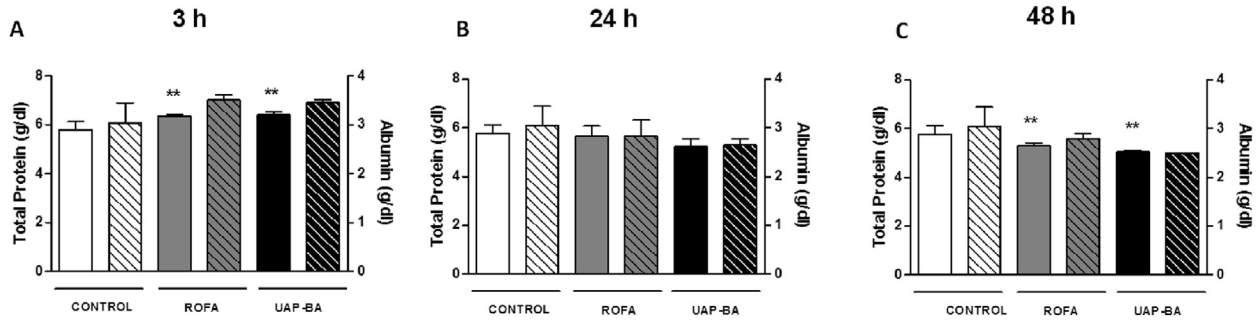

Fig. 8. Total protein and albumin serum concentration. Total protein (□) and albumin (▨) concentrations in serum from middle-aged mice exposed to PBS (white bars), ROFA (gray bars) or UAP-BA (black bars). Results are expressed as mean ± SD. **p < 0.01 compared with controls.

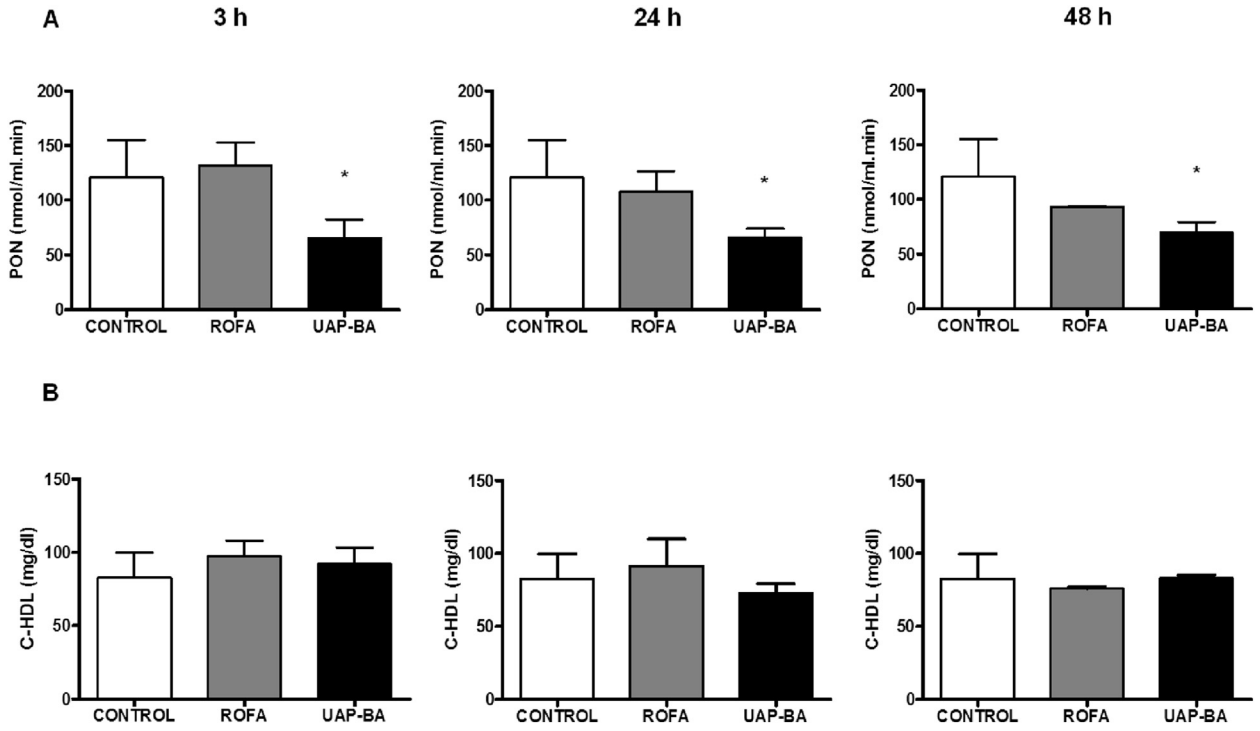

Fig. 9. PON-1 activity and C-HDL serum concentration. PON-1 activity (A) and C-HDL concentration (B) in serum from mice exposed to PBS (white bars), ROFA (gray bars) or UAP-BA (black bars). Results are expressed as mean ± SD. *p < 0.05 compared with controls.

alterations in their cardiorespiratory system. A typical feature that Buenos Aires can share with other megacities is that generally the main source of air pollution is traffic and thus, in the urban area, particle exposure is mostly related to engine combustion derived particles (DEP). In this sense, several authors had previously shown that DEP did not yield significant alterations in markers of inflammation in lung, but did show significant changes in extra-pulmonary tissues (Lund et al., 2009).

One of the advantages of the present study is that we evaluated in ROFA or UAP-BA exposed middle-aged animals a comprehensive set of blood and cardiac biomarkers (total protein, BSA, C-HDL, PON1), local and systemic inflammation (PMN, lymphocytes, IL-6, TNFα), oxidative status (O_2^-, SOD, CAT) and pulmonary and cardiac toxicity (histology) for two distinct morphochemical air pollution particles. Herein, we found that UAP-BA, emitted mainly by vehicular emissions, induced both local lung inflammation and alters heart morphology and serum biomarkers.

Regarding the lung, we found that only ROFA, at the earlier time point assayed, increased the TCN obtained by BAL. This difference could be due to the presence of trace metals in its composition (Antonini et al., 2004). Still, even when TCN from PM-exposed mice showed no differences with control mice, both ROFA and UAP-BA cell differential showed an altered cell subpopulation distribution. BAL polymorphonuclear (PMN) cell percentage from mice exposed to either PMs increased throughout the experiment in agreement with the cell recruitment observed in the lung histological sections. Moreover, the increased numbers of PMNs and lymphocytes found in lung and heart histological sections support the idea of an ongoing inflammation linked to PM exposure. In keeping with our previous studies performed in young mice (Martin et al., 2007), we found histologically in both organs studied, areas of inflammation that depending on the post-exposure time, were seen as focused (3 h) or diffuse (24 and 48 h). It has to be remarked that the signs indicative of an inflammatory process appeared always earlier in UAP-BA than in ROFA-exposed animals. This earlier response could be due to the different morphochemical characteristics of these two environmental particles. Considering their size and shape, we have previously found that Buenos Aires particles are spherical and

ultrafine (Martin et al., 2007). Therefore, they can not only exert their local adverse effect in the lung parenchyma, but can translocate quicker than ROFA to the vascular system, reaching the heart where, in turn, they can induce inflammation (Brook et al., 2004; Brook, 2008). Regarding their chemical composition, we have also previously characterized UAP-BA as a carbon core particle adsorbed by PAHs (Poly Aromatic Hydrocarbons) and PCBs (Poly Chlorinated Biphenyls), two organic compounds declared by IARC as carcinogenic for humans (IARC). These hazardous substances could cause a wide range of health effects including inflammation, necrosis and cancer. Even more, sera from both PM-exposed mice showed changes in total protein and a raise in IL-6 proinflammatory cytokine through time. Our observations are in agreement with Ghio and Devlin (2001) and Riva et al. (2011) who informed that PM increases inflammatory cytokines, raising the systemic and local inflammatory response characterized by PMN cells and mononuclear-macrophages in the lung. In fact, pulmonary pro-inflammatory mediators secreted by activated alveolar macrophages can be involved in the recruitment of other leukocytes modifying lung histoarchitecture (Larsson et al., 2007).

It is well known that PM can cause oxidative stress by inducing a local inflammatory response in the lungs, with the potential to upregulate systemic inflammatory processes that can lead to an organ failure (Rückerl et al., 2014). Therefore, we sought to evaluate the generation of the superoxide anion (O_2^-) in the BAL cells and antioxidant enzymes activities, SOD and CAT, in lung and heart homogenates. Both ROFA and UAP-BA exposure caused similar response in the lung depicting a rise in the lung O_2^- generation. However, the antioxidant enzyme SOD revealed a distinct behavior for each MP: only ROFA particles, containing transition metals, were capable of modulate SOD activity showing a clear adaptive response. On the contrary, no response was observed when middle-aged mice were exposed to UAP-BA.

Antioxidant enzymes are also present in plasma circulation both free and associated to lipoproteins. Among the latter, PON, which is exclusively transported by HDL fraction, seems to be of particular relevance because it confers most of the antioxidant capacity to its carrier (Navab et al., 1998). In the present study, PON activity resulted to be significantly reduced after UAP-BA exposure at all times assayed. Given that HDL levels remained unchanged after exposure, the changes in PON activity seem to be consequence of a direct effect of the PM. Then, HDL particles with deficient antioxidant capacity would not be able to protect low density lipoproteins (LDL) from oxidative damage, which may be considered one of the earliest steps in atherogenesis, becoming also more susceptible to undergo oxidation (Lüscher et al., 2014). Modified HDL, such as oxidized ones, are known to be less protective due to the loss of many direct actions on numerous cell types that influence cardiovascular and metabolic health.

Rizzo et al. (2014) recently described lipid reshaping in lung and extra-pulmonary tissues in young BALB/c mice after exposure to Milan PM. These changes occurred 24 h after a third instillation of 100 μg of PM (instillation was performed on days 0, 3, and 6). In our study using a single instillation of approximately 25–30 μg of UAP-BA (mice weight ~25–30 g) we observed histological changes within 3–24 h. Although the differences could be attributed to the nature of the particle (Milan PM vs UAP-BA) it also suggests that middle-aged animals may have increased susceptibility to extra-pulmonary effects of PM when compared to young animals and partially explain the epidemiological association between air pollution and cardiovalscular disease observed in aged individuals. Our results support the hypothesis that air PM, irrespective of its source of emission and/or its morphochemical characteristics, alters cardiopulmonary function in aged individuals. In particular, our study provides new insight of the local and systemic inflammatory response of the cardiorespiratory system from middle-aged animals exposed to UAP-BA particles.

Funding sources

This work was partially supported by Grants from the National University of San Martin A147 and SJ10/54 and by the PICT 2010-1661 from the National Agency for the Promotion of Science and Technology, Argentina.

Acknowledgments

The authors specially thank Ms. Mariela Lacave and Dr. Patricia Mandalunis for their technical expertise on the histological study and Alejandro Perez de la Hoz for his assistance with the collector sampler.

References

Abrutzky, R., Dawidowski, L., Matus, P., Lankao, R., 2012. Health effects of climate and air pollution in Buenos Aires: a first time series analysis. J. Environ. Prot. 3, 262–271.

Analitis, A., Katsouyanni, K., Dimakopoulou, K., Samoli, E., Nikoloulopoulos, A.K., Petasakis, Y., Touloumi, G., Schwartz, J., Anderson, H.R., Cambra, K., Forastiere, F., Zmirou, D., Vonk, J.M., Clancy, L., Kriz, B., Bobvos, J., Pekkanen, J., 2006. Short-term effects of ambient particles on cardiovascular and respiratory mortality. Epidemiology 17 (2), 230–233.

Antonini, J.M., Taylor, M.D., Leonard, S.S., Lawryk, N.J., Shi, X., Clarke, R.W., Roberts, J.R., 2004. Metal composition and solubility determine lung toxicity induced by residual oil fly ash collected from different sites within a power plant. Mol. Cell Biochem. 255 (12), 257–265.

Baldauf, R.W., Lane, D.D., Marote, G.A., 2001. Ambient air quality monitoring network design for assessing human health impacts from exposures to airborne contaminants. Environ. Monit. Assess. 66 (1), 63–76.

Brook, R.D., 2008. Cardiovascular effects of air pollution. Clin. Sci. Lond 115 (6), 175–187.

Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith Jr., S.C., Tager, I., 2004. Air pollution and cardiovascular disease: a statement for healthcare professionals from the expert panel on population and prevention science of the American heart association. Circulation 109 (21), 2655–2671.

Brunekreef, B., Forsberg, B., 2005. Epidemiological evidence of effects of coarse airborne particles on health. Eur. Respir. J. 26 (2), 309–318.

Delfino, R.J., Staimer, N., Vaziri, N.D., 2001. Air pollution and circulating biomarkers of oxidative stress. Air Qual. Atmos. Health 4 (1), 37–52.

Dockery, D.W., Pope 3rd, C.A., Xu, X., Spengler, J.D., Ware, J.H., Fay, M.E., Ferris Jr., B.G., Speizer, F.E., 1993. An association between air pollution and mortality in six U.S. cities. N. Engl. J. Med. 329 (24), 1753–1759.

Dye, J.A., Lehmann, J.R., McGee, J.K., Winsett, D.W., Ledbetter, A.D., Everitt, J.I., Ghio, A.J., Costa, D.L., 2001. Acute pulmonary toxicity of particulate matter filter extracts in rats: coherence with epidemiologic studies in Utah valley residents. Environ. Health Perspect. 109 (3), 395–403.

Evelson, P., Travacio, M., Repetto, M., Escobar, J., Llesuy, S., Lissi, E.A., 2001. Evaluation of total reactive antioxidant potential (TRAP) of tissue homogenates and their cytosols. Arch. Biochem. Biophys. 388 (2), 261–266.

Ferraro, S.A., Yakisich, J.S., Gallo, F.T., Tasat, D.R., 2011 Dec. Simvastatin pretreatment prevents ambient particle-induced lung injury in mice. Inhal. Toxicol. 23 (14), 889–896. http://dx.doi.org/10.3109/08958378.2011.623195.

Furlong, C.E., Richter, R.J., Seidel, S.L., Costa, L.G., Motulsky, A.G., 1989. Spectrophotometric assays for the enzymatic hydrolysis of the active metabolites of chlorpyrifos and parathion by plasma paraoxonase/arylesterase. Anal. Biochem. 180, 242–247.

Ghio, A.J., Devlin, R.B., 2001. Inflammatory lung injury after bronchial instillation of air pollution particles. Am. J. Respir. Crit. Care Med. 164 (4), 704–708.

Herman, E., Zhang, J., Chadwick, P., Ferrans, V., 2000. Comparison of the protective effects of amifostine and dexrazoxane against the toxicity of doxorubicin in spontaneously hypertensive rats. Cancer Chemother. Pharmacol. 45 (4), 329–334.

IARC. http://monographs.iarc.fr/ENG/Classification/. (accessed November, 2014).

Killingsworth, C.K., Alessandrini, F., Krishna Murty, G.C., Catalano, P.J., Paulauskis, J.D., Godleski, J.J., 1997. Inflammation, chemokine expression, and death in monocrotaline-treated rats following fuel coal ash inhalation. Inhal. Toxicol 9, 541–545.

Larsson, B.M., Sehlstedt, M., Grunewald, J., Sköld, C.M., Lundin, A., Blomberg, A., Sandström, T., Eklund, A., Svartengren, M., 2007. Road tunnel air pollution induces bronchoalveolar inflammation in healthy subjects. Eur. Respir. J. 29 (4), 699–705.

Leong, B.K., Coombs, J.K., Sabaitis, C.P., Rop, D.A., Aaron, C.S., 1998. Quantitative

morphometric analysis of pulmonary deposition of aerosol particles inhaled via intratracheal nebulization, intratracheal instillation or nose-only inhalation in rats. J. Appl. Toxicol. 18 (2), 149–160.
Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193 (1), 265–275.
Lund, A.K., Lucero, J., Lucas, S., Madden, M.C., McDonald, J.D., Seagrave, J.C., Knuckles, T.L., Campen, M.J., 2009. Vehicular emissions induce vascular MMP-9 expression and activity via endothelin-1 mediated pathways. Arterioscler. Thromb. Vasc. Biol. 29, 511–517.
Lüscher, T.F., Landmesser, U., von Eckardstein, A., Fogelman, A.M., 2014. High-density lipoprotein: vascular protective effects, dysfunction, and potential as therapeutic target. Circ. Res. 114 (1), 171–182.
Martin, S., Dawidowski, L., Mandalunis, P., Cereceda-Balic, F., Tasat, D.R., 2007. Characterization and biological effect of Buenos Aires urban air particles on mice lungs. Environ. Res. 105 (3), 340–349.
Mateen, F.J., Brook, R.D., 2011. Air pollution as an emerging global risk factor for stroke. JAMA 305 (12), 1240–1241.
Misra, H.P., Fridovich, I., 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247, 3170–3175.
National Census, 2010 (accesed November, 2014). http://www.censo2010.indec.gov.ar/archivos/censo2010_tomo1.pdf.
Navab, M., Hama, S.Y., Hough, G.P., Hedrick, C.C., Sorenson, R., La Du, B.N., Kobashigawa, J.A., Fonarow, G.C., Berliner, J.A., Laks, H., Fogelman, A.M., 1998. High density associated enzymes: their role in vascular biology. Curr. Opin. Lipidol. 9 (5), 449–456.
Nurkiewicz, T.R., Porter, D.W., Barger, M., Millecchia, L., Rao, K.M., Marvar, P.J., Hubbs, A.F., Castranova, V., Boegehold, M.A., 2006. Systemic microvascular dysfunction and inflammation after pulmonary particulate matter exposure. Environ. Health Perspect. 114 (3), 412–419.
Orona, N.S., Astort, F., Maglione, G.A., Saldiva, P.H., Yakisich, J.S., Tasat, D.R., 2014. Direct and indirect air particle cytotoxicity in human alveolar epithelial cells. Toxicol Vitro 28 (5), 796–802.
Pope 3rd, C.A., 2000. Epidemiology of fine particulate air pollution and human health: biologic mechanisms and who's at risk? Environ. Health Perspect. 108 (4), 713–723.
Rich, D.Q., Kipen, H.M., Huang, W., Wang, G., Wang, Y., Zhu, P., Ohman-Strickland, P., Hu, M., Philipp, C., Diehl, S.R., Lu, S.E., Tong, J., Gong, J., Thomas, D., Zhu, T., Zhang, J.J., 2012. Association between changes in air pollution levels during the Beijing olympics and biomarkers of inflammation and thrombosis in healthy young adults. JAMA 307 (19), 2068–2078.
Riva, D.R., Magalhães, C.B., Lopes, A.A., Lanças, T., Mauad, T., Malm, O., Valença, S.S., Saldiva, P.H., Faffe, D.S., Zin, W.A., 2011. Low dose of fine particulate matter (PM2.5) can induce acute oxidative stress, inflammation and pulmonary impairment in healthy mice. Inhal. Toxicol. 23 (5), 257–267.
Rizzo, A.M., Corsetto, P.A., Farina, F., Montorfano, G., Pani, G., Battaglia, C., Sancini, G., Palestini, P., 2014. Repeated intratracheal instillation of PM10 induces lipid reshaping in lung parenchyma and in extra-pulmonary tissues. PLoS One 9 (9), e106855. http://dx.doi.org/10.1371/journal.pone.0106855 eCollection 2014.
Rückerl, R., Hampel, R., Breitner, S., Cyrys, J., Kraus, U., Carter, J., Dailey, L., Devlin, R.B., Diaz-Sanchez, D., Koenig, W., Phipps, R., Silbajoris, R., Soentgen, J., Soukup, J., Peters, A., Schneider, A., 2014. Associations between ambient air pollution and blood markers of inflammation and coagulation/fibrinolysis in susceptible populations. Environ. Int. 70, 32–49.
Saldiva, P.H., Pope 3rd, C.A., Schwartz, J., Dockery, D.W., Lichtenfels, A.J., Salge, J.M., Barone, I., Bohm, G.M., 1995. Air pollution and mortality in elderly people: a time-series study in Sao Paulo, Brazil. Arch. Environ. Health 50 (2), 159–163.
Samet, J.M., Zeger, S.L., Dominici, F., Curriero, F., Coursac, I., Dockery, D.W., Schwartz, J., Zanobetti, A., 2000. The national morbidity, mortality, and air pollution study. Part II: morbidity and mortality from air pollution in the United States. Res. Rep. Health Eff. Inst. 94 (Pt 2), 5–70 discussion 71–9.
Schwartz, J., Wypij, D., Dockery, D., Ware, J., Zeger, S., Spengler, J., Ferris Jr., B., 1991. Daily diaries of respiratory symptoms and air pollution: methodological issues and results. Environ. Health Perspect. 90, 181–187.
Segal, A.W., 1974. Nitroblue-tetrazolium tests. Lancet 2 (7891), 1248–1252.
Southam, D.S., Dolovich, M., O'Byrne, P.M., Inman, M.D., 2002. Distribution of intranasal instillations in mice: effects of volume, time, body position, and anesthesia. Am. J. Physiol. Lung Cell Mol. Physiol. 282 (4), L833–L839.
Tasat, D.R., de Rey, B.M., 1987. Cytotoxic effect of uranium dioxide on rat alveolar macrophages. Environ. Res. 44 (1), 71–81.
Warnick, G.R., Benderson, J., Albers, J.J., 1982. Dextran sulfate-Mg2+ precipitation procedure for quantitation of high-density-lipoprotein cholesterol. Clin. Chem. 28, 1379–1388.
WHO (World Health Organization), 2014. Ambient (Outdoor) Air Quality and Health. Available: http://www.who.int/mediacentre/factsheets/fs313/en/ (accessed November 2014).