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Time course of systemic oxidative stress and inflammatory response induced by an acute exposure to Residual Oil Fly Ash

T. Marchini [a], N.D. Magnani [a], M.L. Paz [b], V. Vanasco [a], D. Tasat [c], D.H. González Maglio [b], S. Alvarez [a], P.A. Evelson [a,*]

[a] Cátedra de Química General e Inorgánica, Instituto de Bioquímica y Medicina Molecular (IBIMOL UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, C1113AAB Buenos Aires, Argentina
[b] Cátedra de Inmunología, Instituto de Estudios de la Inmunidad Humoral (IDEHU UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, C1113AAB Buenos Aires, Argentina
[c] CESyMA, Facultad de Ciencia Tecnología, Universidad Nacional de General San Martín, Martín de Irigoyen 3100, 1650 San Martín, Buenos Aires, Argentina

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ABSTRACT

It is suggested that systemic oxidative stress and inflammation play a central role in the onset and progression of cardiovascular diseases associated with the exposure to particulate matter (PM). The aim of this work was to evaluate the time changes of systemic markers of oxidative stress and inflammation, after an acute exposure to Residual Oil Fly Ash (ROFA). Female Swiss mice were intranasally instilled with a ROFA suspension (1.0 mg/kg body weight) or saline solution, and plasma levels of oxidative damage markers [thiobarbituric acid reactive substances (TBARSs) and protein carbonyls], antioxidant status [reduced (GSH) and oxidized (GSSG) glutathione, ascorbic acid levels, and superoxide dismutase (SOD) activity], cytokines levels, and intravascular leukocyte activation were evaluated after 1, 3 or 5 h of exposure. Oxidative damage to lipids and decreased GSH/GSSG ratio were observed in ROFA-exposed mice as early as 1 h. Afterwards, increased protein oxidation, decreased ascorbic acid content and SOD activity were found in this group at 3 h. The onset of an adaptive response was observed at 5 h after the ROFA exposure, as indicated by decreased TBARS plasma content and increased SOD activity. The observed increase in oxidative damage to plasma macromolecules, together with systemic antioxidants depletion, may be a consequence of a systemic inflammatory response triggered by the ROFA exposure, since increased TNF-α and IL-6 plasma levels and polymorphonuclear leukocytes activation was found at every evaluated time point. These findings contribute to the understanding of the increase in cardiovascular morbidity and mortality, in association with environmental PM inhalation.

Introduction

Epidemiological studies have shown that an acute exposure to environmental particulate matter (PM) is associated with increased cardiopulmonary mortality rates (Analitis et al., 2006). Daily changes in PM concentration have also been positively correlated with increased hospitalizations due to lower respiratory diseases, ischemic cardiovascular events, arrhythmias, and heart failure (Dominici et al., 2006). In this context, oxidative stress and inflammation have been suggested to play a predominant role in cardiopulmonary PM toxicity (Brook et al., 2010; Gurgueira et al., 2002).

Oxidative stress can be defined as an increase, over physiological values, in the steady-state concentrations of reactive oxygen species (ROS). This situation may lead to changes in the levels of antioxidant defenses, which can be increased as an adaptive response, or depleted due to the action of oxidants (Sies, 1985). Systemic oxidative stress can occur following PM inhalation, leading to oxidative damage to plasma macromolecules, antioxidants depletion and inflammation. Indeed, several studies in humans have found elevated oxidative stress markers and increased circulating proinflammatory mediators in association with environmental PM levels (Brook et al., 2010). Personal exposure to ambient PM concentrations closely paralleled with increased plasma markers of protein oxidation and lipid peroxidation (Sørensen et al., 2003), and of DNA damage (Bräuner et al., 2007). Moreover, short-term PM inhalation positively correlates with increased white blood cell counts, IL-6, and TNF-α levels (Calderón-Garcidueñas et al., 2008; Riediker, 2007). Acute-phase proteins such as C-reactive protein and fibrinogen are also increased in plasma, in association with day-to-day variations in PM (Chuang et al., 2007). Interestingly, the presence of soluble transition metals as PM constituents seems to enhance the

Abbreviations: DAF-2, 4,5-diaminofluorescein; DCF, 2′,7′-dichlorofluorescein; PM, particulate matter; ROS, reactive oxygen species; ROFA, Residual Oil Fly Ash; SOD, superoxide dismutase.

* Corresponding author at: Instituto de Bioquímica y Medicina Molecular (IBIMOL UBA-CONICET), Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 954, C1113AAB Buenos Aires, Argentina. Tel.: +54 011 4964-8249; fax: +54 011 4508-3646.
E-mail address: pevelson@ffyb.uba.ar (P.A. Evelson).

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inflammatory response triggered by PM, due to increased production of ROS via Fenton-like chemical reactions (Chen and Lippmann, 2009).

Airborne PM varies in size, chemical composition and sources of origin. Anthropogenic emissions are the main contributors to the environmental PM burden, and consist mainly of motor vehicle emissions and fossil fuel combustion during power generation and industrial processes (Nel, 2005). The inorganic residue that remains after the incomplete oxidation of such carbonaceous materials contributes to PM in urban air and is termed Residual Oil Fly Ash (ROFA) (Ghio et al., 2002a). Diverse PM surrogates have been assayed in different animal models in order to study the biological effects of PM exposure. Among them, ROFA has been particularly useful given that it is especially rich in soluble transition metals (namely iron, nickel and vanadium), and because of its low concentration of organic compounds (Schroeder et al., 1987). Hence, ROFA is the most frequently used combustion-derived particle in order to evaluate the contribution of such metals on air pollution toxicity (Chen and Lippmann, 2009). Moreover, ROFA particles often present an aerodynamic diameter ≤ 2.5 μm ($PM_{2.5}$), a size that has been shown to be more closely associated with PM adverse health effects than coarser particles ($PM_{10-2.5}$) (Brook et al., 2010).

We have previously reported that the NADPH oxidase homolog Nox2 is activated in mice lung after an acute ROFA exposure (Magnani et al., 2013). This condition contributes to the onset of local oxidative damage, together with direct oxidant production from transition metals coated on ROFA surface, and with an altered mitochondrial function. Moreover, impaired cardiac mitochondrial function and contractile reserve were also observed in the same animal model (Marchini et al., 2013). Of note, the most significant imbalance in lung oxidative metabolism required a shorter exposure time (1 h), in comparison with the time needed to observe those alterations in heart (3 h).

Consistently with our findings, alveolar macrophage activation (Nurkiewicz et al., 2004) and increased macrophage inflammatory protein (MIP)-2 and TNF-α levels (Ferraro et al., 2011; Ghio et al., 2002b) were observed in bronchoalveolar lavages from ROFA-exposed rodents. Increased leukocyte adhesion and rolling, and microvascular oxidative stress have been also reported to be triggered by ROFA inhalation (Nurkiewicz et al., 2006). Interestingly, extracellular superoxide dismutase (SOD) overexpression decreased both lung inflammation and oxidative damage in mice exposed to ROFA (Ghio et al., 2002b). This scenario was also attenuated in IL-$6^{-/-}$ knockout mice (Fujimaki et al., 2006), as well as in toll-like receptor 4 and Nox2 deficient mice (Kampfrath et al., 2011). These findings emphasize the tight link between PM-associated oxidative stress and inflammation in lung, which can in turn trigger a systemic response leading to the observed cardiovascular effects. However, a comprehensive analysis of the time course of the changes in plasma markers of oxidative stress and inflammation, and of the link between the systemic inflammatory response and oxidative stress triggered after an acute ROFA exposure, has not yet been performed.

Taking into account that oxidative stress and inflammation play a predominant role in cardiopulmonary PM toxicity, and the observed lag phase between the onset of pulmonary and cardiac effects, the aim of this work was to evaluate the time changes of systemic markers of oxidative stress and inflammation, in a mouse model of acute exposure to ROFA. The obtained findings could give new insights to the understanding of the biochemical basis of the observed PM-associated adverse health effects.

Methods

Drugs and chemicals

All chemicals were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, US), except HCl, H_2SO_4 and organic solvents which were purchased from Merck KGaA (Darmstadt, Germany), and 2′,7′-dichlorofluorescein diacetate and 4,5-diaminofluorescein diacetate which were provided by Molecular Probes (Eugene, OR, US).

Experimental model

ROFA suspension. ROFA particles were collected from Boston Edison Co., Mystic Power Plant, Mystic, CT, US and were kindly provided by Dr. J. Godleski (Harvard School of Public Health, MA, US) (Killingsworth et al., 1997). ROFA samples from this source have been previously characterized in terms of elemental composition and particle size. Vanadium, nickel and iron are the predominant metals present as water-soluble sulfates, and particle mean aerodynamic diameter is 2.06 ± 1.57 μm (Ostachuk et al., 2008). PM samples were freshly prepared by suspending ROFA particles in sterile saline solution (0.5 mg/mL), followed by a 10 min incubation in an ultrasonic water bath before use.

Animal exposure. Female Swiss mice weighting 20–25 g were anesthetized by an intraperitoneal injection of ketamine (10 mg/kg) and xylazine (0.1 mg/kg), and exposed to ROFA particles (1.0 mg/kg body weight) or saline solution (control group) in a single dose by intranasal instillation. Mice were immobilized in a 60° inclined supine position, while 50 μL of the ROFA suspension was delivered dropwise to the nares by the use of an automatic pipette. After 1, 3 or 5 h of exposure, animals were euthanized and blood samples were collected. Control mice were handled in parallel, instilled with 50 μL of sterile saline solution, and euthanized at the same time points. 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 (Southam et al., 2002). An exposure time of up to 5 h was chosen in order to focus on ROFA-associated short-term effects, and based on previous reports (Marchini et al., 2013). The selected dose falls within the range of concentrations consistently used in several animal studies (Ghio et al., 2002b; Gurgueira et al., 2002; Nurkiewicz et al., 2006, 2004), and would correspond to a weekly human exposure to certain highly polluted environments, such as atmospheric temperature inversions, peak hourly PM levels within certain megalopolis, and occupational PM levels of exposure. Animal treatment was carried following the 6344/96 regulation of the Argentinean National Drug, Food and Medical Technology Administration (ANMAT) guidelines.

Blood samples. Blood samples were obtained by cardiac puncture using heparin as anticoagulant. A 10 min centrifugation at 600 g and 4 °C was carried out in a Thermo Scientific CL31R Multispeed Centrifuge (Thermo Fisher Scientific, Waltham, MA, US) in order to separate the plasma. Samples were kept at −80 °C until use.

Lung homogenates. Lung tissue samples (0.2 g of wet weight) were homogenized in ice-cold 50 mM Tris–HCl, 1 mM EDTA, 0.1% (v/v) Triton X-100, 1 μg/mL pepstatin, 1 μg/mL aprotinin, 1 μg/mL leupeptin, and 0.4 mM PMSE (pH 8.0) with a Potter-Elvehjem glass homogenizer. The obtained suspension was centrifuged at 600 *g* for 10 min at 4 °C to remove nuclei and cell debris. The pellet was discarded and the supernatant was used as tissue homogenate and kept at −80 °C until use.

Protein content. Protein concentration of plasma samples and lung homogenates was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Oxidative damage markers

Thiobarbituric acid reactive substances (TBARS) assay. Oxidative damage to plasma lipids was determined as thiobarbituric acid reactive substances (TBARSs), using a fluorometric assay (Yagi, 1976). Briefly, plasma samples (25 μL) were incubated with 200 μL of 0.1 N HCl, 30 μL of 10% (w/v) phosphotungstic acid, and 100 μL of 0.7% (w/v) 2-