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Respiratory toxicity of repeated exposure to particles produced by traffic and sugar cane burning

Flavia Mazzoli-Rocha[a], Giovanna M.C. Carvalho[a], Manuella Lanzetti[b], Samuel S. Valença[c], Luiz F.F. Silva[d], Paulo H.N. Saldiva[d], Walter A. Zin[a,*], Débora S. Faffe[a]

[a] Laboratory of Respiration Physiology, Carlos Chagas Filho Institute of Biophysics, Universidade Federal do Rio de Janeiro, São Paulo, Brazil
[b] Laboratory of Inflammation, Fundação Oswaldo Cruz, São Paulo, Brazil
[c] Laboratory of Inflammation, Institute of Biomedical Sciences, Universidade Federal do Rio de Janeiro, São Paulo, Brazil
[d] Laboratory of Experimental Air Pollution, Department of Pathology, School of Medicine, Universidade de São Paulo, São Paulo, Brazil

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ABSTRACT

We compared the toxicity of subchronic exposure to equivalent masses of particles from sugar cane burning and traffic. BALB/c mice received 3 intranasal instillations/week during 1, 2 or 4 weeks of either distilled water (C1, C2, C4) or particles (15 μg) from traffic (UP1, UP2, UP4) or biomass burning (BP1, BP2, BP4). Lung mechanics, histology and oxidative stress were analyzed 24 h after the last instillation. In all instances UP and BP groups presented worse pulmonary elastance, airway and tissue resistance, alveolar collapse, bronchoconstriction and macrophage influx into the lungs than controls. UP4, BP2 and BP4 presented more alveolar collapse than UP1 and BP1, respectively. UP and BP had worse bronchial and alveolar lesion scores than their controls; BP4 had greater bronchial lesion scores than UP4. Catalase was higher in UP4 and BP4 than in C4. In conclusion, biomass particles were more toxic than those from traffic after repeated exposures.

1. Introduction

Urban-derived particles are known to cause adverse effects on human health (Schwartz and Dockery, 1992; Hoek and Brunekreef, 1994; Saldiva et al., 1994; Pope et al., 1995; Laden et al., 2000; Braga et al., 2001; Schwartz et al., 2001; Avila et al., 2011; Riva et al., 2011). Unfortunately, the outcomes of the exposure to biomass-derived particles have not yet been studied as extensively as those owing to urban pollution (Long et al., 1998; Phonboon et al., 1999; Arbex et al., 2000; Ribeiro, 2008).

Alcohol participation in the energy matrix has been expanding in Brazil, rising from 6.8% in 1978 to 13.5% in 2004 (Ribeiro, 2008). In Brazil, only 25% of sugar cane harvesting is mechanized and the rest is manually cut and undergoes pre-harvest burning (Ribeiro, 2008), contributing to the deterioration of air quality in the cities located close to sugar cane plantations (Cançado et al., 2006; Arbex et al., 2007). Epidemiologic studies demonstrated a relationship between biomass burning and increased frequency of visits to emergency rooms (Lipsett et al., 1997; Long et al., 1998; Arbex et al., 2000, 2007; Cançado et al., 2006; Rigueira et al., 2011).

In a previous study we demonstrated that a single nasal instillation of sugar cane burning-derived particles caused deleterious changes in lung mechanics and histology. The findings were comparable to those resulting from exposure to an equivalent mass of traffic-derived particles, indicating that biomass-derived particles were at least as toxic as those produced by traffic (Mazzoli-Rocha et al., 2008). However, the outcomes of long-term exposures still deserve some attention. Hence, we aimed at evaluating whether the duration of repeated exposures to urban- and biomass-derived particles may lead to a time-dependent worsening of lung functional, morphological and biochemical profiles, and whether the origin of the particulate matter would influence the results.

2. Methods

2.1. Animal preparation

Ninety-nine female BALB/c mice (25–30 g) were randomly divided into 9 groups. In the control group, animals received three intranasal (*i.n.*) instillations of 15 μL of sterile distilled water per week during 1 (C1, $n = 10$), 2 (C2, $n = 10$) or 4 weeks (C4, $n = 10$). In urban- and biomass-derived particles groups, animals received three *i.n.* instillations of 15 μg of particle mass (suspended in 15 μL

* Corresponding author at: Universidade Federal do Rio de Janeiro, Instituto de Biofísica Carlos Chagas Filho – C.C.S., Av. Carlos Chagas Filho 373, Rm G2-042, Ilha do Fundão, 21941-902 Rio de Janeiro, RJ, Brazil. Tel.: +55 21 2562 6557; fax: +55 21 2280 8193.
E-mail addresses: wazin@biof.ufrj.br, walter_zin@hotmail.com (W.A. Zin).

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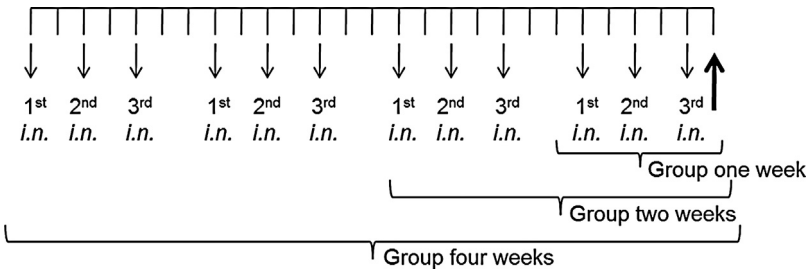

Fig. 1. Timeline of exposure to total particulate matter. Mice received 3 intranasal instillations per week during one, two or four weeks. One-week group underwent instillation when the four-week group was starting the last week of instillation, in order to perform experiments in animals having the same age (8 weeks) and similar weight (25–30 g). *i.n.*, intranasal instillation.

of sterile distilled water) from either an urban area (traffic-derived source, UP) or from sugar cane burning origin (BP) during 1 (UP1, $n = 12$ or BP1, $n = 11$), 2 (UP2, $n = 11$ or BP2, $n = 11$) or 4 weeks (UP4, $n = 12$ or BP4, $n = 12$). All animals were analyzed 24 h after the last instillation (Fig. 1). At the moment of the measurements, all animals had the same age (8 weeks) and similar weight (25–30 g) since the one-week group received the intranasal instillation when the four-week group reached the last week of instillation (Fig. 1). Right before intranasal instillations mice were anesthetized with sevoflurane, and sterile distilled water or suspended particles were gently instilled in their snouts with the aid of a precision pipette. The animals recovered rapidly after instillation.

2.2. Particle sampling and extraction

Particles were collected on fiber glass filters, using a medium volume sampler, which operated at a flow rate of 200 L/min (Handy-Vol, Energetica, Rio de Janeiro, Brazil). Urban-derived particles were sampled in São Paulo (at the intersection of two busy streets), Brazil, while biomass-derived particles were collected in the urban area of Araraquara, Brazil, on sugar cane burning days. Filters were weighed before and after a 24-h collection period and the difference in weight yielded particle mass. During sampling, temperature and relative humidity averaged 24.5 °C and 71.22%, respectively, in São Paulo and 24.6 °C and 43% in Araraquara. Particles were extracted in distilled water by ultrasonication in a water bath during approximately 8 h. Right after that the suspension was separated into small aliquots (approximately 100 μL) that were frozen in 1-mL eppendorf tubes under −20 °C. Before each batch of experiments, a new aliquot was thawed, immediately used and the leftover discarded. A suspension of 15 μg of either biomass or traffic-derived particles in 15 μL of distilled water was instilled in each animal (as previously mentioned in Mazzoli-Rocha et al., 2008) after 15-s ultrasonication.

Part of the material was used for particle granulometry and metal and polycyclic aromatic hydrocarbon analyses. Our previous work, which was done with particulate matter from the same batch, reports the results of these analyses (Mazzoli-Rocha et al., 2008). The former and current studies were done consecutively.

2.3. Pulmonary mechanics

Twenty-four hours after the last distilled water or particle suspension administration the animals were sedated (diazepam, 1 mg *i.p.*), anesthetized (pentobarbital sodium, 20 mg/kg body weight *i.p.*), placed in the supine position on a surgical table, tracheotomized, and a snugly fitting cannula (0.8 mm ID) was introduced into the trachea. The animals were then paralyzed (pancuronium bromide, 0.1 mg/kg), and the anterior chest wall was surgically removed. Hence, airway pressure represents transpulmonary pressure (PL). Lung mechanics was determined as previously described (Mazzoli-Rocha et al., 2008). Briefly, we measured lung resistive ($\Delta P1$) and viscoelastic/inhomogeneous ($\Delta P2$) pressures, static elastance (Est), and viscoelastic component of elastance (ΔE) by the end-inflation occlusion method (Bates et al., 1985, 1988). $\Delta P1$ selectively reflects airway resistance in normal animals and humans and $\Delta P2$ reflects stress relaxation, or viscoelastic properties of the lung, together with a small contribution of time constant inequalities in the lung periphery (Bates et al., 1985; Saldiva et al., 1992).

2.4. Histological study

Heparin (1000 IU) was intravenously injected immediately after the determination of pulmonary mechanics. The trachea was clamped at end-expiration and the animals were euthanized by exsanguination by sectioning of the abdominal aorta and vena cava. The lungs were then removed *en bloc*. The left lung was fixed at end-expiratory lung volume with Millonig's formaldehyde (100 mL HCHO, 900 mL H_2O, 18.6 g NaH_2PO_4, 4.2 g NaOH), routinely prepared for histology, embedded in paraffin, and two 3-μm-thick longitudinal slides were cut and stained with either hematoxylin–eosin, picrosirius or resorcin fuchsin with oxidation for morphometric analyses, and detection of collagen or elastic fibers, respectively.

Morphometric analysis was performed with an integrating eyepiece with a coherent system made of a 100-point and 50-line (1250-μm-long each) grid coupled to a conventional light microscope (Axioplan, Zeiss, Oberkochen, Germany). The fraction areas of collapsed and normal alveoli were determined by the point-counting technique at a magnification of 200× across ten random non-coincident microscopic fields per animal. Points falling on normal or collapsed alveoli were expressed as percentage of points hitting those alveoli (Weibel, 1990).

The bronchoconstriction index (BCI) was determined by counting the number of points that fell onto the airway lumen (NP) and intercepts through the airway wall (NI) using a reticulum and applying the equation: $BCI = NI/\sqrt{NP}$ (Sakae et al., 1994). Only bronchi in which the long diameter did not exceed the short diameter by more than 20% were accepted for measurement.

BCI evaluation was performed at 400× magnification across 5–15 random non-coincident microscopic fields in each animal.

Lung parenchyma and bronchiolar area were analyzed at 100×, 200× and 400× magnifications. The alveoli lesion score varied from 0 to 4, where 0, 1, 2, 3 and 4 indicate no lesions, the presence of parenchymal inflammation, diffuse lesions, parenchymal nodes, and confluent parenchyma nodes, respectively. The bronchiolar lesion score ranged from 0 to 4, where 0, 1, 2, 3 and 4 mean no lesion, focal thickness, bronchiolar node, bronchiolar fibrosis, and bronchiolar obliteration, respectively. The score was attributed considering the worst observed lesion.