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Time-dependency of mice lung recovery after a 4-week exposure to traffic or biomass air pollutants

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ABSTRACT

The time-dependency of lung recovery after 3 intranasal instillations per week during four weeks of distilled water (C groups) or particles (15 μg) from traffic (U groups) or biomass burning (B groups) was observed in BALB/c mice. Lung mechanics [static elastance (Est), viscoelastic component of elastance (ΔE), lung resistive (ΔP1) and viscoelastic/inhomogeneous (ΔP2) pressures] and histology were analyzed 1 (C1, U1, B1), 2 (C2, U2, B2), 7 (C7, U7, B7) or 14 days (C14, U14, B14) after the last instillation. Est, ΔE, ΔP1 and ΔP2 were higher in U1 and B1 than in C1, returning to control values at day 2, except for ΔP1 that normalized after 7 days. Alveolar collapse, bronchoconstriction index and alveolar lesion were larger in U1 and B1 than in C1, however collapse returned to baseline at 7 days, while the others normalized in 2 days. A 4-week exposure to U and B induced lung impairment that resolved 7 days after the last exposure.

1. Introduction

For at least 6 months each year, sugar cane burning is responsible for the generation of large amounts of particles and toxic gases in areas around sugar cane plantation (Arbex et al., 2010). This kind of biomass burning contributes to continuous exposure to air pollution (Cançado et al., 2006; Arbex et al., 2007), differently from the sporadic and short-sustained exposure provoked by forest fires (Duclos et al., 1990; Aditama, 2000; Emmanuel, 2000; Heil and Goldammer, 2001; Kunii et al., 2002; Mott et al., 2005).

Some reports demonstrated public health consequences of biomass burning, including increased emergency room and hospital admissions for inhalation therapy and respiratory symptoms (Long et al., 1998; Cançado et al., 2006; Rigueira et al., 2011); asthma exacerbation (Arbex et al., 2007; Rigueira et al., 2011); hypertension (Arbex et al., 2010); and pneumonia (Arbex et al., 2014). Additionally, experimental studies in mice demonstrated immediate changes in lung mechanics and histology not only after repeated nasal instillations (Mazzoli-Rocha et al., 2014), but also after a single exposure (Mazzoli-Rocha et al., 2008) to sugar cane burning-derived particles.

However, the time required for lung function recovery after repeated exposures to urban and biomass-derived particles remains unclear. Recently, Tsuji et al. (2015) demonstrated that most lung biochemical, histopathological and morphometrical alterations induced by 52 weeks of cigarette smoke exposure were partially restored even after 13 weeks of recovery in a mice model. Hence, the aim of this study was to investigate the time-dependency of lung functional recovery after sub-chronic exposure to urban or biomass particles in BALB/c mice.

2. Methods

2.1. Animal preparation

One hundred and eight female BALB/c mice (8 weeks of age) were randomly divided into 3 major groups: control (C), exposed to either urban area particulate matter (traffic-derived source, U) or sugar cane burning particles (biomass, B). Right before intranasal instillations, mice were anesthetized with sevoflurane via a nasal cone, and sterile distilled water or suspended particles were gen-

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tly instilled in their snouts with the aid of a precision pipette. The animals recovered rapidly after instillation. C animals received 3 intranasal (*i.n.*) instillations of 15 μL of sterile distilled water per week during 4 weeks. In U and B groups, animals received 3 *i.n.* instillations per week of 15 μg of particulate matter (suspended in 15 μL of sterile distilled water) from either an urban area (traffic-derived source) or from sugar cane burning origin (biomass), respectively, during 4 weeks. Animals of the 3 groups were then subdivided according to the time lag between the last 4-week instillation and analyses (temporal group division). Therefore, they were analyzed 1 (C1, n = 4; U1, n = 4; B1, n = 4), 2 (C2, n = 7; U2, n = 11; B2, n = 10), 7 (C7, n = 8; U7, n = 11; B7, n = 12) or 14 days (C14, n = 8; U14, n = 11; B14, n = 12) after the last instillation. During the aforementioned time lag 6 mice died (C1 = 1, U1 = 1, B1 = 1, C2 = 1, B2 = 2), thus yielding a total of one hundred and two mice that provided experimental data. Immediately before the measurements, all animals had similar weights (25–30 g).

2.2. Particle sampling and extraction

Urban- and biomass-derived particles were obtained in São Paulo and Araraquara (both in São Paulo State, Brazil). Particle collection, as well as their granulometry, metal and polycyclic aromatic hydrocarbon contents were previously reported (Mazzoli-Rocha et al., 2008). The present work used particulate matter from the same batch and both studies were done consecutively.

2.3. Pulmonary mechanics

One (C1, U1, B1 groups), two (C2, U2, B2 groups), seven (C7, U7, B7 groups) or fourteen (C14, U14, B14 groups) days after the last instillation of distilled water or particle suspension, 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. A 2-cmH_2O PEEP was applied to the expiratory line of the ventilator (Saldiva et al., 1992) and the animals were then paralyzed (pancuronium bromide, 0.1 mg/kg). A constant-flow ventilator (Samay VR15, Universidad de la Republica, Montevideo, Uruguay) provided artificial ventilation with a frequency of 100 breaths/min, a tidal volume of 0.2 mL, and flow of 1 mL/s. The anterior chest wall was surgically removed and, hence, airway pressure represents transpulmonary pressure. Lung mechanics was determined as previously described (Mazzoli-Rocha et al., 2008). Briefly, we measured lung resistive (ΔP1) and viscoelastic/inhomogeneous (ΔP2) pressures, static elastance (Est), and viscoelastic component of elastance (ΔE) by the end-inflation occlusion method (Bates et al., 1985, 1988). ΔP1 selectively reflects airway resistance in normal animals and humans and ΔP2 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. Lung morphometry

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 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 or picrosirius for morphometric analysis and detection of collagen 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 total points hitting alveoli (Weibel, 1990).

Lung parenchyma area was analyzed at 100×, 200× and 400× magnifications. The alveolar lesion score varied from 0 to 4, indicating the presence of: no lesion (0), parenchymal inflammation (1), diffuse lesions (2), parenchymal nodes (3), and confluent parenchyma nodes (4), respectively. The score was attributed considering the worst observed lesion (Mazzoli-Rocha et al., 2014).

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 relationship between the long diameter did not exceed in 20% the short one were accepted for the measurement. BCI evaluation was performed at 400× magnification across 5–15 random non-coincident microscopic fields in each animal.

Lung parenchyma underwent picrosirius staining to quantify collagen fibers (Montes, 1996) on images captured in a blinded manner across 10 random non-coincident fields (400× magnification). The total tissue area of each field was also computed. The quantification was determined on captured high quality images (2048 × 1536 pixels) using the Image Pro Plus 4.5.1 software (Media Cybernetics, Silver Spring, MD, USA). A single observer blindly performed the morphological measurements. Results are expressed as fiber area/tissue area.

2.5. Statistical analysis

The normality of data distribution (Shapiro-Wilk test) and the homogeneity of variances (Brown-Forsythe test) were firstly tested. A Two-Way ANOVA was used to analyze the effects of different particle exposure and diverse time lags. For all pairwise multiple comparison, we used Holm-Sidak method. The morphometric data, originally expressed as percent, underwent an arcsine transformation. SigmaPlot 11 statistical package (Systat Software, Inc., Chicago, IL, USA) was used. Pearson's correlation test was run to identify associations between functional and morphological data. In all instances $p < 0.05$ was considered as statistically significant.

3. Results

The survival rate was 94.4%, occurring one death in C1, U1, B1 and C2 groups, and 2 deaths in B2 group. All deaths occurred during the time lag between the last instillation and analyses. The lungs seemed normal under macroscopic examination.

Est, ΔE, ΔP1 and ΔP2 were higher in U1 and B1 in relation to C1. These parameters returned to control values in groups U2 and B2, except for resistive pressure that was similar to control only in U7 and B7. No time-dependent difference was observed in C groups (Fig. 1).

The fraction area of alveolar collapse, bronchoconstriction index (BCI) and alveolar lesion score (ALS) were larger in U1 and B1 than in C1. Interestingly, collapsed area returned to baseline at day 7, while BCI and ALS normalized in 2 days. Collagen fiber content was similar in all groups (Table 1 and Fig. 2).