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Kidney damage induced by sub-chronic fine particulate matter exposure

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ABSTRACT

According to the WHO, about 3 million people die each year due to ambient air pollution. Most of the *in vivo* studies on the $PM_{2.5}$ effects have been done on respiratory and cardiovascular tissues. However, little is known about the effects on the tissues involved on xenobiotic removal, such as kidneys. In the present study we assess the harmful effects of sub-chronic exposure to $PM_{2.5}$ on the kidney, by investigating histologic and serum alterations in healthy and hypertensive rat models. Mean $PM_{2.5}$ concentrations during exposures were slightly above the daily WHO standard. Exposed animals showed fibrosis, mesangial expansion, decrease glomerular and tubular lumen volumes in kidneys, with an elevated BUN. Hypertensive animals also exhibited much more severe alterations than healthy animals. We conclude that $PM_{2.5}$ induces minimal or small-scale abnormalities that can be determinant for renal health preservation.

1. Introduction

Epidemiological evidences on air pollution health effects have significantly increased in recent years and have enhanced the notion that it may embody a major environmental risk factor. Indeed, atmospheric particulate matter (PM) having a diameter equal or < 2.5 μm ($PM_{2.5}$) seem to be a reliable indicator to estimate air pollution impact (World Health Organization, 2016), as exposure to small particles has negative health effects even at low concentrations and below the standards suggested by the World Health Organization (Janssen et al., 2011; Riva et al., 2011; Busso et al., 2017).Actually, close to 3 million people die every year due to air pollution related diseases, representing about 16% of the deaths due to non-communicable diseases (World Health Organization, 2016). Furthermore, most of this mortality is linked to cardiovascular complications (Mills et al., 2009). Particularly, several epidemiological (Wellenius et al., 2006; Harrabi et al., 2006; Auchincloss et al., 2008; Franck et al., 2011; Ying et al., 2014) and experimental studies (Brook, 2007; Franklin et al., 2015) have shown a positive correlation between blood pressure and $PM_{2.5}$ exposure, most likely due to vasoconstrictor effects induced by these particles (Mills et al., 2007; Franklin et al., 2008; Aragon et al., 2016). This seems exceedingly important given the toxic effects of $PM_{2.5}$ and the fact that it is estimated that by the year 2020, noncommunicable diseases could account for 60% of the global disease burden, causing a 73% of the deaths (World Health Organization, 2014).

Most of the *in vivo* studies on the $PM_{2.5}$ effects have been done on respiratory (Seagrave et al., 2006; Bonner, 2007; Reed et al., 2008) and cardiovascular tissues (Sun et al., 2005; Wang et al., 2015). In fact, although it is easy to envisage $PM_{2.5}$ gaining access into the respiratory system and translocating into the circulation (Polichetti et al., 2009), the toxicokinetic mechanisms are not fully elucidated and little is known about the $PM_{2.5}$ effects on the tissues involved on xenobiotic removal, such as liver and kidneys. Recently, Miller et al. (2017) have shown that inhaled particles can not only cross the alveolar-capillary barrier thus reaching remote tissues but can also be excreted in the urine. Nevertheless, the relationship between $PM_{2.5}$ chronic exposure and changes in renal function are still poorly understood (Seltenrich, 2016).

Up until now, few epidemiological studies have shown firm evidences for early renal disfunction following particulate matter exposure (Mehta et al., 2016; Bowe et al., 2017; Kim, 2017). Also, current literature shows rather few reports on kidney histologic alterations during particulate matter exposure (Damek-Poprawa and Sawicka-Kapusta, 2003; Aztatzi-Aguilar et al., 2016). Moreover, they do not address human actual exposure to urban atmospheric pollutants, nor the particles natural route of access into the body. Thus, in the present study

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our aim was to assess the harmful effects of sub-chronic exposure to $PM_{2.5}$ on the kidney. For this, we investigated both histologic parameters and serum chemical alterations. Moreover, and taking into account that hypertension is a key world's morbidity cause (World Health Organization, 2013), we also evaluated the $PM_{2.5}$ effects in the Spontaneously Hypertensive Rat model (Kodavanti et al., 2000).

2. Materials and methods

2.1. Sampling site and study design

Sampling site and study design has been previously described by Busso et al. (2017). Briefly, daily $PM_{2.5}$ samples were collected at the roof of the Chemistry Department of the Faculty of Exact, Physical and Natural Sciences (National University of Córdoba, 31°26′11.429″S; 64°11′38.191″W), at 7 m from the ground. At the same place, healthy and spontaneously hypertensive rats were exposed in a mobile animal facility to urban air. After the exposure period, serologic parameters were measured, and the kidneys were removed for histologic examination.

2.2. Exposure protocol

The protocol was completed complying with the Institutional Animal Care and Use Committee guidelines of the USA National Institute of Health. Six 5-week old male Wistar-Kyoto rats (W-K) and six 5-week-old male Spontaneously Hypertensive Rats (SHR) were allocated in an air-filtered bioresources environment at the "J. Robert Cade" Foundation before starting the experiments. The animals in both strains were randomly divided in two equal groups (control and treated) and held in one of the mobile animal facility chambers for a period of three months (2160 h) (Barile, 2013). Each chamber air uptake was connected to a Harvard Impactor (HI). Thus, in the control chamber all particles were removed with an impaction plate and a $PM_{2.5}$ polytetrafluoroethylene (PTFE) filter; whereas in the treatment chamber only large particles ($> PM_{2.5}$) were removed by the impaction plate (no filter was used). A 12.5 L min^{-1} airflow was employed to ensure no hypoxia in exposed animals and a full replacement of the internal atmosphere at least 15 times per hour.

The experimental groups were design as follow: W-K Control; SHR Control; W-K Treatment and SHR Treatment. Water and food were supplied *ad libitum* and beds were renewed twice a week with sieved sawdust. This exposure protocol was repeated three times employing new animals, during the cold seasons (from May to October), in the constructed 2015 and 2016.

2.3. $PM_{2.5}$ sampling and mass determination

Daily $PM_{2.5}$ samples were obtained with an HI coupled to the control chamber in 47-mm PTFE filters with a 2.0 μm pore (*Zefluor*, *Millipore*). Since air flow was lower than that suggested by the HI manufacturer, a cut point slightly over 2.5 μm was expected. The mass of collected particles was verified by gravimetric differences using a microbalance (0.01 mg mass resolution, *Sartorius*) (Busso et al., 2017). Results were expressed as mean ± standard error (SE).

2.4. Blood and tissue collection

To obtain blood and tissue samples, all rats were weighed and then anesthetized using a mixture of xylazine (20 mg/kg^{-1} body weight) and ketamine (100 mg/kg^{-1} body weight) (Busso et al., 2017). Then, 5-mL blood samples were drawn by cardiac puncture for serologic measurements. The kidneys were excised at the hilum and the fasciae removed. In all rats, both kidneys were weighed to estimate the somatic index and then, they were dissected longitudinally through the hilum, washed with cold phosphate buffered saline solution (PBS, 4 °C) and gently compressed to remove excess blood. One fragment was weighed (wet weight, WW) on an analytical balance (resolution 0.1 mg) and dried at 60 °C up to constant weight (DW) for elemental composition measurements. A second fragment was placed in 100 mL of formaldehyde neutral buffer solution for histological assessment (Bancroft et al., 2013).

2.5. Elemental composition

The levels of B, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Cd, Ba, Tl, Pb and Bi were measured in filters with fine particles and renal tissues by Mass Spectroscopy Inductively Coupled Plasma (*Agilent 7500cx*) as previously reported by Busso et al. (2017). High, middle and low concentrations calibration curves were made employing pure standards for all elements (*Sigma*).

2.6. Tissue analysis

The formaldehyde fixed kidneys were dehydrated with ethanol solution, clarified by xylol and embedded in paraffin by an automated tissue processor (*Leica TP1020*). Fragments were then cut with a rotary microtome (2 μm) and mounted on slides. In each rat six tissue slides (3 from each kidney) were obtained. These slides were deparaffinized and stained as follows: one sample with hematoxylin/eosin (HE), a second sample with periodic acid-Schiff (PAS, 2) and a third sample with Masson trichrome (MTS) (Bancroft et al., 2013). 15 fields in each slide were examined at 100, 400 and 1000× (*Olympus CX31*) searching for leucocyte infiltration, fibrosis, mesangial expansion, anisokaryosis, anisocytosis and any other pathologic sign (Kumar et al., 2017; Aztatzi-Aguilar et al., 2016). Each field was analyzed and scored using a scale from 0 to 3 to rank the damage (0: minimum or no observed, 1: mild, 2: moderate and 3: severe) and the slide mean was calculated. Percent of glomerular and tubular lumen (100×, %) and glomerular size (400×, pixels) were also determined in 20 field photographs per animal (400×) by a computer image analysis software (*Bio7*, Freeware). Results of each group were expressed as mean ± SE.

2.7. Serum parameters

Glycemia (enzymatic glycemia AA, *Wiener lab.*), blood urea nitrogen (BUN, enzymatic urea, *Wiener lab.*), uric acid (Uricostat enzymatic AA, *Wiener lab.*), creatinine (AA kinetic creatinine, *Wiener lab.*) and proteinogram (electrophoresis) were measured in the serum samples collected from each animal (Burtis and Bruns, 2007).

2.8. Statistical analysis

Total inhaled mass (TIM, μg) during the 90 days exposure period was calculated summing up daily inhaled mases for each animal. They were calculated multiplying $PM_{2.5}$ concentration (μg m^{-3}) by the maximum ventilation rate (m^3 min^{-1}) and the exposure minutes per day (min) (Sharp and Villano, 2012).

Differences in $PM_{2.5}$ concentration, TIM, and particles elemental composition between expositions were evaluated by ANOVA with LSD Fisher. Student's *t*-test was used to assess statistical differences between treatments (considering strains as independent blocks) and strains (considering treatments as independent blocks). Differences with a p value < 0.05 were considered statistically significant (*IBM SPSS 19.0*, IBM Corp.). In addition, Pearson coefficients were calculated to assess associations between air and tissues elemental composition, as well as between histological and serum parameters.

3. Results and discussion

$PM_{2.5}$ concentrations and elemental compositions for each exposure are shown in Table 1. Overall daily $PM_{2.5}$ mean was