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Repeated exposures to roadside particulate matter extracts suppresses pulmonary defense mechanisms, resulting in lipid and protein oxidative damage☆

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ABSTRACT

Exposure to particulate matter (PM) pollution in cities and urban canyons can be harmful to the exposed population. However, the underlying mechanisms that lead to health effects are not yet elucidated. It is postulated that exposure to repeated, small, environmentally relevant concentrations can affect lung homeostasis. This study examines the impact of repeated exposures to urban PM on mouse lungs with focus on inflammatory and oxidative stress parameters. Aqueous extracts from collected urban PM were administered to mice by 5 repeated intra-tracheal instillations (IT). Multiple exposures, led to an increase in cytokine levels in both bronchoalveolar lavage fluid and in the blood serum, indicating a systemic reaction. Lung mRNA levels of antioxidant/phase II detoxifying enzymes decreased by exposure to the PM extract, but not when metals were removed by chelation. Finally, disruption of lung tissue oxidant-inflammatory/defense balance was evidenced by increased levels of lipid and protein oxidation. Unlike response to a single IT exposure to the same dose and source of extract, multiple exposures result in lung oxidative damage and a systemic inflammatory reaction. These could be attributed to compromised capacity to activate the protective Nrf2 tissue defense system. It is suggested that water-soluble metals present in urban PM, potentially from break and tire wear, may constitute major drivers of the pulmonary and systemic responses to multiple exposure to urban PM.

1. Introduction

Exposure to environmental particles (EPs) in urban locations is known to cause adverse effects on human health (Adamkiewicz et al., 2015; Happo et al., 2010; Lim et al., 2012; Zhu et al., 2006). Yet, biological systems are inherently equipped with defense mechanisms to prevent cellular damage that may cause pathological processes. Thus, whether exposure to urban-derived EPs would initiate a detrimental cascade of events or will be contained and remain locally-confined, depends on the capacity of the biological system to meet the environmental challenge. *In vitro* and *in vivo* studies have identified that exposure to air pollution generates reactive oxygen species (ROS) that increase oxidative stress (Daher et al., 2014; Ghio et al., 2012; Guerra et al., 2013; Verma et al., 2014) and inflammation-associated injury (Dick et al., 2003; Happo et al., 2010). These effects are thought to be the primary biological mechanisms for air pollution-related adverse health effects (Guerra et al., 2013; Happo et al., 2010; Klaine et al., 2008; Liu et al., 2014). Since the main environmental exposure to airborne EPs is via the respiratory system, it is noteworthy that the lungs are particularly rich in non-enzymatic antioxidants, such as reduced glutathione, ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), lycopene, and β-carotene (Sackesen et al., 2008). Several, systems of persistent and inducible antioxidant enzymes are uniquely equipped to reduce intracellular ROS (Deng et al., 2013; Kaspar et al., 2009; Zhang et al., 2012) and restoring cellular homeostasis. The nuclear factor erythroid 2–related factor 2 (Nrf2) is an emerging regulator of cellular resistance to oxidants and critical regulator of

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cellular redox status. Nrf2 controls the basal and induced expression of an array of antioxidant response element–dependent genes to regulate the physiological and pathophysiological outcomes of oxidant exposure (Deng et al., 2013; Kaspar et al., 2009). Indeed, several studies demonstrated that Nrf2 is activated in response to oxidative stress *in vivo* and *in vitro* after exposure to diesel exhaust particles, particulate matter (PM), or to engineered nano-particles (Chan et al., 2013; Pardo et al., 2014). However, the underlying mechanisms are not yet clear. Recently, we demonstrated that a single intra-tracheal instillation (IT) of water-soluble extracts from re-suspended PM collected in a large city (London) caused a confined and temporary (less than 48 h) reaction in the lungs accompanied by changes in inflammatory factors. Yet, anti-oxidative defense systems were activated, a response that associated with the lack of significant evidence for oxidative damage to the lung tissue itself, nor of a "spill-over" manifesting as a systemic inflammatory response (Pardo et al., 2015).

In real world, humans experience repeated exposures to urban EPs. Indeed, whereas short exposure to high levels of PM have been suggested to trigger acute reactions, such as acute myocardial infarction and asthma exacerbation (Brook et al., 2010; Shah et al., 2015; Weinmayr et al., 2010), longer and protracted PM exposures have been associated with chronic diseases such as atherosclerosis, chronic obstructive pulmonary disease, lung cancer, and adverse birth outcomes such as congenital malformations (Beelen et al., 2014; Farhi et al., 2014; Hystad et al., 2013). Intriguingly, various biological defense systems may fail when the insult continues, in some cases by the exact offensive agent, such as ROS. In other words, whereas confined ROS production triggers antioxidant defense, it is conceivable that when oxidative stress persists it may target the defense mechanism(s) themselves, thereby exposing the biological system to oxidative damage. Therefore, in this study we evaluated whether repeated exposure to a dose that *does not* evoke tissue damage and systemic inflammation following single instillation, would nevertheless induce a pathological response. Moreover, we assessed whether this associates with failure to mount an adequate defense mechanism seen in response to a single exposure.

2. Materials and methods

2.1. Sample collection

Large quantities (100's of mgs) of size-resolved aerosol particulate matter (PM) were collected from an urban site in London (UK) in central London UK (Marylebone Road near Baker Street), from May through July 2012. Tisch (TE-230) Hi-Vol Environmental Impactor Samplers were used form sampling. The sampler was fitted with pre-cleaned mixed-cellulose ester (MCE) substrates to obtain two time-segregated samples. Samplers were run continuously for 3–4 days at nominal flow rates of 1.2 m^3 min^{-1}, sampling 5000 m^3 of air, and collecting from 50 to 300 mg of PM on the filter. Total PM Concentrations ranged from less than 10 μg m^{-3} to over 40 μg m^{-3} across the roadside sampling sites.

2.2. PM extract preparation and chelation

Sections of the MCE-filter samples were extracted with high-purity Milli-Q (18 mΩ) water using an initial 15 min sonication followed by 16 h of continuous agitation at room temperature in dark conditions and then finally another 15 min sonication. At the end of the extraction period, an aliquot of the suspension was removed and distributed for various assays/analyses (samples designated "PM extract"). The remaining suspension volume was centrifuged (6600 rpm for approximately 1 min), and then filtered through 0.22 μm polypropylene syringe filter. Soon after filtration, an aliquot of the filtered sample was processed through a miniature column of pre-cleaned Chelex 100 resin (Bio-Rad #143-2832, imino-diacetate in sodium form) to remove/isolate Chelex-labile (truly soluble ions and "loosely" bound) metals (samples designated "Chelex"). The Chelex separations were performed with 0.2 g of Chelex loaded into 1.5 mL polypropylene SPE reservoirs at a sample flow rate of 1 mL/min. Method blanks simulated the processing procedure ("Control"). The extracts and digests of the PM were subjected to a broad range of characterization tools, including: total and water-soluble elements [ICPMS (SF-ICPMS)]; soluble ions (K^+, Na^+, NH_4^+, SO_4^{2-}, NO_3^-, Cl^-) by IC; soluble organic carbon; and several toxicity assays. The TOTAL elemental mass extracted from the London PM (microwave-aided acid digestion) was normalized to the PM mass to enable a direct comparison with similarly normalized data from the unfiltered water extract data. Using a chemical species mass model, the total mass that these elements contribute to the total PM was calculated. The filters show more than 80% recovery of the target metals and inorganic species (Pardo et al., 2015). See Table 1 for metals concentrations in the extracts.

2.3. Mice treatments

Male C57BL/6 (7–8 weeks) mice were housed under standard light/dark conditions and were given access to food and water *ad libitum*. Experiments were approved by the Animal Care and Use Committee of the Weizmann institute of Science. Mice were randomly divided into groups (minimum, n = 6), where different water extracts were used: water extract from urban London PM

Table 1
Metal concentration from filters sampled near roadway in urban London, before and after metal Chelation.

Element	PM extract (μg/Liter)	Chlx (μg/Liter)	% Chelation
Ca	11,909.0 ± 112.0	1.3 ± 3.3	100.0
S	3501.0 ± 26.0	3235.0 ± 52.0	7.6
Fe	1135.0 ± 13.0	17.0 ± 0.3	98.8
Na	556.0 ± 8.0		
Al	523.0 ± 13.0	5.0 ± 4.0	99.0
Mg	463.0 ± 7.0	1.4 ± 0.3	99.7
K	442.0 ± 6.0	9. ± 0.5	98.0
Zn	399.0 ± 4.5	0.0 ± 0.1	100.0
Pb	244.0 ± 4.0	1.7 ± 0.0	99.3
Cu	215.0 ± 0.9	0.7 ± 0.1	99.7
Ba	106.0 ± 2.0	1.2 ± 0.1	98.9
P	92.0 ± 0.6	65.0 ± 1.6	29.3
Y	55.0 ± 1.0	0.2 ± 0.0	99.6
Mn	36.0 ± 1.0	0.0 ± 0.0	100.0
B	29.0 ± 0.6	26.0 ± 0.5	10.3
Sr	29.0 ± 0.2	0.0 ± 0.0	99.9
Ti	9.0 ± 0.3	0.3 ± 0.1	96.7
As	5.0 ± 0.5	4.3 ± 0.3	14.0
Ni	4.9 ± 0.2	0.2 ± 0.2	96.9
Sb	3.8 ± 0.1	2.5 ± 0.0	34.2
Pd	3.8 ± 0.2	0.2 ± 0.0	93.7
Cr	3.6 ± 1.0	0.2 ± 0.0	93.3
Sn	2.0 ± 0.1	0.1 ± 0.0	94.0
V	2.0 ± 0.1	0.0 ± 0.0	98.5
Ce	1.4 ± 0.0	0.0 ± 0.0	97.1
Co	1.0 ± 0.0	0.0 ± 0.0	99.6
LI	1.0 ± 0.0	0.0 ± 0.0	98.0
Rb	1.0 ± 0.0	0.1 ± 0.0	91.0
Mo	0.9 ± 0.1	0.2 ± 0.0	77.8
La	0.5 ± 0.0	0.0 ± 0.0	98.4
Cd	0.4 ± 0.0	0.0 ± 0.0	99.0
Nd	0.3 ± 0.0	0.0 ± 0.0	97.7
Pr	0.1 ± 0.0	0.0 ± 0.0	94.3
W	0.1 ± 0.0	0.0 ± 0.0	42.9