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Toxicity of the readily leachable fraction of urban $PM_{2.5}$ to human lung epithelial cells: Role of soluble metals

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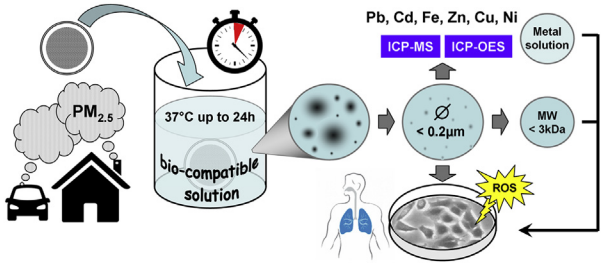
HIGHLIGHTS

- $PM_{2.5}$ low and high MW components are leached fast in a physiological solution.
- Fast leached compounds affect human lung alveolar cells redox status and migration.
- Low MW soluble compounds, including some metal ions, induce cell oxidative stress.
- High MW and/or insoluble compounds promote extracellular homocysteine accrual.
- Pb, Cd, Fe, Zn, Cu and Ni ions do not explain Winter $PM_{2.5}$ soluble fraction effects.

GRAPHICAL ABSTRACT



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ABSTRACT

Fine airborne particulate matter ($PM_{2.5}$) has been repeatedly associated with adverse health effects in humans. The $PM_{2.5}$ soluble fraction, and soluble metals in particular, are thought to cause lung damage. Literature data, however, are not consistent and the role of leachable metals is still under debate. In this study, Winter and Summer urban $PM_{2.5}$ aqueous extracts, obtained by using a bio-compatible solution and different contact times at 37 °C, were used to investigate cytotoxic effects of $PM_{2.5}$ in cultured lung epithelial cells (A549) and the role played by the leachable metals Cu, Fe, Zn, Ni, Pb and Cd. Cell viability and migration, as well as intracellular glutathione, extracellular cysteine, cysteinylglycine and homocysteine concentrations, were evaluated in cells challenged with both $PM_{2.5}$ extracts before and after ultrafiltration and artificial metal ion solutions mimicking the metal composition of the genuine extracts. The thiol oxidative potential was also evaluated by an abiotic test. Results demonstrate that $PM_{2.5}$ bioactive components were released within minutes of $PM_{2.5}$ interaction with the leaching solution.

Abbreviations: ROS, reactive oxygen species; WM, working medium; HEPES, 2-[4-(2-hydroxyethyl) piperazin-1-yl]ethanesulfonic acid; PBS, phosphate buffer saline; NR, neutral red; GSH, glutathione; CYS, cysteine; CYSGLY, cysteinylglycine; HCY, homocysteine; TCEP, tris(2-carboxyethyl)phosphine; TCA, trichloroacetic acid; SBD-F, 7-Fluorobenzofurazan-4-sulfonic acid; DTE, dithioerythritol; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid.

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Oxidative stress
Homocysteine
Thiol oxidation

Among these are i) low MW (<3 kDa) solutes inducing oxidative stress and ii) high MW and/or water-insoluble compounds largely contributing to thiol oxidation and to increased homocysteine levels in the cell medium. Cu and/or Ni ions likely contributed to the effects of Summer $PM_{2.5}$ extracts. Nonetheless, the strong bio-reactivity of Winter $PM_{2.5}$ extracts could not be explained by the presence of the studied metals. A possible role for $PM_{2.5}$ water-extractable organic components is discussed.

1. Introduction

Human exposure to air pollutants reduces life expectancy up to several years and is thought to be responsible for a substantial part of the global burden of both respiratory and cardiovascular diseases (EEA, 2016). Indeed, many epidemiological and experimental studies highlight that both short- and long-term exposure to airborne particulate matter (PM) associates with adverse health effects (Prüss-Ustün et al., 2016). Among the different PM size classes, particles with aerodynamic diameter $\leq$2.5 μm (fine particles, $PM_{2.5}$) pose major concern as an important cause of respiratory and cardiovascular morbidity and premature death (EEA, 2016). Due to their small size, inhaled $PM_{2.5}$ penetrate deeply into the respiratory tree down to the lung alveoli; wherefrom, the smallest particles could enter the bloodstream and diffuse throughout the organism (Kim et al., 2015).

Apart from size, PM pathogenic potential depends on chemical composition. Hence, addressing the still unanswered question as to which PM components pose major health concern would allow for more effective health protection than targeting the whole PM mass (HEI NPACT Review Panel, 2013). It's worth recalling that the high surface-to-mass ratio allows fine particles to carry higher concentrations of adsorbed chemicals than larger ones.

The dangerous effects of PM exposure are thought to be mediated mostly by oxidative damage to epithelial and immune cells, which makes oxidative stress a crucial step in the pathways linking PM exposure to health effects, especially airway damage and inflammation (Bertazzi et al., 2014; Gonzalez-Flecha, 2004; Kim et al., 2017; Moller et al., 2014).

Transition metals in particles, especially Fe, boost the production and release of reactive oxygen species (ROS), which initiate or exacerbate inflammation (Loxham et al., 2015; Kadiiska et al., 1997). Metals are important PM components and several studies point to catalytic metals, Fe and Cu in particular, as the central actors in PM-induced ROS production, the latter being directly related to concentrations of ionizable metals in the water-extractable PM fraction (Adamson et al., 1999; Costa and Dreher, 1997; Ghio et al., 1999; Hetland et al., 2000; Kennedy et al., 1998; Pardo et al., 2015). In a comprehensive research program, PM metals were found to be consistently associated with adverse health effects (HEI NPACT Review Panel, 2013). Nonetheless, the overall weight of evidence does not convincingly implicate transition metals as major contributors to the respiratory and cardiovascular effects associated with airborne PM levels (Gray et al., 2015). Several *in vitro* studies had focused on identifying and quantifying the role of soluble metals in the toxicity of real-world PM, with controversial results (Akhtar et al., 2010; Diabate et al., 2011; Gali et al., 2015). It's important to bear in mind, the majority of these studies were conducted using pure water as a leaching agent. Since pure water far from mimicks human body fluids, it follows that using physiologically relevant leaching media is an absolute requirement for a reliable assessment of the bioaccessibility of PM metals (Wiseman, 2015). Furthermore, harsh, often protracted, extraction procedures were often adopted (mostly vortexing and/or sonication) disregarding leaching kinetics. The fact that PM extracts can undergo significant physico-chemical modifications during the extraction procedure, together with the limitations of the methods used to single out water-soluble and -insoluble portions of PM samples (centrifugation and/or filtration), may account at least in part for the inconsistency of the reported results.

In a previous work from our group, a new comprehensive methodological approach was adopted to thoroughly characterize $PM_{2.5}$ and get more detailed information on the behavior of PM metals in a biological environment (Armiento et al., 2013). In that work, an aqueous saline solution mimicking the lung interstitial environment, combined with mild treatment conditions (pH 7.4, 37 °C, slow back and forth motion) for different periods of time (few minutes to many days) was adopted in the leaching tests.

The same approach was applied in a more recent work to characterize $PM_{2.5}$ sampled during 2013 and 2014 in Rome, the largest Italian city, characterized by a high urban environmental pollution (Mazziotti Tagliani et al., 2017). In that work, it was found that the hosting matrix (mineral phases) of $PM_{2.5}$ plays a crucial role in determining the (bio)availability of $PM_{2.5}$ metals. Cd and Pb were quickly and almost fully released, Cu was only partially released and Fe, consistent with its speciation (FeIII), was not released at all.

In the present study, we went a step further by exploiting the previously characterized extracts to investigate in more depth the cytotoxic effects induced by early leached $PM_{2.5}$ components. Cultured lung epithelial cells (A549, a human lung adenocarcinoma cell line widely used as an *in vitro* model of alveolar toxicity), were exposed to different concentrations of the extracts and various cellular and abiotic endpoints were evaluated (cell viability, oxidative stress and migration, and thiol oxidative potential, respectively). Aiming at better understanding the role of metal ions in the biological effects induced by the $PM_{2.5}$ extracts, water-soluble fractions obtained by ultrafiltration of extracts and artificial solutions with a metal ion composition mimicking that of the genuine extracts were also tested.

2. Materials and methods

2.1. $PM_{2.5}$ sampling

$PM_{2.5}$ samples were collected in the urban area of Rome at a downtown-vehicular traffic oriented site operated by the Istituto Superiore di Sanità (Mazziotti Tagliani et al., 2017). Samplings were carried out in February 2013 (Winter 2013), July 2013 (Summer 2013) and February 2014 (Winter 2014) by means of an automated sequential sampler (Skypost PM HV, Tecora, Cedex, France) working at medium-volume conditions (2.3 $m^3\ h^{-1}$). Each sampling campaign lasted for twenty days, during which $PM_{2.5}$ was continuously collected onto PTFE filters (Ø 47 mm). A total of three loaded filters for each sampling campaign were obtained.