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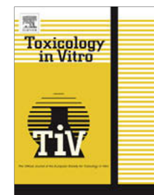

Air pollution particulate matter ($PM_{2.5}$)-induced gene expression of volatile organic compound and/or polycyclic aromatic hydrocarbon-metabolizing enzymes in an *in vitro* coculture lung model

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ABSTRACT

The overarching goals were: (i) to develop an *in vitro* coculture model, including two relevant lung target cells: human alveolar macrophage (AM) isolated from bronchoalveolar lavage fluid, and immortalized cells originated from the normal lung tissue of a human embryo (L132 cell line), as a future strategy for near-realistic exposures to air pollution particulate matter (PM), and (ii) to study the gene expression of volatile organic compound (VOC) and/or polycyclic aromatic hydrocarbons (PAH)-metabolizing enzymes in this *in vitro* coculture model. Human AM and/or L132 cells in mono- and coculture were exposed for 24, 48 and 72 h to Dunkerque City's $PM_{2.5}$ at its lethal concentrations at 10% and 50% (i.e. AM: LC_{10} = 14.93 µg PM/mL and LC_{50} = 74.63 µg PM/mL; L132: LC_{10} = 18.84 µg PM/mL and LC_{50} = 75.36 µg PM/mL), and the gene expression (i.e. *Cytochrome P450 1A1*, *CYP1A1*; *CYP2E1*; *CYP2F1*; *microsomal Epoxide Hydrolase*; *NADPH Quinone Oxydo-Reductase-1*, *NQO1*; and *Glutathione S-Transferase pi-1* and *mu-3*, *GST-π1* and *GST-µ3*) was studied. In human AM in mono- and coculture, and in L132 cells in monoculture, VOC and/or PAH-coated onto PM induced the gene expression of *CYP1A1*, *CYP2E1*, *NQO1*, *GST-π1*, and/or *GST-µ3*. However, there were quiet different outcomes based on the use of L132 cells in mono- vs. coculture: the pattern of VOC and/or PAH-metabolizing enzymes induced by PM in L132 cells in monoculture remained almost unaffected when in coculture with AM. Taken together, these results reinforced the key role of PM-exposed target human AM in the defenses of the human lung from external injuries, notably through their higher capacity to retain PM, and indicated that carbonaceous cores of PM, as physical vector of the penetration and retention of coated-VOC and/or PAH into cells, enabled them to exert a longer toxicity. The use of such a near realistic exposure system could also be a very useful and powerful tool to identify the mechanisms by which air pollution PM induced adverse health effects.

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1. Introduction

There is accumulating evidence from epidemiological studies that airborne particulate matter (PM) is closely associated with dramatically increases in morbidity and mortality (Maier et al., 2008). Long-term exposure to high concentrations of PM has been correlated with hospital admissions and emergency-room visits for treatment of acute respiratory infections, chronic respiratory and/or cardiovascular diseases, and even lung cancers (Brunekreef and Holgate, 2002; Dominici et al., 2007; Elliott et al., 2007; Hales and Howden-Chapman, 2007; Pope, 2004; Samet et al., 2000; Sorensen et al., 2003). Exposure to air pollution PM would also directly or indirectly be responsible for 40,000 deaths/year in Europe or 800,000 deaths/year all over the world (Cohen et al., 2005; Curtis et al., 2006; Kunzli et al., 2000). A rise of 10 $\mu g/m^3$ in PM_{10} concentration would induce increases from 0.5% to 0.6% of the daily mortality, from 1% to 1.5% of hospitalizations for chronic obstructive pulmonary diseases, and from 0.5% to 1.1% of hospitalizations for cardiovascular disorders (Atkinson et al., 2001; Brunekreef and Holgate, 2002; Zanobetti et al., 2000). However, despite intensive investigation, the underlying mechanisms of action by which air pollution PM induced adverse health effects are still not clear. Indeed, researchers are most often face some crucial problems arising from the very complex and heterogeneous nature of PM, on

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the one hand, and the lung cellular heterogeneity, on the other hand.

Air pollution PM is also generally characterized by its highly complex nature and its anthropogenic and/or natural sources of origin (Alfaro-Moreno et al., 2002; De Kok et al., 2006; Harrison and Yin, 2000; Hetland et al., 2004). Epidemiological evidence indicated that the greatest health risks correlate with smaller PM, which has the capacity to reach the distal regions of the lung, and is often associated with the most significant adverse health effects (Diociaiuti et al., 2001; Monn and Becker, 1999; Osornio-Vargas et al., 2003). However, it remains to be elucidated which of the PM size fractions is mainly responsible for adverse health effects, and which particle features might play a significant role in initiating the underlying mechanisms of action (Maier et al., 2008). Now, among the PM-associated parameters actually considered to be determinants, there are particle number, surface area, and surface reactivity, as well as reactive chemicals-coated onto air pollution PM (Maier et al., 2008). Airborne PM is a highly complex and heterogeneous mixture of chemical and/or biological elements (e.g. transition metals; volatile organic compounds, VOC; polycyclic aromatic hydrocarbons, PAH; PolyChlorinated Dibenzo-p-Dioxins and -Furans, PCDD/F; Dioxin-Like PolyChlorinated Biphenyls, DLPCB; PolyChlorinated Biphenyls, PCB; endotoxins, etc.), which can be attached to a carbonaceous core being use as a condensation nuclei (Billet et al., 2008; Carter et al., 1997; Frampton et al., 1999; Monn and Becker, 1999; Nel et al., 2006). Despite their presence at low-doses or very low-doses, some of the above-mentioned PM chemical constituents could in fact be closely involved in PM-induced adverse health effects (Liden et al., 2003; Nel et al., 2001; Osornio-Vargas et al., 2003). Nevertheless, studies have generally been conducted using mixtures, yet their physical and chemical characteristics are not established, and the possible influence of the coated-elements are often neglected (Englert, 2004). A better knowledge of their physical and chemical characteristics constitutes a crucial information to evaluate their toxicity, but reveals to be unsatisfactory to predict their toxicological reactivity (Baulig et al., 2004).

Another difficulty arises from the large cellular heterogeneity of the respiratory tract (Dahl and Lewis, 1993; Hukkanen et al., 2002; Spivack et al., 2003). This primary target organ for inhaled pollutants is composed of more than 40 different cell types, each with variable levels of metabolic competence towards pollutants (Castell et al., 2005; Hukkanen et al., 1997; Maier et al., 2008). Although results should be carefully considered when using *in vitro* models, studies based on the use of alternative models can be very useful approaches to identify the underlying mechanisms of action involved in PM-induced damage in lung cells (Devlin et al., 2005). The major limitation of *in vitro* studies is that cells have been removed from their normal environment: the most of *in vitro* studies are carried out using monoculture systems, thereby excluding neighboring cells to interact with, yet intercellular signaling is central to tissue and organ homeostasis (Carere et al., 2002). The use of *in vitro* coculture systems as a future strategy for near-realistic exposures to airborne PM will therefore be a promising tool to assess toxicity (Carere et al., 2002; Devlin et al., 2005; Maier et al., 2008).

Hence, in this work, in order to improve the knowledge of the underlying mechanisms of action involved in air pollution PM-induced toxicity, we try to develop a coculture model including two relevant lung target cells: human alveolar macrophage (AM) isolated from bronchoalveolar lavage fluid (BALF), on the one hand, and cells originated from the normal lung tissue of a human embryo (L132 cell line), on the other hand. AM also play a critical role in the lung defense against air pollution not only by clearing the airways of deposited PM but also by taking a great part in the detoxification of inhaled organic fraction through its metabolic activation by phase-I and -II xenobiotic-metabolizing enzymes (Castell et al., 2005; Hukkanen et al., 1997; Imrich et al., 2007; Proulx et al., 2007; Saint-Georges et al., 2008). L132 cells present epithelial morphological characteristics, exhibit typical features of pneumocytes, and are highly sensitive to PM-induced adverse effects (Erfle and Mellert, 1996; Dagher et al., 2005, 2006, 2007; Garçon et al., 2006, 2007). Several xenobiotic-metabolizing Cytochrome P450 (CYP) and phase II enzymes (i.e. conjugation enzymes including several transferases) generally involved in the *in situ* activation and/or inactivation of the organic fraction-coated onto airborne PM are present in the human lung and in lung-derived cell lines (Dahl and Lewis, 1993; Hukkanen et al., 2000; Spivack et al., 2003).

Recently, to contribute to a better knowledge of the underlying mechanisms of action involved in airborne PM-induced lung toxicity, we have undertaken an extensive study of the adverse health effects of $PM_{2.5}$, collected in Dunkerque, a French seaside City located on the southern coast of the North-Sea, and characterized by the proximity of important industrial activities and heavy motor vehicle traffic (for review; see Garçon et al., 2007). We have shown that the *in vitro* short term exposure to Dunkerque City's $PM_{2.5}$ induced the metabolic activation of the very low doses of VOC and/or PAH-coated onto the inorganic condensation nuclei in Dunkerque City's PM by primary cultures of human AM isolated from BALF (Saint-Georges et al., 2008). Hence, to improve the understanding of the role of the coated-organic fraction in the lung toxicity, the gene induction of VOC and/or PAH-metabolizing enzymes by Dunkerque City's $PM_{2.5}$ was studied in mono- and coculture model of primary cultures of human AM and/or L132 cells, through the determination of the gene expression of *CYP1A1, CYP2E1, CYP2F1,microsomal Epoxide Hydrolase* (*mEH*), *NADPH Quinone Oxydo-Reductase-1* (*NQO1*), *and Glutathione S-Transferase pi-1* (*GST-π1*), and *mu-3* (*GST-μ3*).

2. Materials and methods

2.1. Chemicals

RPMI 1640 culture medium, fetal bovine serum (FBS), penicillin/streptomycin solution, amphotericin B solution, and sterile phosphate-buffered solution (PBS) were from Invitrogen SARL (Cergy Pontoise, France). Titanium (IV) oxide powder (anatase; purity: 99%; primary particle size: 0.2 μm; surface not coated) was from Acros Organics (Noisy Le Grand, France). Benzene and Benzo(a)Pyrene (B(a)P) were from Sigma–Aldrich (St-Quentin Fallavier, France). Cytotoxicity Detection Kit (LDH) and Cell Proliferation Reagent (WST-1) were from Roche Diagnostics (Meylan, France). RNAqueous-4PCR kit, High Capacity cDNA Archive kit, Taqman fast universal PCR master Mix, No UNG, and TaqMan Gene Expression Assays were provided by Applera France SA (Courtaboeuf, France).

2.2. Methods

2.2.1. PM sampling, physical and chemical characteristics, and outgassing

2.2.1.1. PM sampling. PM was collected in Dunkerque City (51°04′N; 2°38′E; France), a French sea-side City located on the southern coast of the North-Sea,, using a high volume cascade impactor (Billet et al., 2007). Briefly, plates were mounted without any filters and no back up filter was used to maintain a constant aspiration flow rate. No back up filter was used and the lowest stage was doubled to increase the efficiency of smallest particle ($PM_{0.33}$) sampling. Meteorological data (i.e. wind speed, wind direction, temperature) were obtained from Meteo France. With regards to the direction of the prevailing winds, Dunkerque City

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