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Genotoxic potential of Polycyclic Aromatic Hydrocarbons-coated onto airborne Particulate Matter ($PM_{2.5}$) in human lung epithelial A549 cells

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Abstract

To improve the knowledge of the underlying mechanisms of action involved in air pollution Particulate Matter (PM)-induced toxicity in human lungs, with a particular interest of the crucial role played by coated-organic chemicals, we were interested in the metabolic activation of Polycyclic Aromatic Hydrocarbons (PAH)-coated onto air pollution PM, and, thereafter, the formation of PAH–DNA adducts in a human lung epithelial cell model (A549 cell line). Cells were exposed to Dunkerque city's $PM_{2.5}$ at its Lethal Concentrations at 10% and 50% (i.e. $LC_{10} = 23.72$ μg/mL or 6.33 μg/cm^2, and $LC_{50} = 118.60$ μg/mL or 31.63 μg/cm^2), and the study of *Cytochrome P450 (CYP) 1A1* gene expression (i.e. RT-PCR) and protein activity (i.e. EROD activity), and the formation of PAH–DNA adducts (i.e. ^{32}P-postlabeling), were investigated after 24, 48, and/or 72 h. PAH, PolyChlorinated Dibenzo-*p*-Dioxins and -Furans (PCDD/F), Dioxin-Like PolyChlorinated Biphenyls (DLPCB), and PolyChlorinated Biphenyls (PCB)-coated onto collected PM were determined (i.e. GC/MS and HRGC/HRMS, respectively), Negative (i.e. TiO_2 or desorbed PM, dPM; $EqLC_{10} = 19.42$ μg/mL or 5.18 μg/cm^2, and $EqLC_{50} = 97.13$ μg/mL or 25.90 μg/cm^2), and positive (i.e. benzo(*a*)pyrene; 1 μM) controls were included in the experimental design. Statistically significant increases of *CYP1A1* gene expression and protein activity were observed in A549 cells, 24, 48 and 72 h after their exposure to dPM, suggesting thereby that the employed outgassing method was not efficient enough to remove total PAH. Both the *CYP1A1* gene expression and EROD activity were highly induced 24, 48 and 72 h after cell exposure to PM. However, only very low levels of PAH–DNA adducts, also not reliably quantifiable, were reported 72 h after cell exposure to dPM, and, particularly, PM. The relatively low levels of PAH together with the presence of PCDD/F, DLPCB, and PCB-coated onto Dunkerque City's $PM_{2.5}$ could notably contribute to explain the

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borderline detection of PAH–DNA adducts in dPM and/or PM-exposed A549 cells. Hence, remaining very low doses of PAH in dPM or relatively low doses of PAH-coated onto PM were involved in enzymatic induction, a key feature in PAH-toxicity, but failed to show a clear genotoxicity in this *in vitro* study. We also concluded that, in the human lung epithelial cell model we used, and in the experimental conditions we chose, bulky-DNA adduct formation was apparently not a major factor involved in the Dunkerque City's $PM_{2.5}$-induced toxicity.

Keywords: A549 cells; Air pollution Particulate Matter; Polycyclic Aromatic Hydrocarbons; Lung toxicity; Cytochrome P450 1A1; ^{32}P-postlabeling; PAH–DNA adducts

1. Introduction

Long-term exposure to fine Particulate Matter (PM) air pollution is associated with increased incidence of mortality [1–3]. Among the 58 million deaths worldwide identified in 2005, 1.3 million were from lung cancer [4]. Air pollution caused about 5% of trachea, bronchus and lung cancer (i.e. 71,000 deaths) [2,4–6]. The adjusted relative risk associated with a 10 μg/m^3 increase in annual average $PM_{2.5}$ for the 1999–2000 period led to a 13% increase in lung cancer mortality [1].

Even if tobacco use takes a major part in etiology of lung cancer, other explanation like genetic and lifestyle factors, and occupational or environmental exposure to carcinogens have also to be considered. Indeed, one of the most important risk factor that have to be take into account is the relatively low exposure to airborne carcinogens, like Polycyclic Aromatic Hydrocarbons (PAH), generally occurring close to point emissions, such as coal-, wood- and fuel-power plants, steel and petrochemical factories, and/or near mobile sources, such as motor vehicle exhaust [7,8]. The exposure level to PAH emitted from these sources is relatively low as compared to other, as such as diet, occupation, or tobacco smoke [9]. The half-life of airborne PAH is of the order of days but can be longer when they are coated onto ambient PM [9]. Indeed, air pollution PM is a very complex and heterogeneous mixture of chemicals (i.e. metals; salts; carbonaceous material; Volatile Organic Compounds, VOC; PAH; etc.) and/or biological (i.e. bacteria, endotoxins, fungi, etc.) elements, which can in fact be attached to a carbonaceous core being use as a condensation nuclei [10,11].

After their absorption, PAH are distributed into lung cells and/or tissues, where they can be biotransformed. The metabolic activation of PAH occurs via two classes of enzymes: phase I (i.e. oxidation, reduction, hydrolysis) and phase II (i.e. conjugation) enzymes. Cytochrome P450s (CYPs) are essential heme-containing enzymes that play critical functions in the conversion of organic chemicals into water soluble metabolites, thereby helping their excretion. Accordingly, in human lungs, PAH, which require metabolic activation to biologically reactive intermediates to elicit their adverse health effects, are metabolized by the CYP superfamily member CYP1A1 [2]. Certain chemically reactive intermediates arising from PAH metabolic activation in lungs could thereafter interact with DNA target sites to produce adducts, thereby giving rise to mutation, and eventually, tumor initiation [2]. The gene expression of CYP enzymes can be modulate in response to the activation of key transcription factors by specific substrates; in particular, in lung cells, the activation of Aryl hydrocarbon Receptor (AhR) by PAH induces *CYP1A1* mRNA transcription [2,12].

Mutagenic activities of outdoor air pollution from anthropogenic combustion-related sources or its main components have already been shown in *Salmonella* assays, as recently reviewed [13]. Such data have revealed that the PAH present in almost all combustion-related complex mixtures constitute a significant source of genotoxicity among at least 500 mutagenic components of varying chemical classes [13,14]. However, other factors, often neglected, such as PM size, component interactions, secondary chemical reactions in the atmosphere, and/or sampling season are known to affect the genotoxicity of air pollutants [15–18]. The photodegradation and chemical transformation of PAH emissions in the atmosphere during warmer months might also induce the formation of highly biologically reactive products, such as B(*a*)P-7,8-Dihydrodiol-9,10-Epoxide (BPDE), and quinones, able to form DNA adducts [19,20]. Ultimately, in humans, hereditary and acquired susceptibilities have been reported to influence the formation of aromatic DNA adducts [21].