PERSISTENT ORGANIC POLLUTANTS-COATED ONTO AIR POLLUTION PARTICULATE MATTER ($PM_{2.5}$): GENOTOXICITY IN HUMAN LUNG EPITHELIAL A549 CELLS

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Introduction

Long-term exposure to fine Particulate Matter (PM) air pollution is associated with increased incidence of mortality[1,2]. A 10 µg/m^3 increase in annual average $PM_{2.5}$ led to a 13% increase in lung cancer mortality[1]. Air pollution PM is a very complex and heterogeneous mixture of chemicals. It can thus contains metals, salts, carbonaceous material, but also Volatile Organic Compounds (VOC) and Persistent Organic Pollutants (POP) like Polycyclic Aromatic Hydrocarbons (PAH), and PolyChlorinated Dibenzo-p-Dioxins (PCDD), -Furans (PCDF), and PolyChlorinated Biphenyls (PCB). The half-life of airborne pollutants is of the order of days but can be longer when they are coated onto ambient PM. After their absorption, PAH are distributed into lung cells, where they can be biotransformed in order to help their excretion. Accordingly, PAH, which require metabolic activation to biologically reactive intermediates to elicit their adverse health effects, are metabolized by the Cytochrome-P450 (CYP) 1A1[2]. Certain chemically reactive intermediates arising from PAH metabolic activation in lungs could thereafter interact with DNA target sites to produce bulky-DNA adducts, thereby giving rise to mutation, and eventually, tumor initiation[2]. The gene expression of CYPs 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, PCDD/F and/or PCB induces cyp1a1 mRNA transcription[2,3]. Mutagenic Salmonella assays of outdoor air pollution have revealed that the PAH present in almost all combustion-related complex mixtures constitute a significant source of genotoxicity[4]. However, other factors, often neglected, such as PM size, interactions between PM pollutants, secondary chemical reactions in the atmosphere, and/or sampling seasons are known to affect the genotoxicity of air pollutants[5]. Among the frequently used biological markers to monitor PAH exposure, DNA adduct levels are particularly relevant since they are mechanistically linked to the induction of cancer[6]. In this work, we focused our attention on the ability of fine air pollution PM to induce *CYP1A1* gene expression and protein activity and, thereafter, PAH-DNA adduct formation in a human lung epithelial cell model (A549 cell line). We were closely interested in the presence of PM-coated inducible-CYP1A1 enzyme substrates, and, therefore, in the required distinction between PAH, which are able to be converted into reactive metabolites that directly react with DNA to form bulky-DNA adducts, and PCDD/F and PCB, which are not able to form bulky-DNA adducts detectable by post-labelling method[7].

Materials and methods

Atmospheric PM were collected, using high volume cascade impactor, in Dunkerque (51°04'N; 2°38'E), a French City on the southern coast of the North-Sea, characterized not only by lots of industrial activities, but also by a heavy motor vehicle traffic[8]. The most toxicologically relevant physical and chemical characteristics of collected PM were determined, and its potential role in the induction of PAH and/or VOC-metabolizing enzymes were carried out in a human lung epithelial cell model (A549 cell line)[8]. PM size distribution, as carried out by Scanning Electron Microscopy, showed size ranging from 0.33 µm to 5.0 µm (92.15% of $PM_{2.5}$; size ≤ 2.5 µm). Inductively coupled plasma-atomic emission spectrometry showed that Fe (7.84%), Al (5.83%), Ca (4.95%), Na (1.88%), K (0.97%), Mg (0.81%), Pb (0.80%), and Ti (0.51%) were the most abundant inorganic elements.

Gas Chromatography/Mass Spectrometry (GC/MS), after thermal desorption at 500°C and cold trapping, or soxhlet extraction with dichloromethane, allowed notably to identify lots of PAH-coated onto PM. PCDD/F and PCB were analyzed by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry. 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/cm², and LC_{50} = 118.60 µg/mL or 31.63 µg/cm²)[8]. To better elucidate the role of coated PAH, TiO_2 particles and collected PM having undergone a thermal desorption at 400°C under a secondary vacuum and having thereby kept inorganic structures and lost most of organic chemicals were included as negative controls (i.e. TiO_2 or desorbed PM, dPM; $EqLC_{10}$ = 19.42 µg/mL or 5.18 µg/cm², and $EqLC_{50}$ = 97.13 µg/mL or 25.90 µg/cm²). Benzo(a)pyrene (1 µM) was included as positive control in the experimental protocol. The study of *CYP1A1* gene expression (i.e. RT-PCR) and protein activity (i.e. EROD activity) were investigated after 24, 48, and 72h, while the formation of PAH-DNA adducts (i.e. ^{32}P-postlabelling), was considered after 72h.

Results and discussion:

PAH, PCDD/F, DLPCB, and Marker PCB-coated onto PM

The physical and chemical characterization of collected PM showed not only PAH family members but also PCDD/F, Dioxin-Like PolyChlorinated Biphenyls (DLPCB), and marker PCB associated to PM either through adherence to the carbonaceous core particle or as integral components (Table 1-3). PAH concentrations reported in Dunkerque City's $PM_{2.5}$ were equal or smaller than those observed in PM samples collected in two different locations in Paris[9]. With regards to PCDD/F, the concentrations found in Dunkerque City's $PM_{2.5}$ under study were somewhat similar to those reported in airborne PM collected in two sites located downwind from a municipal incinerator, respectively[10]. The presence of this very complex and heterogeneous chemical mixture was also closely related to the anthropogenic emission sources located near the sampling site; apart the heavy motor vehicle traffic, iron and steel industry, aluminum industry, oil refinery, and basic chemistry could be cited.

Effects of PM on Gene Expression and Protein Activity of CYP1A1

A significant induction of *CYP1A1* gene transcript was seen in A549 cells, 24, 48 and 72h after their exposure to dPM, to PM, and to B(a)P, compared to controls. Accordingly, a significant increase in CYP1A1 catalytic activity was observed in A549 cells 24, 48 and 72h after their exposure to PM, to dPM, and to B(a)P. A statistically significant correlation was also reported between the gene transcripts of *CYP1A1* and its EROD activities (Spearman's rho = 0.722, $p<0.001$). This finding suggested that the relatively low-doses of PAH-coated onto collected PM activated the transcription of AhR and induced PAH metabolic activation[2,3]. The PCDD/F, DLPCB, and marker PCB from the airborne PM under study, could also be involved in the induction of the gene expression and the catalytic activity of CYP1A1 in dPM and notably PM-exposed A549 cells.

Effects of PM on DNA Adduct Formation

^{32}P-post-labelling method showed the presence of several DNA adduct spots (I, II, and III) on the autoradiograms from A549 cells 72h after their exposure to dPM at its Eq LC_{50}, to PM at its LC_{50}, and to B(a)P (1 µM), thereby contributing to validate the protocol designed to detect bulky PAH-DNA adducts. (Figure 1). Spot II appeared only after dPM and PM exposure, at a weak, comparable, but not reliably quantifiable, intensity. A comparable spot was seen after A549 cell exposure to B(a)P at 1 µM. It could also be speculated that, if the spot II corresponded to a B(a)P-DNA adduct, it would be greatly more intense in A549 cells exposed to B(a)P than in those exposed to PM. Conversely, if this spot would be closely linked to another PAH, it should be absent in A549 cells exposed to B(a)P. Consequently, we considered that this spot could not be definitively ascribed to PM exposure. Three distinct DNA adduct spots, also included together in the area III, were observed with high intensity 72h after A549 cell exposure to B(a)P (1 µM). The mean calculated RAL for this area III was 89.5×10^{-8} with a standard deviation of 24.1×10^{-8}. The spots in the area III could be identified thanks to a positive control of B(a)P-DNA adduct (B(a)P-7,8-Dihydrodiol-9,10-Epoxide; BPDE).

Taken together, the results reported in dPM and PM-exposed A549 cells revealed an apparent discrepancy between the induction of the gene expression and the catalytic activity of *CYP1A1*, one the one hand, and the low level of bulky PAH-DNA adducts, on the other hand. Some explanations might also be considered. Firstly, it should be emphasized that the B(a)P concentration in A549 cells exposed to PM at their LC_{50}, assuming the whole B(a)P-coated onto PM to be released, would be about 250-fold lower than in A549 cells exposed to B(a)P at 1 µM. Secondly, in other studies assessing the genotoxicity of pollutant mixtures, adduct spots formed by single genotoxic chemicals are too discrete, and only Diagonal Radioactive Zones (DRZ) can be used[5]. However, such DRZ did not appear in our work despite the presence of a lot of PAH-coated onto PM. Thirdly,

man must keep in mind that other authors, carefully studying the ability of various air pollution PM to induce PAH-DNA adducts in different mammalian cell models in culture, confirmed their genotoxic potentials[5,11]. However, these results were reported following cell models exposure to Extractable Organic Matter (EOM) arising from air pollution PM, and also not to ambient PM. There could, therefore, be a major difference between the relatively high doses of PAH present in EOM, on the one hand, and the relatively low doses of PAH-coated onto ambient PM, on the other hand. Fourthly, the time of exposure we chose (i.e. 72h) could perhaps allow the activation of mechanisms of DNA repair; the choice of shorter exposure times could perhaps minimize them. Fifthly, several co-contaminants of PAH, like PCDD/F, DLPCB, and marker PCB are often detected on airborne PM. While these chemicals are powerful AhR agonists and *CYP1A1* gene expression and EROD activity inducers at low doses, they do not form DNA adducts[7]. Indeed, Wu *et al.*[7], studying the effect of TCDD treatment on B(a)P-induced DNA-adduct formation in the liver of female mice, reported a reduced formation of B(a)P-DNA adducts at the lowest dose of B(a)P tested (50 $mg.kg^{-1}$) following TCDD exposure. In agreement with these results, the relatively low levels of PAH together with the presence of other related compounds (i.e. PCDD/F, DLPCB, and PCB)-coated onto Dunkerque City's $PM_{2.5}$ could contribute to explain the very low, and therefore not reliably quantifiable, levels of PAH-DNA adducts detected in dPM and/or PM-exposed A549 cells.

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Figure 1: PAH-DNA adduct patterns

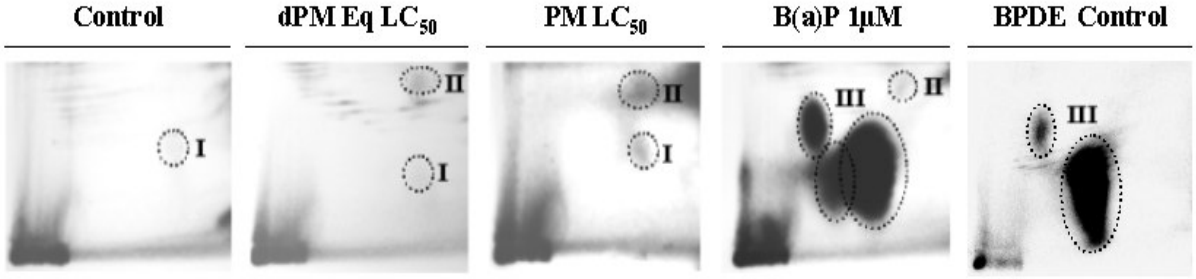

Table 1: PAH detected in collected PM

Compound	Concentration (µg/g)
Naphthalene	38.1
Methylnaphthalene	141.9
Dimethylnaphthalene	90.2
Anthracene	47.1
Phenanthrene	28.3
Dibutylphthalate	110.1
Fluoranthene	4.7
Benzo(b+k)fluoranthene	6.6
Benzo(a)pyrene	7.9
Bis(Ethyl.Hexyl)phthalate	41.4
Acenaphthene	11.1
Fluorene	6.3
Pyrene	4.7
Benzo(a)anthracene or Chrysene	4.9

Table 2: PCDD/F detected in collected PM

Congener	Concentration (pg/g)	I-TEQ (pg I-TEQ/g)
2,3,7,8 TCDD	<5	5.00
1,2,3,7,8 PeCDD	115.28	57.64
1,2,3,4,7,8 HxCDD	139.51	13.95
1,2,3,6,7,8 HxCDD	378.39	37.84
1,2,3,7,8,9 HxCDD	384.43	38.44
1,2,3,4,6,7,8 HpCDD	2644.62	26.45
OCDD	10918.02	10.92
Σ PCDD	**14580.27**	**190.24**
2,3,7,8 TCDF	69.94	6.99
1,2,3,7,8 PeCDF	153.32	7.67
2,3,4,7,8 PeCDF	329.33	164.67
1,2,3,4,7,8 HxCDF	367.05	36.71
1,2,3,6,7,8 HxCDF	395.15	39.52
2,3,4,6,7,8 HxCDF	556.23	55.62
1,2,3,7,8,9 HxCDF	70.11	7.01
1,2,3,4,6,7,8 HpCDF	1645.36	16.45
1,2,3,4,7,8,9 HpCDF	269.62	2.70
OCDF	1058.59	1.06
Σ PCDF	**4914.74**	**338.39**
Σ PCDD/F	**19495.02**	**528.63**

ΣPCDD, ΣPCDF, and ΣPCDD/F were calculated as sum of Tera- (T), Penta- (Pe), Hexa- (Hx), Hepta- (Hp), and Octa (O)-chlorinated PCDD, PCDF, or PCDD/F, respectively

Table 3: DLPCB and Marker PCB detected in collected PM

DLPCB congener	Concentration (pg/g)
PCB 81	<100
PCB 77	1447
PCB 123	<50
PCB 118	16277
PCB 114	<50
PCB 105	9937
PCB 126	<50
PCB 167	2624
PCB 156	5015
PCB 157	1544
PCB 169	<50
PCB 189	912
Σ_1	**37757**

Marker PCB congener	Concentration (pg/g)
PCB 28	51907
PCB 52	50616
PCB 101	16576
PCB 138	32815
PCB 153	23628
PCB 180	21645
Σ_2	**197187**