CYTOTOXIC AND PRO-INFLAMMATORY EFFECTS OF COMBUSTION GENERATED ULTRA-FINE ORGANIC PARTICLES

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Abstract

Combustion processes are considered the major sources of the smallest size fraction of the primary Particulate Matter (PM) present in the atmosphere. Currently, PM is considered the most hazardous air pollutant in urban areas and the size of the particles is the most critical factor in determining the possible impact for their ability to reach deep zones of the respiratory system.

In this work we focus the attention on the possible toxic effects of combustion generated organic ultrafine particles (UFPs) with dimension of few nanometers collected at the exhaust of a commercial diesel engine. The aim is to evaluate the adverse effects of UFPs on in vitro systems. UFPs are collected from a diesel engine operated at stationary conditions when using a paraffinic diesel oil and fuels obtained by adding to the paraffinic diesel oil a fixed amount of compounds of different chemical nature (alkyl benzenes, aromatic compounds, naphthenes, fatty acid methyl esters), in order to analyse the health effect of the UFPs produced by fuels with different chemical structures.

Particles were collected by bubbling in water and condensing the exhausts emitted downstream of the particulate filter from the engine operated at constant speed driving conditions.

Two lung epithelial cell lines were used: the human alveolar epithelial cells (A549) and the human bronchial epithelial cells (BEAS-2B). Biological effects of UFPs were investigated considering the cell viability, assayed with the MTT and Alamar Blue tests, the pro-inflammatory potential assay (ELISA).

Treatment of cells with UFPs generated from the diesel fuel caused a significant reduction of cell viability at the concentration of 10 ppm while a significant increase in the production of inflammatory mediators was found already at concentration of 1 ppm. The presence of additives in the fuel resulted in an increase of both cytotoxicity and pro-inflammatory potential of the UFPs. All samples obtained from doped diesel oils were more cytotoxic. In particular, UFPs originated from the fuel doped with fatty acid methyl esters were found to have the higher toxic responses.

Introduction

Atmospheric fine particulate matter (PM) has been associated with a wide range of health problems [1]. Probable factors affecting PM toxicity are chemical composition, size and solubility, although the causal relationships between measured effects and the fraction of PM responsible for them is still a subject of research [2,3].

Several studies find that the organic fraction of PM causes a stronger toxicological response than the nacked elemental carbon or soot fraction of PM [4,5]. It is generally well-accepted that ultrafine PM (UFPs) is mostly a result of combustion processes. Epidemiological data have also shown a correlation between UFPs and cardiovascular diseases [6] but the relative role of primary organic carbon PM (emitted from combustion sources already in the particle phase) and secondary organic carbon PM (formed in atmospheric gas-to-particle reactions involving organic carbon combustion products) on human health effects is not completely understood.

Although the contribution of combustion generated organic carbon UFPs to the organic fraction of atmospheric PM is not yet well understood, the physico-chemcal characteristics (small size and complex chemical structure) of UFPs emitted from vehicles warrants examination of their reactivity with biological systems.

In this study, we use a water trap procedure to sample UFPs, isolated from soot and semivolatiles, from a diesel engine vehicle exhaust and use these water suspended UFPs for toxicological analysis. The aim is to evaluate the adverse effects on in vitro systems of UFPs emitted by a diesel engine operated in the same conditions with fuels of different chemical composition. Two lung epithelial cell lines were used: the human alveolar epithelial cells (A549) and the human bronchial epithelial cells (BEAS-2B). Biological effects of UFPs were investigated considering the cell viability, assayed with the MTT and Alamar Blue tests, the pro-inflammatory potential assay (ELISA).

Experimental apparatus and procedures

Engine

A commercial, four-cylinder common rail 1.9TD diesel engine, equipped with a diesel particulate filter, has been used for the experiments. The engine was operated at a constant speed of 1500 rpm and low load with all the examined fuels. Measurements of engine parameters and exhaust gas concentrations were performed. Exhaust gases were also analyzed for the determination of the particle size distribution function prior and after the diesel particulate trap.

Fuels

Four fuel oils were tested: a commercial paraffinic-based diesel oil having a cetane number of 52 and three blends obtained by adding 10 vol.% of reference compounds to the commercial diesel oil. The three reference compounds were monoring

alkylated aromatics, polycyclic aromatic hydrocarbons mainly comprising naphthalene, a naphthenic compounds and C17-methyl-oleate, a fatty acid methyl ester. The three reference compounds were chosen to simulate the effect of different chemical structures of the diesel oils on the composition of UFPs.

Particle collection and analysis

Combustion-generated UFPs were collected from the engine exhaust before the diesel particulate trap in order to collect a significant amount of samples for the subsequent biological analysis. A water-based sampling method, which is based on scrubbing as combustion-generated water condenses out of the exhaust flow, was used [4]. A stainless steel suction probe draws out engine products, which are then bubbled through a condenser containing 25 mL of bidistilled laboratory grade water cooled in an ice bath. All volatile components of the sampled species collected in water were removed by rotary evaporation.

The mass concentrations of UFPs collected from the engine and suspended in water were determined by UV-visible light extinction after the volatile species were removed by rotary evaporation. Absorption measurements were performed by the sample in a quartz cuvette with a path length of 1 cm. The concentration was calculated using the Lambert-Beer equation, $A = \varepsilon c L$, where A is the measured absorbance, ε is the particle absorptivity, c the concentration in mg/l, and L is the path-length of light. The value of particle absorptivity, ε= 6E-2 cm^{-1}/mg/l at 250 nm, was derived from the refractive index for nanoparticles of organic carbon produced in rich-premixed flames [7].

Chemical characterization of UFPs collected from premixed ethylene flames has shown that particles have a low degree of three-dimensional order and they are probably aromatic-aliphatic compounds with a loose nonplanar structures. These results have been confirmed by UV-vis absorption, UV-induced fluorescence and Surface Enhanced Raman Spectroscopy [8].

Cell culture and treatment

The human alveolar epithelial cells (A549) and the human bronchial epithelial cells (BEAS-2B) have been maintained at 37°C and 5% CO2, in appropriate medium. Biological effects of UFPs were investigated considering cell viability, assayed with the MTT and Alamar Blue tests and the pro-inflammatory potential (ELISA).

Cells, seeded on a 6 wells multiplate ($1x10^5$ cell/well), were treated the day after with increasing doses of UFPs (1, 3 and 7 ppm) for 24 hours. For three of the available samples, additional doses of exposure were used in order to obtain dose/response curves of cell viability and inflammatory responses. More details on the cell culture and treatments are reported elsewhere [9].

Results and discussion

Treatment with UFPs derived from the base diesel fuel induced a significant reduction of cell viability at the concentration of about 10 ppm in both the cell

lines. In particular in A549 cells (MTT test) a significant reduction occurred at about 11ppm whereas in BEAS-2B cells (Alamar Blue test) the cell viability reduction was significant already at 7ppm (Fig. 1). We have previously shown that carbon black particles did not affect A549 cell viability at concentrations even higher than those used in the present experiments [10].

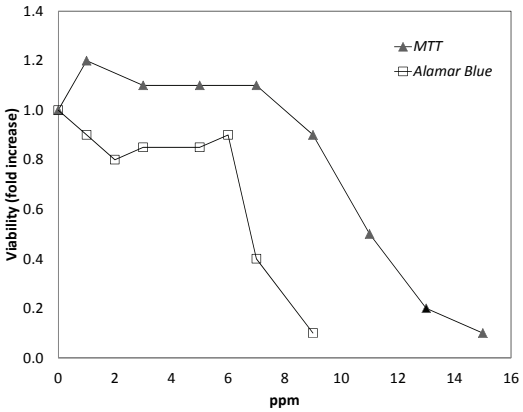

Figure 1. Cell vialibilty reduction of the human alveolar epithelial cells (A549, MTT test) and the human bronchial epithelial cells (BEAS-2B, Alamar Blue test) after 24 hr exposure with increasing doses of UFP generated by the base diesel oil.

A significant increase in the production of the pro-inflammatory mediator IL-6 by the same cells has been found already at concentration of 1 ppm as shown in Fig.2. The dose response release of IL-6 in A549 cells, showed a reduction at higher doses of UFPs treatment, possibly related to the reduced viability of the cells.

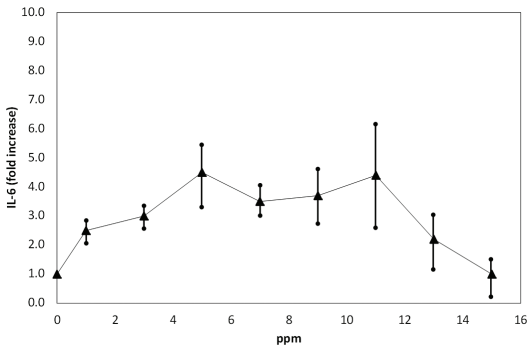

Figure 2. Production of inflammatory mediators IL-6 by the human alveolar epithelial cells (A549) after 24 hr exposure with increasing doses of UFP generated by the base diesel oil.

The presence of additives in the fuel resulted in an increase of both cytotoxicity and pro-inflammatory potential of the UFPs. All samples obtained from doped diesel oils were more cytotoxic than the commercial fuel. In particular, UFPs originated from the fuel doped with fatty acid methyl esters (FAME), generally used as representative of a biodiesel, have been found to have the higher toxic responses. Figure 3 shows the cell viability reduction after 24 hr exposure with increasing doses of UFP generated by the combustion of the base diesel oil, the oil doped with monoring alkylated aromatics (monoring), the polycyclic aromatic compounds (polyclic), the naphthenic compounds (naphtenic) and the fatty acid methyl ester (FAME). There was a significant reduction of cell viability moving from the base diesel oil towards the doped fuels. Interestingly the FAME doped-fuel derived UFPs resulted to be the most toxic and induced a significant cell viability reduction already at the concentration of 0.75 ppm in BEAS-2B cells (Alamar Blue test).

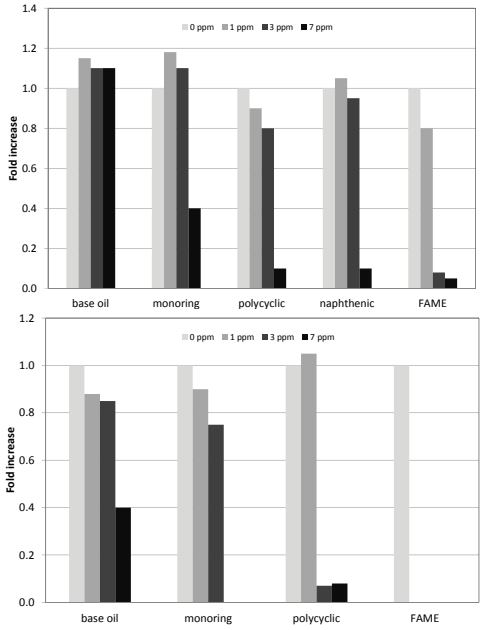

Figure 3. Cell vialibilty reduction of the human alveolar epithelial cells (A549) (MTT test) (top) and the human bronchial epithelial cells (BEAS-2B) (Alamar Blue test) (bottom) after 24 hr exposure with increasing doses of UFP generated by the base diesel oil and the doped diesel oils.

The data obtained show that combustion processes generate organic nanoparticles with high cytotoxic and pro-inflammatory potential. These particles differ from the soot fraction, which is also emitted from the engines, for having dimension smaller than 3nm and an organic carbon structure. The chemical composition of these

organic carbon UFPs seems to be more similar to polyclic aromatic hydrocarbons probably aliphatic-linked with a loose nonplanar structures than to order graphitic-like carbon as the soot particles.UFPs produced by biodiesel combustion were more toxic than those produced by the reference fuel. These findings deserve particular attention and will be subject of further investigations.
Although scientific community has long given attention to diesel combustion derived particles, this study show that UFPs produced in certain phases of the combustion cycle or due to innovations in the engines, may have a potential impact on biological systems. It also appears that the addition of additives in fuel causes a significant increase in toxicity of combustion derived nanoparticles.

Conclusions

The possible toxic effects of combustion generated organic ultrafine particles collected at the exhaust of a commercial diesel engine has been analyzed. UFPs are collected from a diesel engine operated at stationary conditions when using a paraffinic diesel oil and fuels obtained by adding to the paraffinic diesel oil a fixed amount of compounds of different chemical nature (alkyl benzenes, aromatic compounds, naphthenes, fatty acid methyl esters).
The human alveolar epithelial cells (A549) and the human bronchial epithelial cells (BEAS-2B) have been used to determine the biological effects of UFPs. Cell viability and pro-inflammatory potential were investigated and the results showed significant effects of UFPs on these two parameters .
Indeed UFPs caused a significant reduction of cell viability and a significant increase in the production of inflammatory mediators. The presence of additives in the fuel resulted in an increase of both cytotoxicity and pro-inflammatory potential of the UFPs. In particular, UFPs originated from the fuel doped with fatty acid methyl esters have been found to have the higher toxic responses. These results underline the importance of further research on the impacts of combustion derived UFPs on human health.

Acknowledgments

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