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Chemical and microbial components of urban air PM cause seasonal variation of toxicological activity

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ABSTRACT

The chemical and microbial composition of urban air particulate matter (PM) displays seasonal variation that may affect its harmfulness on human health. We studied the *in vitro* inflammatory and cellular metabolic activity/cytotoxicity of urban air particulate samples collected in four size-ranges ($PM_{10–2.5}$, $PM_{2.5–1}$, $PM_{1–0.2}$, $PM_{0.2}$) during four seasons in relatively clean urban environment in Helsinki, Finland. The composition of the same samples were analyzed, including ions, elements, PAH compounds and endotoxins. In addition, microbial contribution on the detected responses was studied by inhibiting the endotoxin-induced responses with Polymyxin B both in the PM samples and by two different bacterial strains representing Gram-positive and -negative bacteria. Macrophage cell line (RAW 264.7) was exposed to the size segregated particulate samples as well as to microbe samples for 24 h and markers of inflammation and cytotoxicity were analyzed. The toxicological responses were dependent on the dose as well as size range of the particles, $PM_{10–2.5}$ being the most potent and smaller size ranges having significantly smaller responses. Samples collected during spring and autumn had in most cases the highest inflammatory activity. Soil components and other non-exhaust particulate emissions from road traffic correlated with inflammatory responses in coarse particles. Instead, PAH-compounds and K^+ had negative associations with the particle-induced inflammatory responses in fine particles, suggesting the role of incomplete biomass combustion. Endotoxin content was the highest in $PM_{10–2.5}$ samples and correspondingly, the largest decrease in the responses by Polymyxin B was seen with the very same samples. We found also that inhibitory effect of Polymyxin B was not completely specific for Gram-negative bacteria. Thus, in addition to endotoxin, also other microbial components may have a significant effect on the toxicological responses by ambient particulate matter.

1. Introduction

Mass concentrations of urban air particles are associated with mortality and morbidity in urban populations (USEPA, 2004; WHO, 2003). However, there have been significant heterogeneities in the concentration-response relationships for particulate-associated hospital admissions (Atkinson et al., 2001) and daily mortality in Europe (Samoli et al., 2005) and the United States (Dominici et al., 2006) and China (Cheng et al., 2013). Moreover, mortality estimates for particulate concentrations (Nawrot et al., 2007), as well as the prevalence of stroke (Kettunen et al., 2007) have had seasonal variation.

Urban air particulate matter is a complex mixture of different chemical and microbial components, originating from distinct sources. In the atmosphere, these particles undergo chemical and physical transformation *via* numerous mechanisms that are largely dependent on the season. There is increasing evidence that in addition to particulate mass concentration, its composition is a major determinant of the adverse health effects (USEPA, 2004; WHO, 2003; Pope and Dockery, 2006). Particle size has been the major determinant of the responses in the toxicological studies (*e.g.* Becker et al., 2003; Happo et al., 2010; Kroll et al., 2013; Steenhof et al., 2011). But even until now the sources and compositions of harmful particles are not fully revealed. Unlike most of the studies, in the present study separate $PM_{1–0.2}$ and $PM_{2.5–1}$ size ranges were included since they have demonstrated different toxicological responses in previous studies (Jalava et al., 2006; Happo et al., 2010). Thus, it is essential to clarify which

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components in inhalable particulate matter are responsible for the toxicity, varying in relation to season or source.

In our previous study (Happo et al., 2010) the very same samples induced significant seasonal variation in the immunotoxic responses in mouse lungs. In a very recent study conducted in a small Finnish town, Kuopio, the particulate sources, compositions and the induced toxicological responses showed considerable seasonal variations (Happo et al., 2014). From the particle sources, engine emissions and other combustion sources have been associated with mortality (Clancy et al., 2002; Hoek et al., 2002), exacerbations of heart disease (Lanki et al., 2006) and increased cardiovascular emergency room visits (Metzger et al., 2004). Respiratory hospital admissions and asthma symptoms seem to be dependent on coarse particle concentrations (Brunekreef and Forsberg, 2005; Meister and Forsberg, 2007; Malig et al., 2013). In addition to natural soil sources the coarse particles are also derived from traffic related resuspension (Amato et al., 2010). In Finland, where roads are sanded during winter, coarse mineral particles cause frequent air pollution episodes in springtime when the snow melts. Other important source of particulate matter in the air in Finland is heating with wood, which produces large amounts of particles during the heating season.

The relevance of microbial constituents in urban air PM size fractions to human health is largely overlooked and current knowledge is mainly limited to the Gram-negative bacterial endotoxins. It is known that endotoxins increase the risk for asthma development (Tavernier et al., 2005) and occupational respiratory symptoms (Douwes et al., 2003). In the toxicological studies, endotoxin has been linked to increased inflammatory responses (*e.g.* Monn and Becker 1999). Endotoxin has been more abundant in the coarse than in the fine particles, and its concentration has shown seasonal variation (Heinrich et al., 2003). However, endotoxin is only one microbial component, it has been estimated that up to 25% of atmospheric particles are of biological origin, *e.g.* fragments of pollens and microbes and all kinds of plant and animal debris (Jones and Harrison, 2004). Aiming to rule out possible role of endotoxin on the detected toxicological responses, Polymyxin B (*e.g.* Salonen et al., 2004) has been used in several studies. However, it may inhibit the toxicological properties of other microbes regardless of the Gram-negative bacterial content, which may lead to false interpretations regarding the role of microbial constituents in detected toxicological characteristics of PM.

We studied toxicological effects of particulate samples collected at the same suburban site in Helsinki during four seasons, which had distinct temperature, precipitation and solar radiation parameters as well as snow cover during winter. The particulate samples were collected in four size ranges; $PM_{10–2.5}$, $PM_{2.5–1}$, $PM_{1–0.2}$ and $PM_{0.2}$. Moreover, Gram-positive and Gram-negative microbial strains were included in the experiments in order to reveal the role of different microbes on the responses. The objectives of the present study were: (1) to investigate the seasonal variation in the inflammatory (NO, TNFa, IL-6) and cellular metabolic (MTT-test) activities of urban air particulate samples in the macrophage cell line (RAW 264.7), (2) to identify particulate constituents and sources contributing to these activities, and (3) to evaluate the role of microbial composition to the observed responses.

2. Material and methods

2.1. Sampling campaigns and particulate sources

The samples were collected in Helsinki, using a modified Harvard high volume cascade impactor (HVCI) (Sillanpää et al., 2003). The $PM_{10–2.5}$, $PM_{2.5–1}$ and $PM_{1–0.2}$ samples were collected on polyurethane foam and the $PM_{0.2}$ samples on glass fiber filters (Jalava et al., 2006).

The sampling site was located in Kallio, Helsinki 2 km north of the downtown and at a distance of 300 m from the nearest major street with average traffic density of 30 000 vehicles/day. The major local sources of particulate pollution during the sampling campaigns were traffic, power plants, ships at city harbors, and residential wood combustion. Majority of the fine particulate mass on the site has been previously attributed to long-range transport of air pollution (Pakkanen et al., 2001; Vallius et al., 2003; Sillanpää et al., 2006).

2.2. Sample preparation for cell studies

The size-segregated particulate samples for the chemical analysis and cell experiments were prepared using previously validated procedures (Jalava et al., 2005; Jalava et al., 2006). Briefly, methanol washed PUF substrates and glass fiber filters were weighted using an analytical balance before and after particulate sampling. The sampled PUF-strips or glass fiber filters were extracted with methanol 2×30 min in an ultrasonic water bath at 20 °C. The methanol extracts of each seasonal sample were pooled together by size range, and the excess methanol was evaporated with a rotary evaporator. Thereafter, the methanol suspension containing $PM_{0.2}$ particles was filtered to remove glass fibers derived from backup filters. Finally, the concentrated suspension was divided into sample tubes on mass basis and the tubes were dried under nitrogen (99.5%) flow and stored at −20 °C. (Happo et al., 2010). Extensive chemical characterization of the particulate samples was made, including analysis of anions and cations, water-soluble elements, PAH-compounds and endotoxinds as previously described in detail by Happo et al. (2010).

2.3. Cell culture

A mouse macrophage cell line RAW264.7 obtained from American Type Culture Collection (ATCC, Rockville, MD, USA) was cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine and 1% penicillin streptomycin (Gibco BRL, Paisley, UK). The cells were cultured at 37 °C and 5% CO_2 atmosphere. For the experiments, the cell suspension was diluted to 5×10^5 cells/ml. One hour before the experiments, 1 ml of fresh medium (37 °C) was changed on the 6-well plates (Costar, Corning, NY, USA). The cells were cultured for 24 h in the experiments.

2.4. Experimental setup

Before the cell experiments, sample and blank tubes were sonicated for 30 min in an ultrasonic water bath to suspend the size-segregated particulate samples into water (Sigma W1503, St. Louis, MO, USA) at a concentration of 5 mg/ml. The exposures were made on equal mass basis to all the particulate samples. Macrophage cell line was separately exposed to the $PM_{10–2.5}$, $PM_{2.5–1}$, $PM_{1–0.2}$ and $PM_{0.2}$ samples at four doses of 15, 50, 150 and 300 μg/ml for 24 h. Three independent experiments were made in duplicate. After adding the sample suspension, the volume of each well was adjusted to 2 ml by adding fresh medium. To rule out the possible contamination, each plate had also an untreated cell control and a sampling substrate blank at a volume corresponding to the dose 150 μg/ml of the particulate samples.

After exposing the macrophages to the particulate samples for 24 h, the cells were resuspended into cell culture medium by scraping. Cytotoxicity (cells' metabolic activity) was measured with the MTT-test from cell suspension (2×100 μl) of each well. The remaining cell suspension was centrifuged to separate the cells