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Ambient ultrafine particles activate human monocytes: Effect of dose, differentiation state and age of donors

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ABSTRACT

Exposure to ambient particulate matter (PM) has been linked to adverse pulmonary and cardiovascular health effects. Activation of both inflammatory and oxidative stress pathways has been observed and may be a probable cause of these outcomes. We tested the hypothesis that in human monocytes, PM-induced oxidative and inflammatory responses are interrelated. A human monocytic cell line (THP-1) was used to determine if dose and differentiation state plays a role in the cellular response after a 24hr exposure to particles. Primary human monocytes derived from eight female, non-smoker donors (aged: 21, 24, 27, 28, 48, 49, 54 & 60yo) were used to determine if the age of donors modulates the response. Cells were treated with aqueous suspensions of ambient ultrafine particles (UFP, defined as smaller than 0.2 µm in size) or a media control for 24hr. After exposure, reactive oxygen species (ROS) formation was increased irrespective of dose or differentiation state of THP-1 cells. In the primary human monocytes, ROS formation was not significantly changed. The release of the proinflammatory cytokine, tumor necrosis factor alpha (TNF-α), was dose-dependent and greatest in differentiated compared to undifferentiated THP-1 cells exposed to UFP. In the Primary human monocytes, TNF-α secretion was increased irrespective of the age of the donor. Our results suggest that after a 24hr exposure to particles, general reactive oxygen species formation was nonspecific and uncorrelated to cytokine secretion which was consistently enhanced. Cytokines play an important role in orchestrating many immune responses and thus the ability of ambient particles to enhance robust secretion of a proinflammatory cytokine from primary human monocytes, and how this may influence the response to pathogens and alter disease states, needs to be further evaluated.

1. Introduction

Particle formation from vehicle exhaust and industrial emissions is a prominent cause of air pollution in the modern urban environment. Epidemiological studies have found a correlation between exposure to ambient particles and increased risk of morbidity and premature mortality (Dockery et al., 1993; Brook et al., 2004; Chen et al., 2013; Chen et al., 2017; Li et al., 2017). PM associated mortality was mainly contributed to cardiopulmonary causes and lung cancer (Pope et al., 1995). According to the World Health Organization (WHO), in 2012, exposure to ambient air pollution was estimated to cause approximately three million deaths. Of these mortalities, 8% was associated with chronic obstructive pulmonary disease; 15% connected with ischemic heart disease; 17% linked to respiratory infection and 25% related to lung cancer (WHO, 2017).

Ambient particulate matter (PM) comprises a wide range of metallic, inorganic and organic compounds, which determine its toxic potential. Many studies show that particles have the capability of enhancing cellular oxidative and inflammatory responses in vitro (Schwarze et al., 2007; Araujo and Nel, 2009; Steenhof et al., 2011; Ghio et al., 2012; Thomson et al., 2016). Particle induced oxidative stress and inflammation also play an important causative role in many diseases including cardiovascular disease (Lawal et al., 2017). The objective of this study was to test the hypothesis that PM-induced oxidative and inflammatory changes in human monocytes are interconnected and influenced by dose, differentiation state, and age of donors.

Inhaled ultrafine particles (UFP, defined as particles smaller than approximately 0.2 µm) have been shown to translocate from the lung to the blood (Nemmar et al., 2001, 2002). Once particles are in the peripheral circulation, monocytes are one of the first cellular defense responders. To test how dose (2 or 20 µg/mL) and differentiated state of monocytes influence response, THP-1 cells were used. This inexpensive and well-characterized in vitro model has been well established to study

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the potential of ambient particles to activate immune-competent cells (Qin et al., 2012; Wu et al., 2014). The concentration chosen is based on other in vitro studies which use a wide range (1–500 μg/mL) for toxicity testing (Bhavaraju et al., 2014; Wu et al., 2014; Thomson et al., 2016). Although reactive oxygen species (ROS) formation was enhanced, this was independent of dose or differentiation state of the cells. On the other hand, differentiated THP-1 cells were more capable compared to undifferentiated cells of releasing the proinflammatory cytokine, tumor necrosis factor alpha (TNF-α), in response to particle exposure. This response was dose-dependent. Primary human monocytes from eight different donors (ages: 21, 24, 27, 28, 48, 49, 54, and 60 years old) were exposed to the lower and more biologically relevant concentration of UFP (2 μg/mL) for 24hr. Although particles did not induce ROS formation in primary monocytes, they caused a robust secretion of TNF-α independent of the age of the donor. Our results indicate that contrary to our hypothesis, the oxidative and proinflammatory response of human monocytes to UFP do not appear to be correlated.

2. Methods

2.1. Particle collection & characterization

UFP (particles with aerodynamic diameter less than 0.2 μm) were collected continuously for approximately 40 days by means of a high-volume ultrafine particle (HVUP) sampler, operating at 400 L/min (Misra et al., 2002). Sampling was conducted in downtown Los Angeles. The sampling site represents a typical urban background site impacted by mostly traffic sources and located about 150 m east of the I-110 freeway (Ning et al., 2007). For the purpose of chemical speciation, the sampler was loaded with zefluor filters (supported PTFE, 3.0 μm pore, 8″ × 10″, Pall Life Sciences). The collected particles were then transferred into an aqueous suspension by soaking of the particle-loaded filter in ultrapure water, followed by 5 min vortexing and 15 min sonication. Aliquots of the UFP slurry samples were analyzed for total organic carbon (TOC) as well as total metals and trace elements. TOC content was determined using a Sievers 900 Total Organic Carbon Analyzer (Stone et al., 2009). Metals and elements were quantified by means of high-resolution magnetic sector inductively coupled plasma mass spectrometry (SF-ICP-MS, Thermo-Finnigan Element 2), following acidification (16N HNO_3) of the slurry sample (Zhang et al., 2008).

Table 1
PM Characterization: mass fraction of total organic carbon (TOC), as well as select metals and elements in the UFP slurry.

Percent (%) Species Fraction	Ultrafine PM
Total Organic Carbon	56.7
Na	6.1
Al	1.5
K	1.1
Mg	1.7
Ca	5.4
Fe	0.5
Mn	0.05
S	6.2
Ti	0.02
V	0.006
Cr	0.014
Cu	0.1
Ni	0.04
Zn	0.5
Ba	0.12
Pb	0.02

2.2. Exposure

2.2.1. THP-1 cells

THP-1 human leukemic monocytes (ATCC) were maintained at 37 °C in 5% CO2. The cells were grown in RPMI-1640 medium (ATCC) containing 0.05 mM 2-mercaptoethanol, 10% fetal bovine serum, and 1% Pen/Strep. THP-1 cells were seeded (10,000 cells/well) in a 96-well plate. For differentiation, cells were incubated with 12-O-tetradecanoylphorbol-13-acetate (TPA) for 48hr (Tsuchiya et al., 1982). Cells were exposed to 0 (control), 2 or 20 μg/mL of UFP for 24hr. Before and after exposure, the cells were visualized and changes were captured using an EVOS FL Cell Imaging System (Life Technologies). After the 24hr exposure, supernatant was collected. The cells were immediately used for ROS assay. Cell cytotoxicity was measured in THP-1 cells by evaluating lactate dehydrogenase (LDH) in the supernatant using the LDH-cytotoxicity colorimetric assay kit from Biovision (Milpitas, CA). The cellTiter-Glo luminescent cell viability assay by Promega (Madison, WI) was used to evaluate cell viability in primary human monocytes at the conclusion of the last kinetic reading for ROS formation.

2.3. Primary monocytes

CD14+ primary monocytes, isolated from peripheral blood, were purchased from StemCell Technologies (Cambridge, MA). Eight different donors were selected as biologically independent samples and each of the samples were run in quadruplicates. The donors were all female Caucasian nonsmokers. The ages were (21, 24, 27, 28, 48, 49, 54 and 60 years old). To better delineate the association of age to particle response, the data is presented per individual as well as divided into two age groups (21, 24, 27, 28 = younger donors and (48, 49, 54, 60 = older donors). Cells were thawed and grown in RPMI 1640 medium supplemented with 10% FBS following the manufacturer's protocol. Cells were seeded at a density of 10,000/well in 96 well plates for 24hr before exposure with 2 μg/mL of UFP. Supernatant was collected and ROS formation was determined followed by CellTiter-Glo luminescent cell viability assay (Promega, Wisconsin). Secreted LDH levels were also quantitated as another measure of cytotoxicity.

2.4. Reactive oxygen species formation

2′,7′-dichlorofluorescein diacetate (DCFH-DA) was used to measure non-specific ROS formation after PM exposure. This dye rapidly diffuses into cells and is hydrolyzed by intracellular esterases to dichlorofluorescein (DCFH). DCFH is oxidized to the fluorescent 2′,7′-dichlorofluorescein (DCF) by generated reactive oxygen species. DCF standard curve was used to quantify ROS formation. BioTek Synergy HT Microplate Reader was used to measure the fluorescence generated kinetically for 1hr at 10 min intervals at an excitation of 485 nm and emission of 528 nm. Results shown are for amount of DCF generated in 30 min and normalized based on viable cells in corresponding well.

2.5. Viability assays

CellTiterGlo-Luminescent cell viability assay was used to measure the number of viable primary human monocytes following the manufacturer's protocol. This assay measures the amount of ATP present in each sample and is based on the assumption that the number of viable cells are directly proportional to the ATP content. Briefly, 100 μl of the CellTiter-Glo reagent is added to each well and the samples are incubated for 10 min at room temperature. The luminescence was recorded and the data was calculated as percent control. Levels of LDH in the supernatant was evaluated for THP-1 cells using the LDH-cytotoxicity colorimetric assay kit provided by Biovision. This assay measures the activity of the enzyme as a marker for membrane damage. Substrate is added to the samples and after a 30 min incubation, the absorption was measured at 450 nm using a BioTek Synergy HT Microplate Reader.

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