NeuroToxicology 58 (2017) 50–57

Contents lists available at ScienceDirect

NeuroToxicology

Full Length Article

Exposure to ambient ultrafine particulate matter alters the expression of genes in primary human neurons

Parrisa Solaimani[a], Arian Saffari[b], Constantinos Sioutas[b], Stephen C. Bondy[c], Arezoo Campbell[a,*]

[a] Department of Pharmaceutical Sciences, Western University of Health Sciences, Pomona, CA, USA
[b] Department of Civil and Environmental Engineering, University of Southern California, Los Angeles, CA, USA
[c] Center for Occupational and Environmental Health, Department of Medicine, University of California, Irvine, CA, USA

ARTICLE INFO

Article history:
Received 5 August 2016
Received in revised form 7 November 2016
Accepted 8 November 2016
Available online 13 November 2016

Keywords:
Air pollution
Ultrafine particulate matter
Human neurons
Noncoding RNA
Neurodevelopment
Metallothionein
Genetic imprinting

ABSTRACT

Exposure to ambient particulate matter (PM) has been associated with the onset of neurodevelopmental and neurodegenerative disorders, but the mechanism of toxicity remains unclear. To gain insight into this neurotoxicity, this study sought to examine global gene expression changes caused by exposure to ambient ultrafine PM. Microarray analysis was performed on primary human neurons derived from fetal brain tissue after a 24 h exposure to 20 μg/mL of ambient ultrafine particles. We found a majority of the changes in noncoding RNAs, which are involved in epigenetic regulation of gene expression, and thereby could impact the expression of several other protein coding gene targets. Although neurons from biologically different lot numbers were used, we found a significant increase in the expression of metallothionein 1A and 1F in all samples after exposure to particulate matter as confirmed by quantitative PCR. These metallothionein 1 proteins are responsible for neuroprotection after exposure to environmental insult but prolonged induction can be toxic. Epidemiological studies have reported that *in utero* exposure to ultrafine PM not only leads to neurodevelopmental and behavioral abnormalities, but may also predispose the progeny to neurodegenerative disease later in life by genetic imprinting. Our results pinpoint some of the PM-induced genetic changes that may underlie these findings.

1. Introduction

Emerging evidence has implicated air pollution exposure as a risk factor for both neurodevelopmental and neurodegenerative abnormalities (Block et al., 2012; Costa et al., 2014; Davis et al., 2013; Genc et al., 2012). Maternal exposure to ultrafine particulate matter (PM) can alter fetal brain development in a manner that predisposes the progeny to neurodevelopmental disorders (Allen et al., 2014; Hougaard et al., 2015). Numerous reports have linked prenatal exposure to air pollution with an increased incidence of autism spectrum disorder (ASD) among children (Kalkbrenner et al., 2015; Raz et al., 2015; Volk et al., 2013). Correlations have been made between air pollution exposure and several other behavioral disorders including anxiety, depression, and schizophrenia (Pedersen et al., 2004; Perera et al., 2012, 2011). Cognitive deficits and structural brain alterations have also been found in children exposed to high levels of air pollution (Calderon-Garciduenas et al., 2011; Freire et al., 2010). Early life exposure may result in genetic imprinting that increases the risk of neurodegenerative disease later in life (Lahiri et al., 2009) including decreased cognitive function, stroke, Parkinson's disease, and Alzheimer's disease (AD) (Block et al., 2012; Chen and Schwartz, 2009; Moulton and Yang, 2012; Ritz et al., 2016). The effects of air pollution on the aging brain may also be direct and a recent population-based cohort study in Taiwan found a 138% increased risk for developing AD when living in areas containing high PM (Jung et al., 2015).

Despite our current knowledge of the adverse CNS effects of air pollution and despite existing EPA standards in the USA, it is still estimated that over 100 million people live in areas that exceed the recommended air quality levels (United States. Environmental Protection Agency, Office of Air Quality Planning and Standards and United States, Environmental Protection Agency, Air Quality Trends Analysis Group). Living under such circumstances is especially prevalent in urban areas. PM is generally the air pollutant that exceeds the recommended air quality standards (Moulton and

* Corresponding author at: Department of Pharmaceutical Sciences, College of Pharmacy, Western University of Health Sciences, 309 E. Second Street, Pomona, CA 91766-1854, USA.
E-mail address: acampbell@westernu.edu (A. Campbell).

http://dx.doi.org/10.1016/j.neuro.2016.11.001
0161-813X/

Yang, 2012). PM is a complex mixture of solid particles and liquid droplets suspended in the air. It originates from a variety of sources including, but not limited to, vehicle emissions and industrial by-products (Hasheminassab et al., 2013). PM is characterized by its size, aerodynamic properties, and chemical composition, which can all influence its biological effects. Ultrafine particles (UFP) in particular are not filtered out during their passage through the nose and bronchioles but can penetrate to the alveoli. From here they are able to enter the blood circulation where they can be distributed throughout the body (Genc et al., 2012). Because of the small size, it is believed that UFP are able to cross the blood brain barrier (Block and Calderon-Garciduenas, 2009). Their large surface area, can enhance both oxidant capacity and greater inflammatory potential. Together with their ability to access the CNS these properties allow ultrafine particles to contribute to the pathogenesis of CNS diseases (Poon et al., 2004).

Although growing evidence implicated air pollution as contributing to adverse health effects on the brain, the underlying mechanisms and sequence of events that culminate in neurotoxicity remain poorly understood (Block et al., 2012; Lucchini et al., 2012). In this study, we evaluated gene expression profiles in human neurons after exposure to UFP. The UFP were collected from a sampling site in downtown Los Angeles, which is heavily impacted by pollution from vehicle emissions. The use of normal primary neurons serves as a good model system for identifying changes in gene expression in the human brain, particularly when observing gene expression changes that may be species-specific. We performed a whole genome wide expression analysis upon neuronal cells derived from fetal tissue of three different samples. Understanding how UFP affects gene expression may help identify early genetic markers of dysregulated pathways that ultimately contribute to CNS abnormalities (Block et al., 2012; Cooper-Knock et al., 2012). This exploratory report may help explain the mechanism by which exposure to UFP can contribute to the etiology of several neurodevelopmental and neurodegenerative diseases.

2. Materials and methods

2.1. UFP collection & characterization

Ambient ultrafine particles (UFP, diameter Dp < 0.18 μm) were collected during January and February of 2014, on Zefluor filters (supported PTFE, 3.0 μm pore size, Pall Life Sciences) using a High-Volume Ultrafine Particle (HVUP) Sampler (Misra et al., 2002) at 400 L/min near downtown Los Angeles, about 120 m downwind of the CA-110 Freeway. The particles were then extracted into an aqueous suspension by 5 min of soaking in Milli-Q ultrapure water (resistivity 18.2 MΩ), followed by vortexing (5 min) and sonication (30 min). The dose of the PM solution was determined by gravimetric analysis. Briefly, the filters were weighed prior to the extraction using a microbalance (Sartorius, model LA 130-F). After the extraction process was completed the filters were dried and weighed again. The difference of the two gravimetric measurements was the mass of the PM that was extracted into the solution. Elemental content of the slurries was quantified using a high-resolution sector field inductively coupled plasma mass spectrometry (ICP-MS, Thermo Finnigan Element 2). Details of this method are described in Herner et al. (Herner et al., 2006). Total organic carbon was also quantified using a Sievers 900 organic carbon analyzer, following the method of Stone et al. (Stone et al., 2009). To investigate whether airborne size characteristic is preserved after extraction in ultrapure water using vortexing and sonication, we measured the aqueous-phase size distribution of PM using Dynamic Light Scattering (DLS). The size distribution of particles in the aqueous-phase by both number and surface area were partitioned in a size range similar to the airborne ultrafine PM, indicating that size distribution is overall well-preserved after water extraction, and the impact of particle agglomeration is trivial.

2.2. Primary neuron cell culture and treatment

Primary human neurons (HN, Cat. #1520) were purchased from ScienCell Research Laboratories and maintained at 37 °C in 5% CO_2 in neuronal medium (NM, Cat. #1521) supplemented with 1% neuronal growth supplement (NGS, Cat. #1562) and 1% penicillin/streptomycin solution (P/S, Cat. #0503). Neurons were plated at a density of 5 × 10^5 cells per well on 6-well cell culture plates that had been pre-treated with 15 μg/mL poly-L-lysine (PLL, Cat. #0413). The neurons were fully differentiated before treatments. Cells were determined to be differentiated by distinct morphological features that are characteristic of normal neurons we have previously reported (Campbell et al., 2014). Neurons were treated for 24 h with either an ultrapure aqueous solution (control) or with 20 μg/mL ultrafine particulate matter suspended in the ultrapure aqueous solution. In our previous studies we demonstrated that ROS and TNF-alpha levels change in primary human neurons exposed to PM within 24 h, and thus continued to use this time point for our current study (Campbell et al., 2014). Primary human neurons came from 3 different fetal samples and were identified by their lot numbers: 10866, 12732 and 13879. UFP were used for exposures because of their unique ability to penetrate the blood-brain-barrier (BBB) and gain access to neurons. In our previous experiments, we had used UFP in the range of 2–20 μg/mL. In this study, the higher dose was selected to better reflect the concentrations used by other investigators. These are in the range of 1–500 μg/mL (Bhavaraju et al., 2014; Campbell et al., 2014; Han et al., 2012).

2.3. Cell viability assays

Cells were lysed and an ATP-dependent cell viability assay was used to determine the viability of the cells after treatments with ultrafine PM. Cell Titer Glo reagent was used according to the manufacturers protocols (Promega, Madison, Wisconsin).

2.4. RNA collection and quantitative real-time PCR

RNA was isolated using RNeasy Mini columns (Qiagen) according to the manufacturer's instructions. Collected RNA was either directly used for microarray analysis, or saved for subsequent confirmation of microarray results by quantitative real time PCR (qPCR) analysis. Briefly, reverse transcription of 1 μg of total RNA was performed with SuperScript III Reverse Transcriptase (Life Technologies) according to the manufacturer's protocols using random hexamer primers (Life Technologies). Synthesized cDNA was then used as a template for qPCR at a 1:10 dilution and quantitative PCR analysis was performed using SYBR Green PCR Master Mix (Applied Biosystems) according to standard protocols on a CFX96 Touch (Bio-Rad) real-time PCR machine. Primers were designed with Primer Express 3.0 (Table S1) using the Transcript Cluster sequence used on the Affymetrix microarray, and then purchased from Fisher Scientific (Pittsburgh, PA). Data was normalized to the HMBS housekeeping gene. We previously determined that the expression of this housekeeping gene was not influenced by exposure to PM, and out of 14 well known housekeeping genes tested HMBS appeared to be best suited to use in primary neurons (data not shown). The housekeeping gene primers came from a set of validated primers whose sequences were proprietary (Prime PCR plate, Bio-Rad, cat# 10025217). Where applicable, a student's 2-tailed *t*-test was performed to