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Exposure to inhaled particulate matter activates early markers of oxidative stress, inflammation and unfolded protein response in rat striatum

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HIGHLIGHTS

- Ultrafine PM induce HO-1 and SOD-2 expression and Nrf-2 activation in CNS.
- Ultrafine PM induce activation of NF-κB and increase of IL-1β and TNFα in striatum.
- Presence of UPR increase of XBP-1S and BiP in striatum after exposure to coarse PM.

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ABSTRACT

To study central nervous system airborne PM related subchronic toxicity, SD male rats were exposed for eight weeks to either coarse (32 μg/m^3), fine (178 μg/m^3) or ultrafine (107 μg/m^3) concentrated PM or filtered air. Different brain regions (olfactory bulb, frontal cortex, striatum and hippocampus), were harvested from the rats following exposure to airborne PM. Subsequently, prooxidant (HO-1 and SOD-2), and inflammatory markers (IL-1β and TNFα), apoptotic (caspase 3), and unfolded protein response (UPR) markers (XBP-1S and BiP), were also measured using real-time PCR. Activation of nuclear transcription factors Nrf-2 and NF-κB, associated with antioxidant and inflammation processes, respectively, were also analyzed by GSMA. Ultrafine PM increased HO-1 and SOD-2 mRNA levels in the striatum and hippocampus, in the presence of Nrf-2 activation. Also, ultrafine PM activated NF-κB and increased IL-1β and TNFα in the striatum. Activation of UPR was observed after exposure to coarse PM through the increment of XBP-1S and BiP in the striatum, accompanied by an increase in antioxidant response markers HO-1 and SOD-2. Our results indicate that exposure to different size fractions of PM may induce physiological changes (in a neuroanatomical manner) in the central nervous system (CNS), specifically within the striatum, where inflammation, oxidative stress and UPR signals were effectively activated.

Abbreviations: ATF6, activating transcription factor 6; BiP, heat shock 70 kDa protein 5 (glucose-regulated protein, 78 kDa); GSMA, gel shift mobility assay; HO-1, heme oxygenase 1; IL-1β, interleukin 1 *beta*; IRE1α, inositol-requiring protein 1; NF-κB, nuclear factor *kappa*-light-chain-enhancer of activated B cells; Nrf-2, nuclear factor (erythroid-derived 2)-like 2; PAHs, polycyclic aromatic hydrocarbons; PERK, eukaryotic translation initiation factor 2-alpha kinase 3; PM, particulate matter; SOD, superoxide dismutase; TNFα, tumor necrosis factor-alpha; UPR, unfolded protein response; VACES, versatile aerosol concentrator system; XBP-1, X-box binding protein 1; XBP-1S, XBP-1 spliced form; XBP-1U, XBP-1 unspliced form.

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1. Introduction

The deleterious effects of air pollution, and especially particulate air pollution, on the lungs and the heart have been well established. Oxidative stress and inflammation have been suggested as some of the underlying mechanisms involved in the toxicity on the cardiopulmonary system induced by exposure to particulate matter (PM) (Katsouyanni et al., 1997; Pope and Dockery, 2006; Araujo, 2011). Exposure to environmental stressors has been reported to also influence the development of neurodegenerative diseases (Dosunmu et al., 2007; Migliore and Coppedè, 2009; Ranft et al., 2009). However the involvement of free radical production and inflammatory processes as stressors in the central nervous system (Abbott, 2011; Block and Calderon-Garciduenas, 2009; Calderon-Garciduenas et al., 2004; Landrigan et al., 2005) is still an area of needing intense research.

Reports have documented increase in neuroinflammation and accumulations of misfolded proteins: β-amyloid and α-synuclein, in the brains of residents living in highly polluted cities (Calderon-Garciduenas et al., 2004). The same authors showed higher particulate deposition (<100 nm) in olfactory bulb neurons and alterations in the blood–brain barrier in the brain of children and young adults living in cities with high air pollution *vs.* those living in conditions of low air pollution (Calderon-Garciduenas et al., 2008). Inhaled particles, or components, that deposit in the nose can access the brain, either by transport along olfactory nerves or possibly by penetration of the blood–brain barrier through systemic distribution of PM (Oberdorster et al., 2002, 2004). Neuroinflammatory events associated with exposure to PM_{10} (particles with mass median diameters $\leq 10\,\mu m$) and $PM_{2.5}$ (particles $\leq 2.5\,\mu m$ mass median diameter), have been observed in experimental murine models as well as in case-control studies from human tissue, where an increase of pro-inflammatory cytokines in the presence of nuclear factor kappa B (NF-κB) activation was observed (Calderon-Garciduenas et al., 2004; Campbell et al., 2005; Gerlofs-Nijland et al., 2010; Kleinman et al., 2008). Additionally, oxidative stress and cerebral vascular damage have also been described as relevant effects induced by exposure to PM, biological effects that may be systemic and local in the different brain regions (Block and Calderon-Garciduenas, 2009).

While production of free radicals and oxidant molecules are part of normal metabolic and inflammatory processes, excessive production of these molecules can lead to cell death and tissue injury. Homeostatic control of these compounds is maintained through the activation of the antioxidant response element (ARE), which is under control of nuclear (erythroid-derived 2)-like 2 (Nrf-2) transcription factor. Nrf-2 activates the transcription of antioxidant enzymes such as SOD and HO-1 in tissues, including the brain (Ghosh et al., 2011). The possibility that Nrf-2 in the brain may be a molecule targeted by exposure to PM has not been extensively explored. A possible consequence of oxidative stress or inflammation in the brain is the accumulation of oxidized proteins in the CNS cells. Such accumulations can produce endoplasmic reticulum (ER) stress. Under normal conditions, ER stress is regulated by the unfolded protein response (UPR), largely through PERK, IRE1α and ATF6 signaling. In addition, under normal physiological conditions, these three molecules, that reside in the ER are kept inactive by BiP chaperone proteins (Kaufman, 2002; Malhotra and Kaufman, 2007; Ron and Walter, 2007; Zhang and Kaufman, 2008). When unfolded or misfolded proteins accumulate, BiP associates with these proteins, thereby allowing them to become active. In addition to their specific functions, these three components stimulate the production of proteins, which control protein folding (Kaufman, 2002; Malhotra and Kaufman, 2007; Ron and Walter, 2007; Zhang and Kaufman, 2008). The presence of protein aggregates in the interior of neurons, largely as a result of oxidation of cytoskeletal and mitochondrial respiratory chain proteins, in patients with neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's disease (PD) has also been documented (Ferrer, 2009; Martínez et al., 2010).

Several PM components induce oxidative stress and inflammation. The pro-oxidant effects of transition metals contained in PM have been extensively described (Valko et al., 2005; Mazzoli-Rocha et al., 2010). In addition, Li et al. (2004) proposed that polycyclic aromatic hydrocarbons (PAH) contained in PM can also induce oxidative stress through quinone and oxygenated PAH metabolism. We, and others, have previously reported that PM collected from the air in Mexico City characteristically contain higher concentrations of minerals, metals and carbonaceous compounds, all which are toxic to cells, capable of inducing apoptosis and DNA damage *in vitro* (De Vizcaya-Ruiz et al., 2006; Mugica et al., 2009), that might be found in ambient air of other cities. There has been a great deal of emphasis given to the role of $PM_{2.5}$ and ultrafine PM (UFP; particles ≤ 100 nm) with respect to human health effects, and less attention has been paid to the effects of coarse PM ($PM_{2.5-10}$). However all three particle size fractions contain components that are cytotoxic and capable of inducing tissue injury. In addition coarse PM has a strong tendency to deposit in the nose, as does UFP. The goal of this project is to examine whether PM-induced oxidative stress and inflammatory processes in the brain, that adversely impact the CNS, acts through mechanisms that lead to ER stress by modifying elements of the unfolded protein response.

Accordingly, we exposed rats to concentrate coarse, fine, and ultrafine PM sampled in Mexico City ambient air and evaluated the effects on the CNS using methods from molecular biology.

2. Materials and methods

2.1. Animal exposure

Six-week-old male Sprague-Dawley rats (purchased from Harlan®, Mexico) were administered food and water *ad libitum*. Animals were housed under barrier conditions using a vented isolation caging system (OneCage® Labproducts, FL) and kept on a strict 12-h light/12-h dark cycle in the animal facility at CINVESTAV-IPN according to institutional guidelines.

2.1.1. Ethics

Experiments described in this study were carried out according to the "Principles of Laboratory Animal Care" (NIH publication #85-23, revised 1985) guidelines and to the "Norma Oficial Mexicana de la Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación" (SAGARPA), subsection: "Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio" (Clave NOM-062-ZOO-1999). Animal handling and exposure was described in protocol ID. 363-06, which was approved by the Institutional Internal Committee for the Use and Care of Laboratory Animals (Comité Interno para el Cuidado y Uso de los Animales de Laboratorio).

2.1.2. Exposure conditions

Animals were exposed to concentrated ambient particulates from Mexico City ambient air by means of size-selective inlets during the warm–dry season between May and June of 2009, 5 h per day, 4 days per week for a total of eight weeks (Table 1). The mass concentration for coarse, fine and ultrafine in the exposure chambers were estimated by using the mass determination of the concentrated PM and of the parallel outside ambient samples collected (collection and mass determination is described in Section 2.2). Outside coarse mass determination was obtained using PM_{10} and $PM_{2.5}$ mass levels, and $PM_{2.5}$ levels from its mass determination, no data for outdoor ultrafine mass levels was available (see Table 1).

Concentrated ambient particles used for the animal exposures were obtained using an inertial particle separator system (Kim et al., 2001a). This system is capable of enriching the concentration of particles by drawing outside ambient air into whole-body animal exposure chambers (Kleinman et al., 2008). Ambient air for the exposures was drawn at a flow rate of 150 L per minute (Kim et al., 2001a,b) through an aluminum duct measuring 2 m in length and 7.5 cm in diameter in order to minimize particle loss due to electrostatic deposition. The duct intake was situated about 3 m above ground level. To control particle flow inline calibrated rotameters were used (Campbell et al., 2005; Kleinman et al., 2005). Concentrated aerosols were delivered to whole-body animal exposure chambers, and each exposure chamber was a sealed unit, sectioned for housing three rats per chamber, and two groups of three rats were exposed to each type of concentrated PM (Kleinman et al., 2005). Temperature and airflow were controlled during the exposures to ensure adequate