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Sex and genetic differences in the effects of acute diesel exhaust exposure on inflammation and oxidative stress in mouse brain

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ABSTRACT

In addition to increased morbidity and mortality caused by respiratory and cardiovascular diseases, air pollution may also contribute to central nervous system (CNS) diseases. Traffic-related air pollution is a major contributor to global air pollution, and diesel exhaust (DE) is its most important component. DE contains more than 40 toxic air pollutants and is a major constituent of ambient particulate matter (PM), particularly of ultrafine-PM. Limited information suggests that exposure to DE may cause oxidative stress and neuroinflammation in the CNS. We hypothesized that males may be more susceptible than females to DE neurotoxicity, because of a lower level of expression of paraoxonase 2 (PON2), an intracellular anti-oxidant and anti-inflammatory enzyme. Acute exposure of C57BL/6 mice to DE (250–300 $\mu g/m^3$ for 6 h) caused significant increases in lipid peroxidation and of pro-inflammatory cytokines (IL-1α, IL-1β, IL-3, IL-6, TNF-α) in various brain regions (particularly olfactory bulb and hippocampus). In a number of cases the observed effects were more pronounced in male than in female mice. DE exposure also caused microglia activation, as measured by increased Iba1 (ionized calcium-binding adapter molecule 1) expression, and of TSPO (translocator protein) binding. Mice heterozygotes for the modifier subunit of glutamate cysteine ligase (the limiting enzyme in glutathione biosynthesis; $Gclm^{+/-}$ mice) appeared to be significantly more susceptible to DE-induced neuroinflammation than wild type mice. These findings indicate that acute exposure to DE causes neuroinflammation and oxidative stress in brain, and suggest that sex and genetic background may play important roles in modulating susceptibility to DE neurotoxicity.

1. Introduction

Air pollution is a mixture comprised of several components, including ambient particulate matter (PM), gases, organic compounds, and metals. While the association between air pollution and morbidity and mortality caused by respiratory and cardiovascular diseases is well established (Brook and Rajagopalan, 2007; Gill et al., 2011), emerging evidence suggests that air pollution may also negatively affect the central nervous system (CNS) and contribute to CNS diseases (Calderon-Garciduenas et al., 2002; Genc et al., 2012; Costa et al., 2014a, 2016). Human epidemiological studies have shown that elevated air pollution is associated with decreased cognitive functions, olfactory and auditory deficits, and depressive symptoms, as well as increased incidence of neurodegenerative disease pathologies, i.e. increased beta-amyloid 42, phosphorylated tau, and alpha-synuclein (Calderon-Garciduenas et al., 2004, 2010, 2011, 2012; Ranft et al., 2009; Fonken et al., 2011; Power et al., 2011; Weuve et al., 2012; Guxens and Sunyer, 2012; Levesque et al., 2011). Among air pollution components, PM is believed to be the most widespread threat, and has been heavily implicated in disease (Brook et al., 2010; Moller et al., 2010; Costa et al., 2014a). PM is broadly characterized by aerodynamic

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diameter (e.g. PM10 and PM2.5, equivalent to <10 um and 2.5 um in diameter, respectively). Ultrafine particulate matter (UFPM; <100 nm) is of much concern, as these particles can more easily enter the circulation and distribute to various organs, including the brain (Oberdoerster et al., 2002, 2004; Genc et al., 2012). Of most relevance is also the fact that UFPM can access the brain through the nasal olfactory mucosa, reaching first the olfactory bulb (Oberdoerster et al., 2004; Peters et al., 2006; Genc et al., 2012; Lucchini et al., 2012). The populations of many countries (e.g. China, India, Middle East, Central America) are commonly exposed for extended periods to relatively high levels of PM (100 $\mu g/m^3$), and such concentration can be easily reached near roads with heavy traffic, and exceeded in certain occupational settings (Van Donkelaar et al., 2015).

Oxidative stress and inflammation are the two cardinal processes by which air pollution is believed to exert its peripheral toxicity (Brook et al., 2010; Lodovici and Bigagli, 2011; Anderson et al., 2012), and the same seems to be true with regard to the CNS, as markers of oxidative stress and neuroinflammation are increased as a result of exposure to air pollution (Calderon-Garciduenas et al., 2008; Genc et al., 2012; Costa et al., 2016).

Traffic-related air pollution is a major contributor to global air pollution, and diesel exhaust (DE) is its most important component (Ghio et al., 2012). DE contains more than 40 toxic air pollutants and is a major constituent of ambient PM, particularly of UFPM; DE exposure is often utilized as a measure of traffic-related air pollution. Few studies have examined controlled acute exposure of humans to DE; for example, acute exposure of humans to DE (300 $\mu g/m^3$) has been shown to induce EEG changes (Crüts et al., 2008). Exposure of mice to DE has been reported to alter locomotor activity and spatial learning and memory (Yokota et al., 2009; Hougaard et al., 2009; Suzuki et al., 2010; Win-Shwe et al., 2008, 2014). Biochemical and molecular studies have evidenced that the most prominent effects of DE exposure on the CNS are oxidative stress and neuroinflammation (MohanKumar et al., 2008; Kraft and Harry, 2011; Win-Shwe and Fujimaki, 2011). Indeed, alterations in some oxidative stress-related genes and other markers of oxidative stress (Hartz et al., 2008; Tsukue et al., 2009; Van Berlo et al., 2010) and increased markers of neuroinflammation (Levesque et al., 2011; Gerlofs-Nijland et al., 2010) have been found in rodents following DE exposure. In vitro, DE particles can activate microglia, and microglia-derived oxidant and/or inflammatory agents cause the demise of neurons (Block et al., 2004; Roqué et al., 2016 in preparation). Altogether, the available evidence suggests that exposure to traffic-related air pollution (and to DE as its major contributor) is associated with adverse CNS effects, with primary mechanisms related to induction of oxidative stress and to neuroinflammation.

Among the factors that can affect neurotoxic outcomes, sex, genetic background, and age are considered the most relevant (Costa et al., 2004; Tiffany-Castiglioni et al., 2005; Weiss, 2011). An aim of the present study was to investigate whether two of these variables (sex and genetic background) would modify susceptibility to DE neurotoxicity. The hypothesis of sex differences was primarily based on recent findings in our laboratory on the differential expression of the enzyme paraoxonase 2 (PON2) between males and females (Giordano et al., 2011, 2013; Costa et al., 2014b). In brain and in other tissues, PON2 was shown to exert antioxidant and anti-inflammatory effects (Ng et al., 2001; Horke et al., 2007; Giordano et al., 2011; Bourquard et al., 2011; Levy et al., 2007), and higher levels of PON2 are associated with decreased susceptibility to oxidative stress and neuroinflammation (Giordano et al., 2013; Costa et al., 2013). We found that females express higher levels of PON2 than males, possibly because this enzyme is modulated by estrogens (Giordano et al., 2013; Costa et al., 2014b). Thus the overall hypothesis was that males would be more susceptible than females to DE neurotoxicity because of a significantly lower level of PON2 expression in brain tissue.

As gene-environment interactions play an important role in toxicology (Costa and Eaton, 2006), we also investigated the possibility that genetic polymorphisms may affect susceptibility to air pollution-induced neurotoxicity. We hypothesized that genetically-based deficiencies in antioxidant defense mechanisms may exacerbate DE neurotoxicity. To test this hypothesis we utilized the *Gclm* mouse, which lacks the modifier subunit of glutamate-cysteine ligase, the first and rate-limiting enzyme in the synthesis of glutathione (GSH), a main player in cellular defense against oxidative stress. *Gclm*$^{-/-}$ mice have very low levels of GSH in all tissues including the brain (Giordano et al., 2006), though they may up-regulate other antioxidant pathways; in contrast, *Gclm*$^{+/-}$ mice have only moderate reductions in GSH but may more closely resemble an human polymorphism of *Gclm* (Nakamura et al., 2002). Interestingly, an enhanced lung inflammation has been observed in *Gclm*$^{+/-}$ mice compared to wild-type mice upon exposure to DE (Weldy et al., 2012).

2. Materials and methods

2.1. Animals

Adult (3 month-old) male and female mice of C57Bl/6 strain background, purchased from Charles River Laboratories (Wilmington, MA) were used in most of these studies. Mice were housed in specific pathogen-free facilities with a 12-h dark–light cycle and unlimited access to food and water. Animals were randomly assigned to exposure to either filtered air (FA) or DE. In some experiments, *Gclm*-null (*Gclm*$^{-/-}$) mice of backcrossed C57Bl/6J (B6.129) strain background (Giordano et al., 2006; McConnachie et al., 2007) were utilized. Male and female mice hemizygous for the *Gclm* deletion (*Gclm*-Hz) were intercrossed, generating wild type, *Gclm*$^{-/-}$, and *Gclm*-Hz mice, in the expected Mendelian ratios. To genotype pups, genomic DNA was isolated from ear punch tissue using a Qiagen DNeasy kit, and mice were genotyped by PCR amplification of the wild type and disrupted *Gclm* alleles (i.e., amplification of β-geo), as previously described (Giordano et al., 2006; McConnachie et al., 2007). As seen previously, all pups developed normally and exhibited no differences in phenotypic landmarks compared to wild type littermates. The number of mice in each experimental group ranged from three to six. The animal use protocols used were approved by the Institutional Animal Care and Use Committee at the University of Washington. All animal experiments were carried out in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals, as adopted by the National Institutes of Health.

2.2. In vivo exposure of mice to diesel exhaust

Individually housed mice were exposed for 6 h to FA or DE (at a $PM_{2.5}$ concentration of 250–300 $\mu g/m^3$). Exposures to either FA or DE were conducted simultaneously under SPF conditions in our diesel exposure facility (Gould et al., 2008; Fox et al., 2015), using an Allentown caging system (Allentown, NJ) with racks modified to receive either diluted DE or FA through their air intakes. DE was derived as described (Gould et al., 2008; Fox et al., 2015), from a Yanmar YDG5500 diesel generator, with a load bank maintaining 75% of rated capacity, using No. 2 undyed, ultra-low sulfur on-highway fuel and Royal Purple Duralec 15W-40 Synthetic crankcase oil. During exposures, DE concentrations were continuously measured and maintained at steady concentrations using a feedback controller monitoring fine particulate levels (Gould et al., 2008; Fox et al., 2015). DE was composed of $PM_{2.5}$ or smaller, with a