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Exposure to traffic-generated air pollutants mediates alterations in brain microvascular integrity in wildtype mice on a high-fat diet

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ABSTRACT

Air pollution-exposure is associated with detrimental outcomes in the central nervous system (CNS) such as cerebrovascular disorders, including stroke, and neurodegenerative diseases. While the mechanisms of these CNS-related outcomes involved have not been fully elucidated, exposure to traffic-generated air pollutants has been associated with altered blood brain barrier (BBB) integrity and permeability. The current study investigated whether inhalation exposure to mixed vehicle emissions (MVE) alters cerebral microvascular integrity in healthy 3 mo old C57BL/6 mice, as well as whether exposure-mediated effects were exacerbated by a high-fat (HF) vs. low-fat (LF) diet. Mice on each diet were randomly assigned to be exposed to either filtered air (FA) or MVE [100 PM/m^3 vehicle emissions mixture: 30 μg PM/m^3 gasoline engine + 70 μg PM/m^3 diesel engine emissions; median size ~ 60 nm; particle mass size distribution median of ~ 1 μm (range: < 0.5–20 μm)] for 6 h/d, 7d/wk, for 30d. Using sodium fluorescein as a tracer, we observed a significant increase in BBB permeability in both HF + MVE exposed and HF + FA animals, compared to LF + FA controls. Exposure to HF + MVE also led to a significant increase plasma ox-LDL and ox-LDL scavenger receptors (LOX-1 and CD-36) expression in the cerebral vasculature. Histological analysis revealed decreased expression of TJ protein, claudin-5, associated with increased matrix metalloproteinase (MMP) − 9 activity and oxidative stress in the cerebral vasculature of HF + MVE mice, compared to LF + MVE. Such findings indicate that inhalation exposure to traffic-generated pollutants, coupled with a HF diet, results in altered BBB integrity and increased ox-LDL signaling in the cerebral vasculature in a wildtype animal model.

1. Introduction

Findings from multiple epidemiological studies over the past two decades have provided a clear correlation between environmental air pollution-exposure and cardiovascular diseases, including stroke and ischemic heart disease Crichton et al., 2016; Du et al., 2016). More recently, air pollution-exposure has been reported to be a major health risk that is associated with neuropathologies such as stroke, Alzheimer's disease (AD), Parkinson's diseases, as well as dementia-related neurotoxicity (Chen et al., 2015; Calderón-Garcidueñas et al., 2016a, b; Tian et al., 2017). The consequences of inhalation exposure to traffic-generated air pollution on the brain are complex, but is known to result in neuroinflammation, generation of reactive oxygen species (ROS), microglial activation, and alterations in blood brain barrier (BBB) permeability (Levesque et al., 2013; Oppenheim et al., 2013). The BBB is a dynamic structure, comprised of microvascular endothelial cells, which are joined by tight junction (TJ) proteins such as occludin and claudin-5, astrocytes, and pericytes that provide a physical and chemical barrier that regulates transport into and out of the brain parenchyma. Importantly, air pollution-exposure mediated alterations in TJ protein expression, in both animal models and human exposures, have been reported to result in decreased BBB integrity and increased BBB permeability that is associated with CNS-related disorders including stroke and AD (Alimohammadi et al., 2016; Calderón-Garcidueñas et al., 2016a and 2016b; Oppenheim et al., 2013). The mechanisms involved in air pollution-exposure mediated alterations in the neurovasculature have not yet been fully elucidated; however, inflammation, ROS, and increased matrix metalloproteinase (MMP)

Abbreviations: 4-HNE, 4-hydroxynonenal; AD, Alzheimer's disease; ApoE$^{-/-}$ mice, Apolipoprotein E null mice; BBB, blood brain barrier; CD-36 receptor, cluster of differentiation 36 receptor; CNS, central nervous system; DIO, diet-induced obesity; FA, filtered air; HF, high-fat diet; LDL, low-density lipoprotein; LF, low-fat diet; LOX-1, lectin-like oxidized LDL receptor; MMP, matrix metalloproteinase; MVE, mixed gasoline and diesel vehicle exhaust; NAD(P)H oxidase, nicotinamide adenine dinucleotide phosphate-oxidase; Na-F, sodium fluorescein; ox-LDL, oxidized low-density lipoprotein; PM, particulate matter; ROS, reactive oxygen species; TIMP, tissue inhibitor of matrix metalloproteinases; TJ, tight junction proteins

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activity have all been associated with exposure-mediated alterations in BBB integrity (Calderón-Garcidueñas et al., 2008).

Several studies have shown that the incidence of ischemic stroke is correlated with increased blood cholesterol levels (Kurth et al., 2007). Specifically, oxidized low-density lipoprotein (ox-LDL) is known to mediate the initiation and/or progression of atherosclerotic plaque growth and microvascular inflammation. The role of ox-LDL in atherogenesis has been reported to occur primarily via signaling through its receptors, including the low-density lipoprotein ox-LDL receptor (LOX-1), the primary ox-LDL receptor in the microvascular endothelium (Chen et al., 2007). Additionally, increased plasma ox-LDL is associated with an increased risk of acute cerebral infarction and/or may also predict the size of the ischemic lesion (Uno et al., 2005). In agreement with this premise, anti-ox-LDL antibody concentrations are significantly higher in patients who have suffered from an atherogenic ischemic stroke, compared to that in healthy individuals (Marta et al., 2014). Increased signaling through another scavenger receptor for ox-LDL, the CD36 receptor, is also associated with endothelial dysfunction and stroke (Cho, 2012). Oxidative lipids such as 4-hydroxy-trans-2-nonenal (4-HNE), which is a major product of lipid peroxidation, have also been found to be elevated in atherosclerosis (Chapple et al., 2013). As plasma ox-LDL typically increased with the consumption of a high-fat (HF) diet (Silaste et al., 2004), understanding the interaction between diet and environmental exposures in mediating the detrimental effects on the microvascular system is critical. This becomes even more imperative when considering the staggering statistics that report more than 1/3 of children and adolescents in U.S. are overweight or obese, often associated with consumption of a high-fat diet, which in itself is a factor for risk of future cardiovascular disease (Freedman et al., 2007; Ogden et al., 2014).

When investigating air pollution-mediated toxicity it is important to also study mixtures of environmental pollutants, including those from traffic-generated sources, as they are a significant component of ambient air pollution. The effects of exposure to components and/or mixtures of vehicle exhaust have emerged from studies reporting detrimental outcomes in the pulmonary, cardiovascular, and nervous system, as well as others. For example, exposure to diesel exhaust particulate matter (PM) was reported to generate the oxidation of LDL (e.g. increase ox-LDL) (Ikeda et al., 1995), higher levels of which are associated with progression of cardiovascular disease, atherosclerotic plaque growth, as well as poorer prognostic outcome in stroke (Ishigaki et al., 2009). Additionally, our laboratory has previously reported that inhalation exposure to a mixture of gasoline and diesel engine exhaust (MVE) resulted in increased circulating ox-LDL in atherosclerotic Apolipoprotein (Apo)$E^{-/-}$ mice, which was associated with increased expression of microvascular LOX-1 and ROS (Lund et al., 2011; Lucero et al., 2017). Furthermore, exposure to air pollution has also been associated with altered BBB integrity and permeability in both laboratory studies and human exposures (Oppenheim et al., 2013; Calderón-Garcidueñas et al., 2016). Exposure to MVE, specifically, has been reported to result in a disruption of the BBB, associated with increased MMP-9 expression and activity and decreased TJ protein (occludin and claudin-5) expression, in the cerebral vasculature of $ApoE^{-/-}$ mice (Oppenheim et al., 2013; Lucero et al., 2017). Importantly, alterations in the integrity of the BBB can lead to exacerbated pathology and/or hemorrhagic transformation during an ischemic stroke (Turner and Sharp, 2016).

We have previously reported the effects of traffic-generated air pollutants (MVE) in mediating microvascular toxicity and BBB disruption, associated with LOX-1 signaling, in atherosclerotic $ApoE^{-/-}$ mice (Oppenheim et al., 2013; Lucero et al., 2017). However, to date, there is very little information on whether inhaled traffic-generated pollutants promote similar detrimental effects on the cerebral vasculature in a wildtype animal model and/or whether a HF diet may exacerbate any of the observed exposure-mediated outcomes. As such, we are investigating the hypothesis that exposure to traffic-generated air pollution (MVE) results in alterations in BBB integrity, related to ox-LDL and LOX-1 signaling pathways, in healthy wildtype (C57BL/6) mice, which is exacerbated by concurrent consumption of a HF diet.

2. Materials and methods

2.1. Animals and inhalation exposure protocol

Three mo-old male C57BL/6 mice were placed on either normal mouse chow or a HF diet (TD88137 Custom Research Diet, Harlan Teklad, Madison, WI; 21.2% fat content by weight, 1.5 g/kg cholesterol content) beginning 30 days prior to initiation of exposure. Mice were randomly grouped to be exposed by whole-body inhalation to a mixture of whole gasoline engine exhaust and diesel engine exhaust (MVE: 30 μg PM/m^3 gasoline engine emissions + 70 μg PM/m^3 diesel engine) or filtered-air (controls) for 6 h/d, 7 d/wk, for a period of 30 d. MVE was created by combining exhaust from a 1996 g gasoline engine and a Yanmar diesel generator system, and exposures chemistries and PM characterized, as previously reported (McDonald et al., 2004, 2008; Lund et al., 2011; Oppenheim et al., 2013; Mumaw et al., 2016; Lucero et al., 2017.). Particle size distribution was measured with a Fast Mobility Particle Sizer (FMPS, TSI, St. Paul, MN) for the ~ 10–500 nm size range and an Aerodynamic Particle Sizer (TSI, St Paul, MN) to measure the 0.5–20 μm size range. Particle mass concentration by gravimetric analysis of Teflon membrane filters at the inlet of the chamber and inside the exposure chamber was conducted once/wk throughout the duration of the exposure protocol. The particle number size distribution for this exposure had a median size of approximately 60 nm; particle mass size distribution had a median of ~ 1 μm (range: < 0.5–20 μm), with total particle mass for the mixture measured at 102.5 ± 20.9 μg/m^3 over the 30 d study. Particle composition was approximately: 60% elemental carbon, 15% organic carbon, 2% nitrates, 5% ammonium, 9% sulfates, 9% elements (metals) (Vedal et al., 2013). Mice were housed in standard shoebox cages within an AAALAC International-approved rodent housing facility (2 m^3 exposure chambers) for the entirety of the study, which maintained a constant temperature (20–24 °C) and humidity (30–60% relative humidity). Mice had access to chow and water *ad libitum* throughout the study period, except during daily exposures when chow was removed. All procedures were approved by the Lovelace Respiratory Research Institute's Animal Care and Use Committee and conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Tissue collection

Animals were weighed (Table 1) and sacrificed within 14–16 h after their last exposure. Mice were anesthetized with Euthasol (0.1 ml per 30 g mouse) and euthanized by exsanguination. The brain tissue was carefully dissected, meninges gently removed, and were either (1) embedded in tissue freezing medium (TFM; VWR Scientific) (n = 3–4 per group; midbrain region) and frozen on dry ice or (2) immediately snap frozen in liquid nitrogen. Whole blood was also collected via cardiac-stick, using a heparinized syringe, centrifuged at 3000 × g for 10 min, and plasma was separated, aliquoted, and stored at − 80C.

2.3. BBB permeability

Changes in BBB permeability were assessed (in a subset of mice, n = 6 per group) using the fluorescent tracer, sodium fluorescein (Na-F). Mice were injected intraperitoneally with 100 μl of 2% Na-F –PBS 30 min prior to the end of their exposure on day 30, as previously described (Oppenheim et al., 2013). At sacrifice, blood was collected via cardiac puncture, and then animals were perfused with PBS until perfusate ran clear. After complete perfusion, brains were removed, the meninges, cerebellum, and brain stem were gently dissected away. The