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Prefrontal white matter pathology in air pollution exposed Mexico City young urbanites and their potential impact on neurovascular unit dysfunction and the development of Alzheimer's disease

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ABSTRACT

Millions of urban children are chronically exposed to high concentrations of air pollutants, i.e., fine particulate matter ($PM_{2.5}$) and ozone, associated with increased risk for Alzheimer's disease. Compared with children living with clear air those in Mexico City (MC) exhibit systemic, brain and intrathecal inflammation, low CSF $A\beta_{42}$, breakdown of the BBB, attention and short-term memory deficits, prefrontal white matter hyperintensities, damage to epithelial and endothelial barriers, tight junction and neural autoantibodies, and Alzheimer and Parkinson's hallmarks. The prefrontal white matter is a target of air pollution. We examined by light and electron microscopy the prefrontal white matter of MC dogs (n: 15, age 3.17 ± 0.74 years), children and teens (n: 34, age: 12.64 ± 4.2 years) versus controls. Major findings in MC residents included leaking capillaries and small arterioles with extravascular lipids and erythrocytes, lipofuscin in pericytes, smooth muscle and endothelial cells (EC), thickening of cerebrovascular basement membranes with small deposits of amyloid, patchy absence of the perivascular glial sheet, enlarged Virchow–Robin spaces and nanosize particles (20–48 nm) in EC, basement membranes, axons and dendrites. Tight junctions, a key component of the neurovascular unit (NVU) were abnormal in MC versus control dogs ($\chi^2 < 0.0001$), and white matter perivascular damage was significantly worse in MC dogs ($p = 0.002$). The integrity of the NVU, an interactive network of vascular, glial and neuronal cells is compromised in MC young residents. Characterizing the early NVU damage and identifying biomarkers of neurovascular dysfunction may provide a fresh insight into Alzheimer pathogenesis and open opportunities for pediatric neuroprotection.

1. Introduction

Clinically healthy Mexico City (MC) children with no known risk factors for neurological or cognitive disorders exhibit cognition deficits, brain metabolic, structural and volumetric changes and the neuropathological and cerebrospinal fluid (CSF) laboratory hallmarks of Alzheimer and Parkinson's diseases i.e., tau hyperphosphorylation with pre-tangles, amyloid beta42 (Aβ42) plaques, low CSF Aβ42, and misfolded α-synuclein accumulation (Calderón-Garcidueñas et al., 2008a, 2010, 2011a, 2012a, 2013a, 2015a).

Brain MRI and MRS studies in MC children and teens versus low air pollution controls show white matter metabolic changes and prefrontal white matter hyperintensities (WMH) (Wardlaw et al., 2013; Calderón-Garcidueñas et al., 2008b, 2011b, 2012b,

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2015a, 2015b), while neuropathology findings reveal cortical disruption of the blood–brain barrier (BBB), endothelial activation, oxidative stress, high concentration of metals associated with combustion, inflammatory cell trafficking along with up-regulated gene network clusters including IL1, NFκB, TNF, IFN, and TLRs (Calderón-Garcidueñas et al., 2003, 2008a, 2009a, 2010, 2011a, 2013a, 2013b). Animal facility healthy MC young dogs also exhibit WMH by MRI, neuroinflammation, DNA oxidative damage, BBB breakdown, and accumulation of combustion-related metals (Calderón-Garcidueñas et al., 2002, 2003, 2008b, 2009a). The prefrontal cortex is a target of air pollution and its damage likely a major contributor to cognitive deficits in Mexico City young residents. Prior research has shown all epithelial and endothelial barriers are compromised in MC children and dogs and the production of high concentrations of endothelin-1 and autoantibodies against tight junction and neural proteins could be playing a role in the diffuse microvascular changes observed in young urbanites (Calderón-Garcidueñas et al., 2001, 2007, 2008c, 2009b, 2015d). In a recent study of 139 clinically healthy MC and control children age 11.91 $\pm$ 4.2 y, serum antibodies against occludin/zonulin 1 and actin IgG, along with myelin oligodendrocyte glycoprotein, myelin basic protein, S-100, and cerebellar IgG were significantly higher in MC children (Calderón-Garcidueñas et al., 2015d). Zonula occludens (ZO) proteins are at the core of the protein networks which are anchored to the TJ-plaque dynamic structures and given that neuroinflammation is associated with BBB dysfunction and loss of tight junctions (Bauer et al., 2014; Elahy et al., 2015; Haseloff et al., 2015), we fully expected brain structural and metabolic changes in MC children.

Extensive data in the literature support human and animal breakdown of the nasal/olfactory, BBB and alveolar-capillary barriers and the expression of detrimental genes associated to urban air pollution (Harkema et al., 2006; Ljubimova et al., 2013; Van Miert et al., 2005; Kaplan et al., 2010; Carson et al., 2013; Bergin and Witzmann, 2013; Garwood et al., 2014). The work by Kamat et al. (2014), Winkler et al. (2014), Hawkes et al. (2014), Cabezas et al. (2014) and Garwood et al. (2014) is of particular interest to us given that their research support damage to brain endothelial cells occurs early in relation to Alzheimer's neuropathology and BBB disruption leads to neuronal damage, reactive gliosis, oxidative stress, neuroinflammation and early neurovascular dysfunction.

Of great concern in polluted environments with high concentrations of ultrafine particulate matter (UFPM, nanosize particles $<$ 100 nm) is that after passage through biological barriers, UFPM end up in contact with the vascular endothelium and can induce damage (Wang et al., 2009; Gehr et al., 2011; Sharma et al., 2013; Ucciferri et al., 2014; Karmakar et al., 2014; Meng et al., 2015). The presence of high affinity autoantibodies against barrier forming proteins in urban children are critical to our understanding of air pollutant mechanistic damage pathways. There is robust evidence nanosize particles can increase endothelial paracellular permeability in vitro and induce endothelial TJ opening (Sharma et al., 2013; Yu et al., 2013; Ucciferri et al., 2014; Karmakar et al., 2014; Li et al., 2015).

The developing brain relies heavily on the delivery of oxygen and nutrients from the blood stream to meet metabolic demands of neural cells and blood supply, thus neural activity and vascular dynamics are tightly coupled (Lecrux and Hamel, 2011; Lacoste and Gu, 2015).The neurovascular unit (NVU) is the anatomical substrate of neurovascular interactions and a complex interaction between endothelial cells, pericytes, astrocytes, microglia and neurons is responsible for optimal delivery of oxygen and nutrients to the brain (Simard et al., 2003; Zlokovic, 2008; Lo and Rosenberg, 2009; Lacoste and Gu, 2015). A key function of this system is to keep a tightly control environment aimed to preserve the brain from toxins, pathogens and harmful chemicals. Neurovascular dysfunction has a relevant focus in Alzheimer's disease (AD) research, particularly regarding BBB integrity, cerebral blood flow (CBF) and glucose transport into the brain (Iadecola, 2004; Keaney and Campbell, 2015; Sweeney et al., 2015). White matter abnormalities are common in dementia and the pathology is the result of a combination of structural alterations of the cerebral vasculature, i.e., arteriolosclerosis, cerebrovascular basement membrane pathology, and amyloid angiopathy, and nonstructural vascular abnormalities (vascular contractility or permeability) and/or neurovascular instability (Love and Miners, 2015).

Very little is known regarding the ultrastructural features of tight junctions (TJs), cerebrovascular basement membranes (Morris et al., 2014), capillaries, arterioles and axons in the prefrontal white matter of young healthy dogs and children with a lifetime exposure to urban air pollution. Our working hypothesis states that healthy, young dogs will have prefrontal vascular and white matter pathology and children living in the same area will share light and electron microscopic (EM) findings with those of dogs' raised in an animal facility in Mexico City.

We have one aim for this study: to document by 1 μm toluidine blue thick sections and EM the integrity of the prefrontal white matter in healthy young dogs resident in MC (n: 9) and in a cohort of MC children and teens (n: 26) autopsy prefrontal samples versus clean air controls (6 dogs and 8 children). Stored brain samples from seemingly healthy children dying suddenly in accidental deaths not involving the cranial cavity and undergoing forensic autopsies were the source of the frontal samples.

Our results identify abnormalities at the endothelial junctional complexes, microbleeds, perivascular lipid accumulation, abnormal cerebrovascular basement membranes, and the presence of ultrafine particles in mitochondria, basement membranes, axons and dendrites. Our study suggests that the integrity of the NVU in the prefrontal white matter is compromised in highly exposed young urbanites and short and long-term brain health consequences are expected.

2. Procedure

2.1. Study cities and air quality

Children's cohorts were selected from the Mexico City Metropolitan Area (MCMA) and control locations consisting of small cities in Mexico (Zacatlán and Huachinango, Puebla; Zitácuaro, Michoacán; Puerto Escondido, Oaxaca). The control cities have $<$ 75,000 inhabitants and because of their small size their levels for the main criteria air pollutants (ozone, particulate matter, sulfur dioxide, nitrogen oxides and carbon monoxide) are lower than the current US EPA standards (Alonso et al., 2007).

Mexico City Metropolitan Area is an example of extreme urban growth and accompanying environmental pollution (Bravo-Alvarez and Torres-Jardón, 2002; Molina et al., 2010; Retama et al., 2015). The metropolitan area of over 2000 km^2 lies in an elevated basin 2200 m above sea level surrounded on three sides by mountain ridges. MCMA's nearly 24 million inhabitants, over 50,000 industries, and 5.5 million vehicles consume more than 50 million liters of petroleum fuels per day, producing an estimated annual emission of 2.3 million tons of particulate and gaseous air pollutants. MCMA motor vehicles produce abundant amounts of primary fine particulate matter ($PM_{2.5}$). The high altitude and tropical climate where the MCMA is settled facilitate ozone production all year and contribute to the formation of $PM_{2.5}$. Children from MCMA were residents in the northern-industrialized and southern-residential zones. Northern children have been exposed to higher concentrations of volatile and toxic organic compounds,