Environmental Research 159 (2017) 186–201

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Combustion-derived nanoparticles, the neuroenteric system, cervical vagus, hyperphosphorylated alpha synuclein and tau in young Mexico City residents

Lilian Calderón-Garcidueñas[a,b,*], Rafael Reynoso-Robles[c], Beatriz Pérez-Guillé[c], Partha S. Mukherjee[d], Angélica Gónzalez-Maciel[c]

[a] The University of Montana, Missoula, MT 59812, USA
[b] Universidad del Valle de México, Mexico City 14370, Mexico
[c] Instituto Nacional de Pediatría, Mexico City 04530, Mexico
[d] Mathematics Department, Boise State University, Boise, Idaho, USA

ARTICLE INFO

Keywords:
Alzheimer
Alpha synuclein
Combustion-derived nanoparticles
Children
GI barrier
Hyperphosphorylated tau
Neuroenteric system
Mexico City
Parkinson
Vagus

ABSTRACT

Mexico City (MC) young residents are exposed to high levels of fine particulate matter ($PM_{2.5}$), have high frontal concentrations of combustion-derived nanoparticles (CDNPs), accumulation of hyperphosphorylated aggregated α-synuclein (α-Syn) and early Parkinson's disease (PD). Swallowed CDNPs have easy access to epithelium and submucosa, damaging gastrointestinal (GI) barrier integrity and accessing the enteric nervous system (ENS). This study is focused on the ENS, vagus nerves and GI barrier in young MC v clean air controls. Electron microscopy of epithelial, endothelial and neural cells and immunoreactivity of stomach and vagus to phosphorylated α-synuclein Ser129 and Hyperphosphorylated-Tau (Htau) were evaluated and CDNPs measured in ENS. CDNPs were abundant in erythrocytes, unmyelinated submucosal, perivascular and intramuscular nerve fibers, ganglionic neurons and vagus nerves and associated with organelle pathology. αSyn and Htau were present in 25/27 MC gastric,15/26 vagus and 18/27 gastric and 2/26 vagus samples respectively. We strongly suggest CDNPs are penetrating and damaging the GI barrier and reaching preganglionic parasympathetic fibers and the vagus nerve. This work highlights the potential role of CDNPs in the neuroenteric hyperphosphorylated α-Syn and tau pathology as seen in Parkinson and Alzheimer's diseases. Highly oxidative, ubiquitous CDNPs constitute a biologically plausible path into Parkinson's and Alzheimer's pathogenesis.

1. Introduction

Mexico City (MC) children, teens and young adults exhibit the neuropathological hallmarks of Alzheimer and Parkinson's diseases i.e., tau hyperphosphorylation with pre-tangles, amyloid beta42 (Aβ42) diffuse and mature plaques, and misfolded α-synuclein olfactory bulb and brainstem accumulation (Calderón-Garcidueñas et al., 2008, 2010, 2011, 2012, 2013a, 2013b; Villarreal-Calderon et al., 2010). Lewy neurites and/or punctuate α-synuclein deposits in the olfactory bulb, trigeminal thalamic tract, mesencephalic V, reticular and raphe nuclei, the glossopharyngeal-vagus complexes and lung and heart autonomic ganglia are seen in MC v control children as young as 11 years old (Calderón-Garcidueñas et al., 2011, 2013a, 2013b). We have shown upregulation of COX2 in the right vagus and of CD14 in both right and left vagus of teens and young MC adults suggesting the vagus nerve plays a role in the brainstem inflammation and neurodegeneration process (Calderón-Garcidueñas et al., 2008). In highly exposed MC Balb-c mice, dorsal vagal complex (DVC) inflammation is a robust finding strongly associated with DVC imbalance in genes associated with antioxidant defenses, apoptosis, and neurodegeneration (Villarreal-Calderon et al., 2010).

Data in the literature support an association between air pollutants (ozone, fine particulate matter $PM_{2.5}$ and/or lead) and higher risk of Parkinson's disease (Kirrane et al., 2015, Santurtún et al., 2016), while others find limited evidence between exposures to ambient particulate matter (PM_{10}, $PM_{2.5}$) or nitrogen oxides (NO_2), and PD risk (Liu et al., 2016; Chen et al., 2017; Calderón-Garcidueñas and Villarreal-Ríos, 2017). Interestingly, the experimental combination of ultrafine carbon black and rotenone -a pesticide used to induce dopaminergic (DA) damage-, work synergistically to activate NADPH oxidase in microglia, leading to DA neurons oxidative damage (Wang et al., 2017). There is no doubt however of the capacity of nanoparticles (NPs) to produce DA

* Correspondence to: The University of Montana, 287 Skaggs Building, 32 Campus Drive, Missoula, MT 59812, USA.
E-mail address: lilian.calderon-garciduenas@umontana.edu (L. Calderón-Garcidueñas).

http://dx.doi.org/10.1016/j.envres.2017.08.008
Received 5 June 2017; Received in revised form 2 August 2017; Accepted 4 August 2017

damage (Hu et al., 2017; Xie et al., 2016; Imam et al., 2015; Levesque et al., 2011a, 2011b, 2013). Of special interest is the work of Levesque and coworkers (Levesque et al., 2011a, 2011b, 2013) showing the effect of diesel NPs upon microglia, neuroinflammation, and DA neurotoxicity. The combination of diesel NPs and lipopolysaccharide (LPS) (2.5 ng/mL) in vitro synergistically amplified nitric oxide production, TNFα release, and DA neurotoxicity. This association is crucial for Mexico City (MC) young residents (Calderón-Garcidueñas et al., 2009a, 2009b, Rosas Pérez et al., 2007; Querol et al., 2008; Vega et al., 2011), since endotoxins (LPS) are an important organic particle component and *healthy* children show an endotoxin tolerance-like state (Calderón-Garcidueñas et al., 2009a, 2009b).

We recently documented by magnetometry, high-resolution transmission electron microscopy (HRTEM), electron energy loss spectroscopy (EELS), and energy dispersive X-ray (EDX) analysis the mineralogy, morphology, and composition of magnetic frontal cortex NPs from MC young residents (Maher et al., 2016) and used transmission electron microscopy to identify combustion-derived nanoparticles (CDNPs) in neurons, glia, choroid plexus, and neurovascular units of MC young residents v matched clean air controls (González-Maciel et al., 2017). CDNPs are spherical nanoparticles associated with pathology in mitochondria, endoplasmic reticulum (ER), mitochondria-ER contacts (MERCs), axons and dendrites. We commented that abnormal MERCs, mitochondria and dilated ER are widespread in brain tissues and CDNPs in close contact with neurofilaments, glial fibers and chromatin are a potential source for altered microtubule dynamics, mitochondrial dysfunction, accumulation and aggregation of unfolded proteins, abnormal endosomal systems, altered insulin signaling, calcium homeostasis, apoptotic signaling, autophagy and epigenetic changes (González-Maciel et al., 2017).

We are very concerned about the passage of NPs through damaged cell junctions in small bowel and the fact MC children have higher levels of antibodies to host proteins involved in cell adhesion (Calderón-Garcidueñas et al., 2015a, 2015b, 2015c, 2015d). MC children and young adults are already showing Parkinsons' disease early neuropathological hallmarks, including accumulation of alpha synuclein in olfactory bulb and brainstem (Calderón-Garcidueñas et al., 2008, 2010, 2013a, 2016a, 2016b, 2016c). Kish and colleagues work is key given the increased gut permeability in mice exposed to particulate matter with a diameter of < 10 μm (PM_{10}) and their conclusions of ingestion of airborne particulate matter altering the gut microbiome and inducing acute and chronic inflammatory responses in the intestine (Kish et al., 2013).

At the core of our work Braak and Del Tredeci proposals (Braak et al., 2003a, 2003b; Tredici and Braak, 2016) "*a putative environmental pathogen capable of passing the gastric epithelial lining might induce α-synuclein misfolding and aggregation*" and the dual-hit hypothesis of Hawkes (Hawkes et al., 2007), both fit the strong possibility *putative environmental pathogens* are indeed swallowed nanoparticles gaining access to the brain through the most vulnerable section of the GI tract: the small bowel (Johansson et al., 2013).

Given that MC children have lifetime exposures to high concentrations of $PM_{2.5}$ and well documented breakdown of epithelial, endothelial and neurovascular barriers (Calderón-Garcidueñas et al., 2008, 2012, 2016a, 2016b, 2016c), and having the experience of studying healthy dogs exposed to the same environment as the children (Calderón-Garcidueñas et al., 2001, 2009a, 2009b), we hypothesized that healthy MC facility dogs will have NPs in the neuroenteric system and similar findings could be seen in children and young MC adults.

There were three primary aims to this study: 1. To study by electron microscopy the integrity of the small bowel epithelium tight junctions, the endothelial barrier and the passage of NPs from circulating red blood cells loaded with particles in blood vessels to the intestinal compartments, 2. To identify and measure NPs and document organelle pathology in the neuroenteric system including the submucosal and myenteric plexus fibers and neurons in duodenal, jejunum and ileum samples of healthy young residents in MC versus controls 3. Since the vagus nerves play a key role in the early neuropathology of PD, we specifically sampled right cervical vagal (X) samples both in dogs and in human autopsies (human samples previously analyzed for mRNA COX2, IL1β and CD14 and found to have high levels versus clean air controls (Calderón-Garcidueñas et al., 2008). Given that highly exposed children and young adults have early features of Parkinson's and Alzheimer diseases (Calderón-Garcidueñas et al., 2008, 2010, 2011, 2012, 2016c, 2017), we did immunohistochemistry for phosphorylated α-synuclein and hyperphosphorylated tau in gastric and vagal samples for the same subjects with right vagal mRNA inflammatory markers (Calderón-Garcidueñas et al., 2008).

Our results identify abnormalities in the apical junctional complexes, desmosomal and gap junctions resulting in interepithelial gaps in the small bowel of MC dogs, along with increased caveolar activity in the luminal and abluminal endothelial cells versus controls. Airborne iron-rich strongly magnetic combustion-derived nanoparticles (CDNPs) are abundant in epithelial and endothelial cells and in enteric neurons, unmyelinated axons and cervical X nerves of MC children and young adults and are associated with pathology in mitochondria, axons and dendrites. Gastric accumulation of hyperphosphorilated α-synuclein (α-Syn) and P-tau is a key finding. Our study shows urban young residents have α-Syn and P-tau pathology of the enteric nervous system and neuronal, axonal and unmyelinated pathology. Combustion-derived nanoparticles likely play a key role in the breakdown of the GI barrier and the α-syn/P-tau neuroenteric pathology. Exposures to CDNPs ought to be contemplated as Parkinson's and Alzheimer's environmental risk factors (Calderón-Garcidueñas et al., 2008, 2010, 2011, 2012, 2016a, 2016b, 2016c, 2017; Maher et al., 2016).

2. Materials and methods

2.1. Study cities and air quality

Mexico City is a prime example of uncontrolled urban growth and sustained severe air pollution (Rosas Pérez et al., 2007; Querol et al., 2008; Vega et al., 2011; Secretaria del Medio Ambiente, 2012, 2012). Driving restriction programs implemented in 2017 have failed to improve air quality (Davis, 2017). 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. MC Metropolitan area nearly 24 million inhabitants, over 50,000 industries, and 5.5 million vehicles consume more than 50 million liters of petroleum fuels *per day*. Northern MC residents have been exposed to higher concentrations of volatile and toxic organic compounds, PM_{10}, and $PM_{2.5}$ including high levels of its constituents: organic and elemental carbon, nitro- and polycyclic aromatic hydrocarbons and metals (Zn, Cu, Pb, Ti, Mn, Sn, V, Ba), while southern residents (exposed dogs in this study are from SW Mexico City) have been exposed continuously to significant and prolonged concentrations of ozone, secondary aerosols (NO_3^-) and particulate matter associated with lipopolysaccharide PM-LPS (Rosas Pérez et al., 2007; Querol et al., 2008; Vega et al., 2011). Studies on the composition of $PM_{2.5}$ with regards to sites and samples collected in 1997 show that composition has not changed during the last decade. Tlaxcala was the selected clean air control with criteria air pollutants typically below the equivalent US EPA air quality standards (Calderón-Garcidueñas et al., 2009a, 2009b).

2.2. Dog small bowel and vagus samples

Previously harvested dog small bowel and right vagus samples for electron microscopy were used for this study (Calderón-Garcidueñas et al., 2009a, 2009b, 2016a, 2016b, 2016c). MC and control mixed beagles were whelped and housed in an outdoor-indoor kennel; husbandry was in compliance with the American Association of Laboratory Animal Certification Standards. Dogs were under daily veterinarian