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The impact of environmental metals in young urbanites' brains

Lilian Calderón-Garcidueñas[a,b,*], Alejandro Serrano-Sierra[a], Ricardo Torres-Jardón[c], Hongtu Zhu[d], Ying Yuan[d], Donna Smith[b], Ricardo Delgado-Chávez[e], Janet V. Cross[f], Humberto Medina-Cortina[a], Michael Kavanaugh[b], Tomás R. Guilarte[g]

[a] Instituto Nacional de Pediatría, Mexico City 04530, Mexico
[b] The Center for Structural and Functional Neurosciences, The University of Montana, Missoula, MT 59812, USA
[c] Centro de Ciencias de la Atmósfera, Universidad Nacional Autónoma de México, Mexico City 04510, Mexico
[d] Biostatistics, University of North Carolina Gillings School of Global Public Health, Chapel Hill, NC 27599, USA
[e] Instituto Nacional de Cancerología, Mexico City 04330, Mexico
[f] Department of Pathology, University of Virginia School of Medicine, Charlottesville, VA 22908, USA
[g] Environmental Health Sciences, Mailman School of Public Health, Columbia University, New York, NY 10032, USA

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ABSTRACT

Air pollution exposures are linked to cognitive and olfaction deficits, oxidative stress, neuroinflammation and neurodegeneration including frontal hyperphosphorylated tau and diffuse amyloid plaques in Mexico City children and young adults. Mexico City residents are chronically exposed to fine particulate matter ($PM_{2.5}$) concentrations (containing toxic combustion and industrial metals) above the annual standard (15 $\mu g/m^3$) and to contaminated water and soil. Here, we sought to address the brain-region-specific effects of metals and key neuroinflammatory and DNA repair responses in two air pollution targets: frontal lobe and olfactory bulb from 12 controls vs. 47 Mexico City children and young adults average age 33.06 ± 4.8 SE years. Inductively coupled plasma mass spectrometry (metal analysis) and real time PCR (for COX2, IL1β and DNA repair genes) in target tissues. Mexico City residents had higher concentrations of metals associated with PM: manganese ($p = 0.003$), nickel and chromium ($p = 0.02$) along with higher frontal COX2 mRNA ($p = 0.008$) and IL1β ($p = 0.0002$) and COX2 ($p = 0.005$) olfactory bulb indicating neuroinflammation. Frontal metals correlated with olfactory bulb DNA repair genes and with frontal and hippocampal inflammatory genes. Frontal manganese, cobalt and selenium increased with age in exposed subjects.

Together, these findings suggest PM–metal neurotoxicity causes brain damage in young urbanites, the olfactory bulb is a target of air pollution and participates in the neuroinflammatory response and since metal concentrations vary significantly in Mexico City urban sub-areas, place of residency has to be integrated with the risk for CNS detrimental effects particularly in children.

1. Introduction

Air pollution is a complex mixture of particulate matter (PM), gases, organic and inorganic compounds present in outdoor and indoor air. Children living in Mexico City (MC) exhibit evidence of chronic inflammation of the upper and lower respiratory tracts, accumulation of ultrafine PM in nasal respiratory epithelium, breakdown of the nasal respiratory epithelial barrier, systemic inflammation, immunodysregulation, brain inflammation, cognitive and olfaction deficits, and brain magnetic resonance imaging (MRI) structural abnormalities (Calderón-Garcidueñas et al., 2001a,b, 2003a,b, 2004, 2007, 2008a,b,c, 2009, 2010, 2011a,c; Block and Calderón-Garcidueñas, 2009). Children and adults exhibit up-regulation of inflammation-associated genes including cyclooxygenase-2 (COX2), interleukin 1 beta (IL-1β), and the key innate immunity receptor CD14 in their olfactory bulbs, frontal cortex, substantia nigra and vagus nerve (Calderón-Garcidueñas et al., 2004, 2008b). The frontal cortex of 40% of children and young adults age 18.37 ± 6.9 years resident in Mexico City exhibited tau hyperphosphorylation with pre-tangle material and 51% had Aβ diffuse plaques compared with 0% in controls (21.8 ± 10.8 years) (Calderón-Garcidueñas et al., 2011b). Data from the same cohorts showed a significant up-regulation of gene network clusters including IL1, NF-κ B, TNF, IFN and TLRs, along with a 15 fold frontal down-regulation of the cellular prion protein (PrP^C) in MC subjects

* Corresponding author at: The Center for Structural and Functional Neurosciences, The University of Montana, 32 Campus Drive, 289 Skaggs Bldg., Missoula, MT 59812, USA. Tel.: +1 406 243 4785; fax: +1 406 243 2807.
E-mail address: lilian.calderon-garciduenas@umontana.edu (L. Calderón-Garcidueñas).

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(Calderón-Garcidueñas et al., 2011b). Our findings suggest that chronic exposure to severe urban air pollution causes a significant imbalance in genes essential for cell proliferation, apoptosis, oxidative stress, inflammation, innate and adaptive immune responses and early Alzheimer's disease-related pathological processes leading to neurofibrillary tangle formation start early in childhood or in early young adulthood. Moreover, clinically healthy Mexico City children performed more poorly across a variety of cognitive tests and had significant differences in white matter volumes compared to children from low polluted areas (Calderón-Garcidueñas et al., 2011c). Thus, exposures to urban pollution also perturb the trajectory of cerebral development (Calderón-Garcidueñas et al., 2011c).

Air pollution in Mexico City is severe and metals are an important component of urban particulate matter (Bravo-Alvarez and Torres-Jardón, 2002; Chow et al., 2002; Molina and Molina, 2004; Molina et al., 2007; Moreno et al., 2008; Rauch et al., 2006; Moffet et al., 2008; Querol et al., 2008; Guzmán-Morales et al., 2011). There is a significant heterogeneity in particle mass, composition and toxicity in PM_{10} (particulate matter < 10 μm in diameter) samples collected in Mexico City (Rosas-Pérez et al., 2007). Further, the biological effects of PM_{10} using in vitro tests vary according to the regions within the city from where they were collected (Alfaro-Moreno et al., 2002, 2007).

There is an extensive literature associating health effects with ambient particulate matter and its components (Sunderman, 2001; Aschner et al., 2007; Maier et al., 2008; Happo et al., 2008; Chen and Lippman, 2009; Afeseh-Ngwa et al., 2009), and studies addressing mechanisms that mediate PM metals toxicology (Ayres et al., 2008; Kodavanti et al., 2008; Nong et al., 2008; Tang et al., 2009; Frick et al., 2011). Accumulation of metal ions in the brain contributes to heightened oxidative stress and neuronal damage (Zatta et al., 2008; Bolognin et al., 2009).

The goals of the present study were as follows: First, we set out to determine, using inductively coupled plasma mass spectrometry (ICP-MS), the content of metals associated with anthropogenic activities as well as essential metals and trace minerals related to normal brain function (V, Ni, Mn, Pb, Cr, Fe, Zn, Se, Cu, Co) in frontal cortex and in the lungs from subjects residing in high- vs. low-pollution areas. A second goal was to investigate if there was an association between the metal content in the frontal cortex and the lungs and gene expression of two inflammatory genes: COX2 and IL1β that have proven to be good markers of exposure to urban air pollution (Calderón-Garcidueñas et al., 2003b, 2004, 2008a, 2011b; Villarreal-Calderon et al., 2010). Thirdly, since the olfactory bulb (OB) is a target and a portal of entry of air pollution components (Ali et al., 2010), we explored the relationship between frontal cortex metal concentrations and OB inflammatory and DNA repair gene responses. Finally, we assessed whether age is related to frontal cortex metal accumulation. Oxidative stress, neuroinflammation, and neurodegeneration are present early in life upon exposure to polluted megacities and environmental exposure to metals could play a critical role for the induction of inflammatory and DNA repair responses in the brain.

2. Materials and methods

2.1. Study cities and air quality data

We selected a polluted megacity and two control cities. Mexico City (MC) was the selected megacity, while Tlaxcala and Veracruz were the low polluted cities. Mexico City represents an extreme of urban growth and environmental pollution (Bravo-Alvarez and Torres-Jardón, 2002; Molina et al., 2007). The Mexico City Metropolitan Area lies in an elevated basin at an altitude of 2240 m above mean sea level and its urbanized area covers around 2000 km^2. The basin is surrounded by high mountain ridges on the east, south, and west but with a broad opening to the north and a gap to the south-southwest. The surrounding mountains combined with the frequent morning thermal inversions contribute to the trapping and accumulation of air pollutants inside the basin. In this geographical setting, 20 million residents, nearly 4 million vehicles, and over 40 000 industries consume more than 40 million L of petroleum fuels per day emitting significant concentrations of primary air pollutants (Molina et al., 2007). The high altitude and tropical climate of the region are highly conducive to fast photochemistry forming secondary pollutants such as ozone (O_3) and fine particulate matter ($PM_{2.5}$).

Control cities: Tlaxcala and Veracruz were selected as the control cities due to their smaller size, low emission sources from industry and cars, and good ventilation conditions. Three additional factors for the selection of the control cities included: (i) altitude above sea level similar to Mexico City (i.e., Tlaxcala), (ii) dog pathology studies from these cities have shown minimal pathology in lungs and hearts (Calderón-Garcidueñas et al., 2001a), and (iii) clinical studies in these cities that have shown healthy children with no evidence of air pollution-associated pathology (Calderón-Garcidueñas et al., 2003a).

2.2. Autopsy population selection

The study protocol was approved by the Institutional Review Boards for Human Studies at the involved Institutions. We studied 59 subjects from 2 cohorts of children and adults, ages 2–87 years, with an average age of 33.06 ± 4.8 years. The control cohort included subjects from low polluted cities (*n*: 12), and the exposed cohort (*n*: 47) from MC. The 59 subjects had complete autopsies and neuropathology examinations, and were included in the real time polymerase chain reaction (RT-PCR) and the inductively coupled plasma mass spectrometry (ICP-MS) studies. All subjects were clinically healthy, had died suddenly in accidents, were dead on arrival and had full autopsies. Autopsy subjects had a negative toxicology screening panel, including drug alkaline and acid/neutral screen, amphetamines, benzodiazepines, cocaine/opiates, alcohol, volatiles and cannabinoids.

Data available for all subjects included age, gender, place of residency, cause of death, and time between death and autopsy. Cause of death was considered for all subjects to rule out the possibility that infection, inflammatory events, drug exposure, brain ischemia and hypoxia might impact the mRNA levels of the inflammatory markers measured in the study.

2.3. Autopsy and tissue preparation

Autopsies were performed 3.9 ± 1.1 h after death. The postmortem period was similar for controls and exposed. The skull was opened and the olfactory bulbs and the brain removed. Selected areas from alternating right and left superior frontal gyri (prefrontal lobe) were dissected and kept at −80 °C for the inductively coupled plasma mass spectrometry (ICP-MS). Frozen tissue for the RT-PCR were taken from the cortex and the white matter in the same regions as the samples for ICP-MS. Sections from the right lung (anterior basal lower right lobe) were also taken for both ICP-MS and RT-PCR.

2.4. Inductively coupled plasma mass spectrometry (ICP-MS)

Frozen tissue was used for ICP-MS analysis using a Perkin-Elmer Sciex ELAN DRCII Inductively coupled plasma mass spectrometer housed in a HEPA filtered metal free laboratory. Each sample was dried, weighed and microwave digested in a closed vessel with nitric acid. The resulting solutions were analyzed directly