Toxicology Letters 220 (2013) 44–52

Contents lists available at SciVerse ScienceDirect

Toxicology Letters

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

Microglial disruption in young mice with early chronic lead exposure☆

Christina Sobin[a,b,c,d,*], Mayra Gisel Flores Montoya[c], Natali Parisi[e], Tanner Schaub[e], Miguel Cervantes[a], Rodrigo X. Armijos[a]

[a] Department of Public Health Sciences, College of Health Sciences, University of Texas, El Paso, USA
[b] Border Biomedical Research Center, College of Science, University of Texas, El Paso, USA
[c] Department of Psychology, University of Texas, El Paso, USA
[d] Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY, USA
[e] Laboratory of Chemical Analysis and Instrumentation, New Mexico State University, Las Cruces, NM, USA

HIGHLIGHTS

- Neuroinflammatory markers and microglia were compared in young mice with and without early chronic Pb exposure.
- Mice with Pb levels from 2.66 to 20.31 μg/dL had dose-dependent reduction of cytokine IL6.
- Mice with Pb levels from 2.66 to 20.31 μg/dL had dose-dependent reduction of detectable microglia.
- No evidence of increased neuroinflammation was found.

ARTICLE INFO

Article history:
Received 27 February 2013
Received in revised form 7 April 2013
Accepted 8 April 2013
Available online 15 April 2013

Keywords:
Pb
Microglia
Neuroinflammation
Neurotoxicity
Dentate gyrus

ABSTRACT

The mechanisms by which early chronic lead (Pb) exposure alter brain development have not been identified. We examined neuroimmune system effects in C57BL/6J mice with Pb exposure, including levels that may be common among children in lower socioeconomic income environments. Pups were exposed via dams' drinking water from birth to post-natal day 28 to low, high or no Pb conditions. We compared gene expression of neuroinflammatory markers (study 1); and microglial mean cell body volume and mean cell body number in dentate gyrus, and dentate gyrus volume (study 2). Blood Pb levels in exposed animals at sacrifice (post-natal day 28) ranged from 2.66 to 20.31 μg/dL. Only interleukin-6 (IL6) differed between groups and reductions were dose-dependent. Microglia cell body number also differed between groups and reductions were dose-dependent. As compared with controls, microglia cell body volume was greater but highly variable in only low-dose animals; dentate gyri volumes in low- and high-dose animals were reduced. The results did not support a model of increased neuroinflammation. Instead, early chronic exposure to Pb disrupted microglia via damage to, loss of, or lack of proliferation of microglia in the developing brains of Pb-exposed animals.

1. Introduction

In children with no observable peripheral symptoms of Pb exposure ("asymptomatic"), blood Pb levels as low as 2.5 μg/dL have been associated with, for example, lower IQ, reduced academic achievement, and poorer memory, attention, motor dexterity and problem-solving, suggestive of altered brain development (Canfield et al., 2003; Chiodo et al., 2004; Lanphear et al., 2005). In mouse and rodent models, early chronic exposure to Pb resulted in decreased memory, and abnormal motor and exploratory behavior (Azzaoui et al., 2009; Kasten-Jolly et al., 2012; Leasure et al., 2008). The mechanisms by which early chronic exposure to Pb alters brain structure and function have not been identified.

Results from in vivo and in vitro studies have suggested that Pb may promote neurotoxicity by disrupting neuroimmune system function (Kraft and Harry, 2011). The neuroimmune system is comprised of microglial cells. Microglia protect brain tissue through constant surveillance and scavenging of debris and foreign substances from the local environment (Schwartz et al., 2006); microglia also facilitate neuronal activity, and interact functionally with astrocytes (Aloisi, 2001). During development, activated

Abbreviations: IBA-1, ionized calcium-binding adapter molecule 1; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin beta 1; IL-6, interleukin 6; IL-10, interleukin 10; IFN-γ, interferon gamma; iNOS, inducible nitric oxide synthase; HO-1, heme oxygenase; ROS, reactive oxygen species; δ-ALA, delta-aminolevulinic acid; DG, dentate gyrus.

* Corresponding author at: Department of Public Health Sciences, College of Health Sciences, University of Texas, El Paso, USA. Tel.: +1 9157478485; fax: +1 9157476553.
E-mail address: casobin@utep.edu (C. Sobin).

0378-4274/$ – see front matter © 2013 Christina Sobin. Published by Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.toxlet.2013.04.003

microglia support and protect neurite development, guide synaptic pruning, and sculpt neural circuits (Paolicelli et al., 2011; Ransohoff and Cardona, 2010; Schafer et al., 2012). The critical neuroprotective role of microglia during early development is suggested by the acute sensitivity of these cells to CNS changes, as indicated by extremely rapid activation and proliferation response times (Dissing-Olesen et al., 2007). Microglia express IBA-1 thus IBA-1 antibody is used in immunohistochemical preparations to label microglia in brain tissue.

Microglia are activated by various agents that trigger a sequence of unique morphologic changes, including cell body enlargement. Activated microglia secrete an array of chemokines, eicosanoids, proteases, complements, and cytokines including, for example, TNF-α, IL-1β, IL-6, IL-10, and IFN-γ. The influence of a given cytokine is not singular, and at different times, might be pro- or anti-inflammatory, and thus have neuro-protective or neuro-destructive effects. Free radicals are increased by up-regulation of iNOS; and astrocytes simultaneously induce HO-1 which promotes reduction of damaging ROS (Min et al., 2006). During activation, microglia proliferate, and proliferation is stimulated by IL1-β and TNF-α (Mander et al., 2006). If microglial activation becomes chronic, microglia synthesize neurotoxic levels of quinolinic acid (Espey et al., 1997) and promote extracellular glutamate concentrations sufficient to cause neuritic beading and cell death (Takeuchi et al., 2005). Pro-inflammatory cytokines inhibit glutamate transporters, which sustain abnormally high levels of extra-cellular glutamate and thus, cyclic re-activation (Minami et al., 1991).

Findings from in vivo and in vitro studies show that Pb exposure alters cellular functions in ways that might be expected to promote chronic microglial activation. Pb accumulation in erythrocytes results in increased brain δ-ALA which enhances and prolongs microglial activation (Kaushal et al., 2007). Moreover, microglia interact functionally with astrocytes, via cytokines (Verderio and Matteoli, 2001), prostaglandins (Mohri et al., 2006) and nitric oxide synthase (Sola et al., 2002). Excess δ-ALA irreversibly inhibits glutamate uptake by astrocytes, via alteration of the glutamate transporter GLT-1 (Emanuelli et al., 2003). Glutamate potentiates astrocytic increases in Ca^{2+} via activation of metabotropic glutamate receptors (Zonta et al., 2003). δ-ALA triggers astrocytic Ca^{2+} waves which in turn activate microglia over large distances (Schipke et al., 2001).

Thus, by way of multiple mechanisms, free-floating Pb in brain tissue and increased brain δ-ALA might be expected to promote neuroimmune system disruption, chronic microglial activation and microglia proliferation, as evidenced by altered levels of pro- and anti-inflammatory markers including TNF-α, IFN-γ, IL6, IL10, iNOS and HO-1, increased microglial mean cell body number, and mean cell body volume.

The aim of this study was to examine evidence of neuroimmune and brain structure differences in young C57BL/6J mice, with and without chronic Pb exposure. In child studies, Pb exposure has been associated with reduced short-term and working memory (see Section 1), which are subserved by dentate gyrus (DG) (Niewoehner et al., 2007), a sub-component of the hippocampal formation. In rodent models, low-level Pb exposure resulted in diminished recognition memory (see Section 1) which is also subserved by dentate gyrus (Jessberger et al., 2009); moreover, DG microglia have been shown to play a critical role in the maintenance of neural genesis and spatial learning and memory (Ziv et al., 2006).

In animals with and without Pb exposure, we compared gene expression levels of neuroinflammatory markers (TNF-α, IFN-γ, IL6, IL10, HO-1, iNOS, and GRP78) in anterior (without hippocampus/DG) and posterior (with hippocampus/DG) brain regions. In a separate study, in animals with and without Pb exposure, we measured IBA-1 labeled microglia mean cell body number and mean cell body volume; and volume of DG. We predicted significant dose-dependent group differences on outcome measures. Only IL6 differed between groups and reductions were dose-dependent. Microglia mean cell body number also differed between groups and reductions were dose-dependent. Microglia mean cell body size differed only among low-dose animals. As compared with controls, dentate gyrus volumes in Pb-exposed animals were reduced.

2. Materials and methods

2.1. Animal methods

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved and annually reviewed by the Institutional Animal Care and Use Committee of the University of Texas at El Paso (NIH Assurance #A3340-01). All surgery was performed under deep Avertin anesthesia and all efforts were made to minimize suffering. C57BL/6J (Jax Mice, Jackson Laboratory, Sacramento, CA) mice were bred and housed at the University of Texas at El Paso Biosciences Research Facility, Animal Vivarium, in clear polycarbonate cages with wood chip bedding, 1 litter per container. Animals were maintained on a 12 h light–dark schedule, vivarium temperature of 21 ± 2 °C, with ad libitum access to food and water. Dams' drinking water was tainted with 99.4% Pb acetate crystals (Sigma–Aldrich). To maximally reduce animal stress, no invasive procedures were conducted during the 28-day exposure period, litters were not culled, and studies included males and females. Natural litters were exposed from birth to one of three possible Pb doses: 0 ppm; 30 ppm; and 230 ppm (study 1) or 0 ppm; 30 ppm; and 330 ppm (study 2). For both studies, the dosing regimen was based on pilot studies demonstrating that 30–40 ppm of Pb acetate in dams' drinking water resulted in a blood Pb level range similar to at least 65% of low-income children tested in our child Pb exposure and behavior studies (unpublished data).

2.2. ICP-MS analysis of Pb

2.2.1. Instrumentation

Analysis by inductively coupled plasma mass spectrometry (ICP-MS) was performed with an Agilent 7500ce ICP/MS equipped with an octopole reaction system and a CETAC ASX-520 autosampler as previously described (Sobin et al., 2011). Briefly, samples were introduced to the plasma through a MicroMist U-series nebulizer (Glass Expansion, Australia) and a double-pass quartz spray chamber (Agilent, Santa Clara, CA). Instrument parameters were: carrier gas, 0.78 L/min; makeup gas, 0.15 L/min; RF power, 1420 W; spray chamber temperature, 2 °C.

2.2.2. Sample treatment and analysis

Certified whole blood standards (Le Centre de Toxicologie du Quebec) were analyzed to determine instrument reproducibility and validate quantitation. Ten solutions were prepared for each of two standards (4.00 μg/dL and 6.59 μg/dL) and each of those were analyzed three times by ICP-MS. Standard concentrations were chosen to approximate low blood Pb values of children in this study. Blood standards were prepared as previously described (Sobin et al., 2011) (Agilent technical note #5988-0533EN). Briefly, 5.58 mL of water (18 MΩ DI, Labconco WaterPro® PS Station, Kansas City, MO) was placed in a polypropylene tube into which 300 μL of whole blood was added, followed by addition of by 60 μL of aqueous internal standard solution (100 ppb each germanium, yttrium and terbium in 5% nitric acid, Fisher Optima) and 60 μL of aqueous 10 ppm gold in 3% hydrochloric acid (EMD Chemicals) solution. The final dilution was twenty-fold, the final internal standard concentration was 1 ppb and the final gold concentration was 100 ppb. A six-point external calibration curve was prepared from a Pb stock solution in 1% nitric acid. ICP-MS standard solutions containing the elements in 2% nitric acid were obtained from Inorganic Ventures (Christiansburg, VA). Samples were vortexed for a few seconds prior to a 1 min centrifugation at 2000 rcf and the supernatant analyzed by ICP-MS. Blank solutions were analyzed after every three samples throughout the analytical sequence and standard check solutions were analyzed five times, interspersed through the sequence. All samples produced signals in excess of the limit of quantitation (i.e. ten-fold greater than the detection limit) for each analyte.

2.3. Gene expression studies

Brain tissue was removed immediately after sacrifice, snap frozen on dry ice and stored at −80 °C until RNA extraction. Cerebellum was removed and the remaining whole brain structure was cut (within 1 min) into anterior and posterior sections; and sections were immediately homogenized (30 s). Anterior segments included at least 90% of basal forebrain, striatum, ventral striatum and septum; and no more than 10% of hippocampus, amygdala, thalamus, and hypothalamus. Posterior sections included at least 90% of the midbrain, hippocampus, amygdala, thalamus, and hypothalamus; and no more than 10% of basal forebrain, ventral striatum, septum, and striatum.

2.3.1. RNA extraction

RNA was extracted using RiboPure™ Kit (Ambion). All procedures were conducted at room temperature unless otherwise specified. Each section was