Toxicology Letters 239 (2015) 97–107

Contents lists available at ScienceDirect

Toxicology Letters

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

Lead exposure induced microgliosis and astrogliosis in hippocampus of young mice potentially by triggering TLR4–MyD88–NFκB signaling cascades

Jin-Tao Liu[a,b,1], Bei-Yu Chen[c,1], Jie-Qiong Zhang[d], Fang Kuang[a,*], Liang-Wei Chen[a,*]

[a] Institute of Neurosciences, Fourth Military Medical University, Xi'an 710032, China
[b] Department of Neurosurgery, Tangdou Hospital, Fourth Military Medical University, China
[c] Department of Orthopedics, Xijing Hospital, Fourth Military Medical University, China
[d] Department of Occupational & Environmental Health, Fourth Military Medical University, China

HIGHLIGHTS

- In this study, we established a mouse model with lead (Pb) exposure.
- Lead exposure induced obvious microglial response, TLR4–MyD88–NFkB signaling activation, inflammatory cytokine generation and MAPK singling activation in the hippocampus.
- Increase of BrdU-incorporated cells and new-born astroglial cells, but no increase of DCX-labeled differentiated neuronal cells occurred in the dentate gyrus of hippocampus.
- Administration of MyD88 inhibitory peptide could relieve above Pb-induced effects.
- Data of this study suggested that lead exposure could induce microgliosis and astrogliogenesis in the hippocampal of young mice possibly by triggering TLR4–MyD88–NFkB signaling cascades.

ARTICLE INFO

Article history:
Received 6 July 2015
Received in revised form 9 September 2015
Accepted 12 September 2015
Available online 16 September 2015

Keywords:
Lead toxicity
Microgliosis
Astrogliosis
Neurogenesis
Hippocampus

ABSTRACT

Proper proliferation and differentiation of neural stem cells or progenitors in hippocampus is critical to learn and memory functions, which might be disturbed by lead toxicity particularly in young individuals. While astroglial and microglial cells are known to play an important role in regulating neurogenesis of hippocampus, their abnormal response and influence on hippocampal neurogenesis remains unclear. In this study, therefore, glial response including microgliosis, astrogliogenesis and mediating involvement of TLR4–MyD88–NFκB signaling cascades were observed in hippocampus of young mice by animal model with lead (plumbum, Pb) exposure. It revealed that (1) significant microglial activation occurred in hippocampus soon following Pb exposure; (2) increased levels of TLR4, MyD88, NFκB expression were concomitantly detected; (3) BrdU-incorporated progenitor cells were observed in dentate gyrus with significantly-increased numbers at d28 in Pb insult group; (4) obvious astrogliogenesis was observed while these doublecortin-labeled differentiated neurons were not significantly changed in hippocampus; (5) administration of MyD88 inhibitory peptide attenuated or relieved above effects; (6) enhanced expression levels of IL-1β, TNFα, p38MAPK and ERK1/2 were also detected in hippocampus, indicating potential implication of inflammatory response and MAPK signaling activation in lead-induced microgliosis and astrogliosis. Data of this study overall have indicated that lead exposure could trigger or induce abnormal microgliosis and astrogliogenesis in the hippocampus of young mice through triggering TLR4–MyD88–NFκB signaling cascades, which might possibly thereafter disturb hippocampal neurogenesis and functional plasticity.

Abbreviations: BrdU, bromodeoxyurinine; DCX, doublecortin; DG, dentate gyrus; GFAP, glial fibrillary acidic protein; IL-1β, interleukin-1β; LPS, lipopolysaccharide; LTP, long-term potentiation; TLR4, toll-like receptor-4; TNFα, tumor necrosis factor-alpha; Pb, plumbum; PBS, phosphate buffered saline.

* Corresponding authors. Fax: +86 29 3246270.
E-mail addresses: jt_superliu@163.com (J.-T. Liu), chenby@fmmu.edu.cn (B.-Y. Chen), zhangjieqiong1@163.com (J.-Q. Zhang), KuangF@fmmu.edu.cn (F. Kuang), chenlw@fmmu.edu.cn, lwchen@fmmu.edu.cn (L.-W. Chen).

[1] Both the authors have equal contribution to this work.

http://dx.doi.org/10.1016/j.toxlet.2015.09.015

1. Introduction

The dentate gyrus (DG) of hippocampus is one distinctive area in the mammalian brains that maintains active the neurogenesis for whole-life, which plays an important role in learn and memory function such as establishment of spatial representations (Cameron and Mckay, 2001; Zhao et al., 2008). Previous studies have indicated a tight correlation between neurogenesis and learning performance (Lemaire et al., 2000; Mohapel et al., 2005), and fewer new-born neurons result in poorer navigation learning and memory (Drapeau et al., 2003; Kempermann, 2002; Kempermann and Gage, 2002). Growing evidence has shown that various factors can influence neurogenesis and plasticity of hippocampus. For instance, astroglial and microglial cells play an important role in regulating neurogenesis of hippocampus. Apparently, astrocytes are fundamental for homoeostasis, development and plasticity of central neurons. Being active partners with neurons at synapses, astrocytes involved in hippocampal metaplasticity. Loss of astroglial function contributed to aging and neurodegenerative diseases. For instance, reactive astrocytes were associated with developing of neurite plaques in Alzheimer's disease (AD), particularly in hippocampal and cortical regions (Jones, 2015; Rodríguez-Arellano et al., 2015). Moreover, microglial cells were also involved in regulation of hippocampal neurogenesis in a temporally and spatially specific manner. Microglia might sense signals from inner and outer environments of brains, become activated and influence neurogenesis (Sato, 2015). Besides, these activated microglia cells present major source of the inflammatory cytokines. Activation of astrocytes and microglial cells exaggerated inflammatory cytokine production and hippocampal deterioration, and inflammatory response might greatly contribute to the impairment of memory function (Barrientos et al., 2015; Ojo et al., 2015). In addition, the hippocampal neurogenesis is also related to cognitive demand in adults (Dupret et al., 2008; Leuner et al., 2004). Accumulating evidences support a view that cognitive function depends on precise neurogenesis in hippocampus, and deficiency of newborn neuronal cells may impair learning and memory functions (Drapeau et al., 2003; Kempermann and Gage, 2002; Lemaire et al., 2000; Mohapel et al., 2005).

Lead (plumbum, Pb) is a heavy metal that widely exists in living surroundings, and lead exposure in high level can cause acute or chronic poisoning such as neuronal injury of the central nervous system (CNS), especially to children whose blood–brain barrier is fragile (Luo et al., 2012). Data showed that lead exposure could suppress generation or differentiation of CNS neurons, inhibit long-term potentiation (LTP), destruct secretion of neurotransmitters and interfere with calcium signaling (Gilbert et al., 2005; Goldstein 1990; Lasley and Gilbert, 2000). Besides, Pb exposure might also cause response of glial cells, which were critical in regulation of neurogenesis and cognitive function in hippocampus (Gilbert et al., 2005; Verina et al., 2007). While they actively functioned in modulation of neurogenesis, neuronal activity and LTP generation (Alvarez-Buylla and Garcı'a-Verdugo, 2002; Bélanger et al., 2011; Sierra et al., 2010), microglial cells and astrocytes appeared susceptive to lead exposure, and cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) were released as a pacemaker of inflammatory response or neuronal injury (Sansar et al., 2011; Strużyńska et al., 2007).

Until now, however, it still remains unclear on related mechanism for Pb-induced microglial response and neurogenesis dysfunction. It is well known that microglial cells can initiate innate immune reaction by toll-like receptors (TLRs) (Takeuchi and Akira, 2010), and TLRs modulate adult hippocampal neurogenesis and TLR deficiency impair neurogenesis in hippocampus (Rolls et al., 2007). But, there was also a report on microglial disruption in the developing hippocampus of young mice with chronic Pb exposure (Sobin et al., 2013). Besides, the adapter protein of most TLRs, myeloid differentiation primary response gene 88 (MyD88) transfers TLR signal to intracellular pathway (Watters et al., 2007; Vogel et al., 2003), and result in NFκB activation and inflammatory responses (Chen and Jiang, 2013). In this study, thus, we focused on mediating role of TLRs–MyD88–NFκB signaling activation in above glial responses of the hippocampus by using young mouse model with Pb exposure. Data of this study has provided new evidence that lead could induce abnormal microgliosis, astrogliosis and even abnormality of neurogenesis in the hippocampus, possibly by triggering TLR4–MyD88–NFκB signaling cascades.

2. Materials and methods

2.1. Animals and animal model preparation

The C57/BL neonatal young mice were used in this study, and provided by the animal center of the Fourth Military Medical University (FMMU), China. These mice with their parents were housed in temperature-controlled, 12 h/12 h light/dark room, and allowed to access freely to pelleted semi-purified mouse chow (Solid, Vital Keao Feed Co., Beijing, China). When they grew to age of 11–12 days and weighing 5–8 g, mice ($n = 64$, totally) were taken for experiment. All animal experiments were carried out in according with the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 80-23) revised 1996. This study was approved by IACUC and the Committee of Animal Use for Research and Education of the Fourth Military Medical University, and all efforts were made to minimize animal suffering and reduce animals used.

The young mice were assigned into following groups. i.e., control, MyD88 inhibitory peptide, Pb and Pb + MyD88 inhibitory peptide. For preparation of Pb model, intraperitoneal injection with lead acetate (15 mg/kg, i.p.) was given to mouse group daily for 3 days, while MyD88 inhibitory peptide was administrated to single MyD88 inhibition or Pb + MyD88 inhibition group by intracerebroventricular injection, and saline injection was used as control. All mice of three groups were processed in two following batches: first animal batch ($n = 48$, d17) was used for serum Pb detection, immunohistochemical and western blot analysis of TLR4, MyD88, NFκB, inflammatory cytokine and MAPK signaling, and second animal batch ($n = 16$, d28) was mainly used for BrdU incorporation assay to progenitor cell proliferation, neuronal and astroglial differentiation in the hippocampus afterwards (Fig. 1a,c).

2.2. Serum Pb detection

For detection of serum Pb concentrations, the mice ($n = 16$) in above four groups were sacrificed in the day after the last injection of lead acetate, blood samples were collected into heparinized syringes and aliquots, which were taken immediately for determination of serum Pb concentration by atomic absorption spectrophotometry with a graphite furnace (AA Scan 1 Thermo Jarrell Ash).

2.3. Immunohistochemistry

The hippocampal samples and sections were prepared for immunohistochemical study. Briefly, mice ($n = 16$) were deeply anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and transcardially perfused with 4% paraformaldehyde in 0.1 M