Journal of Trace Elements in Medicine and Biology 28 (2014) 100–104

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

journal homepage: www.elsevier.de/jtemb

Toxicology

The effects of early life Pb exposure on the expression of IL1-β, TNF-α and Aβ in cerebral cortex of mouse pups

Ning Li[a], FangLi Liu[b], LianJun Song[a], PingAn Zhang[a], MingWu Qiao[a], QiuYan Zhao[a], WenJie Li[b,*]

[a] Food Science Technology College of Henan Agricultural University, Zhengzhou 450002, China
[b] College of Public Health, Zhengzhou University, Zhengzhou 450001, China

ARTICLE INFO

Article history:
Received 17 March 2012
Accepted 9 July 2013

Keywords:
Lead (Pb)
Cerebral cortex
IL1-β
TNF-α
Aβ

ABSTRACT

Objective: To investigate the effects of maternal lead (Pb) exposure on the learning and memory ability and expression of interleukin1-β (IL1-β), tumor necrosis factor (TNF-α) and beta amyloid protein (Aβ) in cerebral cortex of mice offspring.
Methods: Pb exposure initiated from beginning of gestation to weaning. Pb acetate administered in drinking solutions was dissolved in distilled deionized water at the concentrations of 0.1%, 0.5% and 1% groups, respectively. On the PND21, the learning and memory ability were tested by water maze test and the Pb levels were also determined by graphite furnace atomic absorption spectrometry. The expression of IL1-β, TNF-α and Aβ in cerebral cortex was measured by immunohistochemistry and western blotting.
Results: The Pb levels in blood and cerebral cortex of all exposure groups were significantly higher than that of the control group ($P<0.05$). In water maze test, the performances of 0.5% and 1% groups were worse than that of the control group ($P<0.05$). The expression of IL1-β, TNF-α and Aβ was increased in Pb exposed groups than that of the control group ($P<0.05$).
Conclusions: The high expression of IL1-β, TNF-α and Aβ in the cerebral cortex of pups may contribute to the impairment of learning and memory associated with maternal Pb exposure.

Introduction

Pb produces neurotoxic effects, which result into an impairment of learning and memory and other neurological dysfunctions [21]. Neurotoxic effects from exposure to low levels of Pb in the environment are a problem of significant magnitude in the whole world, especially in children and infants [3]. Results from prospective cohort studies provided evidence that low level utero Pb exposure could impair infant growth, development and cognitive function [14]. The extensive studies revealed that prenatal and early postnatal Pb exposure even at low doses is extremely dangerous and can cause varied neurological disturbances [14]. Though numerous studies have shown that Pb could accumulate in the brain when its concentration in the blood is elevated [18], the mechanisms of Pb deposition in brain are not yet fully known.

The research on Pb neurotoxicity has been lasted for long time, but its proinflammatory effects in brain has not been investigated. This kind of effect has been observed in several other tissues and organs. The proliferation of T lymphocytes under Pb action has been observed *in vitro* [8]. It was reported that the young rats treated with Pb could cause the over expression of interleukin (IL)-10, IL-12 and increased numbers of monocytes and T lymphocytes in spleen and thymus [18]. The production of autoantibodies against myelin basic protein and glial fibrillary acidic protein (GFAP) after Pb exposure has been reported elsewhere [18]. This raises the possibility that Pb may affect immune processes in brain. Glial cells, the predominant CNS-antigen presenting cells, are involved in local inflammatory processes by responding to, as well as producing, cytokines such as IL-1β, IL-6, and tumor necrosis factor (TNF)-α, both IL-1β and TNF-α are potent inducers of proteinases in the CNS [18].

The research on Pb neurotoxicity has been lasted for a long time, but its Alzheimer-like effects in brain has few been investigated. Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disease [10]. The neuropathology of AD is characterized by senile plaques (SPs) and neurofibrillary tangles (NFTs). SPs are the extracellular deposits mainly composed of aggregated β-amyloid (Aβ) protein, whereas NFTs are formed due to the intracellular accumulation of hyperphosphorylated tau protein [9]. Furthermore, several studies have previously suggested a link between early-life disturbances (risk factors) and the development of AD [6]. Despite several genetic mutations [24] found in AD patients,

* Corresponding author. Tel.: +86 371 67781869; fax: +86 371 67781869.
E-mail address: lwj@zzu.edu.cn (W. Li).

0946-672X/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jtemb.2013.07.003

more than 90% of AD cases are sporadic [2]. Therefore, it is plausible that environmental exposure may be an etiologic factor in the pathogenesis of AD.

The developing brain is experiencing intensive cellular proliferation, differentiation, and synaptogenesis. It is very sensitive to environmental hazard at this stage. It is known that early life Pb exposure could cause severe consequences for brain development. However, the underlying mechanism of Pb exposure on brain development is still unclear. In order to discover the potential mechanism, this study will test the expression of IL-1β, TNF-α and Aβ in cerebral cortex of Pb exposed mouse pups to know whether these proteins are the media between Pb exposure and brain damage.

Materials and methods

Animals and treatment

All procedures involving animals were carried out in strict accordance with the international standards of animal care guidelines and were approved by the local Care of Experimental Animals Committee. Forty pregnant Kunming female mice were ordered from Henan laboratory animal center. They were randomly divided into four groups and caged individually. Pb acetate was dissolved into distilled at three different concentration levels, 0.1%, 0.5% and 1% for low, moderate and high concentration, respectively. Pb exposure started from beginning of gestation and lasted until weaning (PND21) by drinking Pb containing water. The animals were maintained on a 12–12 h light/dark cycle with food and water available *ad libitum*. All experiments were carried out in accordance with the regulations of the Zhengzhou University Committee on Ethics in the Care and Use of Laboratory Animals. At birth, all litters were culled to eight pups. In the process of experiment, littermates were not used within a group, but they were used across groups.

Determination of Pb concentrations in blood and cerebral cortex samples

Sample collection

The blood samples were collected from the tail of the pups on the 21st postnatal day after wiping the skin to remove the contaminated Pb. Then, the pups were anesthetized under ether inhalation and the brain was dissected. The cerebral cortex was isolated.

Sample preparing for assay

Total of 100 μl of blood was mixed well with 3.9 ml of 0.5 N nitric acid containing 0.01% Triton X-100 by vortexing for 10 s and followed by centrifugation for 10 min at 7500 r/min at room temperature. The supernatant was collected for further analysis of Pb content. The isolated cerebral cortex tissue from each sample was homogenized in a mixture of 0.5 N nitric acid, 0.5 N perchloric acid and 0.01% Triton X-100 to make 1:10 (w/v) of diluted homogenate.

The measurement of Pb concentration in samples was performed as described [7,11,26]. A 20 ml aliquot of each sample was injected into the graphite furnace atomic absorption spectrometry (HITACHI) by an autosampler and 20 ml of 0.2% magnesium nitrate was added as modifier. Pb concentration was measured at 283.3 nm by a programmed heating procedure for blood and brain Pb. Pure argon was used as the purge and protecting gas. The analysis was carried out in duplicate and the mean peak height value was taken for calculation. For the standard curve, 1000 ppm Pb chloride in 0.3 M nitric acid was diluted to 5, 10 and 20 ppm with 0.2% nitric acid containing 0.01% Triton X-100. To examine the recovery rate, standard Pb solution was added (final concentration: 10 ppm) to blood and brain samples as internal standard. The recovery rates for blood and brain homogenate were 97.5% and 98.6%, respectively.

Morris water maze

Spatial learning and memory of the PND 21animals was evaluated in a Morris water maze [5,20]. Animals received 4 trials per day, for a total of 8 test sessions. Data from 4 trials were averaged to represent a performance block. The pool consisted of a white circular fiberglass tank measuring 1.5 m in diameter. Water temperature was maintained at approximately 22 °C and was made opaque by the addition of Funstuff© Liquid Tempera black paint (Reeves & Poole Group,Toronto, ON, Canada). Four points around the edge of the pool were arbitrarily designated as north, south, east and west. A clear plexiglass escape platform (15 cm × 15 cm) was submerged approximately 2 cm below the water surface, and placed initially, in the center of the "eastern" quadrant of the maze (approximately 32 cm from the wall of the pool). A reversal task was then introduced where the platform was moved to the center of the diagonally opposite "western" quadrant for the three days, and finally the platform was returned to its original location. The dependent measures included escape latency(s) and the number of crosses from initiation point to the platform locations.

Sample preparing and Immunohistochemistry

Three pups from each group were selected for histological study [13,15]. The selected pups were anaesthetized by overdose with sodium nembutal (35 mg/kg, i.p.). The whole brain was dissected. The fixed brain samples were cut at 5 μm thickness starting at 3 mm posterior to the anterior pole. The tissue slides were treated by microwave in 10 mM citrate buffer (pH = 6) for 3 min and followed by blockade of non-specific binding by incubation in 0.1 M PBS containing 3% normal goat serum. Sections were then subsequently incubated with primary antibodies overnight at room temperature. The primary antibodies were rabbit anti-IL-1β (Santa Cruz) and rabbit monoclonal anti-Aβ(Santa Cruz). After extensive rinsing steps in 0.1 M PBS, the sections were incubated in biotinylated goat anti-rabbit antibody (Santa Cruz) for 1 h at room temperature and followed by using the Vector ABC system. Subsequent incubation in diaminobenzidine was performed for visualization of the reaction product. For negative controls, the primary antibody was omitted.

Protein isolation and western blotting

The dissected cerebral cortex samples were homogenized in lysis buffer containing 2% SDS, 10% glycerol, 2% 2-mercaptoethanol, 0.002% bromophenol blue in 75 mM Tris-HCl [25]. The samples were heated at 95 °C for 10 min before separating on 10% Tris/Glycine/SDS acrylamide gels. The proteins were subsequently transblotted to polyvinylidene difluoride membranes and blocked in 5% dry milk for 2 h at room temperature. The membrane was incubated with rabbit anti-IL-1β (Santa Cruz Company, USA), anti-TNF-α (Santa Cruz Company, USA) and anti-Aβ antibody (Santa Cruz) for 2 h at 37 °C. After three washes with TBS/0.05%Tween-20, the membrane was incubated with a horseradish peroxidase-conjugated goat anti-rabbit antibody (Santa Cruz) for 1 h at 37 °C [11]. Protein signal was visualized using the SuperSignal West Pico Chemiluminescent Substrate (PIERCE Company) and detected with Imaging System (Syngene Company). β-actin protein was visualized and detected as above.

Statistical analysis

All data were expressed as mean ± SEM. One-way ANOVA and a *post hoc* Bonferroni's test in SPSS12.0 software was used to analyze the differences of IL-1β, TNF-α, Aβ and Pb content in blood and