ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 39 (2015) 221–228

Available online at www.sciencedirect.com

ScienceDirect

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

Effects of selenium on lead-induced alterations in Aβ production and Bcl-2 family proteins

Zhiqiang Deng[a,b], *Hongjun Fu*[c], *Yongmei Xiao*[a], *Bo Zhang*[a], *Guangshun Sun*[a], *Qing Wei*[a], *Baomin Ai*[a], *Qiansheng Hu*[a,*]

[a] *Department of Preventive Medicine, School of Public Health, Sun Yat-Sen University, Guangzhou 510080, China*
[b] *Nanchang Center for Disease Control and Prevention, Nanchang 330038, China*
[c] *The Jackson Laboratory, Bar Harbor, ME 04609, USA*

ARTICLE INFO

Article history:
Received 18 September 2014
Accepted 20 November 2014
Available online 10 December 2014

Keywords:
Selenium
Lead
Amyloid precursor protein
Amyloid beta protein
Bcl-2
Bax

ABSTRACT

Previous studies in humans and animals have suggested that lead (Pb) may increase the expression of amyloid precursor protein (APP) and accumulation of amyloid β protein (Aβ). Our previous studies have revealed that selenium (Se) can partially improve memory deficits induced by Pb exposure. In this study we sought to investigate the effect of Pb and Se on the endogenous expression of APP, $A\beta_{40}$ and Bcl-2 family proteins. *In vitro*, the protein levels of APP and Aβ significantly decreased in SH-SY5Y and PC12 cells co-incubated with Pb-acetate and selenomethionine (SeMet) for 48 h, compared with cells treated with Pb-acetate alone. Furthermore, these reductions induced by Se appeared to be concentration-dependent. In Wistar rats, we observed that the mRNA and protein levels of APP, the protein level of Bax, and the ratio of Bax/Bcl-2 protein significantly increased after Pb treatment at embryonic stage and in neonates. These increases were significantly reversed by the treatment of Se. Taken together, our results suggest that Se can attenuate the alterations in APP expression and Aβ production as well as Bcl-2 family proteins induced by lead exposure in cells and in animals.

1. Introduction

As the most common form of dementia in the elderly, Alzheimer's disease (AD) currently affects about 25 million people in worldwide with increasing 4.6 million new cases every year (Hebert et al., 2003). AD is characterized by a series of changes in the function of nervous system including widespread neuronal cell loss and dysfunction (Smith, 1998). The extracellular accumulation of amyloid beta protein (Aβ) is believed to be an initial feature of AD pathogenesis (Ogomori et al., 1989). Aβ is derived from the proteolytic cleavage of amyloid beta precursor protein (APP), a lager 120 kDa transmembrane protein, by β-secretase and γ-secretase. Furthermore, previous studies suggest that there is a close relationship between Aβ and hyperphosphorylation of tau, another hallmark of AD (Hernandez et al., 2010). Epidemiological investigations found that most of AD cases (>95%) are non-familial and late onset sporadic forms (Migliore and

Abbreviations: Aβ, amyloid β protein; AD, Alzheimer's disease; APP, amyloid precursor protein; SeMet, selenomethionine; BACE, β-amyloid cleaving enzyme; ELISA, enzyme-linked immunosorbent assay; GSH, glutathione; GPX, GSH peroxidase.

* *Corresponding author*. Tel.: +86 20 87332531; fax: +86 20 87332531.
E-mail address: huqsh@mail.sysu.edu.cn (Q. Hu).

http://dx.doi.org/10.1016/j.etap.2014.11.010
1382-6689/

Coppede, 2009), indicating that environmental factors may play an important role in AD.

Lead (Pb) is widely recognized as an environmental central neurotoxin, and low-level Pb exposure could impair the development and the cognitive functions of brain, especially in children and infants (White et al., 2007). Epidemiological investigations have revealed the possible association between low-level Pb exposure and neurobehavioral disorder (Braun et al., 2006). Furthermore, previous studies have established a definite relationship between the Pb exposure and up-regulation of APP protein and the over-accumulation of Aβ, especially $A\beta_{40}$ (Gu et al., 2012; Huang et al., 2011). Exposure to Pb during brain development predetermined the expression and regulation of APP and its amyloidogenic Aβ product followed by increased levels of β-amyloid cleaving enzyme (BACE), oxidative damage to DNA, and a reduction in DNA methyltransferase activity in aged monkey (Wu et al., 2008). More and more studies have provided strong evidence about the linkage between Pb exposure and AD. Even though its current environmental exposure is low, Pb remains one of the most widespread and insidious environmental burdens (White et al., 2007). Thus, it is important to search for effective therapeutics against Pb-induced AD-like pathology.

Selenuim (Se) is an essential and anti-oxidant trace element of our diet with numerous beneficial effects on health (Benton, 2002; Rayman, 2000; Rayman et al., 2006). Changes in Se concentration and selenoprotein activity in blood and brain have been reported in Alzheimer's disease and other brain diseases (Chen and Berry, 2003). Se has been found to attenuate Aβ production and Aβ-induced neurotoxicity (Godoi et al., 2013; Gwon et al., 2010). Furthermore, Se treatment significantly mitigates neurodegeneration and cognitive deficits in animal models with AD-like pathology (Corcoran et al., 2010; Ishrat et al., 2009; Song et al., 2014; van Eersel et al., 2010; Yim et al., 2009a). Therefore, selenium has potential neuroprotective properties in the prevention and/or treatment of AD, but the underlying mechanism and its therapeutic potential remain elusive.

The Bcl-2 family members, such as Bax, Bcl-2 and Bcl-X_L, are involved in the regulation of activities concerned with the survival and death of neurons through apoptosis (Pettmann and Henderson, 1998; Strasser et al., 1997). Bcl-2 belongs to the prosurvival subfamily that prevents death from apoptotic in neurons, while Bax belongs to the pro-apoptotic subfamily that promotes apoptosis. Thus, the ratio of Bcl-2/Bax is important to the fate of cells. Several lines of evidence have demonstrated that neuronal apoptosis play an important role in AD (Wang and Zhang, 2001; Zhu et al., 2004). Moreover, Bcl-2 overexpression protected from loss of cell viability and caspase-9 and 3 activation induced by $A\beta_{25-35}$, showing that Bcl-2 is neuroprotective against apoptotic cell death caused by amyloidogenic peptides (Ferreiro et al., 2007). The treatment of Pb-acetate decreased the expression of Bcl-2 protein and increased the expression of Bax in hippocampus neurons (Tang et al., 2010). Our previous studies suggest that Se may have a neuroprotective effect on Pb-induced impairments in learning and memory (Wang et al., 2013). However, the question as to whether Se has an antagonistic effect against the alterations of Aβ production and Bcl-2 family proteins induced by Pb remains unknown.

In order to explore the relationship between Se and Pb-induced alterations in Aβ production and Bcl-2 family proteins, we exposed cells and Wistar rats to Pb and Se and monitored the expression of APP and Aβ as well as Bcl-2 family proteins.

2. Materials and methods

2.1. Cell culture

SH-SY5Y cells and PC12 cells were obtained from Shanghai Institute of Cell Biology. SH-SY5Y cells were grown in DMEM medium (Invitrogen, CA), while PC12 cells were grown in RPMI1640 medium (Invitrogen, CA) supplemented with 10% fetal bovine serum (Invitrogen, CA), 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin in a CO_2 incubator maintained with 5% CO_2 and 37 °C. The medium was changed every 3 days.

2.2. Pb and Se treatment in cells

Twenty-four hours prior to treatment, human SH-SY5Y cells and PC12 cells were seeded at 1×10^6 cells per well in 6 cm Petri dish. In Pb treatment alone group, cells were incubated with 0, 2.5, 5, 10, and 20 μM of Pb-acetate (Guang Zhou Chemical Agent Factory, China) for 48 h at 37 °C. The co-incubation group exposed to Pb and Se as follows: human SH-SY5Y cells were treated with 20 μM Pb and 0, 0.25, 0.5, 1.0, and 2 μM SeMet, while PC12 cells were incubated with 20 μM Pb and 0, 10, 20, 40, and 80 nM SeMet for 48 h at 37 °C.

2.3. CCK-8 assay

Briefly, 5000 cells/well were seeded on 96-well plates with three replicates per treatment group. Cells were exposed to 20 μM Pb and a series of Se concentrations (SH-SY5Y cells with 0, 0.25, 0.5, 1.0, and 2.0 μM Se; PC12 cells with 0, 10, 20, 40, and 80 nM Se) at 37 °C with 5% CO_2 and 90% humidity. After being treated for 24, 48, or 72 h, 10 μl CCK8 (Bestbio, China) and 90 μl medium was added into each well and incubated for 2.5 h at 37 °C. The absorbance at 450 nm was determined by a Microplate Reader (Bio-Rad 680).

2.4. Animal exposure

Wistar rats were obtained from the Laboratory Animal Center at Sun Yat-sen University (Guang Zhou, China). In each exposure scenario, 2 mM Pb-acetate was added to the drinking water and 60 mg/kg day Se-enriched yeast was intragastrically administrated to each mother rat. The animals were maintained at a constant temperature (21 ± 1 °C) with a 12–12 h light/dark cycle. Twenty-four hours after pregnancy, all mother rats were randomly divided into three groups, namely, Con, Pb–P and Pb + Se–P. The Con group received deionized water during pregnancy and lactation; the Pb–P group was exposed to Pb from 1 day after pregnancy; and the Pb + Se–P group was exposed to Pb and Se (Angel Yeast, China) at the