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The changes of miRNA expression in rat hippocampus following chronic lead exposure

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HIGHLIGHTS

- Lead exposure resulted in the changed expression of 7 miRNAs in rat hippocampus.
- The target genes of up-regulated miRNAs were related to neurophysiological pathways and neurodegenerative diseases.
- The changed miRNAs may play important roles in lead-induced neurotoxicity.

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ABSTRACT

miRNAs have been found to contribute to normal brain functions, nervous system diseases, as well as neurotoxicities induced by external agents. However, whether they are involved in lead-induced neurotoxicities is still not clear. To identify that, a lead-induced chronic neurotoxicity model of rats was built. Both miRNA microarray analysis and qRT-PCR were performed to determine the change of miRNA expression in hippocampus. Then 3 bioinformatics databases were used to analyze the relative target genes of these miRNA, which were further confirmed by qRT-PCR and Western blot. In the present study, lead exposure resulted in the changed expression of 7 miRNAs: miR-204, miR-211, miR-448, miR-449a, miR-34b, and miR-34c were greatly up-regulated while miR-494 was greatly down-regulated. Bioinformatics analysis results showed that the target genes of 6 up-regulated miRNAs were related to neural injury and neurodegeration, axon and synapse function, neural development and regeneration. Correspondingly, the expression levels of mature mRNAs and proteins of three target genes (*Bcl-2*, *Itpr1*, and *Map2k1*) were greatly repressed, verifying the results of bioinformatics analysis. Taken together, our results showed that the expression of several miRNAs reported to be associated with neurophysiological pathways and neurodegenerative diseases changed in rat hippocampus following chronic lead exposure. These miRNAs may play important roles in lead-induced neurotoxicity.

1. Introduction

Lead is an important neurotoxicant. In the past two decades, lead has been legally restricted to be used as an additive to gasoline and paint, and both the number and severity of human lead poisoning cases have declined (Tong et al., 2000). However, as a pervasive heavy metal contaminant in the environment, lead continues to threaten human health, particularly to the development of nervous system (de la Serna et al., 1989; Qian and Tiffany-Castiglioni, 2003). The developmental central nervous system is the most susceptible target to lead (Hirsch et al., 2012). Lead

Abbreviations: 3′-UTR3, ′-untranslated region; Al, aluminum; DEPC, diethyl pyrocarbonate; ECL, enhanced chemiluminescence; KEGG, Kyoto encyclopedia of genes and genomes; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; miRNA, microRNA; NF-κB, nuclear factor-κB; qRT-PCR, quantitative reverse transcription-polymerase chain reaction; SD rat, Sprague Dawley rat; Itpr1, inositol 1,4,5-trisphosphate receptor type 1; Map2k1, mitogen-activated protein kinase kinase 1.

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exposure has been proved to exert adverse effects on learning and memory, neuronal differentiation, synaptic plasticity, neurogenesis, as well as neuronal regeneration (Anderson et al., 2012; Braun et al., 2012; Guilarte et al., 2012; Pawlas et al., 2012; Rahman et al., 2012). Recently, lead has been found to be related to Schizophrenia (Guilarte et al., 2012). Even the blood lead concentrations lower than 10 μg/dL, the "acceptable level" in many countries, lead to cognitive deficits and neurosensory alterations in children (Finkelstein et al., 1998; Peterson et al., 2010; Rothenberg et al., 2002). However, the mechanisms involved in lead-induced neurotoxicity are still not clear.

miRNAs are a family of endogenously expressed small single stranded regulatory RNAs (~22 nucleotides) that post-transcriptionally regulate gene silencing by inhibiting the translation of their target mRNAs through binding to complementary sequences in the 3′-untranslated region (3′-UTR) of target mRNA transcripts (Bartel, 2004; Lee et al., 1993; Liu et al., 2008; Yi et al., 2003). The number of identified miRNAs reaches close to one thousand in human and mice (Bredy et al., 2011). So far, numerous reports regarding the miRNA–mRNA relationships and the resulted functional regulations have been documented (Dugas and Bartel, 2004; Edbauer et al., 2010; Mellios et al., 2011). It is now predicted that as many as 40–50% of mammalian mRNAs could be regulated by miRNAs (Presutti et al., 2006).

In recent years, miRNAs have been found to contribute to normal brain functions and nervous system diseases. In mammals, several hundred distinct miRNAs have been discovered in the brain (Cao et al., 2006). The miRNAs specifically expressed and enriched in the brain have been confirmed to be implicated in maintaining normal neuronal function and homeostasis, such as memory, neuronal differentiation, synaptic plasticity, and neurogenesis (Bredy et al., 2011; Edbauer et al., 2010; Krichevsky et al., 2003). Differential expression of miRNAs has also been reported in neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, ataxia, Huntington's disease, and schizophrenia (Kaur et al., 2012; Lau and de Strooper, 2010; Tal and Tanguay, 2012). More importantly, recent studies have shown that microRNAs can be involved in neurotoxicities induced by external agents (Kaur et al., 2012). Considering that brain is a key target organ of lead with abundant miRNAs, we hypothesized that lead exposure might resulted in the change of miRNA expression in the brain.

2. Materials and methods

2.1. Animal model

All procedures involving animals were in accordance with the procedures outlined in the "Guide for the Care and Use of Laboratory Animals, Eighth Edition" (http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf), and were approved by the institutional animal care and use

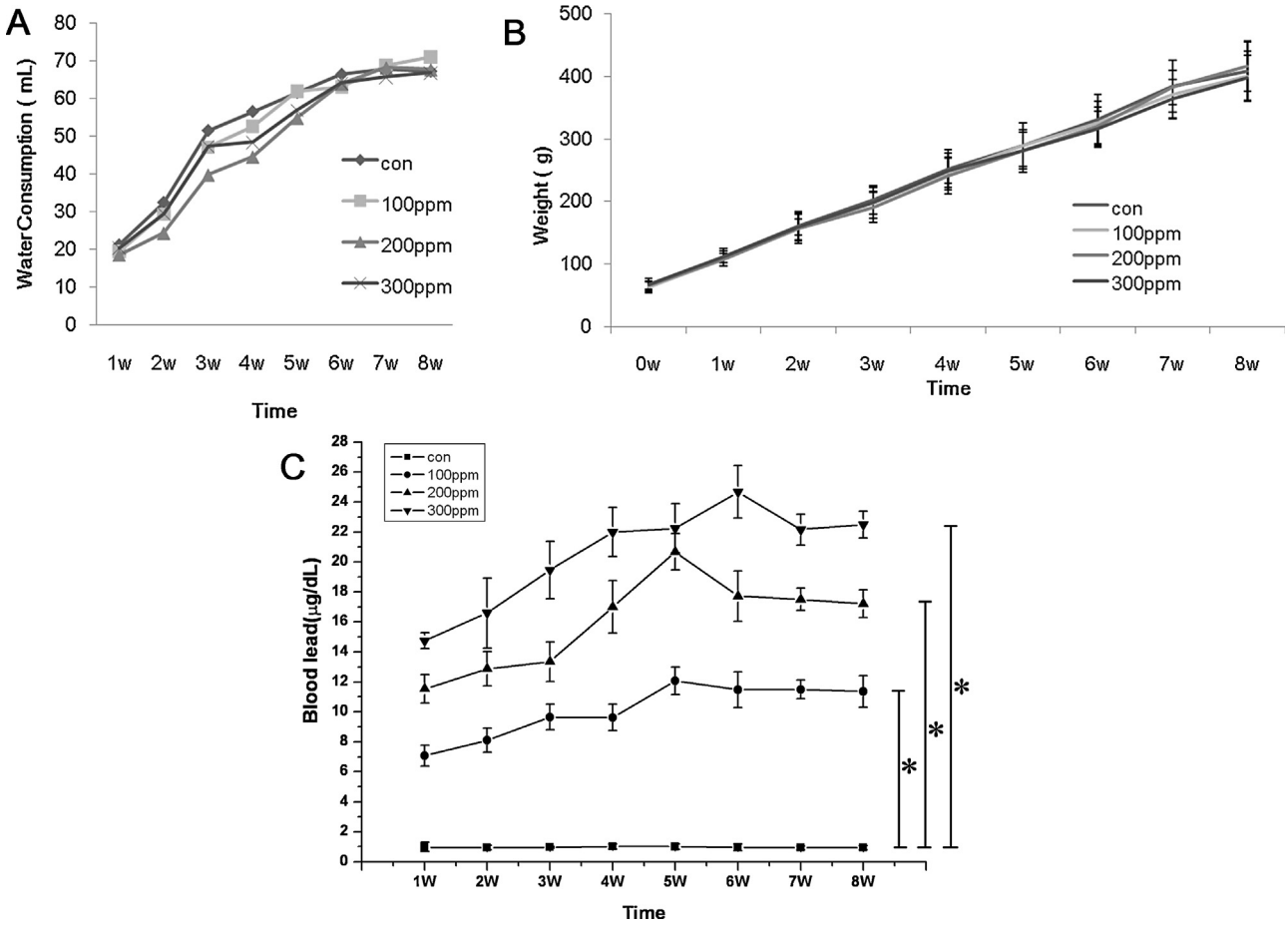

Fig. 1. The establishment of lead-induced chronic rat neurotoxicity model. (A) The water consumption of the rats. (B) The body weights of the rats. (C) The blood lead concentrations of lead-exposed rats from the first week to the end of the research ($n = 10$). Significance (Dunnett's post-hoc comparisons after a repeated measures ANOVA): $^{*}p < 0.01$ vs the control group. Graphs show mean ± SD.