Differential expression of SLC30A10 and RAGE in mouse pups by early life lead exposure

Ning Li (ln8028@163.com)  
Henan Agriculture University

Liuding Wen  
Henan Agriculture University

Yue Shen  
Henan Agriculture University

Tiange Li  
Henan Agriculture University

Tianlin Wang  
Henan Agriculture University

Mingwu Qiao  
Henan Agriculture University

Lianjun Song  
Henan Agriculture University

Xianqing Huang  
Henan Agriculture University

Research Article

Keywords: lead, hippocampus, cerebral cortex, SLC30A10, RAGE, neurotoxicity

Posted Date: October 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2168653/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

It is well known that SLC30A10 and RAGE play a crucial role in regulating the transport and accumulation of Aβ plaques. Our previous studies have shown that early exposure to lead can cause cerebral damage to pups due to the accumulation of Aβ and the deposition of amyloid plaques. However, the effect of lead on the protein expression levels of SLC30A10 and RAGE remains unclear. This study aimed to verify that maternal exposure to lead-containing drinking water during pregnancy would affect the expression of SLC30A10 and RAGE proteins in mice offspring, further verifying the lead-induced neurotoxicity. Four groups of mice were exposed to 0 mM, 0.25 mM, 0.5 mM, and 1 mM of lead for 42 consecutive days from pregnancy to weaning, and the offspring mice were tested on postnatal day 21. The levels of lead in the blood, hippocampus, and cerebral cortex were examined; the learning and memory abilities of the mice were investigated using the Morris water maze; the expression levels of SLC30A10 and RAGE in the hippocampus and cerebral cortex were examined using Western blotting and immunofluorescence. The results showed that the lead concentration in the brain and blood of the mice increased along with the lead content of the mothers during the lead exposure period \((P < 0.05)\). In the Morris water maze test, the spatial memory of the lead exposure group was lower than that of the control group \((P < 0.05)\). Both Immunofluorescence and Western blot analysis showed that the hippocampal and cerebral cortex of the offspring were proportionally affected by differential levels of lead exposure. The expression levels of SLC30A10 were negatively correlated with lead doses \((P < 0.05)\). Surprisingly, under the same conditions, the expression of RAGE in the hippocampus and cortex of offspring was positively correlated with lead doses \((P < 0.05)\). SLC30A10 may play a differential role in aggravated Aβ accumulation and transportation compared with RAGE. A difference in RAGE and SLC30A10 expression in the brain could contribute to lead-induced neurotoxicity.

1. Introduction

Lead (Pb), a ubiquitous heavy metal poison, is widely present in some petroleum products[1]. Lead can accumulate in many organs and tissues of the human body, especially in the nervous system, and is more toxic to the developing nervous system in mammals[2]. Lead can cross the blood-brain barrier (BBB) into the brain and cause neurotoxicity[3]. Some studies have reported that lead exposure causes neural damage, which then leads to decreased learning memory in mice[4, 5]. The most characteristic feature of Alzheimer’s disease (AD) is amyloid beta protein (Aβ) deposition in the brain. It has been suggested that lead exposure upregulates the expression of amyloid precursor proteins (APP), β-secretase (BACE1) and γ-secretase (PS1), which in turn leads to the aggregation of Aβ[6]. Our previous studies have shown that early exposure to lead can cause Aβ to accumulate in the hippocampus of mice[7].

Zinc is regulated through the zinc transporter proteins (ZnTs), of which SCL30A10 is predicted to be the zinc transporter. SLC30A10 is derived from 10 solute carrier family 30 (SLC30) transporter proteins[8]. SLC30A10 has been reported to be associated with the aggregation of Aβ[9]. It was shown that SLC30A10 regulates APP transcription through the regulation of NF-kB and PS1[10, 11]. In addition, SLC30A10 reduces the accumulation of Aβ in the brain by inhibiting the expression of α-secretase[12]. Thus, dysregulation of SLC30A10 in brain tissue may lead to AD. It has been shown that the expression of SLC30A10 is downregulated in the hippocampus of AD patients[13]. It has been shown that the expression of SLC30A10 is
downregulated in the hippocampus of AD patients. It has also been shown that SLC30A10 expression is significantly reduced in the frontal cortex of APP/PS1 mice[9].

Receptors for advanced glycation end products (RAGE) belong to the immunoglobulin superfamily[14]. Abnormal expression of RAGE can lead to certain chronic diseases, such as diabetes, inflammation, and AD[15]. In the pathological development of AD, RAGE acts as a cell surface receptor to bind Aβ to the neurons, blood-brain barrier (BBB), and microglia[16]. In cerebral endothelial cells, RAGE guides Aβ to penetrate the BBB of the cerebrum[17]. RAGE also mediates the influx of circulating Aβ through the BBB[17]. In neurons, RAGE mediates Aβ intraneuronal transport, Aβ-induced oxidative stress and causes mitochondrial dysfunction[14, 18]. The up-regulated expression of RAGE in neurons leads to decreased learning and memory and disturbances in neuronal circuits related to Aβ assembly in APP transgenic mice[19]. In microglia, RAGE enhances Aβ-mediated inflammation[20]. Interestingly, in an Aβ-rich environment, the expression of RAGE is significantly increased in the endothelium and neurons. It can also amplify Aβ-induced adverse effects such as cerebrum and BBB in the cerebrum.

Since both SLC30A10 and RAGE regulate the transport and accumulation of Aβ, it is valuable to study the effect of maternal lead exposure on the expression of SLC30A10 and RAGE in the offspring. This study aimed to characterize the expression of SLC30A10 and RAGE in the brains of offspring when pregnant rats were drink lead-containing water and to understand the potential mechanisms of lead-induced neurotoxicity.

2. Materials And Method

2.1 Animal grouping and handling

Forty pregnant mice (C57BL/6) were purchased from Henan Experimental Animal Center. Upon arrival, the mice were kept in a room with a temperature control of 21 ± 2°C and natural light/dark cycle. Before the experiment, the mice were adapted to the animal center of Zhengzhou University for one week. This study was approved by the Animal Use and Care Ethics Committee of Zhengzhou University, and the relevant standards of animal use laws and regulations were strictly throughout the experiment.

The pregnant mice were randomly divided into four groups of ten each. Three groups of Mice were exposed to lead for 42 consecutive days from pregnancy until weaning and were exposed to either 0.25 mM, 0.5 mM, and 1 mM (low, medium, and high doses) lead acetate, respectively. The remaining group was given lead-free distilled water as a control group. After birth, 12 mice from each group were randomly selected for subsequent experiments.

2.2 Determination of lead levels in blood and brain samples

This study aimed to investigate the mechanism of cerebral damage in the offspring of mice exposed to lead during pregnancy, blood samples were collected from the tail on the 21st day after birth in clean grade PND21 rat pups. The mouse pups were anesthetized with sodium pentobarbital (35mg/kg, i.p.). Hippocampal and cerebral cortical tissues were collected accordingly[21]. Whole blood (100 µl) with 0.5 N ultrapure nitric acid (3.9 ml) (Luoyang Chemical Agent Factory, China) was vortexed for 10 seconds and placed in a centrifuge (7500 r/min) for 10 min at 37°C. The supernatant was used to analyze the lead
concentration. Brain tissues, such as the hippocampus and cerebral cortex, were digested in a digestion solution consisting of 0.5 N perchloric acid, 0.5 N nitric acid, and 0.01% Triton X-100 (Tianjin Chemical Agent Factory, China) with a 1:10 (w/v) tissue/digestion solution ratio.

The method for determining the lead concentration in blood and cerebrum tissue can be found in the literature\[22, 23\]. When necessary, the samples were further diluted with 1.0% (v/v) HNO3 to a concentration range of 0–20 µg/L for reading Hitachi Flameless Graphite Furnace AAS with a wavelength setting of 283.3nm (HITACHI, Japan) was used for scanning the lead concentrations of the samples.

2.3 Water maze detection

The Morris water maze test evaluated the learning and memory of the offspring of 12 PND21 mice exposed to lead during the perinatal period\[24, 25\]. The water maze was a white fiberglass pool with a 1.5 m. The water temperature was kept at around 22°C. The water was made opaque using a non-toxic opaque Funstuff © liquid tempera black paint. Designate four points on the pool’s edge and divide them evenly into four quadrants. The escape platform was a 15 cm² Plexiglas square. First, place it 1 cm below the horizontal and in the center of the first quadrant. The platform was kept in the same position during the learning phase. It was removed during the probe phase.

After the experiment began, mice were tested four times a day for five days. The mice were placed in the water facing the wall, and if they could find the platform within 60 seconds, they were placed on it for 5 seconds. If they could not find the platform, they were guided to the platform and left there for 20 seconds. Moreover, record the time each group of mice spent to reach the platform for these 5 days. The probe test was started on day six by placing the mice in the water facing the wall and recording the number of times the mice crossed the platform position and the time spent in the target quadrant within 60 seconds.

2.4 Immunofluorescence

Immunofluorescence is often used to detect the expression of target proteins, which has been reported in several studies\[26–28\]. The study was performed using immunofluorescence of SLC30A10 and RAGE in mice induced on PMD21 with or without maternal exposure to lead\[29, 30\]. Specifically, three mice samples from each group were randomly selected for IHC. Mice were anesthetized and brain tissue was carefully removed and kept overnight in 4% paraformaldehyde in phosphate buffer (PBS; pH 7.4) at 4°C, followed by immersion in increasing concentrations of sucrose (15% and 30%). Different regions of the brain were subsequently sectioned and collected for storage at -20°C.

Next, brain sections were blocked with PBS containing 5% goat serum for 1 hour at 37°C. Subsequently, brain sections were incubated with SLC30A10 antibody (1:300, Abcam) and RAGE antibody (1:400, Abcam) separately overnight at 4°C. The next day the sections were washed with PBS and incubated with Alexa Flour 488/594 secondary antibody (1:200, Abcam) for 1 hour, followed by staining with DAPI for 5 minutes, finally placed under an inverted fluorescence microscope for visualization.

2.5 Western blotting analysis
Using the tissue lysis buffer (2% SDS, 10% glycerol, 2% 2-mercaptoethanol and 0.002% bromphenol blue), the brain samples at PND21 were homogenized on the ice at a speed of 1000rpm.[31, 32].

After heating at 95°C for about 10 minutes, all samples were separated with 10% Tris-acetate/SDS/Glycine acrylamide gel. Proteins were transferred to PVDF membranes and blocked with 5% skimmed milk for about two hours at room temperature. After washing 3X with 0.5% Tween −20 in TBS and incubated with rabbit anti-SLC30A10 (1:6, Abcam) and rabbit anti-RAGE (1:700, Abcam) for approximately two hours, the PVDF membranes were placed in the second antibody for incubation and immunoglobulin at 37°C for about one hour. The protein signal was captured using Super Signal West Pico Chemiluminescent Substrate (Pierce Chemical Company) and imaging Detection System (Syngene Gene Company). β-actin was visualized using the same method.

### 2.6 Statistical analysis

All data were expressed as mean ± SEM using SPSS 12.0 (IBM). One-way ANOVA was used to analyze the data using the LSD test, and if the variance was not homogeneous, the data were analyzed using Tamhane T2 test. Differences between treatment means were considered statistically significant at $P<0.05$.

### 3 Results

#### 3.1 Lead levels in the hippocampus, cerebral cortex and blood of the offspring

The blood lead level of the offspring in the three lead exposure groups was significantly higher than that of the control group by 9.3, 18.4 and 41.6 times ($P<0.05$) (Table 1). The lead concentration in the hippocampus of the low, medium, and high dose lead exposure group was 16.0, 29.7, and 33.3 times that of the control, respectively ($P<0.05$). In the cerebral cortex, the lead content of the three different dose groups was 8.8, 14.6, and 18.2 times that of the control, respectively ($P<0.05$). Mice exposed to lead in all groups did not have morbidity or death.

#### 3.2 Maternal drinking lead-containing water impairs learning and memory of offspring

The results of the learning and memory abilities of the offspring mice in different groups are shown in Fig. 1. In the Morris water maze test, learning and memory abilities are evaluated by the number of platform site crossings, escape delay, and the time spent to reach the target area.

In training tests, mice exposed to lead during the perinatal period took longer to find the hidden platform than the control group. The period of escape to uncover the platform in lead exposure groups was longer than that of the control ($P<0.05$) on days 2, 3, 4, and 5. On the second and third days, the lead exposure group showed that the concealment period of the platform was more extended than that of the control group ($P<0.05$). There was no statistical difference between the low concentration group and the control group on the second day of training ($P>0.05$) (Fig. 1a). Probe testing showed that mice in the lead-exposed group had fewer platform-site crossings and spent less time in the reach area compared to the control ($P<0.05$) (Fig. 1b, c).
Among all experimental groups, the mice in the high-dose group had the worst spatial memory ability. The above results indicate that lead impairs the spatial learning ability of mice and causes memory impairment.

### 3.3 Effects of lead exposure on SLC30A10 in the hippocampus and cerebral cortex

The immunofluorescence result of SLC30A10 in the hippocampal CA1 area of the offspring is shown in Fig. 2a-b. The SLC30A10 protein is highly expressed in the brain of the control group. However, the fluorescence intensity in the hippocampal CA1 region of the offspring mice exposed to lead (1 mM) was significantly reduced ($P < 0.05$) (Fig. 2c). As shown in Fig. 2d-e, the qualitative and quantitative analysis of SLC30A10 by Western blot further confirmed that at 0.25 mM, 0.5 mM and 1 mM lead doses, the protein expression of SLC30A10 in the hippocampus decreased by 26.7%, 46.7%, and 73.3%, respectively ($P < 0.05$).

This study used the same experimental method to detect the expression level of SLC30A10 in the cerebral cortex. The results of immunofluorescence showed that the expression of SLC30A10 was significantly reduced in the cerebral cortex of the offspring mice in the lead-exposed group compared to the control group (Fig. 3a-c) ($P < 0.05$). The results of western blotting showed that the expression of SLC30A10 in the cerebral cortex of the offspring mice from different lead exposure groups decreased by 30.8%, 68.3%, and 85.0%, respectively, compared with the control group ($P < 0.05$) (Fig. 3d-e).

### 3.4 Effects of lead exposure on RAGE in the hippocampus and cerebral cortex

This study further confirmed the expression of RAGE in the brains of offspring with perinatal lead exposure. Immunofluorescence results are shown in Fig. 4a-b and 5a-b. The results showed that the RAGE protein was expressed in the CA1 region of the hippocampus and cerebral cortex of the offspring. The results of immunofluorescence showed that RAGE expression was significantly elevated in the hippocampus and cerebral cortex of the offspring mice in the lead-exposed group compared to the control group ($P < 0.05$) (Fig. 4c, 5c). It was noted that the RAGE positive signals overlapped slightly with the nuclear staining in the control group (Fig. 4a, 5a). This overlap was more evident in groups exposed to lead (Fig. 4b, 5b).

The analysis of Western blot analysis illustrates the same results (Fig. 4d, 5d). The expression of RAGE was significantly higher in the hippocampus and cerebral cortex of the offspring mice in the lead exposure group compared to the control group ($P < 0.05$). The expression of RAGE in the hippocampus of the offspring of different lead exposure groups increased 7.8, 1.8, and 2.6-fold, respectively, compared to the control group ($P < 0.05$) (Fig. 4e). The expression of RAGE in the cerebral cortex of the offspring of different lead exposure groups increased 3.5, 4.5, and 10.5-fold, respectively, compared with the control group ($P < 0.05$) (Fig. 5e).

### 3.5 Correlation analysis

As shown in Table 2, correlation analysis shows that the expression of SLC30A10 in blood, the hippocampus, and the cerebral cortex is negatively related to lead content and spatial memory, and the difference was statistically significant ($P < 0.05$). However, the level of RAGE was positively correlated with it ($P < 0.05$).
4 Discussion

In this study, the effects of lead exposure in pregnant mice on the expression of SLC30A10 and RGEA in the cerebral cortex and hippocampus of offspring mice were examined by protein blotting and immunofluorescence. The expression of SLC30A10 in the hippocampus and cerebral cortex was negatively correlated with lead concentration. The expression of RAGE in the hippocampus and cerebral cortex was positively correlated with lead concentration. In addition, it was found in the Morris water maze experiment that the higher the concentration of lead exposure, the poorer the learning memory ability of the offspring mice of pregnant lead-exposed mothers.

During pregnancy, childhood exposure or exposure to lead can cause severe brain damage, including coma, cerebral oedema, lead encephalopathy, and convulsions. Many studies have reported early lead exposure is a potential risk factor for AD progression in older adults[33, 34]. Environmental lead exposure has been shown to increase AD progression in mice by targeting the blood-brain barrier (BBB)[35]. Ashok et al. found that exposure to a mixture of As, Cd, and lead induces the amyloid processing pathway of APP, leading to Aβ aggregation and cognitive impairment in young mice through oxidative stress-dependent neuroinflammation[36]. Other studies have found that juvenile lead exposure causes cognitive dysfunction in adult rats[37]. Lead is a widespread environmental neurotoxin, significantly affecting the developing nervous system, but the detailed mechanism of neurotoxicity is poorly understood. All these findings are at the adult or later life phase stage.

Our previous studies have found that lead exposure in early life exacerbates Aβ accumulation and amyloid plaque deposition, inducing AD damage in mice[38]. Juvenile lead exposure causes cognitive dysfunction in adult rats[39]. Some studies have also found that prenatal lead exposure in mothers can cause neurological damage to the offspring[40]. In the current study, we found interestingly that in the Morris water maze test, female mice exposed to lead during the perinatal period had a decrease in the spatial memory of their offspring. Lead exposure interferes with Aβ transport by decreasing SLC30A10 expression and increasing RAGE expression. The toxic effects of lead worsened with increasing lead levels. In addition, the experimental results showed that the differential expression of SLC30A10 and RAGE was closely associated with learning memory ability. The above scenario suggests that the impaired learning memory ability of the offspring mice may be due to neurotoxicity caused by lead exposure.

SLC30A10 exists in the cerebrum, retina, and liver at the tissue level[11]. SLC30A10 has been significantly reduced in patients with Alzheimer's disease, and damage to SLC30A10 contributes to the development of diseases[9]. The publication has shown that SLC30A10 mRNA expression levels are low in the frontal cortex of female APP/PS1 transgenic mice[9]. Reports have also shown that Aβ can be accumulated in mice exposed to the early lead, resulting in impaired learning and memory[38, 41]. In the neuroblastoma cell line model, SLC30A10 was found in the Golgi body, and its mRNA expression was downregulated in the AD cerebrum. In addition, the location of SLC30A10 suggests two possible mechanisms that may induce AD progression by increasing its expression[9]. First, localization to the Golgi body promotes the formation of Aβ by binding zinc to APP and inhibiting the β-secretase. Secondly, since high levels of Zn-induced transport of SLC30A10 to the plasma membrane may be proposed like SLC30A1, the flow of Zn into the extracellular space may provide Zn ions to initiate Aβ deposition and the formation of senile plaques[42]. In this
experiment, the expression of SLC30A10 in the hippocampus and cerebral cortex of the offspring exposed to maternal lead was decreased, and its expression was negatively correlated with lead content and spatial memory capacity.

RAGE plays a crucial role in Aβ transport by regulating the circulation of Aβ; inhibition of RAGE activity protects against the accumulation of Aβ in the cerebral vasculature[43]. Several methods of clearing Aβ from the cerebrum have been reported, including fluid drainage, mesenchymal microglial phagocytosis, and transport of Aβ across the BBB into the bloodstream, which is regulated by RAGE and the lipoprotein receptor-associated protein 1 (LRP1) in the endothelium[44]. As mentioned in the literature, in the brains of AD mice, RAGE increased Aβ expression by increasing β-secretase and γ-secretase[45]. Inflammation has also been reported to play an essential role in AD’s physiological and pathological processes by promoting the aggregation of Aβ[46]. RAGE is a critical player in the inflammatory response cycle, excising a critical role in various processes, including amyloid change, inflammation, cellular stress, and neuronal damage[47]. It has been reported that the binding of RAGE to ligands leads to the death of dopaminergic neurons and that blocking its progression may help to slow the progression of Parkinson's disease[48, 49]. It has also been illustrated that short peptides bound to RAGE protect primary neuronal cells from beta-amyloid toxicity[50]. In this experiment, the expression level of RAGE in the hippocampus and cerebral cortex of the offspring exposed to lead increased, and its expression was positively correlated with lead content and spatial memory capacity. Thus, RAGE may impair the learning and memory abilities of mice by increasing the aggregation of Aβ.

This study provides strong evidence that perinatal exposure to lead in drinking water markedly the expression of SLC30A10 and RAGE in offspring hippocampus and cerebral cortex. In addition, we found that lead exposure resulted in a downregulation of SLC30A10 expression. However, RAGE expression was upregulated. Our results suggest new potential mechanisms for the neuropathologic process of maternal lead exposure.

Declarations

Authors' contributions


Funding

This research was supported by the Excellent Youth Project of the Natural Science Foundation of Henan Province (202300410193) and Young backbone teacher of Henan Province 2021GGJS035. It was also supported by National Natural Science Foundation of China (NNSFC), by grant NNSFC -31201878, U1204804, U1604183 and 32101966. Thanks to Henan Food Processing and Circulation Safety Control
Availability of data and materials

All data generated or analyzed in the course of this study are included in this article or can be obtained from the corresponding authors.

References


37. Wang, Guan, Rui-Li, et al Lead Exposure Impairs Hippocampus Related Learning and Memory by Altering Synaptic Plasticity and Morphology During Juvenile Period


Tables

**Table 1. Lead levels in blood, the hippocampus and the cerebral cortex of different lead-exposed animals at PND21.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood(μg/dL)</th>
<th>hippocampus(μg/dL)</th>
<th>cerebral cortex(μg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.325±0.391</td>
<td>0.003±0.001</td>
<td>0.005±0.002</td>
</tr>
<tr>
<td>L</td>
<td>13.658±1.268*</td>
<td>0.051±0.008*</td>
<td>0.049±0.006*</td>
</tr>
<tr>
<td>M</td>
<td>25.687±2.397*</td>
<td>0.089±0.009*</td>
<td>0.078±0.008*</td>
</tr>
<tr>
<td>H</td>
<td>56.394±5.981*</td>
<td>0.103±0.012*</td>
<td>0.096±0.009*</td>
</tr>
</tbody>
</table>

* indicates statistical significance compared to control group.
Note *: \( P<0.05 \). Data are Mean ± SEM.

**Table 2.** The correlation analysis among the expression of SLC30A10 and RAGE protein, lead level and learning and memory index

<table>
<thead>
<tr>
<th></th>
<th>Lead level in blood</th>
<th>Lead level in hippocampus</th>
<th>Lead level in cerebral cortex</th>
<th>Escape latency(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC30A10</td>
<td>-0.837 0.001</td>
<td>-0.796 0.001</td>
<td>-0.753 0.001</td>
<td>-0.830 0.001</td>
</tr>
<tr>
<td>RAGE</td>
<td>0.926 0.000</td>
<td>0.839 0.002</td>
<td>0.814 0.002</td>
<td>0.861 0.001</td>
</tr>
</tbody>
</table>

Note: \( r \) coefficient of association

**Figures**

![Graph](image)

**Figure 1**
The Water Maze task of offspring in different group. (a) The time taken by the offspring mice to find the platform from day one to day five. (b) Number of platform positions crossed by offspring mice in the probe test. (c) Time spent in the target quadrant by the offspring mice in the probe test (%). Note: * $P<0.05$ when compared with the control group. Data are Mean ± SEM. C, L, M, H refers to control, 0.25 mM, 0.5 mM, and 1 mM lead-exposure, respectively.

**Figure 2**

Immunofluorescence result of SLC30A10 in the hippocampal CA1 region of the offspring following perinatal exposure to lead in drinking water from gestation to PND21 (a-c). Tissues were stained with a mouse anti-SLC30A10 antibody (red) and nuclei were stained with DAPI (blue). (a-b): A representative section from the control group and high dose group. (c): The Quantitative analysis of the fluorescent signals. Western blot analyses of SLC30A10 protein expression in offspring hippocampus following perinatal lead exposure (d-e). (d): A typical gel image using mouse anti-SLC30A10 antibody and with β-actin as a loading control. Labels of C, L, M, and H refer to the control, low dose (0.25 mM), mid dose (0.5 mM), and high dose (1 mM) groups, respectively. (e): Quantitation of SLC30A10 protein expression. Values represent the ratio of SLC30A10/β-actin intensity, *: $P<0.05$. Data are Mean ± SEM.
Figure 3

Immunofluorescence result of SLC30A10 in offspring cerebral cortex following perinatal exposure to lead in drinking water from gestation to PND21 (a-c). Tissues were stained with a mouse anti-SLC30A10 antibody (red) and nuclei were stained with DAPI (blue). (a-b): A representative section from the control group and high dose group. (c): The Quantitative analysis of the fluorescent signals. Western blot analyses of SLC30A10 protein expression in offspring cerebral cortex following perinatal lead exposure (d-e). (d): A typical gel image using mouse anti-SLC30A10 antibody and with β-actin as a loading control. Labels of C, L, M, and H refer to the control, low dose (0.25 mM), mid dose (0.5 mM), and high dose (1 mM) groups, respectively. (e): Quantitation of SLC30A10 protein expression. Values represent the ratio of SLC30A10/β-actin intensity, *: \( P<0.05 \). Data are Mean ± SEM.
Figure 4

Immunofluorescence result of RAGE in the hippocampal CA1 region of the offspring following perinatal exposure to lead in drinking water from gestation to PND21 (a-c). Tissues were stained with a mouse anti-RAGE antibody (green) and nuclei were stained with DAPI (blue). (a-b): A representative section from the control group and high dose group. (c): The Quantitative analysis of the fluorescent signals. Western blot analyses of RAGE protein expression in offspring hippocampus following perinatal lead exposure (d-e). (d): A typical gel image using mouse anti-RAGE antibody and with β-actin as a loading control. Labels of C, L, M, and H refer to the control, low dose (0.25 mM), mid dose (0.5 mM), and high dose (1 mM) groups, respectively. (e): Quantitation of SLC30A10 protein expression. Values represent the ratio of RAGE/β-actin intensity, *: P<0.05. Data are Mean ± SEM.
Figure 5

Immunofluorescence result of RAGE in offspring cerebral cortex following perinatal exposure to lead in drinking water from gestation to PND21 (a-c). Tissues were stained with a mouse anti- RAGE antibody (green) and nuclei were stained with DAPI (blue). (a-b): A representative section from the control group and high dose group. (c): The Quantitative analysis of the fluorescent signals. Western blot analyses of RAGE protein expression in offspring cerebral cortex following perinatal lead exposure (d-e). (d): A typical gel image using mouse anti- RAGE antibody and with β-actin as a loading control. Labels of C, L, M, and H refer to the control, low dose (0.25 mM), mid dose (0.5 mM), and high dose (1 mM) groups, respectively. (e): Quantitation of SLC30A10 protein expression. Values represent the ratio of RAGE/β-actin intensity, *: P<0.05. Data are Mean ± SEM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SLC30A10.tif