Chemosphere 194 (2018) 628–633

Contents lists available at ScienceDirect

Chemosphere

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

Effects of fluoride on synapse morphology and myelin damage in mouse hippocampus

Ruiyan Niu [1], Huijuan Chen [1], Ram Kumar Manthari, Zilong Sun, Jinming Wang, Jianhai Zhang, Jundong Wang*

Shanxi Key Laboratory of Ecological Animal Science and Environmental Veterinary Medicine, Shanxi Agricultural University, Taigu, Shanxi 030801, China

HIGHLIGHTS

- Fluoride induced myelin damage in mouse hippocampus.
- Fluoride shortened the synaptic cleft and thickened the postsynaptic density.
- Fluoride altered the expressions of CREB, BDNF, and NCAM in hippocampus.

ARTICLE INFO

Article history:
Available online 6 December 2017

Handling Editor: A. Gies

Keywords:
Fluoride
Hippocampus
Synapse
Myelin

ABSTRACT

To investigate the fluoride-induced neurotoxicity on mice hippocampus, healthy adult mice were exposed to 25, 50, and 100 mg NaF/L for 60 days. The results showed that medium and high fluoride administration induced ultrastructural alterations in the structure of neuron synapse, including indistinct and short synaptic cleft, and thickened postsynaptic density (PSD). The significant reduced mRNA expressions of proteolipid protein (PLP) in medium and high fluoride groups suggested that myelin damage occurred in hippocampus. The myelin damage in turn was determined by the increased myelin-associated glycoprotein (MAG) level, which is naturally released by injured myelin, in high fluoride group, compared to the medium fluoride group. In addition, high fluoride exposure also reduced the mRNA and protein levels of cAMP response element-binding protein (CREB), brain-derived neurotrophic factor (BDNF), and neural cell adhesion molecule (NCAM). These findings suggested that the alteration in synaptic structure and myelin damage may partly be due to adverse effects of fluoride on the neurotrophy and neuron adhesion in mice hippocampus.

1. Introduction

Long-term fluoride exposure results in its accumulation in different tissues and increases the risk of fluorosis. Besides the geographical and industrial sources of fluoride exposure, there are many other daily fluoride consumptions such as fluoride-containing toothpaste, and mouthwash (Niu et al., 2016). It was reported that only half amount of fluoride consumed each day can be excreted out of the body through urine, sweat, or feces (Mahaboob Basha and Saumya, 2013). Epidemiological investigations from China, India, Iran, and Mexico demonstrated that children residing in the fluoride endemic areas have shown reduced mental ability and lower IQ (Wang et al., 2007; Ding et al., 2011; Poureslami et al., 2011; Trivedi et al., 2012; Bashash et al., 2017). Animal experiments conducted by Mullenix et al. (1995) and Whitford et al. (2009) showed that fluoride deposits in various brain sections, which further confirmed by Basha and Madhusudhan (2010) who reported that five folds of fluoride accumulated in cortex, cerebellum, hippocampus, medulla oblongata in rat brain. The deposition of fluoride in brain could contribute to the deficits in learning and memory. Therefore, the toxic effects of fluoride on central nervous system attract more attention.

The hippocampus is considered to be the primary section in the brain that participates in the process of learning and memory (Norman, 2010). The special structures of hippocampal subfields, synaptic plasticity, and the transmission of nerve signals within the

* Corresponding author.
E-mail address: wangjd53@outlook.com (J. Wang).
[1] Ruiyan Niu and Huijuan Chen contributed equally to this work.

https://doi.org/10.1016/j.chemosphere.2017.12.027
0045-6535/

basic circuit of physiological are the physiological basis of the cognition formation. Earlier works on fluoride neurotoxicity revealed that high fluoride caused alterations in the width of synaptic cleft and postsynaptic density (PSD) thickness (Qian et al., 2013), a significant decrease in the synapses number (Bhatnagar et al., 2002), and pathological changes in the synaptic ultrastructure in hippocampus (Qian et al., 2013), all of which weaken synaptic transmission efficiency. In neuron, brain-derived neurotrophic factor (BDNF) participates in at least the regulation of synaptic plasticity and neurogenesis promoting, while the expression of BDNF is regulated by cAMP response element-binding protein (CREB) (Pruunsild et al., 2011). Neurogenesis is a complex process, which is stimulated by stem cell factor (SCF) (Jin et al., 2002), regulated by neural cell adhesion molecule (NCAM) (Seidenfaden et al., 2006), and recruits the myelination required for the axon elongation (McKerracher and Rosen, 2015). However, the molecular mechanism underlying fluoride induced lesion in synapse and myelin remains unclear. Hence, in the current study, the effects of fluoride on the expressions of neurogenesis related factors (CREB, BDNF, NCAM, SCF) and molecular related to myelin structure in mice hippocampus were also detected to further provide the evidence for fluoride neurotoxicology.

2. Materials and methods

2.1. Establishment of animal model

48 healthy Kunming mice, weighting about 20–25 g, were obtained from the Experimental Animal Center of Shanxi Medical University. After seven days acclimatization to the new environment, mice were randomly allotted to four groups. Animals in control group received distilled water. The other three groups were treated with 25, 50, and 100 mg NaF/L, respectively. During the experimental period, animals have free access to the standard diets and water. The body weight was recorded each week, and compared to the control group, no significant difference was observed in fluoride groups. After 60 days exposure, all mice from each group were sacrificed by cervical dislocation. Hippocampus was removed quickly, then frozen in liquid nitrogen, and stored at −80 °C for total RNA extraction and western blotting analysis. Two samples from each group were fixed in 2.5% glutaraldehyde solution for the transmission electron microscope (TEM) analysis.

2.2. Morphological observation of hippocampus tissue

After 2 h fixation at room temperature (RT), samples were washed with phosphate buffer, and then post-fixed in osmium tetroxide for 2 h at RT, followed by 10 min pre-staining in acetate-barbitone. After dehydration through graded ethanol series, the samples were embedded in Spurr's resin. Sections were prepared and stained with uranyl acetate and lead citrate. Finally, the ultralstructure of neurons in hippocampus was observed in JEM-1400 (JEOL Ltd., Tokyo, Japan) TEM.

2.3. Real-time PCR analysis

Total RNA of hippocampus was isolated by using TRIZOL reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The A260/280 ratio was in the range of 1.8–2.0 using NanoDrop2000 (Thermo Fisher, USA). QRT-PCR was performed on the Stratagene Mx3000P™ qPCR system (Stratagene, La Jolla, CA, USA) using SYBR RT-PCR kit (TaKaRa Bio, Hilden, China). The primer sequences were designed by using Primer 3.0 plus, based on available mice sequence in Genebank for CREB, BDNF, NCAM, SCF, MOG (myelin oligodendrocyte glycoprotein), MAG (myelin-associated glycoprotein), MBP (myelin basic protein), PLP (proteolipid protein), and β-actin (Table 1). Thermal cycling conditions were as follows: 95 °C for 5 min and 40 cycles of 95 °C for 10 s, 60 °C for 30 s, and 72 °C for 6 s. Finally, the melting curve analysis was performed at 95 °C for 1 min, at 55 °C for 30 s, and at 95 °C for 30 s as in the protocol for the three reaction steps.

Table 1
List of quantitative PCR primers.

Gene	GenBank No.	Sequence
β-actin	NM_007393	Forward: gctcttttccagccttcctt Reverse: gatgtcaacgtcacactt
BDNF	NM_001285416	Forward: gcgcccatgaaagaagtaaa Reverse: tcgtcagacctctcgaacct
NCAM	Y00051	Forward: gggaactccatcaaggtgaa Reverse: ttgagcatgacgtggacact
SCF	NM_013598.2	Forward: ccttatgaagaagacacaaacttgg Reverse: ccatcccggcgacatagttgaggg
CREB	MMU46027	Forward: ccagttgcaaacatcagtgg Reverse: ttgtgggcatgaagcagtag
MOG	NM_010814.2	Forward: aaaacaccctgtggtgaagg Reverse: atcctggttggcagaatcac
MAG	NM_010758.2	Forward: gttcctcagctcctcattgc Reverse: ttggggatgtctcctgattc
MBP	NM_001025251.2	Forward: atccaagtacctggccacag Reverse: cctgtcaccgctaaagaagc
PLP	NM_011123.2	Forward: caggctcctgctagaaatgg Reverse: ggtcttcaggagatgcttgc

2.4. Western blotting analysis

The hippocampus was homogenized in a certain volume of RIPA lysis buffer supplemented with 1 mM PMSF (Sigma Chemical, St. Louis, MO, USA) for 30min on the ice. Then the homogenate was centrifuged at 12,000 × g for 10 min at 4 °C and then the supernatantas were harvested. With bovine serum albumin as the standard, the protein concentration of each sample was determined using full form BCA protein assay kit (Beyotime Institute of Biotechnology, Ltd., Shanghai, China). Samples then performed protein electrophoresis analysis by using 10% SDS-PAGE. Following the electrophoresis, the proteins were then transferred to a nitrocellulose (NC) membrane for 60 min at 35 V. Blots were blocked with 5% non-fat dried milk in Tris-buffered saline with 0.05% Tween 20 (TBST) for 2 h at RT. The membrane was then incubated with β-actin (1:1000, Beyotime Institute of Biotechnology, Ltd., Shanghai, China), CREB (1: 1000, proteintech, Ltd, wuhan, china), BDNF (1:100, Santa Cruz Biotechnology, inc., USA), NCAM (1:500, Sangon Biotech, Ltd, shanghai, china), SCF (1:100, Biosynthesis Biotechnology Co., Ltd., Beijing, China) in TBST buffer overnight at 4 °C. After washing 3 times with PBST for 10 min each, the membrane was incubated in horseradish peroxidase (HRP)-linked anti-Rabbit IgG (Biosynthesis Biotechnology Co., Ltd., Beijing, China) at a dilution of 1:4000 or HRP-linked anti-mouse IgG (Beyotime Institute of Biotechnology, Ltd., Shanghai, China) at a dilution of 1:1000 in TBST buffer at RT for 2 h. After 3 times washing with PBST buffer, the blots were shown through the electrochemical luminescence (ECL) methods. FluorChem Q gels image analysis system was used to analyze densitometry of the protein bands.

2.5. Statistical analysis

Each data was expressed as the mean ± SEM. By using GraphPad Prism 5 software (GraphPad Software, Inc., San Diego, CA), data were analyzed using the one-way analysis of variance (ANOVA) test. Values of $P < 0.05$ were considered as statistically significant.