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MiR-132, miR-204 and BDNF-TrkB signaling pathway may be involved in spatial learning and memory impairment of the offspring rats caused by fluorine and aluminum exposure during the embryonic stage and into adulthood

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ABSTRACT

Fluorine and aluminium are nervous system poisons, but it remains unclear whether combined fluorine and aluminium exposure damages spatial learning and memory and, if so, by what mechanism. This study showed that exposure to fluorine and aluminium, either alone or combined, during the embryonic stage and into adulthood caused spatial learning and memory impairment in offspring rats; its mechanism may be associated with increases in miR-132 and miR-204 expression and downregulation of the BDNF-TrkB pathway in the hippocampus. The effects of F were obvious, but the effects of Al were slight. There were antagonistic effects between F and Al, with Al reducing the toxicity of F.

1. Introduction

Excess fluorine (F) exposure leads to fluorosis, which is a worldwide endemia and manifests dental and skeletal fluorosis and other symptoms; the latter category includes symptoms related to central nervous system damage, such as a decline in learning and memory ability (Asawa et al., 2015; Bhagavatula et al., 2016; Kuang et al., 2016; Malin and Till, 2015; Shalini and Sharma, 2015). Aluminium (Al), a ubiquitous substance encountered both in nature (as the third most abundant element) and in human use (including manufacturing, water treatment, food processing, pharmaceuticals and vaccines, etc.), can have biological effects such as neurotoxicity, cognitive dysfunction and impaired learning and memory (Nam et al., 2016; Pan et al., 2015a,b; Wang et al., 2014a,b). Fluorine and aluminium are able to cross the blood-brain barrier and the placental barrier and accumulate in the brain (Dec et al., 2017; Fu et al., 2016; Ghorbel et al., 2016). Less has been reported about the joint effects of fluorine and aluminium on health. Furthermore, some results on the subject have been contradictory (Akinrinade et al., 2015; Dong et al., 2016; Kaur et al., 2009; Wang et al., 2014a,b). Therefore, further research is needed to explore the toxic effects of fluorine combined aluminium. Furthermore, the effect of F and Al exposure on the offspring rats' learning and memory impairment and the mechanism of this effect are complex and still not well understood.

The centres for learning, memory, and neurogenesis are located primarily in the hippocampus. Synapses are the structural foundation for the transmission of information within the nervous system, and synaptic plasticity of neurons is the basis of learning and memory. Neurodevelopment disorders are related to behavior and cognitive function deficits (Gilbert and Man, 2017; Herrera et al., 2018). Early neurobehavioral reflex can reflect the developmental maturity of nervous system.

MicroRNAs are a group of endogenous non-coding RNAs that are important in controlling the developmental processes of different cells through negative regulation of protein-coding gene expression by binding non-translated regions of mRNAs and suppressing translation and/or increasing mRNA degradation; necessary pathways for learning and memory and the effects of epigenetics on normal and pathological brain cognition controlled by them (Saab and Mansuy, 2014). In the nervous system, miR-132 is a neuron-specific microRNA, has functions such as memory-promoting, decisive in synaptogenesis, synaptic plasticity and structural remodelling, and so on (Hansen et al., 2016; Hernandez-Rapp et al., 2017; Wang et al., 2013; Zhang et al., 2017). Hippocampal increasing expression of miR-204 is related to age-

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associated decline in synaptic plasticity and cognitive functions (Mohammed et al., 2016).

Brain-derived neurotrophic factor (BDNF) is an import neurotrophin, its receptor is tyrosine receptor kinase B (TrkB). BDNF-TrkB signal transduction pathway modulates synapse stability, GABAergic signalling, dendritogenesis, and neurogenesis.The improved and damaged learning and memory ability were respectively linked to up-regulation and down-regulation BDNF/TrkB signalling (Yang and Gao, 2017). However, it is unclear whether miR-132, miR-204 and BDNF/TrkB signalling pathway play a role in the effect of F + Al exposure on impaired learning and memory of offspring rats.

In this study, a rat model exposed to F + Al during the embryonic stage and into adulthood was used to determine whether F + Al can lead to impairment of spatial learning and memory. The mechanism was also explored by assessing the effects of F, Al, and F + Al exposure on miR-132 and miR-204 mRNA expression and BDNF and TrkB mRNA and protein expression in the hippocampus.

2. Material and methods

2.1. Chemicals

Sodium fluoride (NaF, > 99% purity) and aluminium trichloride ($AlCl_3$, > 99% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA). The two chemicals were dissolved in drinking water, and the solutions were freshly prepared each day. The standard stock solutions [GBW(E)080,549 F, Al (GBW(E) 080,219)] were provided by the National Center for Standard Reference Materials (Beijing, China).

2.2. Animal treatment

Sprague-Dawley rats were obtained from the Center of Laboratory Animals of Guizhou Medical University (Guiyang, Guizhou, China). The certificate number of the facility was SYXK (Qian) 2012-0001, and the qualitative qualification number was SCXK (Qian) 2012-0001. All animal treatment procedures were approved by the Ethical Committee of Guizhou Medical University (serial number: 1,503,007). The pregnant rats were approximately 250 g in weight and singly housed under controlled temperature (20 ± 5 °C) and lighting (12 h light/12 h darkness) conditions and a relative humidity of 60 ± 2% with food and water *ad libitum*.

The day that a vaginal plug was observed was defined as day 0 of gestation. The 16 pregnant rats were randomly divided into four groups. According to preliminary experimental results, the pregnant rats were exposed to 120 mg/l (1/10 LD_{50}) NaF and/or 600 mg/l (1/10 LD_{50}) of $AlCl_3$ in their drinking water during pregnancy and for the first 21 days after giving birth. The experiment included one control group and 3 experimental groups, generated by adding NaF and $AlCl_3$ to the tap water at the following concentrations: (0, 0), (120, 0), (0, 600), and (120, 600) mg/L, respectively. The groups were designated A, B, C, and D, respectively. From postnatal day (PND) 22 to PND90 (sexual maturity), the offspring rats were treated with the same concentrations of F and Al as the maternal rats through the same procedure.

Then, 8 rats (one female and one male selected randomly from each of four litters) from each group were tested in the Morris water maze (MWM), and urine was collected for 24 h before euthanasia. The rats were sacrificed by rapid decapitation after isoflurane anaesthesia. Blood was collected by heart puncture. The brains and hippocampi were quickly dissected on ice; the hippocampi for RNA extraction were promptly collected in Eppendorf tubes with a sample protector for RNA/DNA (TaKaRa Bio Inc., Japan) and stored in a -80°C freezer. Samples of the right side of the brain were collected so that their organizational structure could be observed. The samples were fixed in 10% neutral formalin, then embedded in paraffin and stained with haematoxylin and eosin (HE) to observe changes by conventional pathological examination. The rest of the brains and hippocampi were immediately transferred to liquid nitrogen until analysis.

2.3. Detection of fluorine and aluminium content

The content of fluorine in urine, serum and brain was tested by a fluorine ion-selective electrode method (PF-1-01 digital ion meter), which is the national health industry standard method in China. The content of aluminium in urine, serum and brain tissue were tested with a ContrAA 700 continuum source atomic absorption spectrometer (Analytical Instruments Co., Ltd of Jena, Germany).

2.4. Observation of physiological development and neurobehavioural manifestations

After birth, the offspring rats were recorded at the same time every 2 weeks. Starting on PND9, the number of offspring that had begun teething was observed every day until all the pups had reached that standard; the age, in days, when the first front tooth erupted through the gums was recorded as the age when the pup reached the standard. Starting on PND10, eye opening was observed every day until all the pups had reached that standard; the age, in days, when fissures were present on both eyes was recorded as the age when the pup reached the standard.

The righting reflex and the cliff avoidance reflex were observed from PND4 until all the rats tested positive for both. The pup was placed supine on a rough testing surface, held in place for 5 s, and then released; the ability of the rat to turn over and bring all four paws in contact with the surface was observed. If the rat righted itself within 2 s, it was recorded as positive for the righting reflex, and its age in days was recorded. For the next test, the offspring rat was placed on the edge of a 30-cm-high platform; if it retreated or turned away from the edge within 60 s, its cliff avoidance reflex was recorded as positive, and its age was recorded.

2.5. Evaluation of spatial learning and memory in the Morris water maze

Spatial learning and memory ability was assessed using the MWM pool and video analysis software (provided by the Institute of Medicine, Chinese Academy of Medical Sciences) by conducting a hidden platform acquisition test and a probe trial test. The former test examines the ability to learn and memorize the water maze, and the latter measures the ability to retain the memory of the spatial position of the platform. The formal experiment lasted five days (4 days for the navigation test and 1 day for the probe trial test). MWM testing was conducted in a round black pool 150 cm in diameter and 70 cm deep. The pool was filled to a depth of 40 cm with tap water, and the pool temperature was maintained at 23 ± 1°C by addition of warm water. There were four trials per day; in each trial, the offspring rats were released from four points (on the north, west, south, and east sides of the pool). The hidden platform was a round black cylinders 5 cm in diameter and 39 cm tall, placed in the centre of one quadrant of the pool; the platform remained in the same position throughout the navigation stage, then was removed from the pool during the spatial exploration stage. The specific experimental procedures were as described in the literature (Jiang et al., 2014).

2.6. Pathological analysis of brain tissue

After being fixed in 10% neutral formalin for 24 h, the tissue of the left side of the brain was cut into approximately 4 μm thickness. The slices were then dehydrated, clarified, embedded in paraffin, and subjected to conventional HE staining; the stained samples were observed under an optical microscope, and the hippocampus was photographed.