Ecotoxicology and Environmental Safety 244 (2022) 114021

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Review

Effects of chronic fluorosis on the brain

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ARTICLE INFO

Edited by Professor Bing Yan

Keywords:
Environmental toxins
Public health
Drinking water
Fluorosis
Brain damage

ABSTRACT

This article reviews the effects of chronic fluorosis on the brain and possible mechanisms. We used PubMed, Medline and Cochraine databases to collect data on fluorosis, brain injury, and pathogenesis. A large number of in vivo and in vitro studies and epidemiological investigations have found that chronic fluorosis can cause brain damage, resulting in abnormal brain structure and brain function.Chronic fluorosis not only causes a decline in concentration, learning, and memory, but also has mental symptoms such as anxiety, tension, and depression. Several possible mechanisms that have been proposed: the oxidative stress and inflammation theory, neural cell apoptosis theory, neurotransmitter imbalance theory, as well as the doctrine of the interaction of fluorine with other elements. However, the specific mechanism of chronic fluorosis on brain damage is still unclear. Thus, a better understanding of the mechanisms via which chronic fluorosis causes brain damage is of great significance to protect the physical and mental health of people in developing countries, especially those living in the endemic areas of fluorosis. In brief, further investigation concerning the influence of fluoride on the brain should be conducted as the neural damage induced by it may bring about a huge problem in public health, especially considering growing environmental pollution.

1. Endemic fluorosis

1.1. Overview

Endemic fluorosis (EF) is a chronic systemic disease characterized by skeletal fluorosis and dental fluorosis due to long-term excessive intake of fluoride, which affects residents living in fluoride-rich environments. A daily fluoride intake of more than 4 mg can cause chronic fluorosis. The disease is currently prevalent in more than 40 countries to varying degrees and poses a serious public health concern. Among Asian countries and areas, China and India are the two countries that are most widespread and severely struck by EF (Fluorides and oral health, 1994). In China, EF is mainly distributed in 14 provinces and municipalities including Guizhou, Shanxi, Gansu, Shandong, and Jiangsu. There are 275 endemic areas of fluorosis (composed of 2026 affected villages) in Jiangsu Province, and the prevalence of skeletal fluorosis is high in 9 counties (including Feng County, Peixian County, and Tongshan County), with Xuzhou City being the most EF-hit area in Jiangsu Province (Ren et al., 2021). There are three main types of EF in China: drinking water-related EF, coal-burning pollution-related EF, and tea-related EF, among which the drinking water-type is the most common. As stated in China's *Criteria for the Classification of Endemic Fluorosis-affected Areas* (GB 17018–1997), the fluoride level in drinking water should not exceed 1 mg/L. According to the updated version of GB 17018–2011, the drinking water-type EF areas are defined as that with a fluoride level of $>$ 1.2 mg/L in domestic water. In 1994, a World Health

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https://doi.org/10.1016/j.ecoenv.2022.114021
Received 8 June 2022; Received in revised form 18 August 2022; Accepted 23 August 2022
Available online 29 August 2022

Organization expert committee recommended that the concentration of fluoride in drinking water should be 0.5–1.0 mg/L (Fluorides and oral health, 1994). The use of fluorinated water as a caries-preventing agent is considered to be an excellent representative of the active interventions for modern public health. After people fully elucidated the toxicokinetics of fluoride entering the brain, more and more studies found that fluoride has neurotoxicity. With the environmental pollution becoming more and more serious, the fluoride pollution of drinking water has become more and more common all over the world. In the train of the situation, the research on the mechanism of fluoride related nervous system damage and the development of prevention and treatment strategies have also become a hot research topic in many countries (especially developing countries). Since the immense and rapid progress of the effects of fluoride on the brain and its mechanisms, it is necessary to frequently review the literature to achieve an up-to-date overview of the subject. In addition, it is also the most noteworthy point that although the role of fluoride on dental and bone health is well known, its neuro-toxicological and suspected carcinogenic effects are debated (Grandjean, 2019; Anon, 2022). Based on above, we conducted this review.

1.2. Physicochemical properties and biological effects of fluorides

Fluorine is the 9th element in the periodic table and belongs to non-metals. As the most electronegative element, fluorine is extremely active and can form compounds with almost all other elements (including some rare gas elements), e.g., hydrogen fluoride, sodium fluoride, calcium fluoride, and ammonium fluoride, which are widely distributed in nature. Human body exposes to fluorides through the water, air, soil, and food. Drinking water is the most important source of fluorine exposure, followed by air and food. The main sources of fluorine in water are fluorine-containing minerals in rocks and sediments, such as fluorite, cryolite, fluoroapatite and hydroxyapatite, and clay minerals (Yadav et al., 2019). Fluorine is an essential trace element for metabolism and can enter all tissues. While being cumulative in the human body, it is mainly deposited in the teeth and bones. Meanwhile, fluoride in human body has a narrow safety range. A proper amount of fluoride can prevent dental caries and promote dental and skeletal health. However, inadequate or excess fluoride intake may undermine the normal physiological function of human body, causing damage not only to organs of the skeletal system but those of non-skeletal systems (such as the digestive system, endocrine system, nervous system, and reproductive system), resulting in acute or chronic fluorosis. Therefore, reducing fluoride in drinking water to the allowable level recommended by international or local organizations is of great significance for health (Baghal Asghari et al., 2017). Common methods include using alternative water sources, improving nutrition and defluoridating water (Lebrahimi et al., 2020; Ardekani et al., 2020). Among them, the research methods of water defluorination include precipitation/coagulation, reverse osmosis, electroflocculation, nanofiltration adsorption and ion exchange, etc (Yadav et al., 2019). Due to limited space and which go beyond the scope of this note, the detailed introduction of these methods will not be expanded in our review. Please refer to other relevant review for details (Solanki et al., 2022).

2. Impacts of chronic fluorosis on the brain

Articles from PubMed, Medline and Cochraine databases whose main subject included effects of fluoride on the brain and its mechanism to provide an overview of the subject and its current extent. The key words that were used as search terms are the following: “environmental toxins, public health, drinking water, fluorosis, brain damage”.

2.1. Effects of chronic fluorosis on fluoride concentration in brain tissue

In 1995, Mullenix et al. for the first time observed that fluoride concentration in some brain structures (e.g., hippocampus) elevated with the increase of fluoride content in the drinking water of rats and concluded that fluoride could cross the blood-brain barrier (BBB) and enter brain tissues (PJ et al., 1995). Niu et al. also found a 250-fold increase in fluoride levels in the brain of mice exposed to high-concentration fluoride (10 mg/L, 25 mg/L, and 50 mg/L) for 8 months comparing to the control mice (Niu et al., 2015a). Afterwards, numerous studies have shown that fluoride can cross the BBB either in ionic form or by binding to albumin. In addition, fluoride can be excreted through the drainage of cerebrospinal fluid to maintain the relative balance of fluoride concentration in the brain. However, long-term intake of high-concentration fluoride can cause fluoride accumulation in the brain, resulting in structural and functional damage to brain tissue. Animal experiments revealed that the fluoride concentration increased in the hippocampus, cerebral cortex, and midbrain of rats with chronic fluorosis, which was positively correlated with the dose of fluoride intake (CSReddy Nallagouni, 2017). Fluoride can penetrate the placental barrier and the BBB, and thus fluoride exposure during the fetal and neonatal periods is dangerous. Du et al. compared the fluoride levels in the brain tissues of 15 fetuses from an EF area that were aborted therapeutically at 5–8 months of gestational age with those of 16 fetuses at the same gestational age from a non-endemic area, and found that brain fluoride levels in fetuses from fluoridated areas (0.28 ± 0.140 μg/g) were significantly higher than those from non-fluoridated areas (0.19 ± 0.06 μg/g), confirming that fluoride can enter the fetus from maternal blood through the placental barrier during pregnancy and then accumulate in the fetal brain tissue after crossing the BBB and affect neurological development (Du, 1992).

2.2. Effects of chronic fluorosis on brain ultrastructure

The nervous system is sensitive to fluoride, and fluoride can accumulate in the brain after crossing the BBB, causing morphological and structural changes in animal and human brain tissues (Holland, 1979). The hippocampus is closely related to functions such as learning, memory and emotion. Niu et al. studied the ultrastructure of hippocampus in mice with long-term intake of high-fluoride drinking water and found significant pathological changes in the ultrastructure of neurons, synapses and myelin damage (Niu et al., 2018a). In addition, the mitochondria and endoplasmic reticulum (ER) in neurons were damaged, along with significantly increased intracellular lipofuscin. The neuronal synapses showed swelling and edema, and the mitochondria were severely degenerated. The electron density in synaptic gaps was increased, and the presynaptic and postsynaptic membranes were fused. Several studies have demonstrated that the accumulation of fluoride in the central nervous system (CNS) can cause morphological changes in neurons (especially changes in microtubule-associated protein expression and Nissl body concentration) as well as changes in neuronal activity and energy metabolism (Akinrinade et al., 2015; Lee et al., 2016; Niu et al., 2015b; Jiang et al., 2014a). Gui et al. investigated the hippocampal tissues of rats with coal-burning pollution-induced fluorosis and found that neuronal Nissl bodies in the hippocampal CA1 region were significantly reduced, indicating that the function of the neurons in synthesizing proteins was diminished (Gui et al., 2011). Significant neurodegenerative changes were found in the hippocampus, amygdala, cortex, and cerebellum of 10-week-old rats with chronic fluorosis, including a reduction in the size and amount of neurons in these regions, demyelination in the cerebral cortex and subcortical areas, and a decrease in the number of Purkinje cells in cerebellum. The altered cell morphologies included cell membrane folding, chromatin condensation, mitochondrial swelling, rough ER expansion, and a decrease in the number of synapses, seriously affecting synaptic function and plasticity (Bhatnagar et al., 2002).

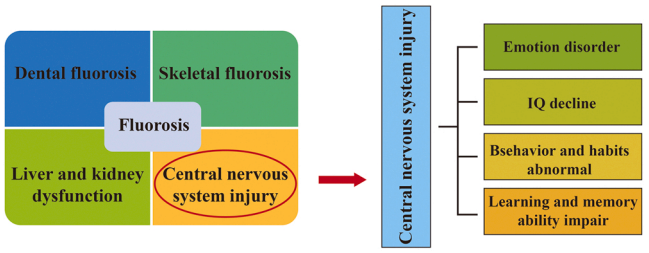

Fig. 1. The damage caused by chronic fluorosis and the specific manifestation of central nervous system injury.

2.3. *Effects of chronic fluorosis on brain function*

Brain tissues such as cerebral cortex, hippocampus, and cerebellum are closely related to learning, memory, emotion, and behavior, and their structural changes or damage can significantly affect the physiological functions of the brain (Fig. 1).

2.3.1. *Impacts of chronic fluorosis on learning, memory, and intelligence quotient (IQ)*

In 1937, Shoutt et al. reported that patients with endemic fluorosis had neurological manifestations such as memory loss, headache, dizziness, tremor, paralysis, and ataxia (Shortt et al., 1937). Since then, an increasing number of studies have focused on the impact of high fluoride level on cognitive function. In 2008, the International Society for Fluoride Research also set its conference theme as "Effects of Fluoride on Bone and Brain". Ekambaram et al. found that fluoride decreased spontaneous activity and rota-rod endurance time in rats, indicating that it decreases self-coordination abilities (Ekambaram, 2001). Mice given high-fluoride drinking water had a lower escape frequency in active avoidance experiments (Chioca et al., 2008). In another study, the effects of fluoride on cognition were investigated using a novel object recognition test (non-spatial cognition) and the Morris water maze (MWM, for spatial learning). It was found that fluoride impaired the retention of non-associative long-term memory rather than habitual persistence. In addition, fluoride exposure resulted in impaired spatial memory in mice (Liu et al., 2014). Wang et al. reported that perinatal fluoride exposure induced learning and memory impairment in offspring mice (Wang et al., 2018). Several experimental studies in rodents have shown that the learning and memory abilities of the high-fluoride-exposure group are significantly lower than those of the control group (Jiang et al., 2014a). Fluoride can also cross the placental barrier and decrease the learning and memory abilities of rats and their offspring.

All these animal experiments indicated that long-term excessive fluoride intake can cause a significant decrease in learning and memory abilities in animals. Similarly, many epidemiological studies supported that chronic high-fluoride exposure in children (e.g., long-term consumption of high-fluoride drinking water) affected children's IQ as well as learning and memory abilities. A meta-analysis involving 7258 children showed that exposure to high-fluoride water was significantly correlated with reduced intelligence levels in children (Duan et al., 2018). A survey on IQ in Indian children aged 10–12 years (Aravind et al., 2016) and a cross-sectional study on the relationship between fluoride and IQ in students aged 6–18 years in India (Das KMondal, 2016) suggested that fluoride concentration of drinking water was negatively correlated with IQ ($r = -0.204$, $r = -0.343$, respectively). In addition, a survey on the intelligence of adults living in endemic areas of chronic industrial fluorosis revealed that fluorotic patients had neurological manifestations including altered olfaction and reduced cognitive function (Calvert et al., 1998). Professor Robert and profess Isaacson presented at the 28th World Conference of the International Society for Fluoride Research that addition of 0.5 mg/L of aluminum fluoride into the drinking water caused Alzheimer's disease (AD)-like changes in the brain tissues of rats. In fact, Varner et al. had already found in 1998 that extended use of fluoride in rats (1 ppm in drinking water) produced damage and formation of β-amyloid plaques, which were similar to those found in patients with AD and dementia, in the brain (Court et al., 2001). Rats chronically exposed to fluoride showed a significant decrease in nicotinic acetylcholine receptors (nAChR) in the brain regions, which is an early event of AD in human (Chen et al., 2003). Thus, evidence from animal and human studies suggests similarities between fluorosis-related abnormalities and AD. An animal experiment showed that exposure of APP/PS1 double-transgenic mice to fluoride by intragastric administration can be early onset of AD and aggravate the phenotypes of AD including the deficits in learning and memory and neuropathological lesions (Cao et al., 2019). Some scholars summarized and put forward that the influence of fluoride on processes of AD initiation and progression is complex, not yet fully understood, and warrants further investigation, especially considering growing environmental fluoride pollution (Goschorska et al., 2018). Then does that mean that the incidence of AD is higher in high-fluoride areas, or with accelerated aging among people exposed to high-fluoride drinking water? So far, there is no epidemiological evidence. Our research group (Ren et al., 2021) conducted a cognitive function screening program for elderly people living in high-fluoride areas in Xuzhou, Jiangsu Province, China in 2017 and found that the abnormality rates of the AD8 scale and the MoCA-B scale were higher in this population than in elderly individuals living in normal drinking water areas. In addition, epidemiological analysis suggested that cognitive function was more sensitive to high blood fluoride concentration (Agalakova and Nadei, 2020), especially during early development (Grandjean, 2019).

Thus, high fluoride level may be associated with increased risks of declined learning and memory abilities, low IQ, mental retardation, and even dementia.

2.3.2. *Impacts of chronic fluorosis on mental symptoms*

In 1994, Spittle et al. reported psychiatric adverse effects in patients with chronic fluorosis, which could manifest as mood disorders such as anxiety and depression, as well as cognitive impairments such as decreased memory function, reduced attention, and thinking difficulties (Spittle, 1994). Since then, more attention has been paid to the role of fluoride neurotoxicity in psychiatry (Liu et al., 2014). Numerous epidemiological studies have shown that residents in the fluorosis areas tend to exhibit more social anxiety, reduced life satisfaction, and higher incidence of depression than those in normal areas, and early life fluoride exposure can lead to mental retardation and autism (Nakamoto and Rawls, 2018). Animal experiments in the newborn mice showed that perinatal fluoride exposure decreased their running distance, frequency, and dwell time in the open area of the Elevated Zero Maze (EZM), suggesting that perinatal fluoride exposure may increase the occurrence of anxiety symptoms in mouse offspring (Li et al., 2019). In another study, after mice were given drinking water containing high concentration of fluoride after weaning, prolonged immobility was observed in the tail suspension test (TST) and forced swim test (FST), and it was assumed that fluoride exposure during development may cause anxiety- and depression-like behaviors in adult mice (Liu et al., 2014). In addition to the neuropsychiatric symptoms caused by fluoride itself, patients with dental fluorosis and bone fluorosis are more likely to develop psychological disorders than normal individuals. A study reported increased risk of mild depression in college students with dental fluorosis comparing to the non-fluorosis group, and it was hypothesized that dental fluorosis might be a risk factor for the development of depression (Pan et al., 2019). A recent psychological survey using the Symptom Check List-90 (SCL90) and the Rosenberg Self-Esteem Scale (SES) in 186 college students with dental fluorosis revealed that they had higher scores on five items compared with the national normal level:

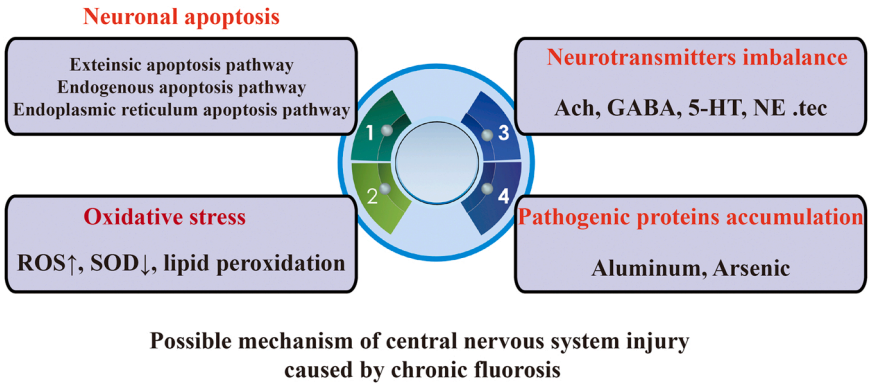

Fig. 2. Possible mechanism of central nervous system injury caused by chronic fluorosis.

interpersonal sensitivity, depression, anxiety, hostility, and paranoia. In addition, there was a negative correlation between the severity of dental fluorosis and the level of self-esteem (Sun et al., 2017a). This indicated that while many studies have focused on the impact of fluoride on cognition, further research is needed to determine the potential effects of long-term consumption of high-fluoride drinking water on emotional behavior. Therefore, attention should be paid to both physical health and mental health of residents in fluoridated areas.

3. Mechanisms of chronic fluorosis-induced brain injuries

The past few decades have witnessed the research advances in the toxic effects of fluoride on the nervous system. However, the exact mechanism by which fluoride reduces learning, memory, and IQ and even causes memory loss, anxiety, and depression has not been clearly defined (Adkins and Brunst, 2021). So far, several mechanisms have been proposed: oxidative stress, apoptosis in CNS, neurotransmitters and their receptors, and interaction between fluorine and other elements (Fig. 2).

3.1. Fluorosis and oxidative stress

The oxidative stress theory has long been one of the hot spots in research on the pathogenesis of chronic fluorosis. Oxidative stress is caused by the disrupted balance between reactive oxygen species (ROS) synthesis and antioxidant enzyme activity. The term ROS encompasses superoxide anion radical (. O_2-), hydroxyl radical (·OH), and hydrogen peroxide (H_2O_2). Antioxidant enzymes basically include superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). It has been observed from cellular experiments, in vivo animal experiments, and epidemiologic studies of chronic fluorosis that fluoride accumulation in the brain leads to increased ROS concentrations, reduced antioxidant enzyme activity, and increased lipid peroxidation. Increased oxidation of lipids and proteins was observed in the cerebral cortex, cerebellum, and medulla oblongata with access to 50 and 150 mg/L fluoride in drinking water (Dec et al., 2017).

In rats exposed to fluoride, the concentrations of fluoride were increased in the serum and brain, along with decreased SOD activity and increased lipid peroxidation (LPO) (Banala and Karnati, 2015). In addition, increased ROS synthesis, disturbed integrity, and altered mitochondrial membrane potential in neuronal mitochondria were observed in rats chronically drinking fluoride-contaminated water (13 mg/kg every 24 h). Antioxidants can protect cells from fluoride-induced lipid peroxidation. Fluoride can induce oxidative stress and regulate intracellular redox homeostasis (Hassan and Yousef, 2009; Mittal and Flora, 2006). Mitochondria are the main source of ROS. Excessive ROS production damages membrane phospholipids and induces lipid peroxidation, mitochondrial membrane depolarization, and apoptosis, which in turn leads to membrane damage, causing disruptions in cellular signaling pathways and triggering membrane lipid release and oxidation. Fluoride can directly interact with antioxidant enzymes and decrease their ability to scavenge free radicals. In hippocampal neuron cultures, after 48 h of incubation with fluoride ion (at concentrations of 40 and 80 mg/L), the ROS synthesis and malondialdehyde (MDA, a lipid peroxidation product and indicator in the organism) content were increased, along with the decrease in the activity of antioxidant enzymes SOD and GPx and in glutathione concentration (Zhang et al., 2007). The activity of CAT was increased in juvenile rats exposed to fluoride in drinking water (with concentrations of 30 and 100 mg/L), suggesting that ROS might have a protective effect against the deleterious activity of oxygen radicals in the organism (Guner et al., 2016). In adult rats exposed to fluoride at a dose of 20 mg/kg every 24 h, decreased glutathione concentration, antioxidant enzymes CAT, SOD, GPX, and glutathione reductase (GR) activities, as well as increased production of free radicals OH and NO were observed in brain tissues (Dec et al., 2017).

Therefore, the mechanism of brain injury caused by fluoride-induced oxidative stress can be summarized as follows: fluoride induces an increase in both ROS synthesis and lipid peroxidation as well as a decrease in antioxidant enzyme activities in neurons and glia, thus attenuating the body's defense mechanisms (Zhang et al., 2015). In addition, oxidative stress can also activate different signaling pathways and initiate the apoptotic program. However, there are still many questions: is oxidative stress a causal factor or parallel phenomenon in the process of brain damage by fluorosis? Is it an initiating step or a triggering link? How do oxidative stress and other mechanisms interconnect and work together? Further research is warranted to answer these questions.

3.2. Apoptosis induced by fluorosis

Apoptosis, or the process of programmed cell death, is essential to maintain homeostasis. Our research showed increased level of apoptosis in PC-12 cells and BV2 microglia exposed to sodium fluoride (Zhang et al., 2017; Chen et al., 2017). The exact mechanism of fluoride-induced apoptosis remains unclear, however studies have shown that G protein-involved signaling pathways, calcium ions, p38 protein, MAP kinase, brain-derived neurotrophic factor (BDNF), and JNK pathway were involved in fluoride-induced apoptosis, and fluoride could induce the extrinsic, intrinsic, and ER-related apoptosis pathways (Dec et al., 2017; Ribeiro et al., 2017).

Extrinsic apoptotic pathway is also known as the death receptor pathway, in which death receptor such as Fas are stimulated by their ligands (e.g. Fas-L) to form a death-inducing signaling complex that activates caspase-8, which in turn triggers downstream caspase-3-mediated apoptosis. The Fas/Fas-L signaling may play a key role in fluoride-induced neural injury. Moreover, the expression of Fas, Fas-L, and caspases (−3 and −8) were increased in human neuroblastoma (SH-SY5Y) cells treated with the high-concentration fluoride (Xu et al.,

2011).

It is well known that TNF-α can activate the apoptosis pathway in neurons. One study found that the expression of TNF-α in the hippocampus of rats treated with fluoride (120 ppm) increased significantly, and microglia were activated to induce neuronal apoptosis and to increase the expression of inflammatory factors (Yan et al., 2016). NF-kB is a transcription factor involved in cell growth, cell cycle regulation, and cellular inflammatory processes. It showed that animals exposed to fluoride (30 mg/L), NF-kB expression was increased, which was correlated with elevated calcium and activated neuronal apoptosis through the extrinsic pathway (Zhang et al., 2011).

The intrinsic apoptotic pathway is also known as the mitochondrial/cytochrome C (Cyt c)-mediated pathway, in which the mitochondria are not only the center of the cellular respiratory chain and oxidative phosphorylation but also the apoptosis-regulation center. In animal experiments, Bcl-2 expression significantly decreased and caspase-12 (the initiator of the ER-related apoptotic pathway) level increased in brain cells of offspring rats exposed to fluoride during pregnancy. In addition, the number of apoptotic cells, the expression level of Cyt c, and the expression levels of caspase-9 and caspase-3 were significantly increased (Sun et al., 2017b). With increased fluoride concentrations, TUNEL assay detected an increase in apoptosis of rat neurons. Bax protein expression was increased and Bcl-2 protein expression was decreased in fluoride-treated rat brains (Yan et al., 2016). It is currently believed that excess intake of fluoride interferes with oxygen metabolism, causing an increase in ROS concentration in cells and alteration in intracellular mitochondrial outer membrane permeability, leading to increased Cyt c released from mitochondria into the cytoplasm, which further activates caspase-9 and caspase-3 and initiates neuronal apoptosis (Su et al., 2017). Another component of apoptotic signaling is the expression/regulation of pro- and anti-apoptotic genes, and abnormal Bcl-2/Bax expression has been shown to be involved in fluoride-induced apoptosis. Based on the close relationship between NF-κB and Bcl-2, Goschorska M et al. presented that fluoride ion in the brain increases apoptosis rate by activating the transcription factors (NF-κB, c-Jun) and proapoptotic proteins Bax, Fas, and P53. Simultaneously fluoride ion inhibits antiapoptotic proteins (Bcl-2 and Bcl-xl) synthesis (Goschorska et al., 2018).

ER plays a key role in protein folding and transport, and in maintaining calcium homeostasis in eukaryotic cells. When ER function is disturbed, the unfolded and misfolded proteins accumulate in the ER lumen, resulting in ER stress and activation of unfolded protein response (UPR), which is an evolutionarily conserved adaptive mechanism (Valenzuela et al., 2016). Prolonged or severe ER stress triggers apoptotic cell death through signaling cascades. ER stress is involved in neuronal disorders, leading to neurodegeneration and cognitive dysfunction (Hossain et al., 2015). ER stress plays an important role in fluoride-induced toxicity (Niu et al., 2018b), and both in vivo and in vitro experiments have shown that sodium fluoride exposure induced ER stress and activated UPR in the rat hippocampus. Excessive ER stress may lead to the failure of UPR-mediated adaptive mechanisms to compensate for the increased need for protein folding, thereby triggering the apoptotic program. Elevated levels of ER stress and associated apoptotic markers IRE1a, GRP78, caspase-12 and caspase-3 in SD rats exposed to sodium fluoride, suggesting the onset of neuronal apoptosis triggered by sodium fluoride. Park et al. found that 4-phenylbutyrate (4-PBA, a chemical chaperone that stabilizes unfolded proteins and promotes their correct folding) treatment decreased the expression of UPR markers in NaF-treated SH-SY5Y cells and also led to a significant decrease in ER stress-induced apoptosis in neurons (Park et al., 2012). Fluoride exposure increased the production of NO, which interferes with disulfide bond formation and leads to accumulation of misfolded proteins in ER, causing ER stress and ROS production (Bülbül and Şireli, 2014). Therefore, the ER stress and associated apoptotic signaling are involved in fluoride-induced neurotoxicity, whereas fluoride induces apoptosis by interfering with signaling processes through multiple mechanisms.

3.3. Neurotransmitters and their receptors

Neurotransmitters are important regulators of brain function. Several studies have shown that fluoride accumulation in the nervous system affects the synthesis of neurotransmitters and the expression of their receptors. Acetylcholine is one of the neurotransmitters that are most closely related to cognition. It was found that the number of nAChR was significantly decreased in the brain of rats with chronic fluorosis and such decrease could be blocked by antioxidants, suggesting that fluoride-induced oxidative stress may be involved in nAChR deficiency in the brain (Spittle, 1994). Neuronal nicotinic receptors play a role in protecting nerves and modulating other transmitters, and are closely related to learning, memory, and cognitive functions. It has been found that chronic fluorosis can cause a decrease in neuronal nicotinic receptors, which occurs mainly at the post-transcriptional level. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are involved in glutamate synthesis, and their activities can be inhibited by excess fluoride (Niu et al., 2009; Yudkoff et al., 1991). Glutamate decarboxylase (GAD) catalyzes the conversion of the excitatory neurotransmitter glutamate to inhibitory neurotransmitter γ-aminobutyric acid (GABA). Fluoride increases GAD activity in the serum, hippocampus, and cerebral cortex of experimental animals, leading to a reduction in glutamate pools in the brain, which results in compromised synaptic transmission and cognitive impairment (Jiang et al., 2014b). Incubation of Bergmann glial cells isolated from rat cerebellum with fluoride caused a significant reduction in cellular lifespan, leading to impaired glutamate metabolism (Flores-Mendez et al., 2014). Glutamate is also involved in memory processes by stimulating specific ionotropic and metabotropic (mGluRs) receptors. Fluoride caused a significant decrease in mGluR5 expression in animal experiments (Jiang et al., 2014b). Increased levels of 5-hydroxytryptamine (5-HT) and norepinephrine (NE) were detected in the striatum, hippocampus, and cerebral cortex of rats after 30 days of administration of drinking water containing NaF (100 mg/L) (Pereira et al., 2011). The concentration of 5-hydroxyindoleacetic acid, a metabolite of 5-HT, was found decreased in the brains of rats chronically exposed to fluoride, and it is speculated that fluoride increases the levels of 5-HT by reducing its metabolism (Cho et al., 1998). Reddy et al., however, had somehow different findings in animals given high-fluoride drinking water: increased levels of neurotransmitters including glutamate, epinephrine, and 5-HT and decreased levels of acetylcholine, norepinephrine, and dopamine in a dose-dependent manner (Reddy et al., 2021, 2014). Therefore, further in-depth studies are needed to verify the effects of neurotransmitters on brain function in patients with chronic fluorosis and the mechanisms involved.

3.4. Interaction of fluoride and other elements

The main source of fluoride for human body is drinking water, in which fluoride can coexist with other elements, altering their kinetic and toxicological properties. It has been noted in the literature that aluminum-fluoride complexes can affect the nervous system and have neurotoxic properties, for instance, an animal experiment showed that long-term consumption of water containing aluminum fluoride and sodium fluoride can affect brain neurons and BBB in rats and cause morphological changes (Varner et al., 1998). Several studies have shown that concentrations of aluminum in drinking water 0.1–0.2 mg/L may increase the risk of AD (Kawahara, 2005). Fluoride may be a protective agent against AD in populations exposed to aluminum and fluoride ions (Colomina MTPeris-Sampedro F, 2017). In addition, both aluminum and fluoride ions are thought to exacerbate or cause neurodevelopmental disorders such as autism, attention deficit/hyperactivity disorder, dyslexia, and other cognitive impairments (Grandjean PLandrigan, 2014; Strunecka et al., 2018).

Arsenic is another element that have potential interaction with

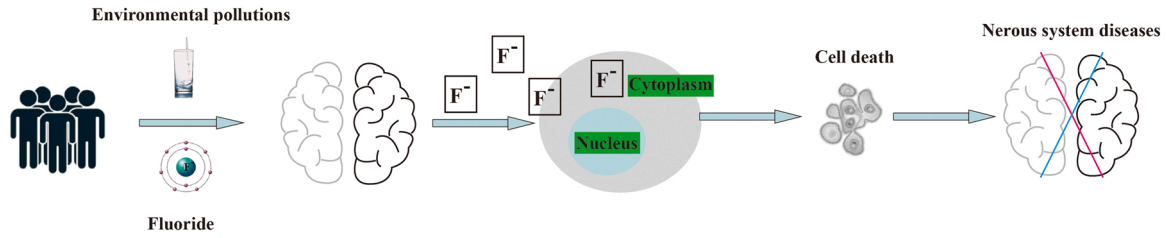

Fig. 3. The table of contents (TOC) graphic-Please pay attention to brain damage caused by fluoride under the background of increasingly severe environmental pollution ! .

fluoride. Although the toxicity of arsenic and fluoride administered alone have been well known, the biological effects and mechanisms of their co-exposure remain controversial, and antagonistic, synergistic, and independent effects have been reported. Several epidemiological studies have indicated that co-exposure to fluoride and arsenic can increase the risk of lowered childhood IQ (Farooqi et al., 2017). Studies based on murine models identified that simultaneous administration of arsenic and fluoride cause less toxicity than given alone (Mittal and Flora, 2006) or exert antagonistic effects (Flora et al., 2009). Therefore, the toxic effects of co-exposure to arsenic and fluoride need to be clarified in future studies.

4. Conclusions

In summary, fluoride is widely present in our daily life. As one of the essential trace elements, fluoride, when taken at a proper amount, is beneficial to human body. However, long-term excessive intake of fluoride can cause the accumulation of fluoride in brain tissue through the placental barrier and/or BBB, which can produce neurotoxicity and damage brain structures and functions, leading to altered cognitive function and psychiatric symptoms such as anxiety and depression. Brain damage caused by fluorosis may involve multiple mechanisms. Once chronic fluorosis develops, the level of oxidative stress increases, various elements and pathways interact with each other to trigger apoptosis, and neurotransmitters and their receptors change. Thus, high fluoride can affect brain function through various pathways, but the exact mechanisms are still unclear. Endemic fluorosis has high incidence in developing countries, and a better understanding of the mechanisms via which chronic fluorosis causes brain damage is of great significance to protect the physical and mental health of people in developing countries, especially those living in the endemic areas of fluorosis (Fig. 3).

Author contributions

C Ren and XC Song: conception and design. XC Song: administrative support. CY Zhang: provision of study materials. C Ren and HH Li: collection and assembly of data. C Ren and HH Li: data analysis and interpretation. All authors: manuscript writing and final approval of manuscript. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Shandong Provincial Key Research and Development Project (2017GSF218043), Natural Science Foundation of Jiangsu Province (BK20151159), and Xuzhou Technological and Scientific Project (KC14SH050).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data was used for the research described in the article.

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