Acta Physiologica Sinica, April 25, 2018, 70(2): 193–200
DOI: 10.13294/j.aps.2018.0024 http://www.actaps.com.cn

Review

High level of Mn in brain is a risk for Alzheimer disease

YU Qin, ZHOU Yuan-Zhong*
School of Public Health, Zunyi Medical University, Zunyi 563000, China

Abstract: Alzheimer disease (AD) is a neurodegenerative disease. Manganese (Mn) is an essential trace element in the human body. It can enter the brain through the blood-brain barrier and blood-cerebrospinal fluid barrier. Excessive accumulation of Mn in the brain may disturb the homeostasis of the central nervous system (CNS) microenvironment and cause severe neuronal damage. The most recent data suggest that excessive Mn is associated with impaired learning and memory in animal models, and may lead to irreversible and progressive mild cognitive impairment and AD. However, the mechanism for the involvement of Mn in AD pathogenesis remains controversial. This paper reviews the effects of Mn on CNS, mitochondrial function, p53 expression, and amyloid precursor protein/β-amyloid metabolism, and analyzes the relationship between these effects and AD pathogenesis.

Key words: Alzheimer disease; manganese; β-amyloid; neurotoxicity

脑内高锰是阿尔茨海默病的危险因素

余 琴，周远忠*
遵义医学院公共卫生学院，遵义 563000

摘 要：阿尔茨海默病(Alzheimer disease, AD)是一种神经系统退行性疾病。锰(manganese, Mn)是人体必需的微量元素。Mn可通过血-脑屏障和血-脑脊液屏障进入脑组织。脑内过量Mn蓄积将会破坏中枢神经系统(central nervous system, CNS)微环境稳态，造成严重的神经损伤。最新的研究提示过量Mn可损害动物的学习记忆功能，引起不可逆转的进行性认知功能减退，最终导致AD的发生。然而，Mn参与AD发病过程的机制仍不清楚，并且存在争议。本文综述了Mn对CNS、线粒体功能、p53表达和β淀粉样蛋白前体或β淀粉样蛋白的作用，并且分析了这些作用在Mn参与AD发病过程中的意义。

关键词：阿尔茨海默病；锰；神经毒性；β淀粉样蛋白
中图分类号：R741.02

Alzheimer disease (AD) is an age-related neurodegenerative disease with progressive deterioration of memory and impairment of cognitive function [1]. Cognitive impairment commonly starts with mild symptoms and gradually worsens [2]. In 2001, more than 24 million people were diagnosed with dementia, and the number of dementia patients is estimated to double every 20 years with an estimated 81 million by 2040 [3]. AD is the most common type of dementia in the elderly [4]. It has affected several million people worldwide, and both the incidence and prevalence of the disease increase with advancing age [3]. In the next several decades, an increase in the lifespan expectancy will raise the number of people affected by the disease. In turn, this will become a significant public health concern in developed and developing countries and a constraint on individuals, families and society [2].

The anatomical characteristics of AD includes the following: (1) lesions made up of senile or neuritic plaques and neurofibrillary tangles in the medial temporal lobe structures and cortical areas of the brain; (2) degeneration of the neurons and synapses [3]. So far,

Received 2017-08-27 Accepted 2017-12-26
*Corresponding author. Tel: +86-851-28642740; E-mail: zhouyuanzhong@163.com

there have been a complicated array of molecular events proposed to explain the pathogenesis of AD, which contains a variety of pathological conditions, such as oxidative stress [5], amyloid-β (Aβ) aggregation, plaque development [6], cell-cycle abnormalities [7], neuroinflammation [8], mitochondrial dysfunction, and energy failure [3, 9]. More than 95% of AD cases are non-familial and late-onset (age > 65 years) sporadic ones that express no clear genetic association [10], suggesting that the environmental factors, such as toxic chemicals exposure, may play a significant role in AD pathogenesis.

It has also been reported that altered homeostasis of some metal elements may be related to the progression of AD. Because of the proposal of the "One Health" concept, which encourages collaboration among medicine, veterinary science, and environmental science around the world to attain optimal health for people, animals, and the environment [11], there has been increasing concern for the relationship between toxicology and the "One Health". It is generally recognized that the manganese (Mn) is a neurotoxin. Over the past few decades, the studies on Mn neurotoxicity have been mostly focused on the impairment of motor function in Parkinson's disease (PD) [12, 13], neglecting that Mn exposure may lead to earlier impaired cognitive function in AD. However, it has not yet been determined whether Mn is responsible for the onset and progression of AD that is observed in the mice exposed to Mn. In this review, we aim to highlight the mechanisms by which Mn overexposure may mediate or trigger AD pathogenesis.

1 Mn and its role in normal physiology

Mn is the 12th most abundant element on the earth and the fifth most abundant metal in the environment [14]. Mn is an essential trace element and plays important roles in normal physiological functions in humans. Mn is involved not only in the metabolism of three major nutrients (lipids, proteins, and carbohydrates), but also in the synthesis and activation of many enzymes as a cofactor or active center [15]. In humans, the minimum daily dietary requirement for Mn ranges from 2.5 to 5.0 mg [16], and the predominant oxidative state of Mn is Mn^{2+}, which is primarily present in the liver, kidney, pancreas, bone, and brain [14]. Some studies have highlighted that the imbalance of Mn in the brain is associated with cognitive impairments, including attention deficits, reduced scores on tests of working memory, lower scores on cognitive tests, impaired learning, and prolonged reaction time [2, 14]. In adults, the normal blood concentration of Mn has been shown to range from 8.6 to 16 ng/mL [16]; the physiological concentration of Mn in the human brain ranges between 5.32–14.03 ng/mg protein, and the pathophysiological threshold of Mn in the human brain ranges between 15.96–42.09 ng/mg protein [17]. Importantly, Mn-induced neurotoxicity is associated with the accumulation of Mn and the susceptibility of different brain regions. As our knowledge of Mn exposure and ensuing toxicity grows, we have discovered that in addition to occupational Mn exposure [12], excessive Mn exposure may also occur in the natural and iatrogenic environments, such as parenteral nutrition or infant formulas [16]. Mn deficiency is rare in humans, whereas Mn-induced toxicity, particularly neurotoxicity, is more prevalent [16]. Chronic Mn exposure produces a neurological syndrome with behavioral, psychiatric, and cognitive features.

2 Mn-induced neurotoxicity

2.1 Mn and the brain barrier system

The blood-brain barrier (BBB) and blood-cerebrospinal fluid (CSF) barrier (BCB) are two indispensable brain barriers that separate the systemic blood circulation from the brain [18, 19]. The BBB consists primarily of cerebral capillary endothelial cells, pericytes, astrocytic end-feet, and basement membranes [18]. The basic components of BBB are brain capillary endothelial cells with highly polarized distinct apical and basolateral compartments, which are involved in the exchange of brain and peripheral substances [20]. The main function of the BBB is to prevent substances from leaving the blood and entering the brain tissues and to maintain the neuronal microenvironment and central nervous system (CNS) homeostasis during physiological and pathological processes [21]. In contrast, the BCB is primarily composed of choroidal epithelial cells [19]. The main function the BCB is to regulate the effusion of molecules from the CSF into the blood or in the reverse direction [22].

Mn plays a significant role in nervous system as a second messenger, is involved in the synthesis of superoxide dismutase and glutathione enzyme, and regulates the expression of genes [17]. Additionally, Mn needs to be maintained at an optimal physiological level to ensure normal functioning of the nervous system [17].

Inhaled Mn can bypass the liver to enter the bloodstream, from there, it can enter the brain via the olfactory tract [23]. Thus it is easy for inhaled Mn to accumulate in the brain [23]. For the ingested Mn, when the level of Mn in the body is within the physiological concentration, Mn can be transported across the brain capillary endothelium into the CNS via specific carriers that are in the luminal and abluminal membranes of the endothelial cells [24, 25]. Mn^{2+} enters the brain capillary endothelium via free ions or non-specific divalent metal transporter-1 (DMT-1), while Mn^{3+} accesses the brain capillary endothelium via a transferrin receptor-mediated mechanism [17]; when the brain lacks of active transport from the CNS to the systemic circulation, Mn easily accumulates in the CNS [17]. The accumulation of Mn in the CNS impairs the mitochondrial electron transport chain and causes neurocytotoxicity [24]. As a carrier membrane protein, DMT1 is also expressed in the choroid plexus epithelial cells and delivers Mn to the CNS [26]. When the concentration of blood Mn is ≥ 78 μmol/L, Mn travels primarily through the choroidal epithelial cells into the CNS [25]. The level of Mn that accumulates in the choroidal epithelial cells is greater than that in the cerebral cortex (150-fold) or CSF (1 000-fold) [25]. The choroidal epithelial cells exposed to Mn increase the uptake of α-synuclein, a key pathogenic factor of neurodegenerative diseases, such as PD and AD [27].

It has been observed that half-life ($t_{1/2}$) of Mn in the rat skeleton was 143 days, and in brain tissue, including the nerve ganglia basal, brainstem, and cerebral cortex, was about 51–74 days [25]. Robison *et al*. [28] have demonstrated by X-ray fluorescence imaging that the accumulation of Mn in the rat brain is ranked as follows: globus pallidus, cerebral cortex, thalamus, substantia nigra, caudate nucleus, axon bundle. In addition to these areas, Mn has also been shown to accumulate in the hippocampus and striatum [29]. The accumulation of Mn will cause different degrees of damage in different targets; for instance, the reduction of myelinated nerve fibers in the globus pallidums and striatum, the atrophy of ventricular volume, the aberrant of dopamine signaling, the reduction of cholinergic neurons, the activation of astrocytes, and the neuronal death or apoptosis [12]. Growing evidence has also shown that overexposure to Mn may impair the integrity of the brain barrier functions, which may affect the normal exchange of material and brain microenvironment homeostasis, causing inflammation, abnormal brain function, and eventual neurodegenerative diseases.

2.2 Mn and dysfunction of neuronal mitochondria

Mitochondria are known as important regulators of the cell cycle and play a central role in ageing [30]. Mitochondrial dysfunction, including oxidative phosphorylation-electron transfer coupling abnormalities and membrane potential changes, eventually leads to the apoptosis of the cells [30, 31]. Mn can accumulate in the mitochondria of neurons, since Mn can enter the mitochondria but has an extremely slow efflux rate from them [2]. Several researchers have suggested that the dysfunction of neuronal mitochondria is heavily implicated in Mn-induced neurotoxicity [2]. In an *in vitro* study, neuronal cell lines, N27 (a rat dopaminergic neuronal cell line from the midbrain) and PC12 (derived from a pheochromocytoma of the rat adrenal medulla), as well as non-neuronal cell lines, Z310 (choroid plexus epithelial cells) and RBE4 (rat brain endothelial cells), were incubated with 100 μmol/L $MnCl_2$ for 24 h. The results suggested that Mn was more likely to accumulate in neuronal cells compared to non-neuronal cells; less than 0.5% Mn accumulated in the mitochondria [29]. Nonetheless, the negative effects of Mn on neuronal mitochondria should not be underestimated [14].

Mitochondrial dysfunction is one of the characteristics of all neurodegenerative diseases. Some findings have emphasized that dysfunction in mitochondrial fission and fusion may underlie both familial and sporadic forms of neurodegenerative disorders [32]. Some studies have also suggested that Aβ peptide, which is causally linked to AD, is involved in mitochondrial fission dysfunction [33]. Zheng *et al*. [34] has suggested that chronic Mn exposure significantly inhibits mitochondrial aconitase activity. Anantharaman *et al*. [35] has further suggested that chronic Mn exposure does not change Mn superoxide dismutase (MnSOD) protein levels in the brain of APP/PS-1 mice compared to wild type mice; in the APP/PS1 mice, MnSOD activity and mitochondrial respiration were limited.

Neurons contain many mitochondria, cytochrome oxidases, mitochondrial DNA (mtDNA), and lipofuscin-associated vacuoles. Some reports have shown a consistent and significant increase in mtDNA and cytochrome oxidase in AD using *in situ* hybridization to mtDNA, immunocytochemical labeling cytochrome oxidases, and morphometry of electron micrographs of biopsy specimens; in contrast, mitochondria were found to be markedly reduced in AD [36]. As the level of

Mn in the brain increases, excess Mn will accumulate in the mitochondria; this could inhibit mitochondrial energy transduction, accelerate the production of free radicals, and promote mutations of the mitochondrial genome [37]. The excess Mn can also inhibit mitochondrial oxidative phosphorylation, three tricarboxylic acid cycle, electron transport chain, and a series of important enzymatic activities [37].

The above evidence indicates that chronic Mn exposure might induce mitochondrial energy metabolism abnormalities, further triggering mitochondrial structure damage, oxidative damage, and brain metabolic abnormalities; these changes may be additional pathogenic mechanisms of AD.

2.3 Mn and Aβ peptide

The accumulation of Aβ and formation of senile plaques and neurofibrillary tangles are important pathophysiological hallmarks of AD [3]. The amyloid cascade hypothesis is the most prominent hypothesis for explaining the pathogenesis of AD (Fig. 1). The hypothesis states that Aβ is a key factor in inducing or triggering AD [3]. Aβ is a natural products that is formed during cellular metabolism and is generated from amyloid precursor protein (APP) cleavage catalyzed by β- and γ-secretase. Beta-site APP cleaving enzyme 1 (BACE1) is a typical aspartyl protease, and γ-secretase is an aspartyl protease complex composed of four individual proteins (presenilin-1, nicastrin, anterior pharynx-defective 1, and presenilin enhancer 2), in which presenilin-1 contains the protease active and working intramembrane sites [38]. It was shown that APP is hydrolyzed by α-secretase to produce soluble APPα (which does not induce neurotoxicity) and APP-C83, whereas APP hydrolyzed by β- and γ-secretase can produce Aβ fragments [38–40]. Aβ self-aggregates spontaneously into oligomers with 2 to 6 Aβ peptides, and the oligomers are more neurotoxic than the Aβ monomers. Aβ peptides can also be arranged into a β-sheet, and ultimately transformed into insoluble fibers of senile plaques. Soluble oligomers and intermediate amyloid assemblies are the predominant neurotoxic forms of Aβ [38]. In the early stage of AD, toxic soluble Aβ oligo-

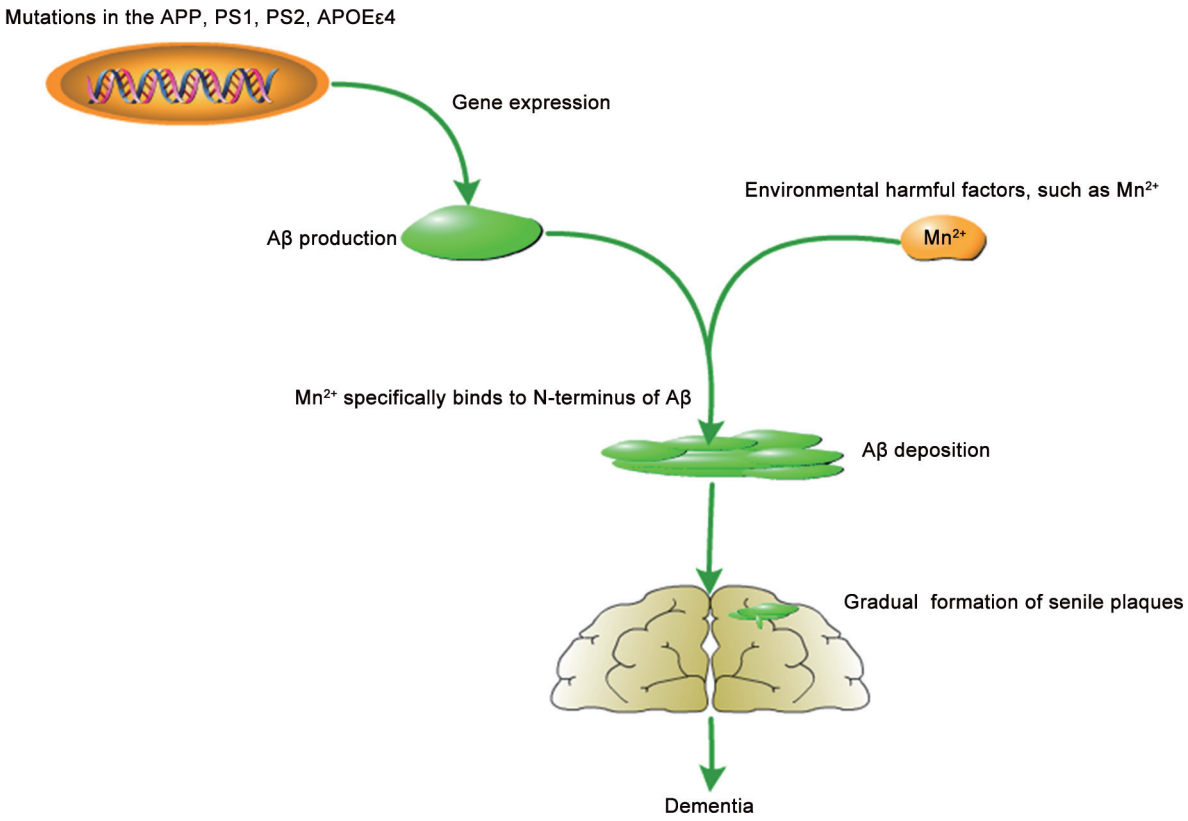

Fig. 1. Aβ cascade hypothesis. According to this hypothesis, the central event in AD disease pathogenesis is an imbalance between Aβ production and clearance, leading to Aβ deposition and gradual formation of senile plaques. Mn^{2+} can specifically bind to the N-terminus of Aβ, resulting in Aβ deposition, senile plaque formation, and eventual AD pathology.

mers damage the structure and function of neuronal synapses. As the amyloid burden increases, these soluble fragments are gradually deposited as insoluble amyloid plaques in the parenchyma and around the vasculature, causing an inflammatory response. By the later stages, aggregations of intracellular neurofibrillary tangles disrupt cellular function and contribute to neuronal death [41]. Aβ missfolding and imbalance of Aβ clearance and deposition are not limited exclusively to the brain parenchyma [42], suggesting that systemic misfolding events could be a consequence of external influences. Anantharaman *et al*. [35] used a APP/PS1 mouse model that recapitulated the natural progression of Aβ pathology, which is similar to that observed in AD patients; their results suggested that Aβ deposition or plaques in the cortex and hippocampus of APP/PS1 mice were age-related and region-dependent.

Growing evidence suggests that Mn homeostasis is disturbed in AD and may be associated with the progression of AD. Using Mn^{2+} as paramagnetic probes in Aβ or micelle, Jarvet *et al*. [43] found that Mn^{2+} was specifically bound to the N-terminus of Aβ. Wallin *et al*. [42] further suggested that Mn^{2+} binds to the N-terminal end of the $A\beta_{1\text{-}40}$ peptide between millimolar to micromolar ranges using nuclear magnetic resonance spectroscopy. Tong *et al*. [44] measured the level of Mn in the whole blood of 40 Chinese elders with different cognitive statuses and neuropsychological tests scores. The results showed that the level of Mn in the whole blood was highly correlated with cognitive function (Mini-Mental State Examination and Clinical Dementia Rating Scale scores) and plasma $A\beta_{1\text{-}42}$ and $A\beta_{1\text{-}40}$ peptide levels. They further determined that high level of Mn has disrupted Aβ metabolism in N2a mouse neuroblastoma cells; high Mn caused a down-regulation of two major enzymes involved in Aβ degradation, neprilysin and insulin degrading enzyme, without altering the APP expression, and there was a dose-dependent effect of Mn on neurotoxicity.

These fruitful works have revealed that Mn has a slightly affinity for Aβ and high level of Mn may be associated with degradation of Aβ. So excessive Mn may accelerate the accumulation of Aβ in the extracellular space of the brain, thereby increasing Aβ neurotoxicity and accelerating the disease progression.

2.4 Mn and p53

p53 has a variety of important functions, such as regulation of gene transcription, DNA synthesis and repairment, cell cycle, cell senescence and apoptosis [45]. Neuronal apoptosis is closely interrelated to changes of mitochondrial ion permeability, release of apoptotic factors and apoptosis inducing factor, activation of caspases, and condensation and degradation of nuclear DNA [45]. It is well known that the activation of p53 in damaged neurons in neurodegenerative diseases, such as AD, is especially harmful. This suggests that the activation of p53 may be involved in the development of neurodegenerative diseases [2, 46]. It has been observed that there are some changes in p53 mRNA and protein expressions in acute impaired neuronal and neurodegenerative diseases. Elevated expression of p53 is mainly present in neurons of the injured cortex and hippocampus [46]. It is another typical feature that p53 gene DNA damage is caused by mutations in presenilin-1 [45]. Thus, the activation of p53 plays an important role in the pathogenesis of AD.

It has been reported that numerous changes in p53 target genes or proteins occur in the frontal cortex of animals exposed to Mn [46]. Activated p53 may play a vital role in altering gene expression of the glial cells in the frontal cortex of Mn-treated animals. p53 immunoreactivity has also been detected in the brain tissue of AD animals models and AD patients [46]. Research by Guilarte *et al*. [47, 48] further indicated that the Mn-induced gene changes are related to activation of p53 gene or interaction with the p53-associated protein product in non-human primates; equivalent to the p53 gene changes, p53 protein levels were increased in the frontal cortex neurons and glial. These results provide a new direction for the study of Mn-induced neurotoxicity. The accumulation of Mn in the frontal cortex is lower than that in the globus pallidus [28]. However, the neurotoxicity related to Mn is not dependent on its accumulated concentration and may be associated with susceptibility of specific brain tissues in different species.

2.5 Mn and APLP1

The APP family consists of APP and the amyloid precursor-like proteins (APLP1 and APLP2) [49]. APP and APLP2 are expressed in almost all tissues of the body, while APLP1 expression appears to be limited in the brain [50]. The human APP gene has been mapped to the long arm of chromosome 21, and its mutation is related to the AD neuropathology [51]. Aβ is a normal product of APP metabolism and can be measured in CSF and plasma. Fundamental researches have showed that the Aβ aggregation is caused by mutations in APP. These

mutations promote the generation of neurotoxicity of Aβ by favoring proteolytic processing of APP [3, 38]. An essential experiment in this area suggested that mutations in the internal Aβ sequence of APP cause self-aggregation of Aβ into amyloid fibrils [51]. APLP1 is enriched in the post-synaptic membrance of cerebral cortex and hippocampus [49], and the elevated expression of APLP1 induces neuronal apoptosis and neurodegeneration disease [50].

A gene expression profiling experiment in the frontal cortex of cynomolgus macaques who received 3.3–5.0 mg/kg of Mn weekly for 10 months showed that 61 out of 6 766 genes were significantly up-regulated compared to those in control macaques, and the most highly up-regulated gene was *APLP1* [47]. Additionally, it was found that APLP1 immunolabeling was increased in subcortical white matter cells in Mn-treated animals, and the morphology of the white matter was similar to that of the interstitial. Immunohistochemistry also indicated that the animals that were exposed to Mn had Aβ plaque accumulation in the frontal cortex [47]. These results relate to the pathogenesis of AD, and non-human primates have a close genetic relationship with humans. Therefore this study provides substantial evidence for Mn-induced neuronal apoptosis and AD in humans.

3 Conclusion

In conclusion, Mn mediates or triggers a neurological syndrome with psychiatric and cognitive symptoms, including dysfunction of mitochondria, p53, and APP/Aβ metabolism. The toxic effects of Mn are dose-dependent; the longer the exposure to Mn, the greater the damage is [44]. Even if the Mn exposure is terminated, the damage is increasing, indicating that Mn-induced nervous system damage is irreversible [52]. It is well known that the causes of AD are multifactorial, and may involve neuroinflammation, p53 expression changes, and APP/Aβ-related metabolic disorder, mitochondrial energy metabolic imbalance, and other processes.

AD is most common in the elderly diseases, but its associated morbidity does not necessarily begin at old age; the average latency of AD is 15, or even up to 20 years [53, 54]. With the continuous development of industrial processes and the extensive use of ferromanganese alloy and methylcyclopentadienyl manganese tricarbonyl (MMT), most people may be exposed to more than the physiological concentration of Mn. Since the changes of motor function and emotional induced by Mn exposure are most noticeable, there is a lack of awareness about cognitive effects of Mn exposure, which has not yet received widespread public attention. In fact, Mn-induced neurotoxicity not only damages dopamine neurons and induces PD-like motor function problems, but also induces AD-like neuropathy by accumulation of Mn in the choroid plexus, hippocampus, cortex and other brain areas. Unfortunately, the mechanism by which Mn induces or mediates AD pathogenesis remains to be determined. Therefore, future studies should use animal and cell-based experiments to explore the imbalance in mitochondrial energy metabolism, neuronal damage and synaptic loss, hippocampal metabolism and structural dysfunction, and other AD-related mechanisms that involve Mn.

* * *

ACKNOWLEDGEMENTS: This review was supported by the National Natural Science Foundation of China (No. 8166120272).

REFERENCES

1 Gu H, Wei X, Monnot AD, Fontanilla CV, Behl M, Farlow MR, Zheng W, Du Y. Lead exposure increases levels of beta-amyloid in the brain and CSF and inhibits LRP1 expression in APP transgenic mice. Neurosci Lett 2011; 490(1): 16–20.

2 Prakash A, Dhaliwal GK, Kumar P, Majeed AB. Brain biometals and Alzheimer's disease - boon or bane? Int J Neurosci 2017; 127(2): 99–108.

3 Scheltens P, Blennow K, Breteler MM, de Strooper B, Frisoni GB, Salloway S, Van der Flier WM. Alzheimer's disease. Lancet 2016; 388(10043): 505–517.

4 Berr C, Wancata J, Ritchie K. Prevalence of dementia in the elderly in Europe. Eur Neuropsychopharm 2005; 15(4): 463–471.

5 Agostinho P, Cunha RA, Oliveira C. Neuroinflammation, oxidative stress and the pathogenesis of Alzheimer's disease. Curr Pharm Design 2010; 16(25): 2766–2778.

6 Hua S, Zhen W. A brief review on studies of Alzheimer's disease in China: Its mechanism, imaging and therapy. Sci China Life Sci 2013; 56(12): 1142–1144.

7 Moh C, Kubiak JZ, Bajic VP, Zhu X, Smith MA, Lee HG. Cell cycle deregulation in the neurons of Alzheimer's disease. Results Probl Cell Differ 2011; 53: 565–576.

8 Sudduth TL, Schmitt FA, Nelson PT, Wilcock DM. Neuroinflammatory phenotype in early Alzheimer's disease. Neurobiol Aging 2013; 34(4): 1051–1059.

9 Hroudová J, Singh N, Fišar Z. Mitochondrial dysfunctions in neurodegenerative diseases: Relevance to Alzheimer's disease. Biomed Res Int 2014; 2014: 175062.
10 Migliore L, Coppedè F. Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. Mutat Res 2009; 667(1): 82–97.
11 American Veterinary Medical Association. One health: A new professional imperative. One Health Initiative Task Force Final Report 2008; 1–76.
12 Gorell JM, Johnson CC, Rybicki BA, Peterson EL, Kortsha GX, Brown GG, Richardson RJ. Occupational exposure to manganese, copper, lead, iron, mercury and zinc and the risk of Parkinson's disease. Neurotoxicology 1999; 20(2–3): 239–247.
13 Finkelstein M, Jerrett M. A study of the relationships between Parkinson's disease and markers of traffic-derived and environmental manganese air pollution in two Canadian cities. Environ Res 2007; 104(3): 420–432.
14 O'Neal SL, Zheng W. Manganese toxicity upon overexposure: a decade in review. Curr Environ Health Rep 2015; 2(3): 315–328.
15 Lebda MA, El-Neweshy MS, El-Sayed YS. Neurohepatic toxicity of subacute manganese chloride exposure and potential chemoprotective effects of lycopene. Neurotoxicology 2012; 33(1): 98–104.
16 Zheng W, Kim H, Zhao Q. Comparative toxicokinetics of manganese chloride and methylcyclopentadienyl manganese tricarbonyl (MMT) in Sprague-Dawley rats. Toxicol Sci 2000; 54(2): 295–301.
17 Peres TV, Schettinger MR, Chen P, Carvalho F, Avila DS, Bowman AB, Aschner M. "Manganese-induced neurotoxicity: a review of its behavioral consequences and neuroprotective strategies". BMC Pharmacol Toxicol 2016; 17(1): 57–76.
18 Kandel EE, Schwartz J Kandel ER, Schwartz JH, Jessell TM, Siegelbaum SA, Hudspeth AJ, eds. Principles of Neural Science. 5th ed. McGraw-Hill Companies, Inc., 2013.
19 Gu H, Zhong Z, Jiang W, Du E, Dodel R, Liu J, Farlow MR, Zheng W, Du Y. The role of choroid plexus in IVIG-induced beta-amyloid clearance. Neuroscience 2014; 270(6189): 168–176.
20 Chow BW, Gu C. The molecular constituents of the blood-brain barrier. Trends Neurosci 2015; 38(10): 598–608.
21 Zheng W, Aschner M, Ghersi-Egea JF. Brain barrier systems: a new frontier in metal neurotoxicological research. Toxicol Appl Pharmacol 2003; 192(1): 1–11.
22 Monnot AD, Zheng W. Culture of choroid plexus epithelial cells and *in vitro* model of blood-CSF barrier. Methods Mol Biol 2012; 945: 13–29.
23 Peres TV, Parmalee NL, Martinez-Finley EJ, Aschner M. Untangling the manganese-α-synuclein web. Front Neurosci 2016; 10: 364.
24 Aschner M. The transport of manganese across the blood-brain barrier. Neurotoxicology 2006; 27(3): 311–314.
25 O'Neal SL, Hong L, Fu S, Jiang W, Jones A, Nie LH, Zheng W. Manganese accumulation in bone following chronic exposure in rats: steady-state concentration and half-life in bone. Toxicol Lett 2014; 229(1): 93–100.
26 Wang X, Li GJ, Zheng W. Upregulation of DMT1 expression in choroidal epithelia of the blood-CSF barrier following manganese exposure *in vitro*. Brain Res 2006; 1097(1): 1–10.
27 Bates CA, Fu S, Ysselstein D, Rochet JC, Zheng W. Expression and transport of α-synuclein at the blood-cerebrospinal fluid barrier and effects of manganese exposure. ADMET DMPK 2015; 3(1): 15–33.
28 Robison G, Zakharova T, Fu S, Jiang W, Fulper R, Barrea R, Marcus MA, Zheng W, Pushkar Y. X-ray fluorescence imaging: A new tool for studying manganese neurotoxicity. PLoS One 2012; 7(11): e48899.
29 Kalia K, Jiang W, Zheng W. Manganese accumulates primarily in nuclei of cultured brain cells. Neurotoxicology 2008; 29(3): 466–470.
30 Lin MT, Beal MF. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 2006; 443 (7113): 787–795.
31 Green DR, Kroemer G. The pathophysiology of mitochondrial cell death. Science 2004; 305(5684): 626–629.
32 Wang X, Su B, Lee HG, Li X, Perry G, Smith MA, Zhu X. Impaired balance of mitochondrial fission and fusion in Alzheimer's disease. J Neurosci 2009; 29(28): 9090–9103.
33 Knott AB, Bossy-Wetzel E. Impairing the mitochondrial fission and fusion balance: a new mechanism of neurodegeneration. Ann N Y Acad Sci 2008; 1147(1): 283–292.
34 Zheng W, Ren S, Graziano JH. Manganese inhibits mitochondrial aconitase: a mechanism of manganese neurotoxicity. Brain Res 1998;799(2): 334–342.
35 Anantharaman M, Tangpong J, Keller JN, Murphy MP, Markesbery WR, Kiningham KK, St Clair DK. Beta-amyloid mediated nitration of manganese superoxide dismutase: implication for oxidative stress in a APPNLH/NLH X PS-1P264L/P264L double knock-in mouse model of Alzheimer's disease. Am J Pathol 2006; 168(5): 1608–1618.
36 Hirai K, Aliev G, Nunomura A, Fujioka H, Russell RL, Atwood CS, Johnson AB, Kress Y, Vinters HV, Tabaton M, Shimohama S, Cash AD, Siedlak SL, Harris PL, Jones PK, Petersen RB, Perry G, Smith MA. Mitochondrial abnormalities in Alzheimer's disease. J Neurosci 2001; 21(9): 3017–3023.
37 Brown S, Taylor NL. Could mitochondrial dysfunction play a role in manganese toxicity? Environ Toxicol Phar 1999;

7(1): 49–57.

38 Querfurth HW, Laferla FM. Alzheimer's disease. N Engl J Med 2010; 362(4): 329–344.

39 Zheng W. Neurotoxicology of the brain barrier system: new implications. J Toxicol Clin Toxicol 2001; 39(7): 711–719.

40 Hosono T, Nishitsuji K, Nakamura T, Jung CG, Kontani M, Tokuda H, Kawashima H, Kiso Y, Suzuki T, Michikawa M. Arachidonic acid diet attenuates brain Aβ deposition in Tg2576 mice. Brain Res 2015; 1613: 92–99.

41 Allemang-Grand R, Scholz J, Ellegood J, Cahill LS, Laliberté C, Fraser PE, Josselyn SA, Sled JG, Lerch JP. Altered brain development in an early-onset murine model of Alzheimer's disease. Neurobiol Aging 2015; 36(2): 638–647.

42 Wallin C, Kulkarni YS, Abelein A, Jarvet J, Liao Q, Strodel B, Olsson L, Luo J, Abrahams JP, Sholts SB. Characterization of Mn(II) ion binding to the amyloid-β peptide in Alzheimer's disease. J Trace Elem Med Bio 2016; 38: 183–193.

43 Jarvet J, Danielsson J, Damberg P, Oleszczuk M, Gräslund A. Positioning of the Alzheimer Aβ(1-40) peptide in SDS micelles using NMR and paramagnetic probes. J Biomol NMR 2007; 39(1): 63–72.

44 Tong Y, Yang H, Tian X, Wang H, Zhou T, Zhang S, Yu J, Zhang T, Fan D, Guo X, Tabira T, Kong F, Chen Z, Xiao W, Chui D. High manganese, a risk for Alzheimer's disease: high manganese induces amyloid-beta related cognitive impairment. J Alzheimers Dis 2014; 42(3): 865–878.

45 Culmsee C, Mattson MP. p53 in neuronal apoptosis. Biochem Biophys Res Commun 2005; 331(3): 761–777.

46 Morrison RS, Kinoshita Y. The role of p53 in neuronal cell death. Cell Death Differ 2000; 7(10): 868–879.

47 Guilarte TR, Burton NC, Verina T, Prabhu VV, Becker KG, Syversen T, Schneider JS. Increased APLP1 expression and neurodegeneration in the frontal cortex of manganese-exposed non-human primates. J Neurochem 2008; 105(5): 1948–1959.

48 Guilarte TR. APLP1, Alzheimer's-like pathology and neurodegeneration in the frontal cortex of manganese-exposed non-human primates. Neurotoxicology 2010; 31(5): 572–574.

49 Kim TW, Wu K, Xu JL, Mcauliffe G, Tanzi RE, Wasco W, Black IB. Selective localization of amyloid precursor-like protein 1 in the cerebral cortex postsynaptic density. Brain Res Mol Brain Res 1995; 32(1): 36–44.

50 Tang X, Milyavsky M, Goldfinger N, Rotter V. Amyloid-beta precursor-like protein APLP1 is a novel p53 transcriptional target gene that augments neuroblastoma cell death upon genotoxic stress. Oncogene 2007; 26(52): 7302–7312.

51 Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. Science 2002; 297(5580): 353–356.

52 Bowler RM, Gysens S, Diamond E, Nakagawa S, Drezgic M, Roels HA. Manganese exposure: Neuropsychological and neurological symptoms and effects in welders. Neurotoxicology 2006; 27(3): 315–326.

53 Jack JC Jr, Wiste HJ, Lesnick TG, Weigand SD, Knopman DS, Vemuri P, Pankratz VS, Senjem ML, Gunter JL, Mielke MM, Lowe VJ, Boeve BF, Petersen RC. Brain β-amyloid load approaches a plateau. Neurology 2013; 80(10): 890–896.

54 Bateman RJ, Xiong C, Benzinger TL, Fagan AM, Goate A, Fox NC, Marcus DS, Cairns NJ, Xie X, Blazey TM. Clinical and biomarker changes in dominantly inherited Alzheimer's disease. N Engl J Med 2012; 367(9): 795–804.