Potential Prevention and Treatment of Maifanite for Alzheimer's Disease Based on Behavior Test, Oxidative Stress Assay, and Trace Element Analysis in Hippocampus of Aβ$_{(25–35)}$-Induced AD Rats

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Abstract This study aimed to assess whether maifanite can improve the learning and memory, and antioxidant abilities of Alzheimer's disease (AD) rats. The 70 rats were divided into seven groups: [A] normal control group, [B] AD model group, [C] sham group, [D] positive control group (donepezil), [E] low-dose maifanite group, [F] middle-dose maifanite group, [G] high-dose maifanite group. For [B], [D], [E], [F], and [G] groups, Aβ$_{(25–35)}$ ventricle injection was carried out, then respective medicine were administered once a day for 60 consecutive days. The step-down and step-through test were used to measure learning and memory ability. The hippocampus levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA) were assayed. The hippocampus contents of Al, Fe, Cu, Zn, Se, and Mn were analyzed by inductively coupled plasma–atomic emission spectrometer. Maifanite decreased the acquisition errors and the retention errors while prolonging the step-down latency, and decreased the number of electric shocks while prolonging the first latency of AD rats. Aβ$_{(25–35)}$ ventricle injection initiated the decrease of SOD and GSH-Px activities and the increase of MDA content, and triggered the rise of Al, Fe, and Cu levels and the decline of Mn, Zn, and Se levels. The SOD and GSH-Px activities were enhanced followed by reduced MDA level, and the levels of Mn, Zn, and Se increased accompanied by Al, Fe, and Cu decreased in the maifanite treat groups. Maifanite could improve the learning and memory, and the antioxidant abilities of AD rats. Maifanite had the potential prevention and treatment for AD.

Abbreviations

AD	Alzheimer's disease
MDA	Malondialdehyde
SOD	Superoxide dismutase
GSH-Px	Glutathione peroxidase
ICP-AES	Inductively coupled plasma–atomic emission spectrometer

Introduction

Alzheimer's disease (AD), an age-related neurodegenerative condition, was associated with an early impairment in memory and was the major cause of dementia in the elderly [1], and was pathologically characterized by oxidative stress, amyloid-β (Aβ) oligomerization [2], metal dyshomeostasis, etc. [2–5]. These three phenomena shared a common instigating factor reactive transition metal aberration [6]. Therefore, to find a suitable metal homeostasis reagent was one of the effective ways of prevention and treatment of AD. Maifanite might be the ideal mineral medicine, which was a granitoid silicate and was used in many fields for a long time in East Asia countries such as China and Japan, and had no toxicity [7, 8]. Both the loose porosity structure and the very small size on the surface were caused as a result of the long-term weathering and denudation [8]. Therefore,

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many mineral elements were produced after the primary maifanite mineral was immersed into water [8]. The metal element concentration in aqueous extract of maifanite was related to lattice energy of mineral, hydration energy of mineral, size of mineral, temperature, pH value, etc. [9]. Though the total percentage of SiO_2 and Al_2O_3 in maifanite mineral was more than 70 %, both Si and Al concentrations were near to zero at this condition, because the bond energy of Al-O and Si-O was 582 and 464 KJ/mol, respectively [9]. The major elements in the aqueous extract of maifanite were K, Na, Ca, Mg, Sr, Zn, Cu, Fe, Mn, Se, etc. [7–9]. The animal experiments demonstrated that maifanite possessed the obvious pharmacological affect on anti-aging, anti-hypoxia, and so on, which might be related to mineral elements in maifanite [8, 10].

Establishment of suitable AD animal model was very important to find the effective prevention and treatment drug for AD. $A\beta$ ventricle injection model was one of the AD animal models, this model was earliest built in 1994 using $A\beta_{(1–40)}$ continuous ventricular perfusion for 2 weeks [11]. Subsequent reports had also demonstrated, ventricle injection of $A\beta_{(1–40)}$ was a success method in making AD animal model [1, 12]. Based on recent studies, the peptide $A\beta_{(25–35)}$ was also reported to build AD model [13–16]. Investigations with the use of $A\beta_{(25–35)}$ in animal models would provide a convenient model, which was also adopted in our research.

Materials and Methods

Main Drugs and Reagents

The maifanite was purchased from the Guifeng Maifanite Company in Guangxi Guiping City, China. The aqueous extract of maifanite was obtained as follows: (1) the 10 g of maifanite (100 no. mesh) was immersed into deionized water (100 ml) for 30 min at room temperature, and then it was refluxed at 80 °C for 60 min. It was filtered to prepare the aqueous extract of maifanite, represented by the MF_{high}. (2) The MF_{high} was diluted by ten times and 100 times, respectively, represented by the MF_{middle} and MF_{low}. Using ICP-AES, the element concentration (μg/ml) of the MF_{high} is determined, which is Fe 0.068, Cu 0.022, Mn 0.049, Zn 0.037, Se 0.015, and Al 0.001.

$A\beta_{(25–35)}$ was purchased from Sigma Corp., and was dissolved in sterile deionized saline at a concentration of 10 μg/ml, and incubated at 37 °C for 72 h to obtain the aggregated form before being use. The other chemicals were analytical reagent grade and obtained from Shanghai Chemical Reagent Corp. (China).

The test kits of malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) were all purchased from Nanjing Jiancheng Bioengineering Institute (China).

Animals

The male Sprague–Dawley (SD) rats weighed 250–300 g at the beginning of the experiments were provided by Lab Animal Central of Guangxi Medical University. They were maintained under a 12/12-h light–dark cycle (light beginning at 8:00 a.m.) and in a controlled temperature (22±2 °C), relative humidity of (60±10)%. The rats had free access to food and deionized water, except during the experiments. Animals were given a 1-week acclimation period to the lab condition. The protocols for the animal care and treatment were approved by the Ethics Committee of Guangxi University of Chinese Medicine.

Establishment of AD Rats Model

The rats were divided into seven groups: [A] normal control group, [B] AD model group, [C] sham group, [D] positive control group (donepezil), [E] low-dose maifanite group, [F] middle-dose maifanite group, [G] and high-dose maifanite group. Each experimental group consisted of ten animals, and each animal was used once.

The normal control group received no treatment, the other five groups were anesthetized with 2 % sodium pentobarbitol (50 mg/kg bw) by intraperitoneal injection. The heads of rats were set on the stereotaxis instrument (Jiangwan type I animal cranium stereotaxis instrument). For AD model group, positive control group, low-dose maifanite group, middle-dose maifanite group, and high-dose maifanite group, the animal's skull was exposed. With reference to *The Rat Brain in Sterotaxic Coordinates* [17], the lateral ventricle is located (AP=−1.1 mm, ML=1.7 mm, DV= 3.9 mm from Bregma). Five microliters of $A\beta_{(25–35)}$ was injected by Hamilton micro-syringe. The injection lasted for 10 min, and the needle was left on the injection site for another 2 min. Rats in the sham group were injected in an identical manner with the same amount of sodium chloride. To avoid infection, all surgically treated rats were given antibiotic, penicillin, once daily for the first 3 days after the surgery. Each group began intragastric administration after 7 days, positive control group was administered donepezil 0.05 mg/kg, low-dose maifanite group was administered MF_{low} 3 ml, middle-dose maifanite group was administered MF_{middle} 3 ml, and high-dose maifanite group was administered MF_{high} 3 ml. The normal control group, AD middle group, and sham group were administered the same dose 3 ml of deionized water. The rats were given the reagent once daily for 60 consecutive days. Additionally, all efforts were made to minimize unwanted stress or discomfort to the rats during experimental procedures.

Behavior Tests [18]

After 60 days administration, behavior tests, including step-down test and step-through test were carried out.

Step-down test: the rats were tested on a step-down test in a passive avoidance chamber. The floor of the chamber consists of copper rods and a well-insulated platform made of rubber in one corner of the chamber. The rats were placed in the chamber for a 3-min adaptation period and were then placed on the platform. Their latency to step down on the grid with all four paws was measured. Upon stepping down on the copper bars, the rats received an immediate mild electrical shock (36 V). To avoid the shock, rats demonstrate an instinctive reaction to jump back onto the platform. Rats were tested in this manner for 5 min. The number of times rats stepped down from the platform within 5 min was considered acquisition errors. After 24 h, this procedure was repeated, and the step-down latency was used as a measurement of memory retention. The number of times the rats stepped down onto the platform within the 3-min interval was recorded as retention errors.

Step-through test: a step-through-type passive avoidance test was carried out to evaluate the effect of maifanite on learning and memory. The shuttle box ($60 \times 50 \times 80$ cm) consisted of two compartments of equal size ($20 \times 12 \times 60$ cm). Each compartment was divided into a light and a dark chamber by a guillotine door (3×4 cm). The light chamber was equipped with an illuminator. All the rats were habituated to the light and dark chamber three times for 3 min until they entered the dark compartment within 15 s (training trial). The rats that did not enter the dark compartment within 15 s after the three habituations would be rejected. The test consisted of acquisition and retention sessions carried out 24 and 48 h, respectively, after the training trial. For the acquisition trial, the rats were placed in the illuminated chamber opposite the guillotine door and allowed to move freely. When all four paws were on the grid floor of the dark compartment, a scrambled constant-current foot shock (constant voltage 50 V) was delivered to the grid. The number of times the rats stepped into the dark chamber, the time spent in the dark chamber, and latency to move into the dark chamber within 5 min were recorded automatically by computer. Twenty-four hours after the acquisition trial, the rats were again placed in the light chamber for the retention trial. If a mouse did not enter the dark chamber within 5 min, the latency was recorded as the cutoff time of 5 min. Rats were removed manually from the light chamber when the cutoff time was reached.

Lipid Peroxidation, GSH-Px, and SOD Analyses

After the behavior tests, the rats were sacrificed, and the brains were quickly removed, followed by dissection of the hippocampus on ice. In order to determine the SOD and GSH-Px

activities and the MDA content, the five random rats hippocampus were weighed and homogenized with a buffer consisting of 10 mM sucrose, 10 mM Tris–HCl, and 0.1 mM EDTA (pH 7.4) and then the homogenate was centrifuged at 3,000 rpm for 10 min at 4 °C. The supernatant was used for bioassays.

The activities of SOD and GSH-Px, and the content of MDA were assayed strictly according to the detection kit specification as follows.

Protein assay: (1) steps and methods: 0.05 ml distilled water was added to blank tube, 0.05 ml standard solution was added to respective standard tube and determination tube. Then the mixed solution rests for 10 min after 3 ml CBB_{G250} was added to the three tubes. Take distilled water tube as zero. Read the absorbance at 595 nm, with 1 cm optical path. (2) Calculation: protein level (g/L)=absorbance of test tube/absorbance of standard tube×the concentration of standard solution (g/L).

MDA assay: (1) steps and methods: firstly, 0.1 ml 10 nmol/ml standard sample was added to standard tube, 0.1 ml anhydrous ethanol was added to standard blank tube, and 0.1 ml sample was added to respective test tube and test blank tube. Secondly, 0.1 ml no. 1 reagent was added to the respective four tubes after mixing, then 3 ml no. 2 reagent was added. Thirdly, 1 ml no. 3 reagent was added to respective standard tube, standard blank tube, and test tube, while 1 ml 50 % glacial acetic acid was added to test blank tube. Mix each tube by vortex mixer, then close them, and incubated at 95 °C for 40 min. Remove tubes and cool to room temperature by running water. Centrifuge all sample tubes at 3,000 rpm for 15 min. Remove the supernatant from samples for further analysis. Take distilled water tube as zero. Read the absorbance at 532 nm, with 1 cm optical path. (2) Calculation: MDA level (pmol/mg prot)=(absorbance of test tube−absorbance of blank tube)/(absorbance of standard tube−Absorbance of standard blank tube)×10 nmol/ml×dilution multiple/protein level (mg prot/ml).

SOD assay: (1) steps and methods: firstly, 1.0 ml no. 1 reagent was added to respective test tube and control tube. Secondly, 30 μl sample solution and 0.5 ml distilled water are added to the test tube, 0.53 ml distilled water was added to the control tube. Thirdly, each 0.1 ml no. 2, no. 3, and no. 4 was successively added to each tube. Mix each tube by vortex mixer, then incubated at 37 °C for 40 min. Lastly, 2 ml chromogenic agent was added to each tube. The mixed solution rests for 10 min. Take distilled water tube as zero. Read the absorbance at 550 nm, with 1 cm optical path. (2) Calculation: SOD activity (U/mg prot)=(absorbance of control tube−absorbance of test tube)/absorbance of control tube/50 %×dilution multiple/protein level (mg prot/mL).

GSH-Px assay: (1) steps and methods: firstly, 0.2 ml 1 mmol/L GSH was added to respective control tube and test tube, then 0.2 ml sample solution was added to test tube. Mix each tube then incubated at 37 °C for 5 min. Secondly,

0.1 ml no. 1 reagent was added to respective control tube and test tube. Then mix each tube and incubated at 37 °C for 5 min. Thirdly, 2 ml no. 2 reagent was added to respective control tube and test tube. Then 0.2 ml sample solution was added to control tube. Mix each tube then centrifuge all sample tubes at 3,000 rpm for 10 min. The supernatant was used for further assays. Fourthly, 1 ml GSH standard sample solvent was added to blank tube, 1 ml 20 µmol/L GSH standard solution was added to standard tube, and 1 ml supernatant was added to respective control tube and test tube. Fifthly, 1 ml no. 3, 0.25 ml no. 4, and 0.05 ml no. 5 were successively added to each tube. The mixed solution rests for 15 min. Take distilled water tube as zero. Read the absorbance at 412 nm, with 1 cm optical path. (2) Calculation: GSH-Px activity (U/mg prot)=(absorbance of control tube−absorbance of test tube)/(absorbance of control tube−absorbance of blank tube)×20 µmol/L×dilution multiple/reaction time/(sample quantity×protein level).

Metal Elements Analysis

To transfer the hippocampus into liquid in order to measure metal elements concentration by the inductively coupled plasma-atomic emission spectroscopy (ICP-AES; IRIS Intrepid II XSP, USA Thermo Elemental), a microwave sample preparation system (MD6, Beijing Haotianhui trade Ltd. Corp.) was used. First, the hippocampus (1,000 mg, wet weight) was mixed with 2 mL of 10 % hydrogen nitrate and 1 ml of 30 % hydrogen peroxide. They then were put into the microwave oven for wet digesting for about 1 h, after which we obtained the liquid. By ICP-AES, the elements Fe, Cu, Zn, Mn, Se, and Al in the hippocampus of rats were analyzed. The conditions were 1,200 W from the radio-frequency generator, a plasma argon flow rate of 15 L/min, a cooling gas flow of 14 L/min, a carrier gas flow of 1.0 L/min, a 20-µm entrance slit, a 30-µm exit slit, a height of observation of 15 mm, and an integration time lapse of 5 s.

Statistical Analysis

All statistical analysis was performed using SPSS statistical software package version 13.0. Data were expressed as mean±SD. One-way analysis of variance and LSD test were used for the tests between two or more groups. A P value< 0.05 was considered to be statistically significant.

Results

Step-Down Test

The result of step-down test was shown in Table 1. Compared with normal control group, the acquisition errors and

Table 1 Results of step-down test (mean±SD, n=6)

Group	Acquisition errors	Step-down latency (s)	Retention errors
[A]	0.9±0.3	165±36.7	0.6±0.4
[B]	1.8±0.7*	102±50.2*	0.8±0.6*
[C]	1.1±0.5**	163±31.1**	0.7±0.6**
[D]	0.9±0.4***	149±52.1***	0.6±0.4***
[E]	0.8±0.5***	128±33.3***	0.5±0.3***
[F]	0.7±0.4***	133±29.4***	0.4±0.2***
[G]	0.6±0.3***	147±31.2***	0.3±0.3***

[A] normal control group, [B] AD model group, [C] sham group, [D] positive control group, [E] low-dose maifanite group, [F] middle-dose maifanite group, [G] high-dose maifanite group

*P<0.01, significantly different from [A]; **P>0.05, no significant difference from [A]; ***P<0.05, significantly different from [B]

retention errors increased (P<0.01), but the step-down latency shortened (P<0.01) in AD model group. Compared with AD model group, the acquisition errors and retention errors in low-, middle-, and high-dose maifanite group, and positive control group decreased (P<0.01), but the step-down latency prolonged (P<0.01). There had no significant difference (P>0.05) between sham group and normal control group in the acquisition errors, step-down latency, and retention errors.

Step-Through Test

The result of step-through test was shown in Table 2. Compared with normal control group, the first latency and the latency 24 h later shortened (P<0.01), the number of electric shocks in 5 min 24 h later increased (P<0.01) in AD model group. Compared with AD model group, the first latency and the latency 24 h later prolonged (P<0.05), the number

Table 2 Results of step-through test (mean±SD, n=6)

Group	First latency(s)	Latency(s) 24 h later	Number of electric shocks in 5 min (n) 24 h later
[A]	23.7±10.1	32.8±19.5	6.2±1.7
[B]	13.9±8.2*	21.9±24.2*	7.8±1.5*
[C]	33.7±15.3**	37.9±20.3**	5.4±1.1**
[D]	24.6±17.4***	29.8±23.1***	5.9±2.2***
[E]	15.9±21.6***	24.9±18.9***	7.2±1.2***
[F]	24.1±14.8***	30.7±27.5***	6.5±1.1***
[G]	30.8±14.6***	35.5±17.9***	5.8±1.2***

[A] normal control group, [B] AD model group, [C] sham group, [D] positive control group, [E] low-dose maifanite group, [F] middle-dose maifanite group, [G] high-dose maifanite group

*P<0.01, significantly different from [A]; **P>0.05, no significant difference from [A]; ***P<0.05, significantly different from [B]

of electric shocks in 5 min 24 h later decreased ($P<0.05$) in low-, middle-, and high-dose maifanite groups, and in positive control group. There was no significant difference ($P>0.05$) between sham group and normal control group.

Biochemical Markers of Oxidative Stress in Hippocampus

The levels of the SOD, GSH-Px, and MDA was illustrated in Table 3. Compared with normal control group, the activities of SOD and GSH-Px were decreased, and the content of MDA was increased in AD model group, the differences had statistically significant ($P<0.01$). There were no significant difference between sham group and normal group ($P>0.05$). Compared with AD model group, the activities of SOD and GSH-Px were increased, the content of MDA was decreased in positive control group, but there is no significant difference ($P>0.05$). Compared with AD model group, the activities of SOD and GSH-Px were increased, the content of MDA was decreased in low-, middle-, and high-dose maifanite groups, the differences had statistically significant ($P<0.05$).

Element Analysis in Hippocampus

The hippocampus levels of Fe, Cu, Zn, Mn, Se, and Al of different groups were shown in Table 4. Compared with normal control group, the levels of Cu, Fe, and Al were higher ($P<0.01$) followed by the levels of Mn, Zn, and Se were lower ($P<0.01$) in AD model group. All the element levels showed no significant difference ($P>0.05$) between sham group and normal control group. Compared with AD model group, the levels of Cu, Fe, and Al were lower followed by the levels of Mn, Zn, and Se were higher in positive control group, but there is no significant difference

($P>0.05$). Compared with AD model group, the levels of Al, Fe, and Cu in low-, middle-, and high-dose maifanite groups decreased ($P<0.05$), but the level of Mn, Zn, and Se were increased ($P<0.05$). The imbalance levels of Fe, Cu, Zn, Mn, Se, and Al were regulated by maifanite. The levels of Fe, Cu, Zn, and Mn in high-dose maifanite group were near to those of normal control group. The level of Al in high-dose maifanite group still doubled that of normal control group. The Se level in high-dose maifanite group was higher than that in normal control group.

Discussion

Compared with normal control group, $A\beta_{(25-35)}$ ventricle injection induced significant elevation in hippocampus MDA level accompanied by significant reduction of the activities of GSH-Px and SOD in AD model group. The results suggested that $A\beta_{(25-35)}$ ventricle injection could cause a defect in the antioxidant defense system that was incapable of responding to increased free radical production, which might lead to oxidative damage. Compared with AD model group, the activities of GSH-Px and SOD in hippocampus were obviously enhanced followed by evidently reduced MDA level in the maifanite treatment groups. All these findings suggested that maifanite might relieve oxidative stress in AD rats and thus played a potential prevention and treatment role in AD.

AD presents marked accumulations of oxidative stress-induced damage, and increasing evidence points to aberrant transition metal homeostasis as a critical factor in its pathogenesis [6]. In order to clarify the potential mechanism, the hippocampus levels of Fe, Cu, Zn, Mn, Se, and Al were determined by ICP-AES technique. The results showed that hippocampus Al, Cu, Fe, Mn, Se, and Zn were variable in $A\beta$-induced AD rats. Moreover, it was cleared that markedly increased Al concentration affected the concentration of other elements by increasing the concentration of Fe and Cu and decreasing the concentration of Mn, Se, and Zn. Trace Al levels cross the blood–brain barrier and progressively accumulate in large pyramidal neurons of the hippocampus, cortex, and other brain regions vulnerable in AD [19]. As a prooxidant, Al causes oxidative damage both on its own and in synergy with Fe [19]. Our results also demonstrated that the increase Cu and Fe in $A\beta$-induced AD rats. Cu is an essential element for the activity of cytochromeC oxidase, Cu/ZnSOD, and dopamine-beta-hydroxylase, which is critical in scavenging ROS. The increased Cu generates reactive oxygen species which leads to oxidative stress and contributes to the cell death pathway [20]. Fe plays a key role in oxidative stress [21] and readily

Table 3 Activities of SOD and GSH-Px, content of MDA in hippocampus of rats (mean±SD, $n=6$)

Group	SOD (U/mg prot)	GSH-Px (U/mg prot)	MDA (pmol/mg prot)
[A]	90.6±22.5	77.6±31.9	4.3±2.9
[B]	47.4±19.8*	36.7±15.5*	9.2±1.4*
[C]	87.7±13.8**	80.2±15.7**	4.1±1.3**
[D]	58.8±20.1***	47.9±15.3***	8.8±1.1***
[E]	63.1±12.3****	53.4±16.7****	7.2±1.2****
[F]	78.2±8.8****	64.9±18.6****	6.3±2.2****
[G]	81.4±5.9****	73.6±19.5****	5.6±1.9****

[A] normal control group, [B] AD model group, [C] sham group, [D] positive control group, [E] low-dose maifanite group, [F] middle-dose maifanite group, [G] high-dose maifanite group

*$P<0.01$, significantly different from [A]; **$P>0.05$, no significant difference from [A]; ***$P>0.05$, no significant difference from [B]; ****$P<0.05$, significantly different from [B]

Table 4 Results of elementary analysis in hippocampus of rats (mean±SD, $n=6$)

Group	Element (µg/g wet weight)					
	Fe	Cu	Zn	Mn	Se	Al
[A]	41.36±9.01	1.61±0.41	26.67±1.92	1.71±0.61	0.186±0.049	0.028±0.004
[B]	54.29±5.83*	2.05±0.29*	13.76±2.86*	0.97±0.51*	0.087±0.011*	0.399±0.144*
[C]	28.5±9.55**	1.58±0.45**	29.38±2.52**	2.13±0.44**	0.159±0.059**	0.032±0.011**
[D]	33.01±8.88***	1.87±0.45***	17.77±5.57***	1.12±0.49***	0.098±0.075***	0.343±0.045***
[E]	49.95±11.37****	1.96±1.01****	15.29±3.44****	1.22±0.45****	0.109±0.099****	0.311±0.057****
[F]	46.75±9.34****	1.79±0.44****	21.19±3.34****	1.39±0.88****	0.133±0.084****	0.225±0.087****
[G]	43.97±11.33****	1.69±1.32****	24.38±4.23****	1.82±0.45****	0.169±0.087****	0.103±0.058****

[A] normal control group, [B] AD model group, [C] sham group, [D] positive control group, [E] low-dose maifanite group, [F] middle-dose maifanite group, [G] high-dose maifanite group

*$P<0.01$, significantly different from [A]; **$P>0.05$, no significant difference from [A]; ***$P>0.05$, no significant difference from [B]; ****$P<0.05$, significantly different from [B]

reduces hydrogen peroxide to liberate the reactive and unselective hydroxyl radical, capable of inflicting severe oxidative damage [22]. Our results also demonstrated the decrease concentrations of Mn, Zn, and Se in Aβ-induced AD rats. Mn is an essential trace element required for ubiquitous enzymatic reactions. Chronic over-exposure to this metal may promote potent neurotoxic effects. The mechanism of Mn toxicity is not well established, but several studies indicate that oxidative stress play major roles in the Mn-induced neurodegenerative processes [23]. Zn is an antioxidant metal required as a cofactor for Cu/ZnSOD and Zn–thionein [24]. Increasing evidence suggests that the etiology of AD may involve disruptions of Zn homeostasis [25]. Zn has critical functions in the brain, and lack of Zn can cause neuronal death [26]. Se, a nutritionally essential trace element with known antioxidant potential, protects the brain from oxidative damage in various models of neurodegeneration [27]. AD has an important relation with Se deficiency [28, 29].

Based on the results of ICP-AES, it was notable that there was an internal relationship among the elements that maintain the homeostasis of biological system. What is more, Aβ$_{(25–35)}$ ventricle injection could disturb the element homeostasis, and the change of external environment or the abnormalities of a single element concentration would affect the total element distribution pattern in the biological system. In the present study, the maifanite was used to regulate the dyshomeostasis of elements in Aβ-induced AD rats. Compared with AD model group, the hippocampus levels of Mn, Se, and Zn in low-, middle-, and high-dose maifanite groups increased, and those of Al, Fe, and Cu decrease. The larger the concentration of maifanite was, the stronger the regulating effect was. The levels of Fe, Cu, Zn, and Mn in high-dose maifanite group were near to those of normal control group. The level of Al in high-dose maifanite group still doubled that of the normal control group. Al could not thoroughly be cleared. The other most intriguing finding in this study might be that the Se level in high-dose maifanite group was higher than that in normal control group. These results were very encouraging and deserved further research.

Conclusion

Aβ$_{(25–35)}$ ventricle injection caused the impairment of learning and memory of rats, resulted in the decrease of the activities of SOD and GSH-Px and the increase of the level of MDA, and triggered the rise of Al, Fe, and Cu levels and the decline of Mn, Zn, and Se levels. Maifanite could improve the learning and memory abilities of AD rats. Moreover, the activities of SOD and GSH-Px were enhanced; the level of MDA reduced and the levels of Mn, Se, and Zn increased. And at the same time Al, Fe, and Cu decreased in the maifanite treat groups. The intake of maifanite promoted the unbalance of trace elements in AD rats to reach towards balance state, which was helpful to increase the activities of SOD and GSH-Px. Sequentially, the free radicals could be effectively eliminated, oxidation effect be weakened, MDA level be reduced. Based on these studies, it could be concluded that maifanite could balance the dyshomeostasis of trace elements in Aβ-induced AD rats, had the potential prevention, and treatment effect on AD.

References

1. Stéphan A, Phillips AG (2005) A case for a non-transgenic animal model of Alzheimer's disease. Genes Brain Behav 4:157–172
2. Castellani RJ, Rolston RK, Smith MA (2010) Alzheimer disease. Dis Mon 56:484–546
3. Ozcelik D, Uzun H, Nazıroglu M (2012) N-acetylcysteine attenuates copper overload-induced oxidative injury in brain of rat. Biol Trace Elem Res 147(1–3):292–298
4. Takahashi S, Takahashi I, Sato H, Kubota Y, Yoshida S, Muramatsu Y (2001) Age-related changes in the concentrations of major and trace elements in the brain of rats and mice. Biol Trace Elem Res 80(2):145–158
5. Nazıroglu M, Dikici DM, Dursun S (2012) Role of oxidative stress and Ca(2+) signaling on molecular pathways of neuropathic pain in diabetes: focus on TRP channels. Neurochem Res 37:2065–2075
6. Bonda DJ, Lee HG, Blair JA, Zhu X, Perry G, Smith MA (2011) Role of metal dyshomeostasis in Alzheimer's disease. Metallomics 3:267–270
7. Juan L, Zhang PY, Gao Y, Song XG, Dong JH (2008) Overview of maifanshi: its physico-chemical properties and nutritious function in drinking water. Environ Sci Technol 31:63–66
8. Zhang BG (2005) The progress of pharmaceutical research on the maifanite. Chinese Traditional Patent Med 27:1205–1208
9. Yao RZ (1991) The discussion on dissolution mechanism of trace elements in maifanite. Henan Geology 9:10–15
10. Liu ZY, Liu Q, Ma JK, Zhang CW (1986) Study on pharmacologic action of China maifanite. Jilin J Traditional Chinese Med 4:28–30
11. Nabeshima T, Nitta A (1994) Memory impairment and neuronal dysfunction induced by beta-amyloid protein in rats. Tohoku J Exp Med 174:241–249
12. Hashimoto M, Hossain S, Agdul H, Shidao O (2005) Docosahexaenoic acid-induced amelioration on impairment of memory learning in amyloid beta-infused rats relates to the decreases of amyloid beta and cholesterol levels in detergent-insoluble membrane fractions. Biochim Biophys Acta 1738:91–98
13. Diaz A, Limon D, Chávez R, Zenteno E, Guevara J (2012) Aβ25-35 injection into the temporal cortex induces chronic inflammation that contributes to neurodegeneration and spatial memory impairment in rats. J Alzheimers Dis 30:505–522
14. Huang TC, Lu KT, Wo YY, Wu YJ, Yang YL (2011) Resveratrol protects rats from Aβ-induced neurotoxicity by the reduction of iNOS expression and lipid peroxidation. PLoS One 6:e29102
15. Díaz A, De Jesús L, Mendieta L, Calvillo M, Espinosa B, Zenteno E, Guevara J, Limón ID (2010) The amyloid-beta25-35 injection into the CA1 region of the neonatal rat hippocampus impairs the long-term memory because of an increase of nitric oxide. Neurosci Lett 468:151–155
16. Limón ID, Díaz A, Mendieta L, Chamorro G, Espinosa B, Zenteno E, Guevara J (2009) Amyloid-beta(25–35) impairs memory and increases NO in the temporal cortex of rats. Neurosci Res 63:129–137
17. Paxinos G, Watson C (2005) The rat brain in sterotaxic coordinates, 5th edn. Elsevier, Sydney
18. Xu SY, Bian RL, Chen X (2002) Experimental protocols in pharmacology, 3rd edn. People's Medical Publishing House, Beijing
19. Walton JR (2012) Aluminum disruption of calcium homeostasis and signal transduction resembles change that occurs in aging and Alzheimer's disease. J Alzheimers Dis 29:255–273
20. Carrí MT, Ferri A, Cozzolino M, Calabrese L, Rotilio G (2003) Neurodegeneration in amyotrophic lateral sclerosis: the role of oxidative stress and altered homeostasis of metals. Brain Res Bull 61:365–374
21. Dai XL, Sun YX, Jiang ZF (2006) Cu(II) potentiation of Alzheimer Abeta1-40 cytotoxicity and transition on its secondary structure. Acta Biochim Biophys Sin (Shanghai) 38:765–772
22. Huang X, Atwood CS, Hartshorn MA, Multhaup G, Goldstein LE, Scarpa RC, Cuajungco MP, Gray DN, Lim J, Moir RD, Tanzi RE, Bush AI (1999) The A beta peptide of Alzheimer's disease directly produces hydrogen peroxide through metal ion reduction. Biochemistry 38:7609–7616
23. Chtourou Y, Trabelsi K, Fetoui H, Mkannez G, Kallel H, Zeghal N (2011) Manganese induces oxidative stress, redox state unbalance and disrupts membrane bound ATPases on murine neuroblastoma cells in vitro: protective role of silymarin. Neurochem Res 36:1546–1557
24. Mustak MS, Rao TS, Shanmugavelu P, Sundar NM, Menon RB, Rao RV, Rao KS (2008) Assessment of serum macro and trace element homeostasis and the complexity of inter-element relations in bipolar mood disorders. Clin Chim Acta 394:47–53
25. Lyubartseva G, Lovell MA (2012) A potential role for zinc alterations in the pathogenesis of Alzheimer's disease. Biofactors 38:98–106
26. Brewer GJ (2012) Copper excess, zinc deficiency, and cognition loss in Alzheimer's disease. Biofactors 38:107–113
27. Ishrat T, Parveen K, Khan MM, Khuwaja G, Khan MB, Yousuf S, Ahmad A, Shrivastav P, Islam F (2009) Selenium prevents cognitive decline and oxidative damage in rat model of streptozotocin-induced experimental dementia of Alzheimer's type. Brain Res 1281:117–127
28. Loef M, Schrauzer GN, Walach H (2011) Selenium and Alzheimer's disease: a systematic review. J Alzheimers Dis 26:81–104
29. Cardoso BR, Ong TP, Jacob-Filho W, Jaluul O, Freitas MI, Cozzolino SM (2010) Nutritional status of selenium in Alzheimer's disease patients. Br J Nutr 103:803–806