Geniposide Attenuates Mitochondrial Dysfunction and Memory Deficits in APP/PS1 Transgenic Mice

Cui Lv¹,²,³,⁴, Xiaoli Liu¹,²,³,⁴, Zhi Li¹,²,³,⁴, Hongjuan Liu¹,²,³,⁴, Tong Chen⁵ and Wensheng Zhang¹,²,³,⁴,*

¹State Key Laboratory of Earth Surface Processes and Resource Ecology, Beijing Normal University, Beijing 100875, China; ²Beijing Area Major Laboratory of Protection and Utilization of Traditional Chinese Medicine, Beijing 100088, China; ³Engineering Research Center of Natural Medicine, Ministry of Education, Beijing 100088, China; ⁴College of Resources Science Technology, Beijing Normal University, Beijing 100875, China; ⁵School of Pharmaceutical Sciences and Yunnan Key Laboratory of Pharmacology for Natural Products, Kunming Medical University, Kunming 650504, China

Abstract: Oxidative stress and mitochondrial dysfunction appear early and contribute to the disease progression in Alzheimer’s disease (AD), which can be detected extensively in AD patients brains as well as in transgenic AD mice brains. Thus, treatments that result in attenuation of oxidative stress and mitochondrial dysfunction may hold potential for AD treatment. Geniposide, a pharmacologically active component purified from gardenia fruit, exhibits anti-oxidative, anti-inflammatory and other important therapeutic properties. However, whether geniposide has any protective effect on oxidative stress and mitochondrial dysfunction in AD transgenic mouse model has not yet been reported. Here, we demonstrate that intragastric administration of geniposide significantly reduces oxidative stress and mitochondrial dysfunction in addition to improving learning and memory in APP/PS1 mice. Geniposide exerts protective effects on mitochondrial dysfunction in APP/PS1 mice through suppressing the mitochondrial oxidative damage and increasing the mitochondrial membrane potential and activity of cytochrome c oxidase. These studies indicate that geniposide may attenuate memory deficits through the suppression of mitochondrial oxidative stress. Thus, geniposide may be a potential therapeutic reagent for halting and preventing AD progress.

Keywords: Alzheimer’s disease, APP/PS1 transgenic mice, cytochrome c oxidase, geniposide, mitochondrial dysfunction, oxidative stress.

INTRODUCTION

Alzheimer’s disease (AD) is a neurodegenerative disease, which is characterized by age-dependent memory loss and impairments of multiple cognitive functions [1-4]. The typical pathological features of AD are the presence of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs), progressive synapse and neuronal loss, the proliferation of reactive astrocytes and microglia, oxidative stress and mitochondrial dysfunction [5]. To date, many hypotheses have been proposed to explain the origins of AD. A large number of evidence demonstrate that oxidative stress is an early event and plays a key pathogenic role in AD. It occurs prior to the appearance of cytopathology, such as amyloid plaques and NFTs [6-9]. Extensive free radical-induced oxidative damage, particularly to nucleic acids [10], lipids [11] and proteins [12, 13] were found in brains, biologic fluids and peripheral tissues of AD patients, as well as in transgenic AD mice models.

Mitochondria are critical for meeting the high energy demands of the brain, but they also generate the majority of intracellular reactive oxygen species (ROS), which can cause oxidative damage to important cellular structures. In AD, damaged mitochondria have been observed in brains, fibroblasts and blood cells of AD patients, as well as in AD transgenic mice models [14-20]. Moreover, it is reported that molecular indices of mitochondrial dysfunction occur early in AD and worsen with its progression [21, 22]. These findings suggest that early antioxidant therapy targeting at mitochondria may have potential therapeutic advantages for halting the onset and progression of AD.

Geniposide is an iridoid glucoside isolated from the gardenia fruit (Gardenia jasminoides Ellis, Rubiaceae), and has diverse pharmacological capabilities including anti-inflammatory [23], anti-oxidation [24] and anti-tumor [25] effects as well as neurotrophic and neuroprotective properties. Importantly, some studies have demonstrated geniposide could cross the blood brain barrier and reported its protective property in brain [26]. However, whether geniposide has any protective effect on oxidative stress and mitochondrial dysfunction in AD transgenic mice models has not yet been reported. In this study, we assessed the effects of geniposide on the oxidative stress and mitochondrial dysfunction associated with memory deficits in APP/PS1 mice. Our results clearly show that geniposide treatment significantly improves mitochondrial function contributing
to attenuated memory impairment at the early stage of AD in APP/PS1 mice.

MATERIAL AND METHODS

Reagents

Geniposide (Purity: >96%, Lot. Q/ZX003-2009, Fig. 1) was purchased from Lin Chuan Zhi Xin Biotechnology Co., Ltd. (Jiangxi, China), and was free of endotoxin. Monoclonal antibodies to Cytochrome c Oxidase (CcO), voltage-dependent anion channels (VDAC), lysosomal-associated membrane protein 1 (LAMP1) and calnexin were purchased from Abcam (Abcam, Cambridge, UK). Goat anti-rabbit and goat anti-mouse antibodies were provided by GE Healthcare (Buckinghamshire, UK). The kits employed to isolate the mitochondria and detect the levels of MDA, the levels of mitochondrial oxidative damage and Cytochrome c Oxidase (CcO) activity were purchased from Shanghai GENMED pharmaceutical technology Co., Ltd. (Shanghai, China). 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) and rhodamine 123 (Rh-123) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals were of reagent grade.

Fig. (1). Chemical structure of geniposide.

Animals and Drug Administration

Experimental male mPrP-APPswe/PS1dE9 AD transgenic mice (3 months, male), weighing 25-30g, were purchased from the Beijing HFK Bio-Technology. Co., LTD. Animals were housed 1 per cage at an ambient temperature of 23 ± 1 °C and relative humidity 50 ± 5% under a 12-h light/dark cycle, and allowed free access to water and food. Before experimentation, mice were housed under these conditions for 2–3 days to allow them to adapt to the environment. APP/PS1 mice and age-matched C57BL/6 mice were randomly split between treatment groups. APP/PS1 mice were treated with either geniposide (12.5 mg/kg/d, 25 mg/kg/d and 50 mg/kg/d; n = 15/each group) or water (n = 15) for three months via intragastric administration. Age-matched C57BL/6 mice were fed with water as the vehicle control (n = 15). Geniposide was dissolved in water before use within 24 hours. An equal volume of liquid was given to each group once per day for 3 months before being sacrificed. All animal procedures followed the “Guide for the care and use of laboratory animals” [27] and Beijing Normal University Laboratory Animals Care and Use Committee approved the study protocol.

Morris Water Maze Test

Place Navigation Test. We evaluated the spatial learning and memory ability in response to treatment with geniposide in APP/PS1 mice using the Morris water maze test [28, 29]. For 2 days prior to the experiments, mice were familiarized with the water maze environment in the morning and afternoon. For each training trial, the mice were placed into the maze from the southeast, northeast, southwest or northwest. Mice were given four trials per day from day 3 today 7. A quadrant was selected randomly, and the mice were placed into the water along the wall with their back against the platform. Escape latency was designated as the length of time that mice took to reach the platform. If mice could not find the platform within 90 sec, they were led to it by the experimenter, and the latency was recorded as 90 sec. The swimming trajectory and movement distance were also recorded to judge learning capacity. All experiments were carried out at approximately the same time each day. The investigator was blinded to mouse genotypes until behavioral testing was completed.

Spatial Probe Test. On the 8th day of the experiment, the platform was withdrawn. A quadrant was selected randomly, and the mice were placed into the water with their face toward the wall. The swimming trajectory and the number of crossings of the original platform within 1 min were recorded to judge memory capacity of mice.

Visible-Platform Test. For the visible-platform test, on day 9, the platform was raised above the surface of the water and placed in a different position than the hidden-platform. Mice were given four trials. If mice floated rather than swam, they were disqualified from statistical analysis.

Measurement of ROS

ROS was measured with 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA), which detects most ROS including hydrogen peroxide, peroxyl radical, and peroxy-nitrite anion. DCFH-DA can be oxidized to dichlorofluorescein (DCF) by ROS, which is an intensely fluorescent chemical. Therefore, the fluorescence intensity of DCF reflects the level of ROS. In brief, mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) and flushed-perfused transcardially with 0.9 % saline. The brains were removed and stored in a –80°C freezer. Cerebral cortex were weighed and homogenized in 3 volumes of ice-cold saline and then further homogenized by DOUNCE homogenizer on ice. The protein concentrations of homogenate samples were determined by the BCA method, and then samples were diluted with PBS for determination of ROS. The samples from different groups (0.5 mg protein) were incubated with 10 μM DCFA at 37 °C for 60 min, and the fluorescence intensity of DCF was measured with excitation at 485 nm and recording at 528 nm on the fluorescence microplate reader (BioTek Senergy HT, Vermont, USA). The results of ROS levels were presented as arbitrary fluorescence units (AFU).

Measurement of MDA

The contents of malondialdehyde (MDA) were measured by using commercially available assay kits according to the instruction recommended by the manufacturers. In brief, cerebral cortex were weighed and homogenized in 3 volumes of ice-cold saline and then further homogenized by sonication using 5 pulses of 30 seconds each on ice. The homogenates were centrifuged at 3000 g for 20 min at 4 °C to obtain
the supernatant. The protein concentrations of homogenate samples were determined by the BCA method, and then samples were diluted with the assay buffer solution in the kit for determination of MDA. The optical density (OD) of the microplate was read at 532 nm.

Isolation of Mitochondria

Mitochondria were isolated from the cerebral cortex of mice using the mitochondria isolation kit according to the instruction recommended by the manufacturers. Briefly, tissues were homogenized five times the volume of ice-cold isolation buffer, and then the homogenate was centrifuged at 1500 × g for 10 min and the resultant supernatant was subjected to centrifugation at 10,000 × g for 10 min. The pellet was collected and resuspended in ice-cold storage solution and stored at −80 °C to be used for the biochemical measurements.

Western Blot Analysis

The purity of mitochondria was determined by the presence of mitochondrial markers (CcO and VDAC (the components of the inner and outer membrane)), and relative absence of endoplasmic reticulum and lysosomal markers (Calnexin and LAMP1, respectively) via western blot assay. Mitochondria isolated from the brain tissues of mice were prepared in lysis buffer according to the instruction recommended by the manufacturers. After the determination of protein concentration by the BCA method, the samples were analyzed by western blot according to standard protocols. In brief, equal amount of proteins (40 μg/10 μl) were loaded and separated by SDS-PAGE and transferred to nitrocellulose membrane (Millipore). Membrane was blocked in PBST buffer (20 mM Tris–HCl, 150 mM NaCl, 0.1% Tween-20) containing 5% nonfat dry milk (Santa Cruz) for 1 h at room temperature on a rotary shaker, and then incubated and gently shaken overnight (at 4 ºC ) with primary antibodies against CcO (1:500), VDAC(1:500), LAMP1(1:2000) or Calnexin (1:1000) in PBST containing 0.1% Tween-20) containing 5% nonfat dry milk (Santa Cruz) for 1 h at room temperature on a rotary shaker, and then incubated and gently shaken overnight (at 4 ºC ) with primary antibodies against CcO (1:500), VDAC(1:500), LAMP1(1:2000) or Calnexin (1:1000) in PBST containing 5% nonfat dry milk; this was followed by incubation with corresponding secondary antibody for 1 h at room temperature. Blots were washed twice with PBST and then developed with an Infrared Imaging System (Odyssey). Intensity of blots was analyzed and compared using the NIH Image J program.

Measurement of the Level of Mitochondrial Oxidative Damage

The Nonyl-Acrydine Orange (NAO) assay was used to measure the level of mitochondrial oxidative damage. Cardiolipin is one of the main components of the mitochondrial membrane which is particularly sensitive to intracellular oxidation. The lipolysis of mitochondria causes the significant reduction of cardiolipin, resulting in serious damage of mitochondria. NAO is a kind of specific fluorescent dye staining on the cardiolipin of mitochondria. Once the quality of mitochondrial membrane is reduced, oxidized or damaged, its fluorescence will decrease significantly. The measurements were performed with commercial kit according to the manufacturer’s instruction. The fluorescence signal of NAO was measured on a fluorescence microplate reader (BioTek Senergy HT, Vermont, USA) with λex 580 nm and λem 630 nm.

Measurement of Cytochrome c Oxidase (CcO) Activity

CcO activity was measured according to the method reported previously [30]. In brief, mitochondria (2 μg) were added to a cuvette containing 0.95 ml of 1× assay buffer (10 mM Tris-HCl, pH 7.0, and 120 mM KCl), and the reaction volume was brought to 1.05 ml with 1× enzyme dilution buffer (10 mM Tris-HCl, pH 7.0). The reaction was then initiated by the addition of 50 μl of ferrocytochrome c substrate solution (0.22 mM) (from Sigma, USA). The change in the absorbance of cytochrome c at 550 nm was measured using a UV-2450 spectrophotometer (Shimadzu company, JAPAN). The reading was recorded every 10 sec during the first 3 minutes. Background levels were measured without mitochondria.

Measurement of Mitochondrial Membrane Potential

Mitochondrial membrane potential (ΔΨM) was evaluated with fluorescent dye rhodamine 123 (Rh-123) according to the method reported previously [31, 32]. The uptake of Rh-123 reflects ΔΨM because of the electrophoretic accumulation of Rh-123 into energized mitochondria in response to their negative inside membrane potential. In brief, mitochondria isolated from brains of the mice (0.5 mg protein) were added into the media (150 mM sucrose, 5 mM MgCl2, 5 mM potassium phosphate, and 20 mM KOH–HEPES pH 7.4) containing 0.3 μM Rh-123. After 5 min of equilibrium at 37 °C, ΔΨM was assessed at an excitation wavelength of 505 nm and emission wave length of 528 nm on the fluorescence plate reader (BioTek Senergy HT, Vermont, USA). As described by Chen et al. [32], “the fluorescence of the suspension was measured and the contents of the well were centrifuged at 15,000 g to pellet the mitochondria. The Rh-123 concentration remaining in the media ([Rh-123] out) in, nmol/μL) was calculated from the fluorescence values of the supernatant. The initial total amount of Rh-123 in the well ([Rh-123] total) and the amount remaining in the media ([Rh-123] out) were used to calculate the total amount of Rh-123 taken up by mitochondria ([Rh-123] mit, in nmol/mg protein) by subtracting ([Rh-123] mit = [Rh-123] total - [Rh-123] out). The concentration of free Rh-123 in the matrix ([Rh-123] in, in nmol/μL) was calculated using the following equation, and the binding partition coefficients at 37 °C (Ki = 26 μL/mg, Ko = 120 μL/mg) [33]: [Rh-123] mit = Ki [Rh = 123] in + Ko [Rh = 123] out. Mitochondrial membrane potentials (negative inside) were calculated by the electrochemical Nernst–Guggenheim equation: ΔΨM = 59 log ([Rh-123] in / [Rh-123] out).”

Statistical Analyses

The results were processed for statistical analysis using the SPSS (version 13.0 for Windows). All values are presented as the means ± SEM. In Fig. (2), statistical comparisons between groups of the escape latencies in Morris water maze test were performed by applying two-way repeated-measures ANOVA, following by Fisher's Protected Least Significant Difference (LSD) test for post-hoc comparisons. The rest of the biochemical data were analyzed using a one-
way ANOVA (Dunnett's t-test) and two-tailed Student's t-test. Statistical significance was defined as p < 0.05 or p < 0.01.

RESULTS

Geniposide Attenuates the Memory Impairment at the Early Stage of AD in APP/PS1 Mice

To examine the effect of geniposide on the learning and memory at the early stage of AD, we used 3-month-old APP/PS1 transgenic mice in our studies. After three-month intragastric administration, the five groups of mice (vehicle-treated C57 mice, vehicle-, and geniposide (12.5, 25, 50 mg/kg/d) -treated APP/PS1 mice) were subjected to the Morris water maze test to detect memory ability. As shown in Fig. (2A), the mean escape latency of APP/PS1 mice was significantly increased compared to wild-type mice (P < 0.01), while 25 or 50 mg/kg/d geniposide-treated APP/PS1 mice showed significant improvements compared to APP/PS1 mice after the training periods (P < 0.01). There were no significant differences in swimming speed and escape latency among all the groups (P > 0.05, Fig. 2B,C.), suggesting that the memory impairments of APP/PS1 mice were not due to visual or motor dysfunctions or motivational shortage.

After the platform test, all mice were subjected to a spatial probe test to evaluate their memory retention and spatial exploration ability. The number of crossings of the platform of vehicle-treated APP/PS1 mice was significantly reduced, while after treatment with geniposide, the number was significantly increased (Fig. 2D). Moreover, geniposide-treated APP/PS1 mice spent more time in the target quadrant than APP/PS1 mice (Fig. 2E). Importantly, all of the improvements by geniposide were in a dose-dependent manner. Taken together, these results demonstrated that geniposide treatment attenuated the memory deficits significantly at the early age of AD in APP/PS1 mice.

Geniposide Suppresses Oxidative Stress in the Brain of APP/PS1 Mice

Several studies have now determined oxidative stress as one of the primary events in the course of AD. This stress is manifested by damage to lipids, proteins and nucleic acids. It is known that the brain is particularly vulnerable to free radical attack for many reasons, such as its high oxygen concentrations and low antioxidant protection. To characterize geniposide’s ability to inhibit oxidative stress in the brains of APP/PS1 mice, the levels of ROS and marker of lipid peroxidation (Malondialdehyde, MDA) were examined. The production of ROS in the cerebral cortex of APP/PS1 mice were significantly increased by 89.98% (P < 0.01) relative to age-matched wild-type mice. While the ROS levels in APP/PS1 mice treated with geniposide were reduced in a dose-dependent manner relative to APP/PS1 mice treated with vehicle (Fig. 3A). As shown in Fig. (3B), MDA levels in the cerebral cortex of APP/PS1 mice were significantly increased by 84.6% (P < 0.01) relative to age-matched wild-type mice. While MDA levels in APP/PS1 mice treated with geniposide were respectively reduced 13.9%, 23.6% and 37.5% relative to APP/PS1 mice treated with vehicle.

Geniposide Protects Against Mitochondrial Dysfunction in APP/PS1 Mice

Mitochondria are primary sites of ROS generation and mitochondrial dysfunction is an early event in the process of AD. To evaluate the effect of geniposide on mitochondrial function and properties in the brain of APP/PS1 mice, we measured the level of mitochondrial oxidative damage, mitochondrial membrane potential and mitochondrial enzyme activity associated with the respiratory chain, cytochrome c oxidase (CcO). To verify the preparation of mitochondrial fractions, mitochondrial (lane 1), and non-mitochondrial (lane 2) fractions (Fig. 4A) were subjected to immunoblotting with antibodies specific to LAMP-1 (lysosome marker), Calnexin (endoplasmic reticulum marker), VDAC (mitochondrial outer membrane protein) and CeO (mitochondrial inner membrane protein). Mitochondrial fractions had strong immunoreactive bands for mitochondrial markers (VDAC and CeO) but not for the other protein markers (LAMP-1 and Calnexin). These data indicate that the mitochondrial fractions were free from other organelle contamination. Next, the mitochondrial fractions were subjected to the further study.

Firstly, we evaluated the level of mitochondrial oxidative damage was measured using NAO, a kind of specific fluorescent dye staining on the cardiolipin of mitochondria. Once the quality of mitochondrial membrane is reduced, oxidized or damaged, the fluorescence of NAO will decrease significantly. As shown in Fig. (4B), a significant mitochondrial oxidative damage was found in APP/PS1 mice (~40% of WT mice), which could be alleviated after geniposide treatment in a dose-dependent manner (~45%, ~55% and ~80% of WT mice).

Secondly, we evaluated the level of mitochondrial membrane potential (ΔΨM) using Rh-123 as an indicator. As shown in Fig. (4C), APP/PS1 mice showed a statistically significant decrease in ΔΨM when compared to the WT mice. The ΔΨM of WT mice was 187.20 ± 8.12 mV, while this value dropped to 131.98 ± 8.32 mV in APP/PS1 mice. However, treatment with varying doses of geniposide showed a dose-dependent increase in ΔΨM as compared to vehicle-treated APP/PS1 mice (138.86 ± 5.73 mV, 152.40 ± 9.29 mV and 164.10 ± 7.46 mV).

Lastly, we also analyzed mitochondrial respiratory function by measuring CcO activity. CcO (complex IV) is one of the enzyme complexes that compose the mitochondrial respiratory chain. It is vital to the mitochondrial respiratory function. However, “defects in CcO activity have been shown in early stage AD brains from AD mouse models and Aβ-treated neurons” [30]. APP/PS1 mice demonstrated significantly decreased CcO activity (~60% of WT mice, Fig. 4D). Notably, geniposide treatment significantly increased CcO activity as compared to vehicle-treated APP/PS1 mice in a dose-dependent manner (~70%, ~83.3% and ~90% of WT mice).

DISCUSSION

This study demonstrated for the first time that geniposide could attenuate memory deficits, oxidative stress and mitochondrial dysfunction in APP/PS1 mice. Geniposide attenuates the extent of increased mitochondrial oxidative damage,
Fig. (2). Geniposide treatment reverses spatial memory deficits in 6-month-old APP/PS1 mice in the Morris water maze test. A. Escape latency in the hidden-platform test. Vehicle-treated APP/PS1 mice showed significantly longer escape latencies in the hidden-platform test when compared with WT mice (\(^*\) \(p < 0.01\)) and the geniposide-treated APP/PS1 mice (\(##\) \(p < 0.01\) geniposide-treated APP/PS1 mice vs vehicle-treated APP/PS1 mice). B. Escape latency in the visible-platform test. No significant differences were detected among groups during any of 4 sessions. C. Swimming speed during the Morris water maze test. D. The number of annulus crossings; vehicle-treated APP/PS1 mice performed significantly worse than geniposide treated or WT mice in the probe trials. (\(^*\) \(p < 0.01\) vs WT mice; \(\#\) \(p < 0.05\), \(##\) \(p < 0.01\) vs vehicle-treated APP/PS1 mice). E. Time spent in the target vs. opposite quadrants. (\(^*\) \(p < 0.01\) vs WT mice; \(\#\) \(p < 0.05\), \(##\) \(p < 0.01\) vs vehicle-treated APP/PS1 mice). Data represent mean \(\pm\) SEM (\(n = 15\)).
Fig. (3). Effect of geniposide on the levels of ROS and MDA in the cerebral cortex of APP/PS1 mice. The levels of ROS (A) and MDA (B) were significantly elevated in vehicle-treated APP/PS1 mice compared to WT mice, and partially restored in geniposide-treated APP/PS1 mice in a dose-dependent manner. Data represent mean ± SEM (n = 7–9 mice per group). **p < 0.01 vs WT mice, ^p < 0.05, ^^^p < 0.01 vs vehicle-treated APP/PS1 mice.

Fig. (4). Geniposide suppresses mitochondrial dysfunction in the cerebral cortex of APP/PS1 mice. A, Characterization of isolated mitochondrial fractions. To verify the preparation of mitochondrial fractions, mitochondrial (lane 1), and non-mitochondrial (lane 2) fractions were subjected to immunoblotting with antibodies specific to LAMP-1 (lysosome marker), Calnexin (endoplasmic reticulum marker), VDAC (mitochondrial outer membrane protein) and CcO (mitochondrial inner membrane protein). B, The level of mitochondrial oxidative damage of mitochondria from the brain of mice. C, The mitochondrial membrane potential levels in the mitochondrial fractions. D, CcO activity was assayed by spectrophotometer. Data represent mean ± SEM (n = 7–9 mice per group). **p < 0.01 vs WT mice; ^p < 0.05, ^^^p < 0.01 vs vehicle-treated APP/PS1 mice.
decreased CcO activity and mitochondrial membrane potential in the brain of APP/PS1 mice, contributing to the decrease of oxidative stress and improvement of memory impairment at the early stage of AD.

“The onset of memory impairment progressing to dementia is the main clinical symptom of AD”[34]. It has been demonstrated that the cognitive deficits occur prior to Aβ plaques in APP transgenic animal models [35-38]. Evidence suggests that increased oxidative damage may participate in triggering the memory deficits at the early stage of AD [39, 40]. Geniposide has diverse pharmacological capabilities including anti-oxidation, anti-inflammatory and anti-tumor effects as well as neurotrophic and neuroprotective properties. In addition, it has been demonstrated that geniposide could cross the blood brain barrier and reported protective property in brain. Therefore, we first determined the effect of geniposide on the memory deficits at the early stage of AD in APP/PS1 mice. Some studies provide evidence that memory deficits occur early in 3.5-month-old preplaque APP/PS1 mice [4, 41]. Thus, we employed the 3-month-old APP/PS1 mice in this study. The data from Morris water maze test clearly shows that spatial learning and memory deficits are significantly improved in APP/PS1 mice treated with geniposide for 3 months (from 3 to 6 months of age) in a dose-dependent manner.

It has been well documented that “brain cells are particularly vulnerable to reactive oxygen and reactive nitrogen species-mediated oxidative damage because of their high utilization of oxygen, high content of easily oxidizable polyunsaturated fatty acids, high content of metals catalyzing free radical formation, and low concentrations of antioxidants to combat oxidative stress” [42]. However, oxidative damage to lipids and proteins can lead to the disruption of membrane integrity and inactivation of enzymes, which result in the cell impairment and even apoptosis. Based on the anti-oxidation effect of geniposide, we may infer that geniposide has neuroprotective effects by decreasing the oxidative stress in the brain of APP/PS1 mice. To address this question, we assessed the level of ROS and one widely accepted and sensitive measure of oxidative damage, the lipid peroxidation product MDA in the brains of APP/PS1 mice. We showed that ROS and MDA levels in the cerebral cortex were increased dramatically in the brains of APP/PS1 mice, while geniposide treatment could reduce both of them in a dose-dependent manner.

Oxidative stress originating from the mitochondria has been proposed to be an important pathogenic trigger in the progression of AD. In AD, mitochondrial dysfunction appears early. Therefore, the inhibition of mitochondrial oxidative stress may have potential therapeutic advantages for AD. In this study, we found the increased mitochondrial oxidative damage and decreased CcO activity and mitochondrial membrane potential in the cerebral cortex of APP/PS1 mice. Our observations are consistent with the previous report on the mitochondrial function in AD mouse model [43, 44]. Intriguingly, geniposide significantly attenuates the compromised mitochondrial function. It is reported that “Changes in mitochondrial oxidative damage levels and impaired mitochondrial membrane potential are related to the mitochondrial electron transport chain (ETC). The CcO defect is central in AD mitochondria among the several mitochondrial enzyme activities in the ETC. CcO activity reduction occurs at all stages of AD, including mild cognitive impairment (MCI)”[30]. In this study, we found geniposide up-regulated CcO activity in a dose-dependent manner. Therefore, we conclude that geniposide can preserve the ETC by protecting CcO, thereby increasing mitochondria membrane potential and decreasing mitochondrial oxidative damage.

However, further investigation is required to elucidate the detailed mechanisms underlying the geniposide-mediated pathways to improve the CcO activity. It may be improved through the change in its protein subunits or mtDNA [45], or other pathways such as anti-oxidant mechanisms.

CONCLUSIONS

The present study demonstrates that geniposide, a pharmacologically active component purified from gardenia fruit, has a protective effect in the maintenance of mitochondrial function in addition to attenuating oxidative stress and memory deficits in APP/PS1 mice. The specific pharmacological mechanism of the geniposide on mitochondrial dysfunction needs further investigation. This study demonstrates geniposide has great potential to develop to be a drug for the treatment of age-related diseases including AD.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by grants from National Natural Science Foundation of China (No.81274118, No.81230010), and the Key New Drug Creation and Development Program of China (No.2012ZX09103-201).

REFERENCE


