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Andrographolide sulfonate improves Alzheimer-associated phenotypes and mitochondrial dysfunction in APP/PS1 transgenic mice

Ji Geng[a,b], Wen Liu[c], Yuyun Xiong[d], Hongqun Ding[a], Chunhong Jiang[e], Xiaoling Yang[e], Xiang Li[c], Ahmed Elgehama[c], Yang Sun[c], Qiang Xu[c,*], Wenjie Guo[c,*], Jing Gao[a,*]

[a] School of Pharmacy, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China
[b] Jiangsu Key Laboratory of Translational Research and Therapy for Neuro-Psycho-Diseases, School of Pharmaceutical Science, Soochow University, Suzhou 215123, China
[c] State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, 163 Xianlin Avenue, 210093, Nanjing, China
[d] Department of Clinical Laboratory, Affiliated Hospital of Jiangsu University, Zhenjiang, Jiangsu 212001, China
[e] State Key Laboratory of Innovative Nature Medicine and TCM Injections, Jiangxi Qingfeng Pharmaceutical Co., Ltd., Ganzhou, China

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ABSTRACT

Alzheimer's disease is a neurodegenerative disorder with Amyloid-β plaques onset, synaptic damage, and cognitive decline. Aβ deposits cause pathological events including oxidative stress, mitochondrial dysfunction, and neuron death. In this study, APPswe/PSENΔ9 double transgenic mice model was used to imitate Alzheimer's disease and the effect and possible mechanism of Andrographolide sulfonate were examined. Andrographolide sulfonate was given to the mice for 7 months before the onset of Aβ plaque. Spatial memory test showed that Andrographolide sulfonate treatment prevented cognitive decline. Aβ deposits were not affected while hippocampus and synapse damage was significantly alleviated. Mechanism studies showed that oxidative stress and mitochondrial swelling was reduced after Andrographolide sulfonate administration. These findings suggest that Andrographolide sulfonate, which has been applied in clinical medicine, might be a promising therapeutic agent for AD therapy via mitochondria protection.

1. Introduction

Alzheimer's disease (AD), the most common form of dementia which occurs mainly in the elderly, is characterized by memory deficits, cognitive decline as well as mental derangement. The major pathological features of the AD are neurofibrillary tangles (NFT) in cells involved in tau hyperphosphorylation, extracellular senile plaques mediated by Amyloid-β (Aβ), and the loss of neurons [1]. The APP/PS1 mice with transgenic of familial AD mutant forms of the human amyloid precursor protein (APPsw) and presenilin 1 (m146L) are often used to study the mechanisms of the neuropathology of AD and the therapeutic effects of drugs on Alzheimer's disease [2].

Energy metabolism disturbance and mitochondrial dysfunction, which cause ATP deletion, ROS generation, and apoptosis, have been implicated in the pathogenesis of AD. Mitochondrial dysfunction has been detected earlier than major pathological features of the AD, which promote APP expression and procession in sporadic and late-onset AD [3]. MtDNA mutation is also increased in the hippocampus of early-stage AD [4]. The accumulation of Aβ peptide results in feedback inhibition of peptidase, which leads to mitochondrial dysfunctions [5]. Impaired mitochondrial energy metabolism disturbs epigenetic regulation of chromatin, which is also observed in AD [6]. Thus, alleviation of mitochondrial dysfunction might promisingly slow down the progress of AD.

Andrographolide is a bicyclic diterpenoid lactone distributed in medicinal herb *Andrographis paniculata* and has been reported to exhibit anti-viral, anti-inflammatory, anti-tumor, and anti-cardiovascular properties in several diseases [7–10]. A pharmacokinetic study showed that Andrographolide can enter into the brain [11] and has protective effects against MPTP-induced Parkinson's disease [12] as well as cerebral ischemia-reperfusion [13]. In an animal model of the AD, Andrographolide can also significantly improve cognitive impairment through decreasing Aβ levels and recovering synaptic plasticity [14,15]. Andrographolide sulfonate, a water-soluble form of Andrographolide by sulfonate reaction (trade name: Xi-Yan-Ping Injection), has been applied to treat inflammatory disease. What's more, Andrographolide sulfonate displays a stronger inhibition effect on LPS-induced sepsis than Andrographolide itself [16]. In this study, we found out that Andrographolide sulfonate administration can improve the cognitive impairments and maintain mitochondrial structure and

* Corresponding authors.
E-mail addresses: molpharm@163.com (Q. Xu), guowj@nju.edu.cn (W. Guo), jinggao@ujs.edu.cn (J. Gao).

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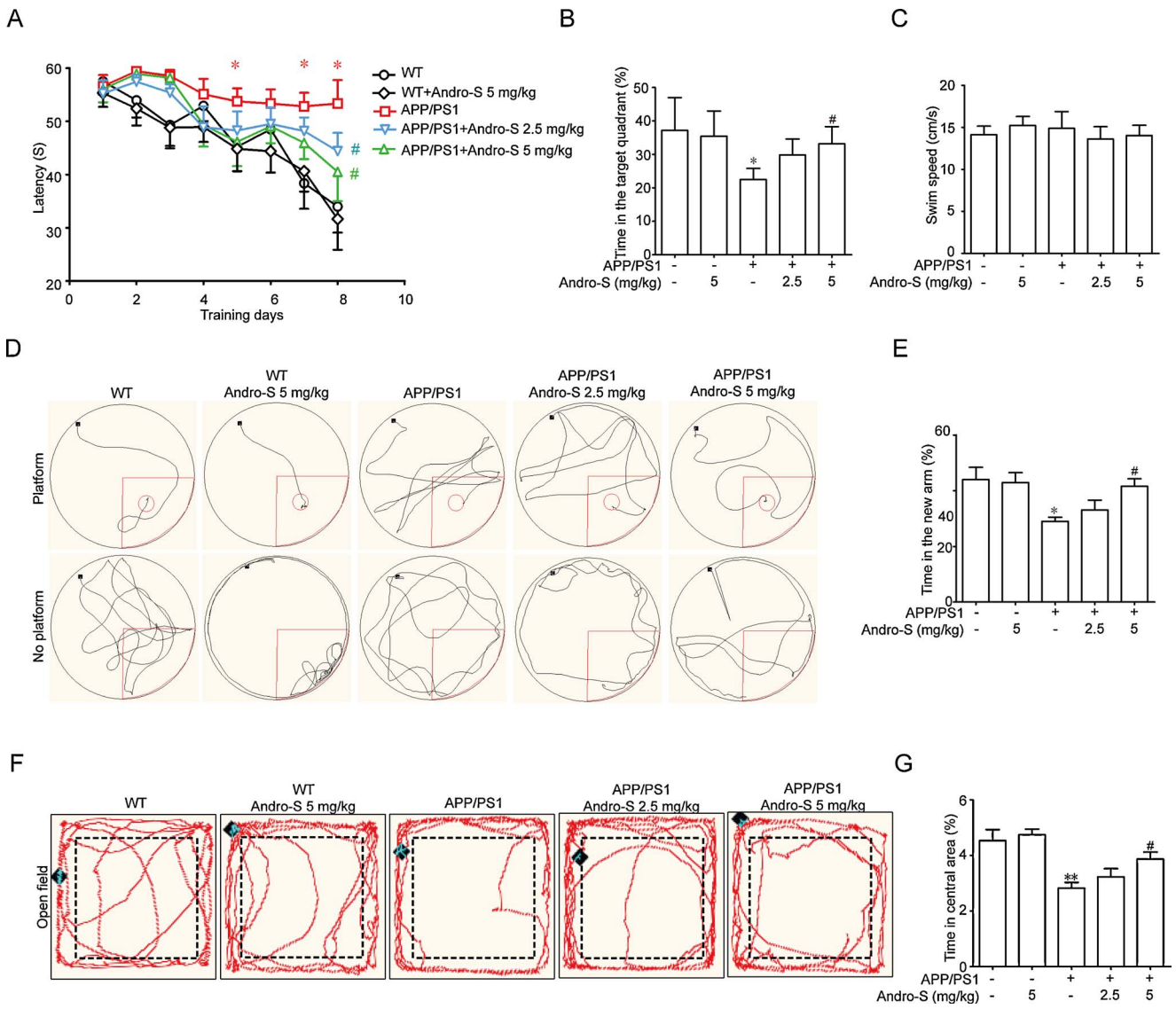

Fig. 1. Andrographolide sulfonate improves cognitive impairment in APP/PS1 mice. (A) Andrographolide sulfonate treatment significantly lowered the latency of APP/PS1 transgenic mice to Escape a submerged platform in the Morris water maze test during the 1 min training phase. Escape latencies were expressed as means ± SEM. (B) Andrographolide sulfonate-treated APP/PS1 mice spent more time on the platform located quadrant after removing the platform. (C) Swimming speed was measured when the visible platform is above the water surface in the Morris water maze. No significant differences were observed among different groups. (D) Representative search traces during 1 min in the final training (upper) and in the Morris maze removing platform (lower). (E) Y maze task. Spatial memory was measured by the percentage of new arm choice during 6 min. (F) The representative movement traces of each group in the open field were recorded during 5 min. (G) The percentage of time spent in the central area of the open field was calculated. Data are expressed as the mean ± SEM of values obtained from eight mice. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with WT group, #P < 0.05, ##P < 0.01 compared with APP1 mice. Andro-S: Andrographolide sulfonate.

function in APP/PS1 mice.

2. Materials and methods

2.1. Materials

Andrographolide sulfonate injection (5 mg5 mg/15 ml) were purchased from Jiangxi Qingfeng Pharmaceutical Co., Ltd (Ganzhou, China). Thioflavin T (ThT, T3516) was procured from Sigma-Aldrich (St. Louis, Missouri, USA). TRIzol was purchased from Life Technologies (Grand Island, New York, USA). TUNEL staining, Nissl staining kit (C0117) and JC-1 dye (C2005) were purchased from Beyotime Biotechnology (NanTong, China). ATP kit (A095-1), Catalase activity kit (A006-2), Superoxide Dismutase (SOD) assay kit (WST-1 method) (A001-3), Malondialdehyde (MDA) assay kit (TBA method) (A003-1) were all obtained from the Institute of Biological Engineering of Nanjing Jiancheng (Nanjing, China). Anti-SYP (sc-9116), anti-PSD95 (sc-28914) were bought from Santa Cruz Biotechnology, anti-NeuN (ab177487) were purchased from Abcam, HRP conjugated Goat anti-Rabbit IgG (7074) and RIPA cell lysate (9806) were purchased from Cell Signaling Technology corporation.

2.2. Animal treatment

Animal research was approved by the Animal Ethical and Welfare Committee (AEWC) of the Jiangsu University. Male WT mice and male APP/PS1 mice (8–12 weeks old, 18–20 g, n = 40) were purchased from Model Animal Research Center of Nanjing University (Nanjing, China), and housed under constant conditions (12 h light/dark cycle, room temperature 21 ± 1 °C) with water and food available ad libitum.

In this study, mice were divided into five groups of 8 mice (WT group received water, WT group received 5 mg/kg/day Andrographolide sulfonate, APP/PS1 group, APP/PS1 group received 2.5 mg/kg/day Andrographolide sulfonate, APP/PS1 group received

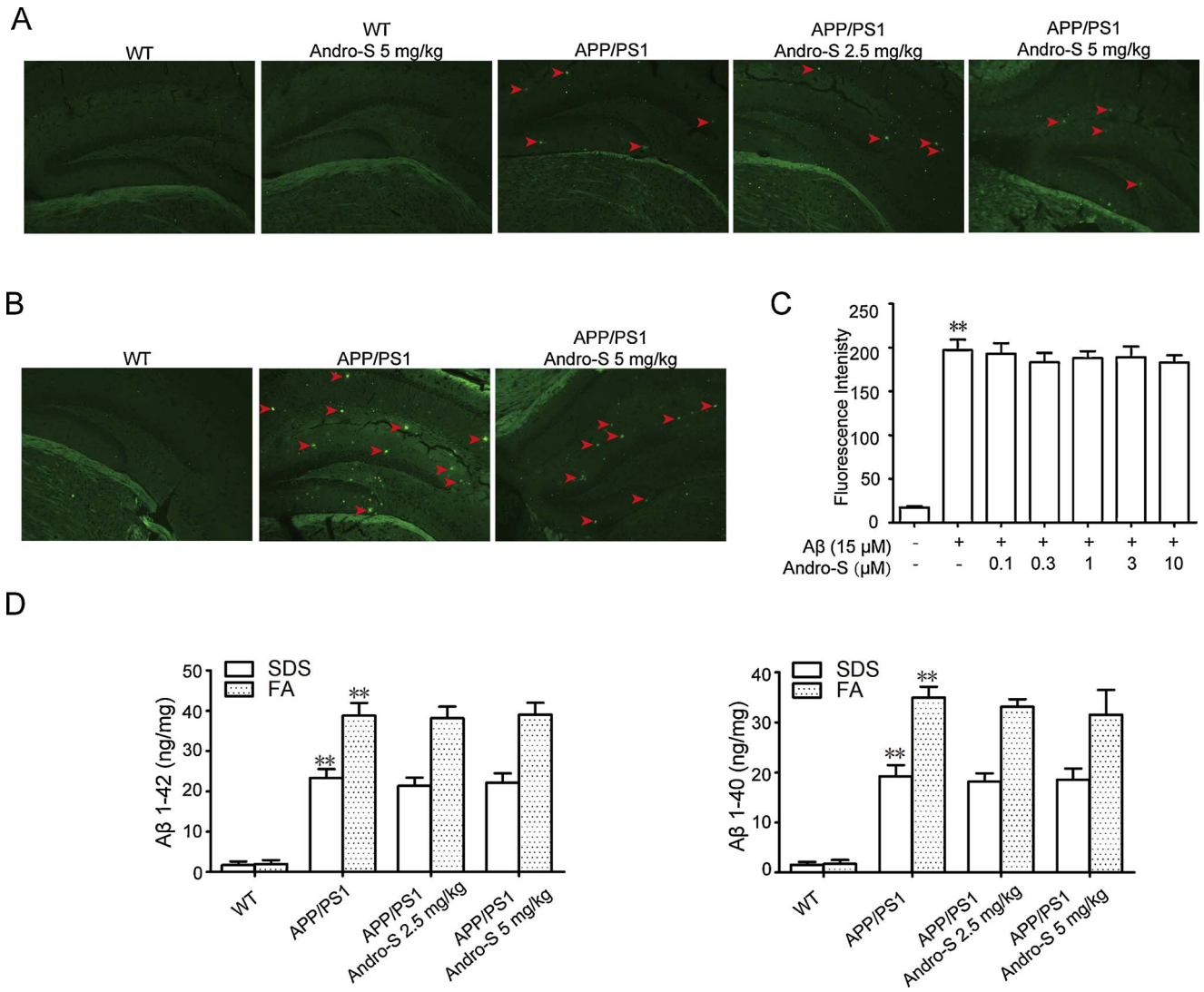

Fig. 2. Andrographolide sulfonate has no effect on the generation of toxic Aβ aggregates and Aβ deposition in vivo and in vitro. The hippocampus sections of mice treated with Andrographolide sulfonate for 7 months (A) or 10 months (B) were stained with ThT. (C) Various concentrations of Andrographolide sulfonate were mixed with Aβ1-42 (15 μM) and kept at 37 °C for 3 days before coincubated with ThT solution for 5 min5 min. Fluorescence intensity was detected at an excitation wavelength of 435 nm and an emission wavelength of 485 nm using a fluorescence microplate reader. (D) SDS-soluble and FA-soluble Aβ1-40 and Aβ1-42 in the hippocampus of each group were measured using ELISA. Data are expressed as the mean ± SEM of values obtained from eight mice. **P < 0.01 compared with WT group. Andro-S:: Andrographolide sulfonate.

5 mg/kg/day Andrographolide sulfonate).

It has been reported that each mouse is estimated to drink 1.5 ml of water/10 grams of body weight/day [17], which is almost the same volume as we have monitored. As the average body weight of mice was about 25 g, the volume of drinking water was 3.75 ml. To reach the dose of 2.5 mg kg^{-1}/day and 5 mg kg^{-1}/day, 0.017 mg ml^{-1} and 0.034 mg/ml Andrographolide sulfonate in drinking water was given in cage respectively. Fresh Andrographolide sulfonate was changed every five days. The administration was started from 2-month-old mice and lasted for 7 months. Mice behavioral test was carried out before sacrifice and brains were collected for immunohistochemistry and western blot analysis.

2.3. Morris Water Maze

Morris water maze was applied to examine the learning and memory capability as previously described. The water maze, located in a bright room, was a white circular pool (2 m in diameter, 60 cm in height) filled with water (25 °C) to a depth of 30 cm and a circular white Escape platform (0.15 m in diameter) submerged 2 cm below the water surface. The maze was separated into four equal quadrants and there are red triangular, green circular and blue square-shaped spatial cues in three quadrants. EthoVision (Noldus, Netherlands) was used to measure the escape latency and traveled distance for the animals to reach the platform.

Mice were placed in the behavioral room for 30 min habituation before the test. On the first day, the platform was deployed above the water surface and swimming speed and a vision were examined.

Mice received four trials per day for eight consecutive days with the platform located in a fixed position. The starting quadrants were randomly assigned every day. Each trial was ended as soon as the mice had climbed onto the platform. Any mouse that missed the platform within 60 s was brought to the platform and assigned to stay on the platform for 60 s. Time spent to find the platform and swimming paths were analyzed and recorded by a computer software.

On the 9th day, a 60 s probe trial without the platform in the tank was performed. Time spent to find the platform and swimming paths were analyzed and recorded by a computer software.

2.4. Y maze

As described previously with little modification [18], the Y maze

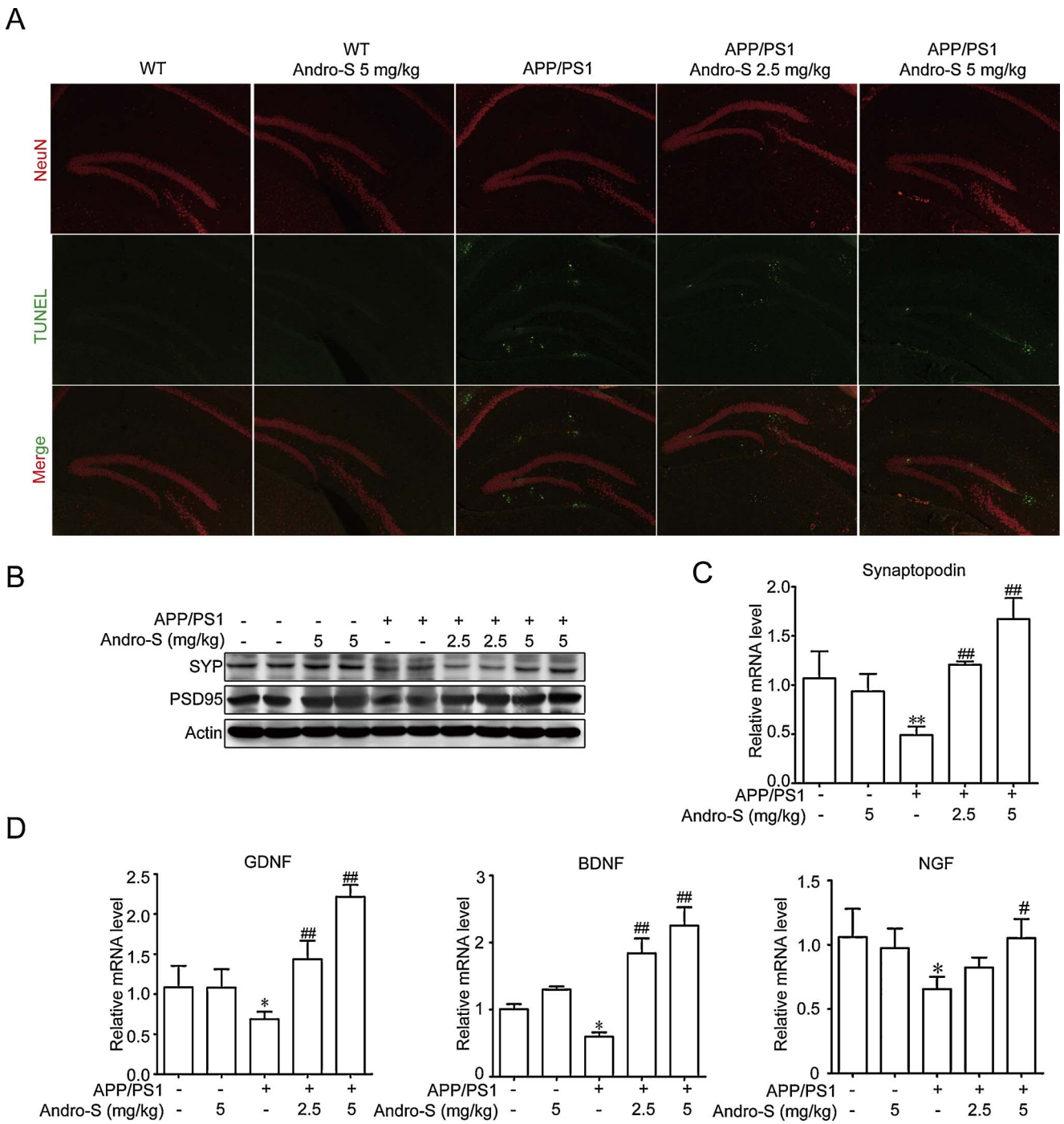

Fig. 3. Andrographolide sulfonate protects against amyloid-β-mediated hippocampus damage and synapse damage. (A) Brain section was stained with antibodies against NeuN and TUNEL for apoptosis. (B) Synapse proteins SYP and PSD95 in the hippocampus of APP1 mice are detected by western blot. of Synaptopod (C) and neurotrophic factor transcripts GDNF, BDNF and NGF (D) in the hippocampus were examined by RT-PCR. Data are presented as the mean ± SEMmean ± SEM of values obtained from eight mice. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with WT group, #P < 0.05, ##P < 0.01 compared with APP/PS1 mice. Andro-S: Andrographolide sulfonate.

was made of white Plexiglas (10 cm width, 45 cm length, 10 cm height) consisted of three identical arms with 120°. Different visual cues placed on the walls of each arm enable spatial orientation. The arms were designated into open arm, novel arm, and another arm. Mice were placed on the open arm facing the wall to explore the maze. The novel arm was blocked during the first trial but open during the second trial. First, Mice moved freely in the open arm and another arm for 6 min. Second, the maze was cleaned with 70% ethanol. Then, mice moved freely in three arms for 6 min. The time spent in the novel arm was calculated with EthoVision (Noldus, Netherlands).

2.5. Open field

The open field test can assess ambulatory movement and anxiety-like behaviors. The amount of time spent in the center zone is a reflection of anxiety. The test was similar to that described in ref [19] with little modified. A rectangle box (45 cm 45 cm 20 cm) with a 35 cm 35 cm central square was placed in an open, bright environment. The mice were put on the center to unstructured activities for 5 min to adaptation. Then, the total movement paths and the time spent in the center were recorded during 5 min by EthoVision (Noldus, Netherlands). Each mouse performs only one trial, and 75% alcohol was used

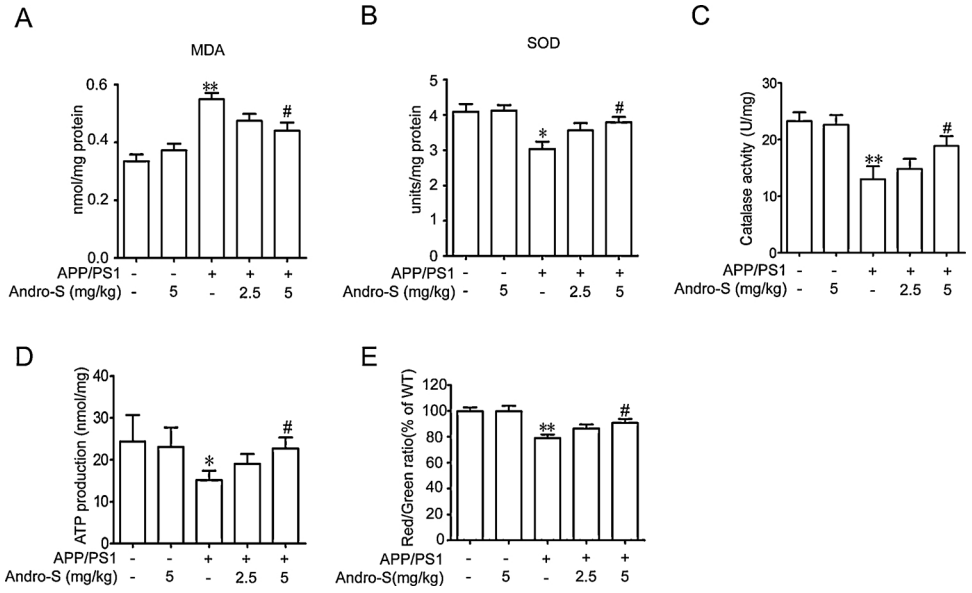

Fig. 4. Andrographolide sulfonate attenuates oxidative stress and maintains mitochondrial membrane potential. Andrographolide sulfonate treatment decreased the concentration of MDA (A), and increased SOD (B) and Catalase (C) in the hippocampus of mice in each group. Compared with Andrographolide sulfonate treated or WT mice, ATP generation was reduced in APP/PS1 mice (D). Mitochondrial membrane potential of isolated mitochondria was measured using JC-1 dye. The fluorescence intensity ratio of JC-1 aggregates (Red) to monomers (Green) was calculated as an indicator of membrane potential. The values were normalized against the WT mice, set to 100%. Data are presented as the mean ± SEM of values obtained from eight mice. *P < 0.05, **P < 0.01 compared with WT group, #P < 0.05 compared with APP/PS1 mice.

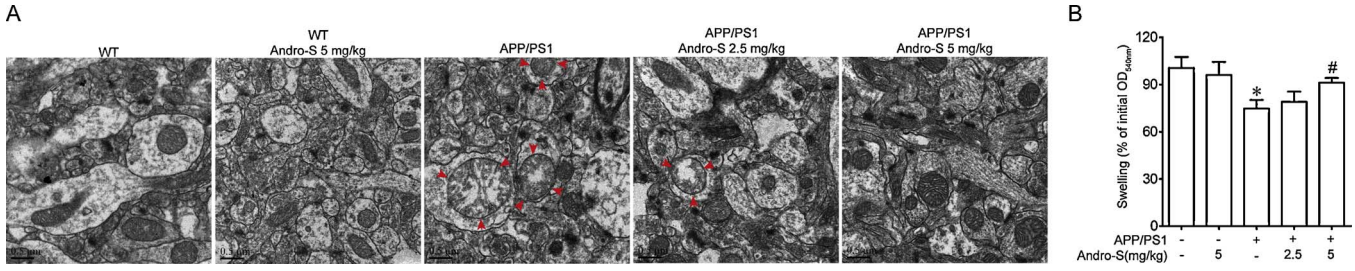

Fig. 5. Andrographolide sulfonate indirectly attenuates mitochondria swelling. (A) The morphology of mitochondrion was observed in APP/PS1 mice using TEM. (B) Quantification of swelling for mitochondria isolated from 3 mice in each group was detected as the absorbance at 540 nm using a microplate reader. (C) Mitochondria were pretreated with Andrographolide sulfonate for 30 min, followed by exposure to Aβ1-42 oligomers for another 30 min. Mitochondria swelling traces were recorded in absorbance at 540 nm. Data are presented as the mean ± SEM of values obtained from eight mice. *P < 0.05, **P < 0.01 compared with WT group, #P < 0.05 compared with APP/PS1 mice. Andro-S: Andrographolide sulfonate.

to clean the box between trials.

2.6. Thioflavin T fluorescence (ThT) measurement

Aβ1-42 was dissolved in PBS and incubated for 3 days at 37 °C to form Aβ1-42 oligomers. 15 μM Aβ1-42 was dissolved in PBS and incubated with different concentrations of Andrographolide sulfonate for 3 days at 37 °C. 10 μM stock solution of ThT was prepared in 10 mM sodium acetate buffer (pH 5). ThT stock solution was preincubated with Aβ1-42 oligomers to a final concentration of 5 μM at room temperature for 5 min before measurements. Fluorescence intensities were detected using a fluorescence microplate reader (Molecular device, USA) at an excitation wavelength of 435 nm and an emission wavelength of 485 nm.

2.7. ThT staining

The 10 μm brain tissues section were equilibrated with PBS for 0.5 h and then incubated with 0.3% ThT in distilled water for 5 min followed by differentiation in 70% ethanol for 5 min and rinsing in water.

2.8. TUNEL staining

For TUNEL assay, samples were stained using an In Situ Apoptosis Detection Kit (Clontech, Mountain View, CA) to detect in situ DNA fragmentation according to the manufacturer's protocol, and FITC signals were visualized with a Confocal Laser Scanning Microscopy (Carl Zeiss, Oberkochen, Germany)

2.9. Extraction and Detection of Aβ1-40 and Aβ1-42

Initial sonication of the brain took place in 2% SDS with protease inhibitors and the resultant pellet was then extracted with 70% formic acid in water, which can extract the soluble and insoluble Aβ. The concentrations of human Aβ1-40 and Aβ1-42 in the hippocampus of APP/PS1 mice were measured by ELISA according to the manufacturer's guidelines (Exp Cell Bio).

2.10. Western blot analysis

After administration for 7 months, APP/PS1 mice were sacrificed and brain tissue was homogenized using a lysis buffer containing protease cocktail inhibitors (Roche). The homogenate was centrifuged at 14,000 g for 15 min at 4 °C and the protein concentration was determined using BCA kit. Whole tissue lysate (10 μg) was loaded onto 10% SDS-PAGE. Electrophoresis was performed using a stacking gel at 80 V for 20 min and a separating gel at 110 V for 70 min. The proteins were transferred to PVDF membranes (Millipore, MA, USA) using an electro-blotting apparatus (Bio-Rad, CA, USA) at 300 mA for 90 min. The membranes were blocked for 1 h in TBST containing 0.1% Tween-20 and 5% dry milk and then incubated overnight with primary antibodies. After washing for 3 times in TBST for 5 min each, the membrane was incubated for 2 h with horseradish peroxidase-conjugated secondary antibodies. Then membranes were washed for 3 times in TBST

for 5 min each. The optical densities of the antibody-specific bands were analyzed using a Luminescent Image Analyzer (Alpha, USA).

2.11. Mitochondria swelling

The test was similar to the ref [20] with little modified. Mitochondria of each group mice were isolated according to the manual of mitochondria isolation kits (Beyotime Biotechnology). The concentrations of isolated mitochondria were measured using BCA kits (Beyotime Biotechnology). The changes of mitochondrial swelling were reflected in light scattering at 30 °C by a microplate reader (SpectraMax Plus, Molecular Devices, USA).

2.12. TEM

The brain of mice from each group was fixed with 2.5% ice-cold glutaraldehyde overnight. The tissue was cut into 1 μm^3 and fixed in OsO4, then sliced into 1 μm sections. tissues from 3 mice in each group were sliced and 5 fields at each section were randomly chosen and photographed.

2.13. Statistical analysis

All experiments were repeated at least three times and presented as mean ± SEM. The statistical analysis was performed using GraphPad Prism 5 (San Diego, CA, USA). The statistical evaluation of the data was performed by one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Andrographolide sulfonate ameliorates cognitive impairment in APP/PS1 mice

APP/PS1 transgenic mice that express mutant APPswe (K595N/M596L) and PS1 (PSEN1ΔE9) were utilized to imitate AD, which generates Aβ plaques in the cortex and hippocampus in an age-dependent manner. After administrated with Andrographolide sulfonate, behavior tests were taken out to assess whether it can ameliorate cognitive impairment in APP/PS1 mice. Morris water maze and Y maze were applied to measure spatial memory of APP/PS1 mice. After consecutive training, APP/PS1 performed impaired learning and spatial orientation in the submerged platform phase. Interestingly, the spatial deficits of APP/PS1 were alleviated by Andrographolide sulfonate (Fig. 1A). After removing the platform, the mice treated with Andrographolide sulfonate spent much more time in the platform quadrant compared to APP/PS1 mice (Fig. 1B). There is no obvious difference among mice in swimming velocity, implying that Andrographolide sulfonate treatment has no effect on change locomotor activity (Fig. 1C). The swimming trace of mice in water maze was recorded by the camera during 1 min (Fig. 1D). Andrographolide sulfonate treated mice stayed longer in the novel arm of Y maze than APP/PS1 mice (Fig. 1E). Anxiety symptoms are observed in dementia [21–23], and open field was used to assess the anxious symptoms in APP/PS1 mice. As shown in Fig. 2F and 2E, 5 mg/kg Andrographolide sulfonate treatment led to increased movement and time in the central area, suggesting alleviation of the anxious symptom.

3.2. Andrographolide sulfonate has no effect on the generation of toxic Aβ aggregates and Aβ deposition in vivo and in vitro

Decreasing Aβ generation and deposition is a promising therapeutic strategy to improve cognitive impairment. ThT staining was applied to detect the Aβ deposits. As shown in Fig. 2A and B, APP/PS1 mice treated with Andrographolide sulfonate for 7 months or 10 months showed unchanged Aβ deposits in cortex and hippocampus compared to APP/PS1 mice. Further, the results of Aβ1-42 oligomers-Thioflavin T fluorescence (ThT) assay also showed that Andrographolide sulfonate cannot inhibit the formation of Aβ oligomers in vitro as depicted in Fig. 2C. Aβ1-40 and the more virulent Aβ1-42 are the most important components in Aβ plaques. The soluble and insoluble Aβ1-40 and Aβ1-42 were extracted by using SDS and formic acid, and the levels of Aβ1-40 and Aβ1-42 were measured by ELISA. As exhibited in Fig. 2D, Andrographolide sulfonate has no effect on the levels of Aβ1-40 and Aβ1-42.

3.3. Andrographolide sulfonate reduced amyloid-β-mediated hippocampus and synapse damage

With the progress of AD, neuron would be gradually damaged. Except for decreasing the Aβ plaques, neuron protection may also contribute to cognitive improvement. The apoptosis was detected using TUNEL. As shown in Fig. 3A, the TUNEL-positive cells are scattered in the hippocampus of APP/PS1 mice. 5 mg/kg Andrographolide sulfonate administration can significantly inhibit apoptosis. Behavioral memory is mediated by plasticity in the synaptic connections. Synaptic protein such as SYP and PSD95 were examined using western blot. As shown in Fig. 3B, SYP, and PSD95 expression were decreased in APP/PS1 mice while Andrographolide sulfonate treatment can restore its expression. Synaptic plasticity recover was further confirmed that by increased mRNA level of Synaptopodin in Andrographolide sulfonate treated mice (Fig. 3C). Transcriptional levels of GDNF, BDNF and NGF were also promoted by Andrographolide sulfonate, which may contribute to neuron protection against Aβ induced injury (Fig. 3D).

3.4. Andrographolide sulfonate treatment attenuated oxidative stress and maintains mitochondrial membrane potential

Oxidative stress is accompanied with the progress of AD. As exhibited in Fig. 4A-C, imbalanced redox reactions arise in APP/PS1 mice as evidenced by increased MDA, and decreased SOD/catalase level. 5 mg/kg Andrographolide sulfonate treatment significantly reduced MDA content and increased the activities of SOD and catalase. Mitochondria are an important source of ROS, and ROS, in turn, can impair mitochondria function. Loss of mitochondrial function in the hippocampus of APP/PS1 mice were reflected by a marked decrease of ATP levels and reduction in mitochondrial membrane potential as showed in Fig. 4D. 5 mg/kg Andrographolide sulfonate maintained the ATP content nearly to a normal level. Mitochondrial membrane potential was measured using JC-1 dye. Compared with JC-1 monomers (green), JC-1 accumulates in mitochondria to form aggregates (red) depending on mitochondrial membrane potential. As shown in Fig. 4D, the mitochondrial membrane potential of isolated mitochondria from APP/PS1 was reduced, while mitochondria from 5 mg/kg Andrographolide sulfonate-treated group remained at a high level.

3.5. Andrographolide sulfonate treatment attenuates mitochondria swelling

Based on the above results, TEM was applied to observe the ultrastructure of mitochondria. As shown in Fig. 5A, mitochondria in WT mice administrated with Andrographolide sulfonate is similar to WT mice. However, swollen mitochondria with broken cristae and membrane are observed in APP/PS1 mice. Andrographolide sulfonate can significantly decrease the abnormal mitochondria in the hippocampus. As shown in Fig. 5B, the isolated mitochondria from Andrographolide sulfonate treated APP/PS1 mice exhibits a higher absorbance than APP/PS1 mice at 540 nm, suggesting less extent of mitochondria swelling. Taken together, Andrographolide sulfonate treatment can attenuate mitochondria swelling in APP/PS1 mice.

4. Discussion

As a transmembrane protein containing the C-terminus of the Aβ region located within the intramembranous domain, APP is cleaved by β-secretase to form secreted APPβ (sAPPβ) and β-C-terminal fragment binding membrane (CTFβ), then the remaining CTFβ is cleaved by γ-secretase, an intramembranous protein complex composed of Aph1, Pen2, Nicastrin, and Presenilin 1, to generate different population of Aβ with C-terminal heterogeneity. The most abundant population is Aβ40 followed by Aβ42. Aβ42 is more hydrophobic and is the principal species deposited in the brain [24]. Earlier diagnosis of the AD is beneficial for prevention and clinical therapies. Detection of progressive brain atrophy using magnetic resonance imaging (MRI) [25], cognitive function [26], biomarkers such as Aβ42 and tau protein from peripheral blood and cerebrospinal fluid, and amyloid PET radiotracers by (^{18}F)-florbetaben (FBB) [27] have been applied in AD diagnosis. However, soluble oligomeric forms of Aβ predates overt amyloid plaque to damage neuron, providing critical evidence that neuronal damage is earlier than Aβ deposits and neuron protection, suggesting neuronal protection should be a promising therapeutic strategy for AD [28].

As one of the earliest and most prominent features in vulnerable neurons, abnormal mitochondrial dysfunction causes oxidative stress, synaptic dysfunction and neuron death in the brain of AD patients [29]. Mitochondria function is closely associated with its structure. Mitochondrial swelling was observed using transmission electron microscopy in APP/PS1 mice. Moreover, Aβ peptide can directly localize to mitochondrial cristae through Tom import machinery [30]. Mitochondrial swelling is facilitated by the opening of the mitochondrial permeability transition pore (mPTP) [31]. There are several proteins participated in the formation of mPTP, including the voltage-dependent calcium channel (VDAC), the adenine nucleotide translocase (ANT), cyclophilin D (CypD), cytochrome-c, hexokinase II (HKII) as well as Bax [32]. Aβ oligomers promote an open and irreversible conformation of mitochondrial mPTP via interaction with CypD [20]. The opening mPTP permeates the mitochondrial inner membrane, increase the exchanges between mitochondrial matrix and the cytoplasm and results in mitochondrial perturbations, such as the collapse of mitochondrial membrane potential, the release of pro-apoptotic factors, ROS generation, and calcium deregulation. Andrographolide sulfonate treatment decreased mitochondrial swelling and restore the balance of oxidation and redox. However, whether proteins associated with mPTP is involved or not remains unclear.

Considering the cognitive improvement function of Andrographolide sulfonate, we tested the potential mechanism of Andrographolide sulfonate, including Aβ generation and deposits, the neuronal protection. Even though Andrographolide has been reported to reduce Aβ deposits, there is no significant decrease of Aβ plaques in Andrographolide sulfonate treated group. Andrographolide sulfonate can obviously improve the cognitive impairments in APP/PS1 mice through alleviating mitochondrial dysfunction and maintaining the ultrastructure of mitochondria while the detailed mechanism needs further exploration.

In conclusion, the results of our study demonstrate that clinically used Andrographolide sulfonate can restrain AD disorder through inhibiting mitochondrial swelling, reducing neuron cell death and cognitive decline, suggesting its possible use for AD therapy.

Conflicts of interest

Authors acknowledged that there are no conflicts of interest

Acknowledgments

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References

[1] W.V. Graham, A. Bonito-Oliva, T.P. Sakmar, Update on Alzheimer's disease therapy and prevention strategies, Annu. Rev. Med. 68 (2017) 413–430.
[2] F. Trinchese, S. Liu, F. Battaglia, S. Walter, P.M. Mathews, O. Arancio, Progressive age-related development of Alzheimer-like pathology in APP/PS1 mice, Ann. Neurol. 55 (6) (2004) 801–814.
[3] R.H. Swerdlow, J.M. Burns, S.M. Khan, The Alzheimer's disease mitochondrial cascade hypothesis: progress and perspectives, Biochim Biophys Acta 1842 (8) (2014) 1219–1231.
[4] J.G. Hoekstra, M.J. Hipp, T.J. Montine, S.R. Kennedy, Mitochondrial DNA mutations increase in early stage Alzheimer disease and are inconsistent with oxidative damage, Ann. Neurol. 80 (2) (2016) 301–306.
[5] D. Mossmann, F.N. Vogtle, A.A. Taskin, P.F. Teixeira, J. Ring, J.M. Burkhart, N. Burger, C.M. Pinho, J. Tadic, D. Loreth, C. Graff, F. Metzger, A. Sickmann, O. Kretz, N. Wiedemann, R.P. Zahedi, F. Madeo, E. Glaser, C. Meisinger, Amyloid-beta peptide induces mitochondrial dysfunction by inhibition of preprotein maturation, Cell Metab. 20 (4) (2014) 662–669.
[6] A. Salminen, A. Haapasalo, A. Kauppinen, K. Kaarniranta, H. Soininen, M. Hiltunen, Impaired mitochondrial energy metabolism in Alzheimer's disease: impact on pathogenesis via disturbed epigenetic regulation of chromatin landscape, Prog. Neurobiol. 131 (2015) 1–20.
[7] J. Zhou, S.E. Hu, S.H. Tan, R. Cao, Y. Chen, D. Xia, X. Zhu, X.F. Yang, C.N. Ong, H.M. Shen, Andrographolide sensitizes cisplatin-induced apoptosis via suppression of autophagosome-lysosome fusion in human cancer cells, Autophagy 8 (3) (2012) 338–349.
[8] Z. Yu, B. Lu, Y. Sheng, L. Zhou, L. Ji, Z. Wang, Andrographolide ameliorates diabetic retinopathy by inhibiting retinal angiogenesis and inflammation, Biochim. Biophys. Acta. 1850 (4) (2015) 824–831.
[9] W. Guo, Y. Sun, W. Liu, X. Wu, L. Guo, P. Cai, X. Wu, X. Wu, Y. Shen, Y. Shu, Y. Gu, Q. Xu, Small molecule-driven mitophagy-mediated NLRP3 inflammasome inhibition is responsible for the prevention of colitis-associated cancer, Autophagy 10 (6) (2014) 972–985.
[10] P. Panraksa, S. Ramphan, S. Khongwichit, D.R. Smith, Activity of andrographolide against dengue virus, Antiviral Res. 139 (2017) 69–78.
[11] R. Bera, S.K. Ahmed, L. Sarkar, T. Sen, S. Karmakar, Pharmacokinetic analysis and tissue distribution of andrographolide in the rat by a validated LC–MS/MS method, Pharm. Biol. 52 (3) (2014) 321–329.
[12] Z. Zhang, D. Lai, L. Wang, P. Yu, L. Zhu, B. Guo, L. Xu, L. Zhou, Y. Sun, S.M. Lee, Y. Wang, Neuroprotective effects of the andrographolide analog AL-1 in the MPP (+)/MPTP-induced Parkinson's disease model in vitro and in mice, Pharmacol. Biochem. Behav. 122 (2014) 191–202.
[13] C.M. Chern, K.T. Liou, Y.H. Wang, J.F. Liao, J.C. Yen, Y.C. Shen, Andrographolide inhibits PI3K/AKT-dependent NOX2 and iNOS expression protecting mice against hypoxia/ischemia-induced oxidative brain injury, Planta Med. 77 (15) (2011) 1669–1679.
[14] F.G. Serrano, C. Tapia-Rojas, F.J. Carvajal, J. Hancke, W. Cerpa, N.C. Inestrosa, Andrographolide reduces cognitive impairment in young and mature AbetaPPswe/PS-1 mice, Mol. Neurodegener 9 (2014) 61.
[15] D.S. Rivera, C. Lindsay, J.F. Codocedo, I. Morel, C. Pinto, P. Cisternas, F. Bozinovic, N.C. Inestrosa, Andrographolide recovers cognitive impairment in a natural model of Alzheimer's disease (Octodon degus), Neurobiol. Aging 46 (2016) 204–220.
[16] W. Guo, W. Liu, G. Chen, S. Hong, C. Qian, N. Xie, X. Yang, Y. Sun, Q. Xu, Water-soluble andrographolide sulfonate exerts anti-sepsis action in mice through down-regulating p38 MAPK, STAT3, and NF-κB pathways, Int. Immunopharmacol. 14 (4) (2012) 613–619.
[17] E.D. Olfert, The biology, and medicine of rabbits and rodents, Can J. Comp. Med. 47 (4) (1983).
[18] Z.L. Cai, C.Y. Wang, Z.J. Jiang, H.H. Li, W.X. Liu, L.W. Gong, P. Xiao, C.H. Li, Effects of cordycepin on Y-maze learning task in mice, Eur J. Pharmacol. 714 (1–3) (2013) 249–253.
[19] M.L. Seibenhener, M.C. Wooten, Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice, J. Vis. Exp. (96) (2015) e52434.
[20] M.S. Arrazola, E. Ramos-Fernandez, P. Cisternas, D. Ordenes, N.C. Inestrosa, Wnt signaling prevents the Abeta Oligomer-induced mitochondrial permeability transition pore opening preserving mitochondrial structure in Hippocampal Neurons, PLoS One 12 (1) (2017) e0168840.
[21] M.H. Breitve, M.J. Hynninen, K. Bronnick, L.J. Chwiszczuk, B.H. Auestad, D. Aarsland, A. Rongve, A longitudinal study of anxiety and cognitive decline in dementia with lewy bodies and Alzheimer's disease, Alzheimer's Res. Ther. 8 (2016) 3.
[22] N.C. Kaiser, L.J. Liang, R.J. Melrose, S.S. Wilkins, D.L. Sultzer, M.F. Mendez, Differences in anxiety among patients with early- versus late-onset Alzheimer's disease, J. Neuropsychiatry Clin. Neurosci. 26 (1) (2014) 73–80.
[23] L. Ferretti, S.M. McCurry, R. Logsdon, L. Gibbons, L. Teri, Anxiety and Alzheimer's disease, J. Geriatr. Psychiatry Neurol. 14 (1) (2001) 52–58.
[24] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2) (2001) 741–766.
[25] C. Davatzikos, P. Bhatt, L.M. Shaw, K.N. Batmanghelich, J.Q. Trojanowski, Prediction of MCI to AD conversion, via MRI, CSF biomarkers, and pattern classification, Neurobiol. Aging 32 (12) (2011) e19–e27 2322.
[26] J.J. Gomar, M.T. Bobes-Bascaran, C. Conejero-Goldberg, P. Davies, T.E. Goldberg, Utility of combinations of biomarkers, cognitive markers, and risk factors to predict

conversion from mild cognitive impairment to Alzheimer disease in patients in the Alzheimer's disease neuroimaging initiative, Arch. Gen. Psychiatry 68 (9) (2011) 961–969.
[27] H. Barthel, O. Sabri, Florbetaben to trace amyloid-beta in the Alzheimer brain by means of PET, J. Alzheimers Dis. 26 (Suppl. 3) (2011) 117–121.
[28] R. Kayed, E. Head, J.L. Thompson, T.M. McIntire, S.C. Milton, C.W. Cotman, C.G. Glabe, Common structure of soluble amyloid oligomers implies common mechanism of pathogenesis, Science 300 (5618) (2003) 486–489.
[29] X. Zhu, G. Perry, M.A. Smith, X. Wang, Abnormal mitochondrial dynamics in the pathogenesis of Alzheimer's disease, J. Alzheimers Dis. 33 (Suppl. 1) (2013) S253–S262.
[30] C.A. Hansson Petersen, N. Alikhani, H. Behbahani, B. Wiehager, P.F. Pavlov, I. Alafuzoff, V. Leinonen, A. Ito, B. Winblad, E. Glaser, M. Ankarcrona, The amyloid beta-peptide is imported into mitochondria via the TOM import machinery and localized to mitochondrial cristae, Proc. Natl. Acad. Sci. U. S. A. 105 (35) (2008) 13145–13150.
[31] H. Du, S.S. Yan, Mitochondrial permeability transition pore in Alzheimer's disease: cyclophilin D and amyloid beta, Biochim. Biophys. Acta. 1802 (1) (2010) 198–204.
[32] R. Kumarswamy, S. Chandna, Putative partners in Bax mediated cytochrome-c release: ANT, CypD, VDAC or none of them? Mitochondrion 9 (1) (2009) 1–8.