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Original Paper

Bajijiasu Ameliorates β-Amyloid-Triggered Endoplasmic Reticulum Stress and Related Pathologies in an Alzheimer's Disease Model

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Key Words
Alzheimer • Disease • Aβ • Bajijiasu • ER Stress

Abstract
Background/Aims: Alzheimer disease (AD) is a common neurodegenerative disease that is characterized by the deposition of beta-amyloid peptide and formation of intracellular neurofibrillary tangles. Due to the failure of various clinical trials of novel drugs for AD, effective drugs for AD treatment are urgently required. ***Methods:*** In this study, we used the classic APP/PS1 mouse model to explore the neuroprotective effects of a new compound, bajijiasu, and the mechanisms involved. Behavioral tests and western blotting were performed to assess the beneficial effects of bajijiasu in APP/PS1 mice. ***Results:*** Morris water maze and Y-maze test results showed that oral administration of bajijiasu (35 mg/kg/day and 70 mg/kg/day) improved learning and memory abilities in APP/PS1 mice. Bajijiasu reduced ROS and MDA levels in both the hippocampus and cortex. Moreover, western blotting results showed that bajijiasu protected neurons from apoptosis, elevated the expression levels of neurotrophic factors, and alleviated endoplasmic reticulum stress in both the hippocampus and cortex. ***Conclusion:*** These results indicate that the mechanisms underlying the effects of bajijiasu on AD might be related to beta-amyloid-downstream pathologies, particularly endoplasmic reticulum stress.

Introduction

Alzheimer disease (AD), the most common neurodegenerative disorder, is defined by slowly progressing cognitive impairment and memory loss [1]. At the neuropathological level, AD is characterized by widespread oxidative stress, neuroinflammation, aggregation, and deposition of misfolded proteins, particularly aggregated β-amyloid (Aβ) peptide [2]

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and hyperphosphorylated tau protein [3-6]. Numerous sources of evidence have confirmed the central role of Aβ and its oligomers in the pathogenesis of AD [7-9]. Almost all approved treatments for AD are geared toward symptom management and do not target the underlying neuropathology. Unfortunately, all phase III clinical trials testing therapeutics directed at the neuropathological substrates of AD (i.e., Aβ) have failed [10, 11]. Therefore, novel drugs are urgently required to treat this complex disease. Additionally, amyloid deposits and tangles trigger subsequent pathologies, such as synaptic degeneration, oxidative stress, neuroinflammation, neurite degeneration, and endoplasmic reticulum (ER) stress [12-14]. These pathologies can themselves form vicious cycles and accelerate disease progression [15-17]. Thus, multiple targeted treatments are needed.

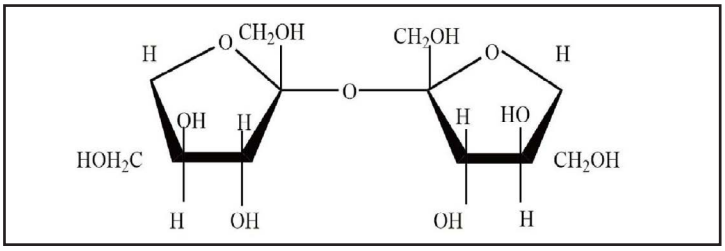

Fig. 1. Chemical structure of bajijiasu.

Morinda officinalis is a common medicinal herb in Southern China. It is a component herb that contains some potential active ingredients, including hexasaccharide and heptasaccharide, which have been shown to attenuate symptoms of depression [18-21]. Bajijiasu (previously known as bajisu), which is isolated from *Morinda officinalis*, has a dimeric fructose structure [β-D-fructofuranosyl (2-2)] (Fig. 1). Bajijiasu has been shown to be effective in ameliorating disease and pathological mechanisms such as oxidative stress [22, 23], reinforcing population spikes and long-term potentiation [19], attenuating D-galactose-induced cognitive dysfunction in mice, and protecting against neuronal damage or death induced by ischemia. Bajijiasu can also protect against $A\beta_{25-35}$-induced neurotoxicity in PC12 cells [24]. The protective effects of bajijiasu might involve enhanced anti-oxidative abilities, elevated intracellular Ca^{2+}concentrations, and reduced neuronal apoptosis [25]. These results indicate that bajijiasu plays an effective role in protecting against neuronal damage or death. However, we still have limited knowledge on the mechanisms involved.

In this study, we investigated the effect of bajijiasu on cognitive dysfunction in APP/PS1 mice. We treated the mice with bajijiasu (35 mg/kg/day and 70 mg/kg/day) for 4 weeks and evaluated the cognitive dysfunction. We explored the neuroprotective effects and levels of oxidative and ER stress.

Materials and Methods

Materials

Bajijiasu (purity > 98%) was extracted from *Morinda officinalis* root and donated by College of Chinese Material Medical, Guangzhou University of Chinese Medicine (Guangzhou, China). The purity of the compound was analyzed by high-performance liquid chromatography as previously described [22].Primary antibodies—PKR-like ER kinase (PERK), phosphorylated PERK (P-PERK), inositol-requiring enzyme (IRE-1α), phosphorylated IRE-1α (P-IRE-1α), phosphorylated eukaryotic initiation factor 2 (P-eIF-2α), eukaryotic initiation factor 2 (eIF-2α), binding immunoglobulin protein (BIP), protein-disulphide isomerase (PDI), C/EBP homologous protein (CHOP), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), Bcl-2, and caspase-3—were purchased from Cell Signaling Technology, Inc. Anti-Bax antibody was purchased from Santa Cruz Biotechnology, Inc. Anti-β-actin was purchased from Sigma-Aldrich. All secondary antibodies (horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG) were purchased from Cell Signaling Technology, Inc. All other reagents were of the highest grade available commercially.

Animals and treatment

APP/PS1 (APPswe/PSEN1dE9) double Tg mice were purchased from the Model Research Centre of Nanjing University, with wild-type mice (non-Tg mice) with the same background and age used as a control

group. Animals were housed at a standard temperature (22 ± 2 °C) with automatic light cycles (12-h light/dark) and a relative humidity of 40-60 %. All procedures involving mice were performed according to the protocols of the Guiding Principles for the Care and Use of Laboratory Animals adopted and promulgated by the United States National Institutes of Health. Eight-month-old mice were randomly divided into 4 groups: the vehicle control group (wild-type [WT], 0.9% saline, n = 10), APP/PS1 group (n =10), low-dose bajijiasu group (APP/PS1, bajijiasu 35 mg/kg/day, n = 10), and high-dose bajijiasu group (APP/PS1, bajijiasu 70 mg/kg/day, n = 10). Mice were treated with saline or bajijiasu by gavage once a day for 4 weeks.

Morris water maze test

Spatial learning-memory ability was assessed by the Morris water maze, performed according to the Morris method [26]. The water maze equipment (Guangzhou Feidi Biology Technology Co., Ltd., Guangzhou, China) consisted of a black circular tank filled with water at 24°C, a hidden platform, and a recording system. The pool was spatially divided into 4imaginary quadrants (target, opposite, left, and right) by a computerized tracking/image analyzer system. A circular transparent escape platform (10 cm diameter) was positioned 1-2 cm below the opaque water surface in the middle of the target quadrant of the pool. The learning and memory abilities of mice were assessed using the Morris water maze test in a dark room. Mice were given orientation navigation tests for 6 consecutive days. Before the measurement, mice were trained once to find the platform. For each daily trial, there were 4 sequential training trials beginning with placement of the animal in the water facing the wall of the pool with the drop location changing for each trial randomly; the recording system then started to record the time. The escape latency and the swim path tracking until the mice landed on the platform were recorded on video tape. If the mouse failed to locate the platform within 60 s, it was guided to the platform and kept there for 10 s. For the probe trials, the mice were allowed to swim freely in the pool for 60 s with platform removal. The time required to cross to the original platform position, the time spent in the target quadrant, and the swimming speed were measured.

Y-maze tests

Y-maze tests were used to assess cognitive changes, short-term spatial working memory (by spontaneous alternation), and exploratory activity (by total number of arm choices) of mice placed into a black Y-maze [27, 28]. The Y-maze is a three-arm horizontal maze (40 cm long and 10 cm wide with 12-cm-high walls) in which the arms are symmetrically disposed at 120° angles from each other. The task was carried out on day 1 of the behavioral tests. Mice were placed at the end of one arm and allowed to move freely through the maze during a 5-min session. Alternation was defined as successive entries into the three arms on overlapping triplet sets. The number of total arm choices and the sequence of arm choices were recorded. The percent alternation is defined by the proportion of arm choices differing from the last two choices. Before each trial, the interior of the maze was sprayed with a 70% ethanol solution to erase any scent cues.

Reactive oxygen species and malondialdehyde levels

The tissues were centrifuged at 12, 000 ×g for 10 min at 4 °C with ice-cold saline. We used the supernatant to detect the levels of reactive oxygen species (ROS) and malondialdehyde (MDA). ROS were measured using the radiosensitive fluorescent dye DCFH-DA. In the presence of ROS, nonfluorescent DCFH-DA converts to fluorescent dichlorofluorescein (DCF), which is measured on a microplate reader. The fluorescence emission intensity of DCF (538 nm) was measured in response to 485 nm excitation. The level of intracellular ROS is expressed as the percentage of control cultures incubated in DCFH-DA. The level of MDA was detected according to the manufacturer's instructions. The absorbance was read at 550 nm using a Universal Microplate Spectrophotometer (Bio-Rad, Hercules, CA, USA).

Western blot analysis

Western blotting was used to analyze the levels of proteins, including ER-related proteins, apoptosis-related proteins, and synapse-related proteins. The tissues were homogenized and lysed in sample buffer (0.5 M Tris/HCl pH 6.8, 50 % glycerol, 10 % sodium dodecyl sulphate [SDS], 1:100 inhibitor proteases, and a phosphatase cocktail). We centrifuged the lysate at 12, 000 ×g for 10 min at 4 °C and then boiled it at 100 °C with 1:4 loading buffer. The lysate (30 μg protein) was fractionated by SDS-polyacrylamide gel electrophoresis and then transferred onto 0.2-μM polyvinylidene fluoride sheets (PVDF) membranes.

After being blocked with 5% skim milk dissolved in TBST for 1 h at room temperature, transferred PVDF membranes were incubated at 4 °C overnight with the antibodies, including anti-PERK, anti-P-PERK, anti-IRE-1α, anti-P-IRE-1α, anti-eIF-2α, anti-P-eIF-2α, anti-BIP, anti-PDI, anti-CHOP, anti-BDNF, anti-NGF, anti-Bax, anti-caspase-3, anti-Bcl-2, anti-Bax, and mouse anti-β-actin. The membrane was then incubated with secondary antibody (anti-rabbit or anti-mouse) for 1.5 h at room temperature. Protein loading was detected by using super-enhanced chemiluminescence reagent (Applygen Technologies Inc., Beijing, China).

Statistical analysis

Experimental values are presented as the mean ± standard error of the mean (SEM). All statistical analyses were performed with SPSS 19.0 statistical software (IBM, Endicott, NY). Two-way analysis of variance was applied to analyze differences in data for the biochemical parameters among the different groups, followed by Dunnett's significant post-hoc test for pair-wise multiple comparisons. The level of statistical significance for all tests was $P < 0.05$.

Results

Bajijiasu rescues cognitive deficits in APP/PS1 mice

As shown in Fig. 2, the Morris water maze test demonstrated the effect of bajijiasu on cognitive deficits in APP/PS1 mice. Compared with the WT control group, the incubation period was markedly longer in the APP/PS1 group. After bajijiasu treatment, the mice in the high-dose group spent less time in the water than those in the APP/PS1 group (Fig. 2A). The change in the total swimming distance in each group was similar to the change observed for the latency period (Fig. 2B). On the seventh day, we conducted the probe trial with the platform removed, allowing the mice to swim freely, to estimate their spatial working memory. The mice in the APP/PS1 group spent less time in the target quadrant and had shorter crossing times than those in the WT group (Fig. 2C and 2D). The bajijiasu-treated groups had longer platform crossing times and spent more

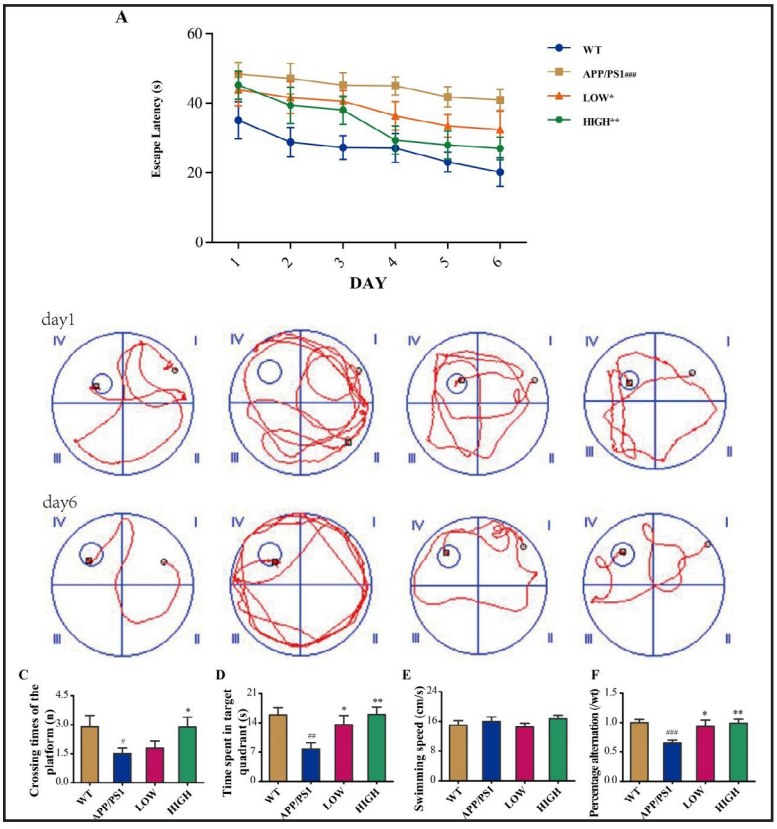

Fig. 2. Bajijiasu ameliorates cognitive dysfunction in behavioral testing in APP/PS1 mice.(A) Escape latency of five consecutive daily tests. (B) Swimming paths of the respective groups on the first and fifth day. (C) Target platform crossing times in the probe trial. (D) Time spent in the target quadrant in the probe trial. (E) Swimming speed in the probe trial. (F) Percentage alternation in the Y-maze test. WT: wild-type; LOW: bajijiasu (35 mg/kg/day); HIGH: bajijiasu (70 mg/kg/day). Data represent the mean ± SEM (n = 10 per group). $^{\#}P<0.05$, $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$ vs. WT; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ vs. APP/PS1.

time in the target quadrant than those in the APP/PS1 group. The average swimming speed was similar among all groups (Fig. 2E, $P > 0.05$). Cognitive ability was also determined as the percentalternation in the Y-maze. The results showed that the mice in the APP/PS1 group had a lower percentage of alternation than the WT group (Fig. 2F). After bajijiasu treatment, the percentage was significantly improved when compared with the APP/PS1 group (Fig. 2F).

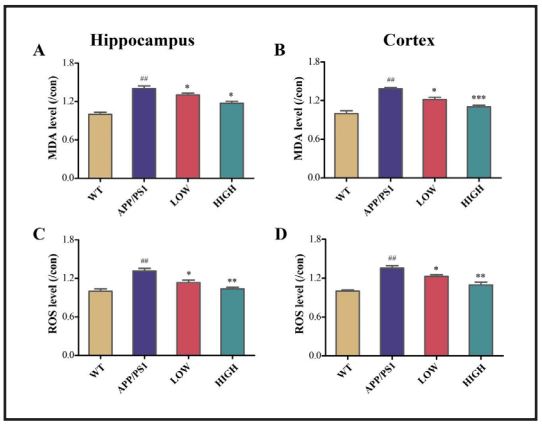

Fig. 3. Bajijiasu attenuates oxidation stress in APP/PS1 mice. (A) Level of MDA in both the hippocampus and cortex. (B) Level of ROS in both the hippocampus and cortex. WT: wild-type; LOW: bajijiasu (35 mg/kg/day); HIGH: bajijiasu (70 mg/kg/day). Data represent the mean ± SEM (n = 10 per group). #P<0.05, ##P<0.01, ###P<0.001 vs. WT; *P<0.05, **P<0.01, ***P<0.001 vs. APP/PS1.

Bajijiasu alleviates oxidative stress in the brain of APP/PS1 mice

To determine the effect of bajijiasu on oxidative stress status, we tested the levels of MDA and ROS in both the hippocampus and cortex. The levels of MDA and ROS were higher in the APP/PS1 group than in the WT group. The levels of MDA and ROS were decreased sharply after 4-week oral administration of bajijiasu compared with the APP/PS1 group (Fig. 3A and B).

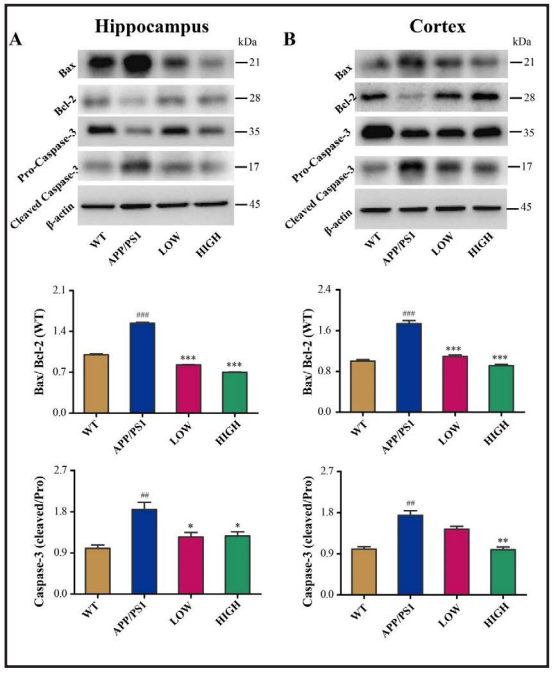

Fig. 4. Bajijiasu hinders apoptosis in both the hippocampus and cortex. (A and B) Expression of Bax, Bcl-2, and caspase-3 was detected with western blotting in both the hippocampus and cortex. WT: wild-type; LOW: bajijiasu (35 mg/kg/day); HIGH: bajijiasu (70 mg/kg/day). Data represent the mean ± SEM (n = 10 per group). #P<0.05, ##P<0.01, ###P<0.001 vs. WT; *P<0.05, **P<0.01, ***P<0.001 vs. APP/PS1.

Bajijiasu decreases neuronal apoptosis in APP/PS1 mice

As shown in Fig. 4A and B, we tested the state of apoptosis in the hippocampus and cortex. The expression levels of the proapoptotic proteins Bax and cleaved caspase-3 increased while that of Bcl-2 (an inhibitor of apoptotic proteins) decreased in APP/PS1 when compared with the WT group. Administration of bajijiasu increased Bcl-2 expression and decreased Bax and cleaved caspase-3 expressions compared with the APP/PS1 group.

Bajijiasu increases neurotrophic factor levels in APP/PS1 mice

As shown in Fig. 5, the protein expressions of the neurotrophic factors NGF and BDNF were sharply decreased in APP/PS1 mice compared with the WT group. After treatment with bajijiasu, the levels of NGF and BDNF returned to the normal level (Fig. 5A and B) in

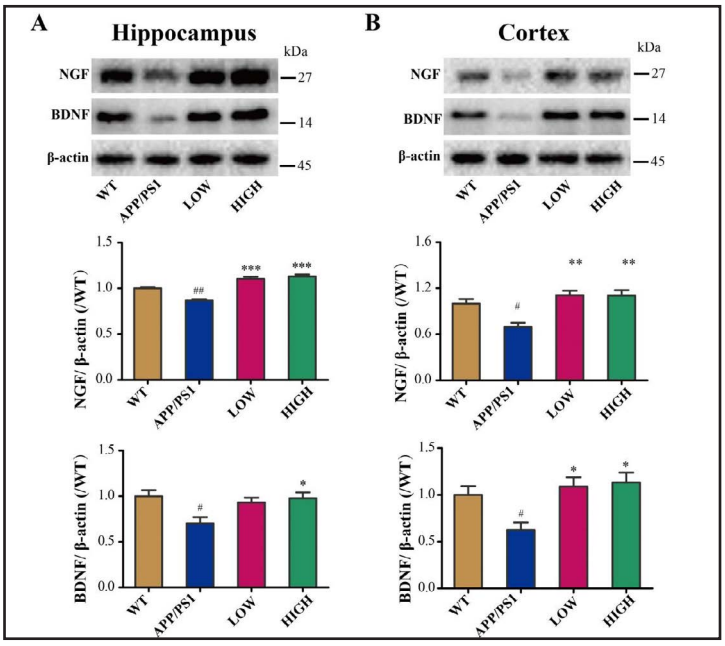

Fig. 5. The effect of bajijiasu on neurodegeneration in APP/PS1 mice. Western blotanalysis of NGF and BDNF in the hippocampus (A) and cortex (B). WT: wild-type; LOW: bajijiasu (35 mg/kg/day); HIGH: bajijiasu (70 mg/kg/day). Data represent the mean ± SEM (n = 10 per group). #P<0.05, ##P<0.01, ###P<0.001 vs. WT; *P<0.05, **P<0.01, ***P <0.001 vs. APP/PS1.

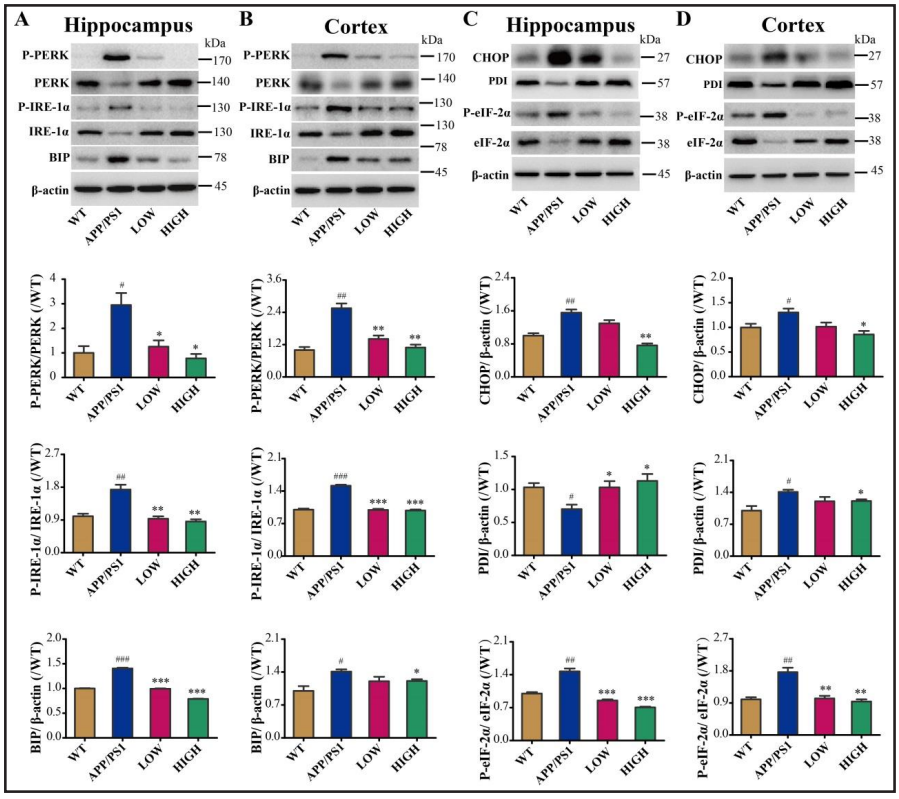

Fig. 6. Bajijiasu ameliorates endoplasmic reticulum (ER) stress in APP/PS1 mice. (A) Expression of ER-associated proteins in the hippocampus and cortex. (B) Expression of PKR-like ER (PERK), phosphorylated PERK (P-PERK), binding immunoglobulin protein (BIP), inositol-requiring enzyme (IRE-1α), and phosphorylated IRE-1α (P-IRE-1α) in the hippocampusand cortex. Expression of proteins related to apoptosis in the ER: protein-disulfide isomerase (PDI), C/EBP homologous protein (CHOP), initiation factor 2α (eIF-2α), and phosphorylatedeIF-2α(P-eIF-2α) in the hippocampus (C) and cortex (D).WT: wild-type; LOW: bajijiasu (35 mg/kg/day); HIGH: bajijiasu (70 mg/kg/day). Data represent mean the ± SEM (n = 10 per group). #P<0.05, ##P<0.01, ###P<0.001 vs. WT; *P <0.05, **P<0.01, ***P<0.001 vs. APP/PS1.

both the hippocampus and cortex.These results indicated that bajijiasu could ameliorate neurodegeneration in APP/PS1 mice.

Bajijiasu attenuates ER stress in APP/PS1 mice

We next measured the levels of two ER stress transducers, PERK and IRE-1α, and the expression of the chaperone BIP (Fig. 6A and B). There was an increase in the active forms of the effectors of the unfolded protein response (UPR;i.e., P-PERK, P-IRE-1α) and in BIP in the APP/PS1 group compared with the WT group, but a decrease after the administration of bajijiasu. To further evaluate the consequences of ER stress, we studied the expression levels of CHOP, PDI, and eIF-1α. Both CHOP and P-eIF-1α were higher in APP/PS1 mice than in WT mice, but decreased after the bajijiasu intervention. The expression of PDI significantly decreased in APP/PS1 mice, but increased in the bajijiasu groups in both the hippocampus and cortex (Fig. 6C and D). These results indicated that bajijiasu could attenuate ER stress in APP/PS1 mice.

Discussion

In this study, we demonstrated that bajijiasu is able to mitigate cognitive dysfunction in APP/PS1 mice. Our experiments involved 4-week oral administration of bajijiasu to 8-month-old mice. The results indicated that bajijiasu ameliorates learning and memory abilities and that the neuroprotective effects of bajijiasu on cognitive dysfunction in APP/PS1 mice are related to protection against apoptosis, oxidative stress, and ER stress (Fig. 7).

The ER, the organelle in eukaryotic cells, is responsible for protein folding and transport. When proteins become misfolded in the ER, the UPR is elicited to maintain homeostasis [29, 30]. ER stress and activated UPR signaling are detected in the brains of both AD patients and AD animal models [31, 32]. AD is a very complex disease caused by a complicated interaction among genetic and environmental factors [33].The amyloid hypothesis suggests that accumulation of Aβ in the brain is critical to AD pathogenesis. Numerous studies have demonstrated that Aβ disturbs the function of the ER, leading to the accumulation of unfolded proteins in the ER and resulting in ER stress, which triggers the UPR [34-38]. Considerable evidence demonstrates that the occurrence of AD mutations is closely related to ER stress. PERK and eIF-2α are positively correlated with Aβ plaque aggregation in the brains of AD patients and APP/PS1 mice [14, 39-41]. Molecular alteration in translational machinery through phosphorylation of eIF-2α may play a key role in the pathogenesis of neurodegenerative diseases [40, 42]. Accordingly, we hypothesized that bajijiasu, a potential therapy for AD, may help to ameliorate cognitive dysfunction related to Aβ and ER stress. There is a strong resemblance between PERK and IRE-1α: both have a cytoplasmic kinase domain involving serine/threonine kinase and an N-terminal luminal domain [42]. Moreover, PERK and IRE-1α are activated through a similar mechanism, which demands oligomerization of monomers into dimers or higher structures [43]. When the level of unfolded and misfolded proteins in the ER is sufficiently high, the ER chaperone protein BIP is titrated away from PERK/IRE-1α monomers [42, 44].

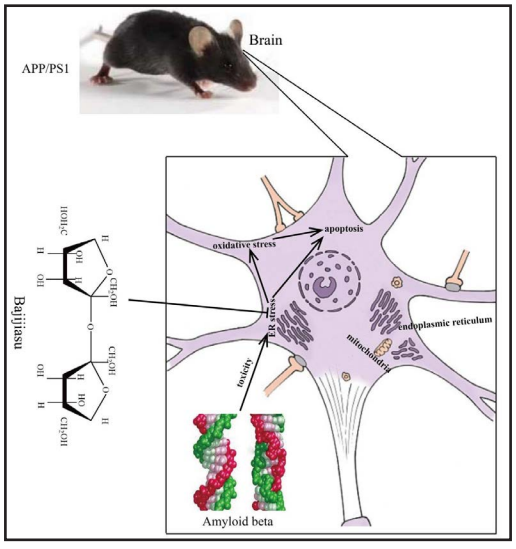

Fig. 7. Schematic summary illustrating bajijiasu amelioration of endoplasmic reticulum stress, oxidative stress, and apoptosis induced by β-amyloidin APP/PS1 mice. A characteristic of Alzheimer disease is the aggregation of β-amyloid (Aβ) peptide. Aβ aggregation can induce ER stress, oxidative stress, and apoptosis in the brain of APP/PS1 mice. Bajijiasu ameliorates ER stress, oxidative stress, and neurotoxicity induced by Aβ.

In this study, we found higher expression levels of P-PERK (an ER stress sensor),P-IRE-1α,P-eIF-2α, and BIP in the hippocampus and

cortex of the APP/PS1 group than the WT group, which demonstrates that the brain of APP/PS1 mouse is under ER stress conditions. ER stress was attenuated by bajijiasu treatment, which was accompanied by high expression of PERK. Above all, we can prove the occurrence of ER stress in the brain of APP/PS1 mice. After 4-week administration of bajijiasu, these proteins returned to the normal level, which demonstrate the attenuation of ER stress. When ER stress is prolonged or overwhelming, the UPR fails to restore ER homoeostasis and the apoptotic cascade is activated [45, 46]. Long-term activation of the PERK/eIF-2α signaling pathway will induce upregulation of the proapoptotic transcription factor CHOP, indicating apoptosis. This apoptosis in APP/PS1 mice is accompanied by high expression of CHOP and low expression of PDI, indicating the activation of PERK/eIF-2α, an apoptotic signaling pathway related to ER stress. Moreover, the neurotoxicity of Aβ induces synaptic dysfunction and apoptotic and necrotic cell death [47, 48]. In this study, the expression levels of Bax/Bcl-2 and cleaved caspase-3/pro-caspase-3 were significantly higher in APP/PS1 mice. Compared with the APP/PS1 group, bajijiasu groups showed apoptosis inhibition. These results demonstrate that bajijiasu can attenuate Aβ-induced ER stress and apoptosis.

Hippocampal and cortical neurons are the most severely affected cells in AD [49]. Aβ exerts strong detrimental effects by inducing excitotoxicity [50] and synaptic dysfunction [51-53]. Synapses, composed of presynaptic axonal terminals and postsynaptic dendritic spines [54, 55], mediate the transmission of information between neurons. NGF and BDNF carry out a variety of actions in these neurons and are involved in the clinical and pathophysiological signs of AD [56, 57]. A reduction in neurotrophin levels occurs in some areas of the CNS in AD [58]. Here, a significant reduction was found in NGF and BDNF in the hippocampus and cortex of APP/PS1 mice. Neurotrophic factors have been used in clinical trials to prevent or reduce neuronal cell loss or to improve hippocampus neurogenesis in adult and aged male rats [59]. After treatment with bajijiasu, the expression levels of NGF and BDNF were increased in both the hippocampus and cortex compared with the APP/PS1 group. The restoration of neurotrophic factor levels demonstrates the recovery of neuronal function and the protective effect of bajijiasu in neurodegeneration diseases.

The brains of AD patients are exposed to oxidative stress during the process of the disease [60]. Aβ peptides exacerbate the overexpression of ROS, which damage proteins, DNA, lipids, and other compounds [19, 61]. Many studies consider oxidative stress and ER stress as closely linked events playing crucial roles in cell homeostasis and apoptosis [62, 63]. As shown here, ER stress response can alter the cellular respiration and perturb mitochondrial bioenergetics, inducing ROS overproduction [64]. Long-term activation of ER stress with ROS generation activates cell death [65]. MDA is a naturally occurring organic compound that is a marker of oxidative stress [66]. In this study, administration of bajijiasu robustly decreased ROS and MDA levels, demonstrating the ability of bajijiasu to inhibit the formation of reactive oxygen radicals and eliminate lipid-free radicals. The occurrence of oxidative stress mediates damage to neurons and eventually leads to dramatic neuronal loss and cognitive dysfunction [67]. Our results showing that bajijiasu improves the learning and memory abilities of APP/PS1 mice might be related to its ability to ameliorate oxidative stress.

In this study, we showed the neuroprotective effects of bajijiasu on cognitive dysfunction in APP/PS1 mice. We also determined that the underlying mechanism might be related to Aβ and downstream pathologies such as ER stress. However, given the complexity of AD pathology and its uncertain pathogenesis, we need to obtain a more complete and deeper understanding of the effect of bajijiasu on AD.

Acknowledgements

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Disclosure Statement

The authors certify that there is no conflicts of interest.

References

1 Rathmann KL, Conner CS: Alzheimer's disease: clinical features, pathogenesis, and treatment. Drug Intell Clin Pharm 1984;18:684-691.
2 Selkoe DJ: Alzheimer's disease: Genes, proteins, and therapy. Physiol Rev 2001;81:741-766.
3 Grundke-Iqbal I, Iqbal K, Tung YC, Quinlan M, Wisniewski HM, Binder LI: Abnormal phosphorylation of the microtubule-associated protein tau (tau) in Alzheimer cytoskeletal pathology. Proc Natl Acad Sci U S A 1986;83:4913-4917.
4 Wyss-Coray T: Inflammation in Alzheimer disease: driving force, bystander or beneficial response? Nat Med 2006;12:1005-1015.
5 Schoonenboom NSM, Pijnenburg YAL, Mulder C, Rosso SM, Van Elk EJ, Van Kamp GJ, Van Swieten JC, Scheltens P: Amyloid beta(1-42) and phosphorylated tau in CSF as markers for early-onset Alzheimer disease. Neurology 2004;62:1580-1584.
6 Sobow T, Flirski M, Liberski PP: Amyloid-beta and tau proteins as biochemical markers of Alzheimer's disease. Acta Neurobiol Exp (Warsz) 2004;64:53-70.
7 Selkoe DJ, Hardy J: The amyloid hypothesis of Alzheimer's disease at 25years. Embo Mol Med 2016;8:595-608.
8 Barage SH, Sonawane KD: Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. Neuropeptides 2015;52:1-18.
9 Korczyn AD: The amyloid cascade hypothesis. Alzheimers Dement 2008;4:176-178.
10 Mullane K, Williams M: Alzheimer's therapeutics: Continued clinical failures question the validity of the amyloid hypothesis-but what lies beyond? Biochem Pharmacol 2013;85:289-305.
11 Gauthier S, Feldman HH, Schneider LS, Wilcock GK, Frisoni GB, Hardlund JH, Moebius HJ, Bentham P, Kook KA, Wischik DJ, Schelter BO, Davis CS, Staff RT, Bracoud L, Shamsi K, Storey JMD, Harrington CR, Wischik CM: Efficacy and safety of tau-aggregation inhibitor therapy in patients with mild or moderate Alzheimer's disease: a randomised, controlled, double-blind, parallel-arm, phase 3 trial. Lancet 2016;388:2873-2884.
12 Mucke L, Selkoe DJ: Neurotoxicity of Amyloid beta-Protein: Synaptic and Network Dysfunction. CSH Perspect Med DOI: 10.1101/cshperspect.a006338."
13 Walsh DM, Selkoe DJ: Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron 2004;44:181-193.
14 Devi L, Ohno M: PERK mediates eIF2 alpha phosphorylation responsible for BACE1 elevation, CREB dysfunction and neurodegeneration in a mouse model of Alzheimer's disease. Neurobiol of Aging 2014;35:2272-2281.
15 Zawia NH, Lahiri DK, Cardozo-Pelaez F: Epigenetics, oxidative stress, and Alzheimer disease. Free Radic Biol Med 2014;35:2272-2281.
16 Guglielmotto M, Giliberto L, Tamagno E, Tabaton M: Oxidative stress mediates the pathogenic effect of different Alzheimer's disease risk factors. Front Aging Neurosci DOI: 10.3389/neuro.24.003.2010.
17 Tamagno E, Guglielmotto M, Monteleone D, Tabaton M: Amyloid-beta Production: Major Link Between Oxidative Stress and BACE1. Neurotox Res 2012;22:208-219.
18 Li YF, Li Y, Xu YK, Yang M, Zhao YM, Luo ZP: Antistress effect of oligosaccharides extracted from Morinda officinalis in mice and rats. Acta Pharmacol Sin2001;22:1084-1088.
19 Li YF, Liu YQ, Yang M, Wang HL, Huang WC, Zhao YM, Luo ZP: The cytoprotective effect of inulin-type hexasaccharide extracted from Morinda officinalis on PC12 cells against the lesion induced by corticosterone. Life Sci 2004;75:1531-1538.
20 Li J, Zhang H-L, Wang Z, Liang Y-M, Jiang L, Ma W, Yang D-P: Determination content of the antidepressant extraction and analysis the trace elements from Morinda officinalis. J Chin Med Mater 2008;31:1337-1340.
21 Jun LI, Hualin Z, Zhe W, Yuming L, Lin J, Wei MA, Depo Y: Determination Content of the Antidepressant Extraction and Analysis the Trace Elements from Morinda officinalis. J Chin Med Mater 2008;31:1337-1340.

22 Chen D-L, Zhang P, Lin L, Zhang H-M, Deng S-D, Wu Z-Q, Ou S, Liu S-H, Wang J-Y: Protective effects of bajijiasu in a rat model of A beta(25-35)-induced neurotoxicity. J Ethnopharmacol 2014;154:206-217.
23 Chen D-l, Li N, Lin L, Deng S-d, Zhang H-m, Liu S-h: Method to detect the variants of the erythrocyte in a rat model of A beta(25-35)-induced neurotoxicity based on micro-Raman spectroscopy. J Biomed Opt 2013;18:
24 Chen D-L, Zhang P, Lin L, Shuai O, Zhang H-M, Liu S-H, Wang J-Y: Protective Effect of Bajijiasu Against beta-Amyloid-Induced Neurotoxicity in PC12 Cells. Cell Mol Neurobiol 2013;33:837-850.
25 Fang-hua LIN, Li LIN, Feng-xia X, Xiao-han LIU, Jin-yu W: Effect of Bajijiasu on mating capability and immune organ coefficients in healthy male mice. Chin J N Drugs 2008;17:1924-1926.
26 Himeno E, Ohyagi Y, Ma L, Nakamura N, Miyoshi K, Sakae N, Motomura K, Soejima N, Yamasaki R, Hashimoto T, Tabira T, LaFerla FM, Kira J-i: Apomorphine Treatment in Alzheimer Mice Promoting Amyloid-beta Degradation. Ann Neurol 2011;69:248-256.
27 Meunier J, Ieni J, Maurice T: The anti-amnesic and neuroprotective effects of donepezil against amyloid beta(25-35) peptide-induced toxicity in mice involve an interaction with the sigma(1) receptor. Br J Pharmacol 2006;149:998-1012.
28 Tsunekawa H, Noda Y, Mouri A, Yoneda F, Nabeshima T: Synergistic effects of selegiline and donepezil on cognitive impairment induced by amyloid beta (25-35). Behav Brain Res 2008;190:224-232.
29 Kim I, Xu W, Reed JC: Cell death and endoplasmic reticulum stress: disease relevance and therapeutic opportunities. Nat Rev Drug Discov 2008;7:1013-1030.
30 Vembar SS, Brodsky JL: One step at a time: endoplasmic reticulum-associated degradation. Nat Rev Mol Cell Bio 2008;9:944-U930.
31 Endres K, Reinhardt S: ER-stress in Alzheimer's disease: turning the scale? Am J Neurodegener Dis 2013;2:247-265.
32 Hoozemans JJM, van Haastert ES, Nijholt DAT, Rozemuller AJM, Scheper W: Activation of the Unfolded Protein Response Is an Early Event in Alzheimer's and Parkinson's Disease. Neurodegener Dis 2012;10:212-215.
33 Hampel H, Prvulovic D, Teipel S, Jessen F, Luckhaus C, Froeliche L, Riepe MW, Dodel R, Leyhe T, Bertram L, Hoffmann W, Faltraco F: The future of Alzheimer's disease: The next 10 years. Prog Neurobiol 2011;95:718-728.
34 Ferreiro E, Resende R, Costa R, Oliveira CR, Pereira CMF: An endoplasmic-reticulum-specific apoptotic pathway is involved in prion and amyloid-beta peptides neurotoxicity. Neurobiol Dis 2006;23:669-678.
35 Ghribi O: The role of the endoplasmic reticulum in the accumulation of p-amyloid peptide in Alzheimer's disease. Cur Mol Med 2006;6:119-133.
36 Chafekar SM, Hoozemans JJM, Zwart R, Baas F, Scheper W: A beta(1-42) induces mild endoplasmic reticulum stress in an aggregation state-dependent manner. Antioxid Redox Sign 2007;9:2245-2254.
37 Chafekar SM, Zwart R, Veerhuis R, Vanderstichele H, Baas F, Scheper W: Increased A beta(1-42) Production Sensitizes Neuroblastoma Cells for ER Stress Toxicity. Curr Alzheimer Res 2008;5:469-474.
38 Pinkaew D, Changtam C, Tocharus C, Thummayot S, Suksamrarn A, Tocharus J: Di-O-demethylcurcumin protects SK-N-SH cells against mitochondrial and endoplasmic reticulum-mediated apoptotic cell death induced by A beta(25-35). Neurochem Int 2015;80:110-119.
39 O'Connor T, Sadleir KR, Maus E, Velliquette RA, Zhao J, Cole SL, Eimer WA, Hitt B, Bembinster LA, Lammich S, Lichtenthaler SF, Hebert SS, De Strooper B, Haass C, Bennett DA, Vassar R: Phosphorylation of the Translation Initiation Factor eIF2 alpha Increases BACE1 Levels and Promotes Amyloidogenesis. Neuron 2008;60:988-1009.
40 Ohno M: Roles of eIF2 alpha kinases in the pathogenesis of Alzheimer's disease. Front Mol Neurosci DOI: 10.3389/fnmol.2014.00022."
41 Wu Q, Ye X, Xiong Y, Zhu H, Miao J, Zhang W, Wan J: The Protective Role of microRNA-200c in Alzheimer's Disease Pathologies Is Induced by Beta Amyloid-Triggered Endoplasmic Reticulum Stress. Front Mol Neurosci 2016;9:
42 Hugo Cornejo V, Hetz C: The unfolded protein response in Alzheimer's disease. Semin Immunopathol 2013;35:277-292.
43 Cui W, Li J, Ron D, Sha B: The structure of the PERK kinase domain suggests the mechanism for its activation. Acta Crystallogr D 2011;67:423-428.
44 Rozpedek W, Markiewicz L, Diehl JA, Pytel D, Majsterek I: Unfolded Protein Response and PERK Kinase as a New Therapeutic Target in the Pathogenesis of Alzheimer's Disease. Curr Med Chem 2015;22:3169-3184.

45 Tabas I, Ron D: Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress. Nat Cell Bio 2011;13:184-190.
46 Jing G, Wang JJ, Zhang SX: ER Stress and Apoptosis: A New Mechanism for Retinal Cell Death. Exp Diabetes Res DOI: 10.1155/2012/589589."
47 Obulesu M, Lakshmi MJ: Apoptosis in Alzheimer's Disease: An Understanding of the Physiology, Pathology and Therapeutic Avenues. Neurochem Res 2014;39:2301-2312.
48 Hardy J, Selkoe DJ: Medicine - The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. Science 2002;297:353-356.
49 Allen SJ, Dawbarn D: Clinical relevance of the neurotrophins and their receptors. Clin Sci 2006;110:175-191.
50 Harkany T, Abraham I, Timmerman W, Laskay G, Toth B, Sasvari M, Konya C, Sebens JB, Korf J, Nyakas C, Zarandi M, Soos K, Penke B, Luiten PGM: beta-Amyloid neurotoxicity is mediated by a glutamate-triggered excitotoxic cascade in rat nucleus basalis. Eur J Neurosci 2000;12:2735-2745.
51 Walsh DM, Klyubin I, Fadeeva JV, Cullen WK, Anwyl R, Wolfe MS, Rowan MJ, Selkoe DJ: Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. Nature 2002;416:535-539.
52 Leuner B, Shors TJ: Stress, Anxiety, and Dendritic Spines: What Are the Connections? Neuroscience 2013;251:108-119.
53 Howland JG, Wang YT: Synaptic plasticity in learning and memory: stress effects in the hippocampus; in Essence of Memory. 2008;169:145-158.
54 Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA: Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 2011;476:458-U112.
55 Miller DB, O'Callaghan JP: Aging, stress and the hippocampus. Ageing Res Rev 2005;4:123-140.
56 Williams BJ, Eriksdotter-Jonhagen M, Granholm A-C: Nerve growth factor in treatment and pathogenesis of Alzheimer's disease. Prog Neurobiol 2006;80:114-128.
57 Arancio O, Chao MV: Neurotrophins, synaptic plasticity and dementia. Curr Opin Neurobiol 2007;17:325-330.
58 Tuszynski MH, Thal L, Pay M, Salmon DP, Sang UH, Bakay R, Patel P, Blesch A, Vahlsing HL, Ho G, Tong G, Potkin SG, Fallon J, Hansen L, Mufson EJ, Kordower JH, Gall C, Conner J: A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nat Med 2005;11:551-555.
59 Frielingsdorf H, Simpson DR, Thal LJ, Pizzo DP: Nerve growth factor promotes survival of new neurons in the adult hippocampus. Neurobiol Dis 2007;26:47-55.
60 Fukui K, Takatsu H, Shinkai T, Suzuki S, Abe K, Urano S: Appearance of amyloid beta-like substances and delayed-type apoptosis in rat hippocampus CA1 region through aging and oxidative stress. J Alzheimers Dis 2005;8:299-309.
61 Foyet HS, Asongalem AE, Oben EK, Cioanca O, Hancianu M, Hritcu L: Effects of the Methanolic Extract of Vitellaria paradoxa Stem Bark Against Scopolamine-Induced Cognitive Dysfunction and Oxidative Stress in the Rat Hippocampus. Cell Mol Neurobiol 2016;36:1139-1149.
62 Ashraf NU, Sheikh TA: Endoplasmic reticulum stress and Oxidative stress in the pathogenesis of Non-alcoholic fatty liver disease. Free Radic Res 2015;49:1405-1418.
63 Ri M: Endoplasmic-reticulum stress pathway-associated mechanisms of action of proteasome inhibitors in multiple myeloma. Int J Hematol 2016;104:273-280.
64 Kaufman RJ, Malhotra JD: Calcium trafficking integrates endoplasmic reticulum function with mitochondrial bioenergetics. BBA-Mol CellRes2014;1843:2233-2239.
65 Manole E, Bastian AE, Butoianu N, Goebel HH: Myositis non-inflammatory mechanisms: An up-dated review. J Immunoass Immunoch 2017;38:115-126.
66 Del Rio D, Stewart AJ, Pellegrini N: A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovas 2005;15:316-328.
67 Sanchez PE, Zhu L, Verret L, Vossel KA, Orr AG, Cirrito JR, Devidze N, Ho K, Yu G-Q, Palop JJ, Mucke L: Levetiracetam suppresses neuronal network dysfunction and reverses synaptic and cognitive deficits in an Alzheimer's disease model. Proc Natl Acad Sci U S A 2012;109:E2895-E2903.