The effects of Danggui-Shaoyao-San on neuronal degeneration and amyloidosis in mouse and its molecular mechanism for the treatment of Alzheimer’s disease

Cong Yang1,2,†, You-Sheng Mo1,2,†, Hui-Fang Chen1,2, Yan-Hui Huang1,2, Shao-Ling Li1,2, Hong Wang1, Shui-Qing Huang1,2, Xiang Chang3, Qun Du1,6, Qi Wang1,2,†

1 Science and Technology Innovation Center, Guangzhou University of Chinese Medicine, 510405 Guangzhou, Guangdong Province, China
2 Institute of Clinical Pharmacology, Guangzhou University of Chinese Medicine, 510405 Guangzhou, Guangdong Province, China
3 Xi’an Hospital of Traditional Chinese Medicine, 710021 Xi’an, Shaanxi Province, China

*Correspondence: duqun@gzucm.edu.cn (Qun Du); wangqi@gzucm.edu.cn (Qi Wang)
† These authors contributed equally.
DOI: 10.31083/j.jin2002025
This is an open access article under the CC BY 4.0 license (https://creativecommons.org/licenses/by/4.0/).
Submitted: 30 September 2020 Revised: 6 December 2020 Accepted: 1 February 2021 Published: 30 June 2021

The abnormal deposition of the extracellular amyloid-β peptide is the typical pathological hallmark of Alzheimer’s disease. Strategies to reduce the amyloid-β deposition effectively alleviate the neuronal degeneration and cognitive deficits of Alzheimer’s disease. Danggui-Shaoyao-San has been considered a useful therapeutic agent known for the treatment of Alzheimer’s disease. However, the mechanism of Danggui-Shaoyao-San for the treatment of Alzheimer’s disease remains unclear. We investigated Danggui-Shaoyao-San’s effect on amyloidosis and neuronal degeneration in an APP/PS1 mouse model. We found Danggui-Shaoyao-San alleviated the cognitive deficits in APP/PS1 mice. Additionally, Danggui-Shaoyao-San ameliorated the neuronal degeneration in these mice. Danggui-Shaoyao-San reduced the amyloidosis and amyloid-β1–42 deposition in APP/PS1 mouse brain and down-regulated the receptor for advanced glycation end products, and up-regulated the level of low-density lipoprotein receptor-related protein-1. However, the protein expression of the β-amyloid precursor protein, β-Secretase and presenilin-1 (PS1) in the amyloid-β production pathway, and the expression of neprilysin and insulin-degrading enzyme in the amyloid-β degradation pathway were not altered. Our findings collectively suggest that Danggui-Shaoyao-San could ameliorate the amyloidosis and neuronal degeneration of Alzheimer’s disease, which may be associated with its up-regulation lipoprotein receptor-related protein-1 and down-regulation of the receptor for advanced glycation end products.

Keywords
Danggui-Shaoyao-San; Alzheimer’s disease; Cognitive deficits; Amyloidosis; Lipoprotein receptor-related protein-1; RAGE

1 Introduction
Alzheimer’s disease (AD) is a common neurodegenerative disease that is age-related and features progressive memory decline [1]. Its pathologic characteristic is the deposition of extracellular amyloid-β (Aβ) peptides and the formation of intracellular neurofibrillary tangles (NFTs). Although the exact mechanism of AD is not fully clarified, accumulating evidence supports a crucial role of Aβ in the process of neurodegeneration and cognitive changes of AD [2–4]. The overproduction and accumulation of Aβ in the brain are tightly related to neuronal cell death in AD. It is also reported that the abnormal deposition of Aβ peptides in the brain can induce oxidative stress and neuroinflammation and initiate a cascade of pathologic events, such as tau-hyperphosphorylation, neurite degeneration and neuronal loss [5-7].

Aβ is the product of sequential proteolytic processing of Aβ precursor protein (APP) by β and γ secretases. Aβ1–40 and Aβ1–42 are the primary forms of Aβ peptide. Compared with the former, Aβ1–42 is considered more amyloidogenic and toxic in the brain [8]. Aβ can be removed from the brain through enzymatic degradation, cell uptake and blood-to-brain (BBB) transport [9, 10]. Low-density lipoprotein receptor-related protein-1 (LRP1) and the receptor for advanced glycation end products (RAGE) are the primary transporters in BBB Aβ transport. LRP1 modulates the outflow of Aβ from the brain tissues to peripheral blood, while RAGE is the primary carrier of soluble Aβ from peripheral blood circulation to the brain [11, 12]. There is growing evidence that the clearance of Aβ is significantly impaired in most patients with AD [13, 14]. There is reported down-regulation of LRP1 and a decrease in the amount of Aβ efflux in AD patients. Studies also showed that the level of RAGE increases in many AD brain regions, and the blockade of RAGE could alleviate the amyloidosis [13]. Therefore, regulating LRP1 and RAGE expression, responsible for Aβ transportation, may be an effective strategy to reduce the abnormal Aβ deposition in AD.

To date, many therapies targeting Aβ production have failed in phase III clinical trial, including β-secretase and γ-secretase inhibitors and Aβ monoclonal antibody [2]. Traditional Chinese medicine research recently attracted atten-
tion in AD treatment [15–17]. Danggui-Shaoyao-San (DSS) is a well-known Chinese herbal medicine consisting of Radix angelicae sinensis, Radix paeoniae alba, and Rhizoma Ligustici, Poria, Rhizoma atractylodis macrocephalae and Rhizoma Alismatis.

Studies suggest that DSS may be an effective treatment for AD [18–20]. Our earlier research demonstrated that DSS effectively alleviates cognitive impairment in APP/PS1 mice [21]. We also found that DSS can enhance the expression of LRP1 in rats with vascular cognitive dysfunction [22]. Although previous studies have proved DSS is effective to reduce the neurotoxicity induced by Aβ, it remains unknown whether DSS is effective to alleviate the Aβ deposition of AD, and if so, what is the molecular mechanism. We aim to examine the effects of DSS on neuronal damage and amyloidosis and explore the possible molecular mechanism of DSS treatment on AD in an APP/PS1 transgenic mouse model.

2. Materials and methods

2.1 Antibodies and reagents

The primary antibodies: Rabbit monoclonal Aβ1-42 (#39377), Rabbit monoclonal anti-APP (#32136), Rabbit monoclonal anti-PS1 (#76083), Rabbit monoclonal anti-BACE1 (#183612), Rabbit monoclonal anti-PSD95 (#18258), Rabbit polyclonal anti-NGF (#6199), Rabbit monoclonal anti-BDNF (#108319), Rabbit polyclonal anti-NEP (#227195), Rabbit monoclonal anti-ID1 (#133561), Rabbit polyclonal anti-RAGE (#3611) and Rabbit monoclonal anti-LRP1 (#92544) were purchased from Abcam, Inc. Mouse monoclonal anti-β-actin (#A1978) was purchased from Sigma-Aldrich. Rabbit monoclonal anti-SYN (#4329s) and all secondary antibodies: Horse anti-mouse (#7076), Goat anti-rabbit IgG HRP-linked antibody (#7074) and Alexa Fluor 594 Goat anti-Rabbit IgG (#8889) were purchased from Cell Signaling Technology, Inc. Thioflavin S (ThS) was purchased from Sigma-Aldrich.

2.2 Preparation of DSS

The six raw herbs of DSS, including Radix angelicae sinensis, Radix paeoniae alba, Rhizoma Ligustici, Poria, Rhizoma atractylodis macrocephalae and Rhizoma Alismatis were obtained from Kangmei pharmaceutical co., ltd (Guangdong, P.R. China). They were mixed at a ratio of 3 : 16 : 8 : 4 : 8 : 4. The DSS extraction process was completed based on our previous study [21]. The mixed dried herbs were briefly immersed with 8 times (v/w) distilled water for 1 hour and were then decocted for 1 hour. The filtrate was collected, and the residue was again decocted with six times (v/w) distilled water for 1 h. The filtrates were mixed, then concentrated. Finally, it was determined that 1 mL of DSS contained 0.64 g of crude herbs. The mixture was stored at -20 °C and then recomposed with distilled water for use.

2.3 Animals and drug treatment

Male APPswe/PSEN1dE9 (APP/PS1) double-transgenic mice and wild-type mice (Non-Tg mice, WT) were 3-months old. All mice were purchased from Nanjing University (Nanjing, P. R. China). All mice were reared in a standard SPF environment (22 °C-25 °C, 40% - 60% relative humidity).

After three months of feeding, mice in APP/PS1 group were divided into three groups randomly: APP/PS1 group (model group), APP/PS1+DSS group (DSS group), and an APP/PS1 + Donepezil group (Donepezil group). The Donepezil group (3 mg/kg/day) was used as a positive drug group, and the wild-type mice were set as the control group (n = 10–12 per group). The dose of DSS 6.4 g/kg/d used in the present study was based on our previous study [21]. Mice in the drug treatment groups received DSS or Donepezil treatment by oral gavage once a day for 8 weeks. Mice in control and model groups were gavaged with distilled water.

2.4 Behavioral measurement

2.4.1 Morris water maze (MWM) test

The Morris water maze test protocols were carried out with our previous studies with few modifications [23]. The Morris water maze consists of a circular pool with a circular platform. The circular pool was 120 cm in diameter and 50 cm deep. The swimming pool was divided into four virtual quadrants. A platform, which is 10 cm in diameter and 35 cm in height, was placed in the middle of one of the quadrants. The platform is 1 cm below the water surface. The water in the pool was controlled at 22 ± 1 °C. After drug treatment, mice in all groups were given an acquisition trail for 4 consecutive days. For each trial, the mouse was sent into the water at starting points and allowed to swim freely until they found the hidden platform within 60 s. The time to reach the platform is defined as escape latency. If the mouse failed to find the platform with 60 s, the escape latency was recorded as 60 s. The escape latency and cumulative path were recorded with the corresponding software (Guangzhou Feidi Biology Technology Co., P.R. China). On the fifth day, the platform was removed, and the mouse was released at the opposite point of the platform positioned. The mouse was also allowed to swim 60 s, the time spent in the target quadrant and the numbers of each mouse to cross the platform were recorded.

2.4.2 Y-maze test

The Y-maze test was conducted as described in our previous study [21]. The Y-maze was made of three identical arms. Each arm in the Y maze was 35 cm in length, 5 cm in width and 15 cm in height. The three arms were placed at 120° from each other. The mice were gently placed at the end part of one arm. The times and sequences of each mouse entering the three arms in a 5-min period were recorded. Alternation was defined as the continuous entries into the three arms, with no overlapping triplets, such as ABC, CAB or BCA. The following formula calculated the percentage of alternation:

\[
\text{Alternation}\% = \frac{\text{[times of alternations]/(total times of arm entries - 2)]}}{100}
\]
2.4.3 Open field test

The open field apparatus was composed of a black open field box (50 × 50 × 50 cm chamber). The box was divided into twenty-five squares of 10 × 10 cm. The central areas were defined as the nine square areas in the middle of the open field. Mice were placed at the open field and allowed to explore for 30 min. The time traveled in the central area was recorded with a video-tracking system (Shanghai XinRuan Information Technology. Co. Ltd, P.R. China).

2.5 Tissue preparations

After behavioral examination, all mice were anesthetized with a phenobarbitone (0.5 mg/kg, i.p). All mice were transcardially perfused with 50 mL ice-cold phosphate-buffered saline (PBS) and sacrificed by decapitation. After that, the brains were taken out immediately, then the hippocampus and the cortex were separated on an ice clod board, and they were gathered and stored at -80 °C subsequently for western blotting assays. Mice used for morphological examination also received an additional 4% paraformaldehyde (PFA) perfusion. Four brain samples were gathered per group and then fixed in 4% PFA overnight; following that, they were subsequently transferred to 15% and 30% sucrose solutions and fixed in 4% PFA overnight; following that, they were adjusted with uniform concentration. The cortex samples were briefly lysed by SDS lysis buffer (10% SDS, 0.5 M tris-HCl PH6.8 and glycerin) containing protease inhibitor and phosphatase inhibitors. They were subsequently sonicated for 2 min with a probe sonicator and allowed to stand on ice for about 30 min to lyse fully. After that, they were centrifuged at 4 °C for 10 min. The supernatant was collected, and BCA assays determined the protein concentration. Following that, they were adjusted with uniform concentration.

The total protein (30 μg) were separated with 10%-12% SDS-PAGE gel and subsequently transferred to PVDF membranes at 300 mA for 2 h. Following that, the membranes were incubated with corresponding primary antibodies: anti-APP (1 : 3000), anti-PS1 (1 : 2500), anti-BACE1 (1 : 3000), anti-PSD95 (1 : 3000), anti-SYN (1 : 3000), anti-NGF (1 : 3000), anti-BDNF (1 : 3000), anti-NEP (1 : 3000), anti-IDE (1 : 3000), anti-RAGE (1 : 3000) and anti-LRP1 (1 : 4000) overnight before incubation with 7% skim milk in 1 × TBST for 1 h. After three incubations in TBST of 10 min each, the membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-mouse (1: 4000) or Goat anti-rabbit IgG (1 : 4000) for 2 h at room temperature. Then, the blots were visualized by using a chemiluminescence kit (WBKLS0500, Millipore). The densitometry of the band was analyzed with Image J software (NIH, Bethesda, Maryland).

2.9 Western blot assay

Western blot assay protocols were performed as previously described with few modifications [25]. The cortex samples were briefly lysed by SDS lysis buffer (10% SDS, 0.5 M tris-HCl PH6.8 and glycerin) containing protease inhibitor and phosphatase inhibitors. They were subsequently sonicated for 2 min with a probe sonicator and allowed to stand on ice for about 30 min to lyse fully. After that, they were centrifuged at 4 °C for 10 min. The supernatant was collected, and BCA assays determined the protein concentration. Following that, they were adjusted with uniform concentration.

The total protein (30 μg) were separated with 10%-12% SDS-PAGE gel and subsequently transferred to PVDF membranes at 300 mA for 2 h. Following that, the membranes were incubated with corresponding primary antibodies: anti-APP (1 : 3000), anti-PS1 (1 : 2500), anti-BACE1 (1 : 3000), anti-PSD95 (1 : 3000), anti-SYN (1 : 3000), anti-NGF (1 : 3000), anti-BDNF (1 : 3000), anti-NEP (1 : 3000), anti-IDE (1 : 3000), anti-RAGE (1 : 3000) and anti-LRP1 (1 : 4000) overnight before incubation with 7% skim milk in 1 × TBST for 1 h. After three incubations in TBST of 10 min each, the membranes were incubated with horseradish peroxidase (HRP)-conjugated anti-mouse (1: 4000) or Goat anti-rabbit IgG (1 : 4000) for 2 h at room temperature. Then, the blots were visualized by using a chemiluminescence kit (WBKLS0500, Millipore). The densitometry of the band was analyzed with Image J software (NIH, Bethesda, Maryland).

2.10 Statistic analysis

Statistic Package for Social Science (SPSS, v.22.0) statistical software was used for the data analysis. Repeated measures analysis of variance (ANOVA) was used to analyze escape latency data in the Morris water maze. One-way ANOVA followed by post hoc Bonferroni or Tamhane’s T2 test for the analysis of other data. The statistical significance was set at P < 0.05.

3. Results

3.1 DSS ameliorates the cognitive disorders in APP/PS1 mice

The cognitive impairment of APP/PS1 mice was determined by behavioral testing following DSS treatment. As shown in Fig. 1A, there is a substantial increase in escape latency in mice’s navigation test in the model group (P < 0.05). In contrast, DSS treatment remarkably reduced the navigation test’s escape latency (P < 0.05). However, Donepezil treatment did not affect escape latency. The swimming path of mice of each group on the fourth day is shown in Fig. 1B. On the fourth day, mice’s path in the APP/PS1 group is com-
Fig. 1. DSS alleviates the cognitive disorders in APP/PS1 mice. (A–D) Morris water maze test: Escape latency (A) and pathway (B) during platform trials, time spent in the target quadrant (C) and the number of crossing through the platform (D) in the probe test. Percentage of alternation (E) in the Y-maze test. The time spent in the central area (F) in the open field test. The escape latency was shown as mean ± SEM. Other data were shown as mean ± SD. Compared with the control group, *P < 0.05 or **P < 0.01, compared with the model group, *P < 0.05 or **P < 0.01. N = 10–12 in each group.

plex, while mice in the DSS and Donepezil group find the platform easily (Fig. 1B). As shown in Fig. 1C, D mice in the APP/PS1 group exhibited a shorter time in the target quadrant and a lower frequency of crossing the probe test platform than the model group (P < 0.01). However, DSS and Donepezil treatment could improve this condition (P < 0.05 or P < 0.01). These data collectively indicated that DSS exerted beneficial effects on learning and memory in APP/PS1 mice.

The Y-maze test was used to assess working memory. We found that mice in the APP/PS1 group presented a decrease of alternation percentage relative to those in the control group.
In contrast, the alternation percentage increased remarkably after DSS treatment ($P < 0.01$, Fig. 1E). The open-field test evaluated Anxiety-related behavior. We found that mice in the APP/PS1 group exhibited an approximately 50% decrease in movements in the central area in comparison with mice in the control group ($P < 0.01$), while both DSS and Donepezil enhanced movements in the central area of the open field ($P < 0.05$ or $P < 0.01$, Fig. 1F). These data indicated that DSS could ameliorate the anxiety-related behavior of APP/PS1 mice.

3.2 DSS alleviates the neuronal degeneration of APP/PS1 mouse brain

The effect of DSS on the neuronal degeneration of AD was assessed with Nissl staining. We observed numerous vacuoles (the Nissl bodies were disappeared, which was indicated with yellow arrows) in the neurons of APP/PS1 mouse brains (not only the cortex area, but also hippocampus CA1, CA3 and DG area), and the number of Nissl-positive neurons in these regions were reduced remarkably ($P < 0.01$, Fig. 2A). This suggests the occurrence of neuronal loss and necrosis in APP/PS1 mice. In contrast, DSS and Donepezil treatment decreased the vacuoles and increased the Nissl-positive neurons in APP/PS1 mice ($P < 0.01$, Fig. 2A,B).

Also, the protein levels of PSD95 and SYN, which are related to the neuronal development and synaptic function and the protein levels of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), which belong to nerve growth factors, were detected by Western blotting (Fig. 2C). We found that PSD95 and SYN levels in the cortex of APP/PS1 mice were reduced compared with that in the control group ($P < 0.01$, Fig. 2D). Similarly, the expression of NGF and BDNF was also down-regulated in APP/PS1 mice ($P < 0.01$, Fig. 2E). Interestingly, DSS and Donepezil treatment enhanced these proteins’ levels in APP/PS1 mice ($P < 0.01$, Fig. 2D,E). These results indicate that DSS can ameliorate neuronal and synaptic damage in the APP/PS1 mouse brain.

3.3 DSS mitigates the amyloidosis in the brain of APP/PS1 mice

We detected Aβ plaque deposition and Aβ1–42 levels in the brain by Ths staining and immunofluorescence assays (Fig. 3). We observed a large amount of Aβ plaque deposition in APP/PS1 mice (Fig. 3A). Correspondingly, the number of Aβ1–42 positive plaques in the brains of APP/PS1 mice was also increased (Fig. 3B). However, following an 8-week administration of DSS and Donepezil, the deposition of Aβ plaques and the number of Aβ1–42 immunoreactivity plaques was markedly reduced compared to that in APP/PS1 group ($P < 0.01$, Fig. 3C,D). These data indicate that DSS treatment can alleviate amyloidosis in APP/PS1 mice.

3.4 The effect of DSS treatment on production, degeneration and transport of Aβ of APP/PS1 mice

Proteins including APP, PS1, BACE1 for Aβ production, NEP and IDE for Aβ degeneration, RAGE and LRP1 for Aβ transportation were detected by Western blot.

We observed the level of RAGE, which is responsible for Aβ influx into the brain, was increased, whereas the level of LRP1, which is responsible for Aβ transferring out of the brain, was reduced in APP/PS1 mice ($P < 0.01$, Fig. 4A,B). It is noteworthy that DSS and Donepezil intervention down-regulated the level of RAGE and up-regulated the level of LRP1 in APP/PS1 mice ($P < 0.01$, Fig. 4A,B). We also observed increased protein expression of APP, PS1 and BACE1 ($P < 0.01$, Fig. 5A,B) and decreased protein levels of NEP and IDE in APP/PS1 mice ($P < 0.01$, Fig. 6A,B). Unfortunately, the protein levels of APP, PS1, BACE1 in the Aβ production pathway, NEP and IDE in the Aβ degeneration pathway were not altered following DSS treatment (Fig. 5 and Fig. 6). These findings indicate that DSS might have no effect on Aβ production and degeneration, yet can regulate the transport of Aβ.

4. Discussion

Several studies have demonstrated that DSS is effective against AD. However, the mechanisms of DSS in AD are still largely unclear. This study explored the possible mechanisms of DSS on amyloidosis and neuronal degeneration in APP/PS1 transgenic mice. We found DSS alleviated the cognitive deficits and neuronal degeneration of APP/PS1 mice. Moreover, DSS reduced the amyloidosis in the APP/PS1 mouse brain and down-regulated the level of RAGE and up-regulated the level of LRP1. However, DSS did not alter the primary protein levels of Aβ production and degeneration. These findings collectively demonstrate DSS ameliorates the amyloidosis, and neuronal degeneration of AD is associated mainly with its up-regulation of LRP1 and down-regulation of RAGE.

The APP/PS1 transgenic mouse is a classic model to mimic the pathology of Aβ deposition and behavioral changes of AD [26]. This study evaluated the effects of DSS on cognitive deficits and neuronal degeneration in APP/PS1 mice. As a result, we found DSS could ameliorate the cognitive deficits, including spatial memory, working memory and anxiety-like behavior of APP/PS1 mice, which is consistent with previous reports in AD animal models [21, 27–29]. Meanwhile, we also observed that the improvement effect of DSS on neuronal survival and neuronal function in APP/PS1 mice. DSS promoted neuronal survival by decreasing vacuoles in neurons and increased the overall number of Nissl-positive neurons in APP/PS1 mouse brains. Also, DSS enhanced the protein levels of PSD95 and SYN, which are near related to neuronal development and the protein expressions of NGF and BDNF, which are belong to nerve growth factors of APP/PS1 mice. These results collectively indicate the potential of DSS to treat AD.

Accumulating studies indicated that the imbalance between Aβ production and clearance leads to extracellular Aβ accumulation, which is considered to trigger AD’s cognitive dysfunction [30, 31]. However, clinical trials that target inhibiting Aβ production have been declared a failure [2].
Fig. 2. DSS ameliorates the neuronal degeneration in the brain of APP/PS1 mice. Representative images and quantification of Nissl-positive cells in the cortex and hippocampal CA1, CA3 and DG region by Nissl staining (A–B). Scale bar: 50 μm (n = 4). The protein bands and quantification data of PSD95, SYN, NGF and BDNF in the brain (C–E) (n = 3 per group). All data were shown as mean ± SD. Compared with the control group, *P < 0.05 or **P < 0.01, compared with the model group, *P < 0.05 or **P < 0.01.
Fig. 3. DSS reduces Aβ amyloidosis in the brain of APP/PS1 mice. Representative images of Aβ plaques (A) and Aβ1–42 immunoreactivity plaques (B) were examined by Ths staining and immunohistochemistry assays. Scale bar: 200 μm (n = 4, four sections per mouse). The statistical data of Aβ and Aβ1–42 immunoreactivity plaques (C–D). All data were shown as mean ± SD. Compared with the control group, *P < 0.05 or **P < 0.01, compared with the model group, *P < 0.05 or **P < 0.01.

Fig. 4. DSS increases the level of LRP1 and decreases RAGE level in APP/PS1 mice’s brain. The protein bands and quantification data of LRP1 and RAGE (A–B) in the cortex (n = 3 per group). All data were shown as mean ± SD. Compared with the control group, *P < 0.05 or **P < 0.01, compared with the model group, *P < 0.05 or **P < 0.01.
Nowadays, promoting Aβ clearance to reduce its aggregation in the brain to alleviate the amyloidosis and cognitive deficits have attracted more and more attention [14, 32].

We found DSS treatment can alleviate the deposition of Aβ plaques and Aβ1–42 levels in the brain of APP/PS1 mice. LRP1 and RAGE are the primary transport receptors of Aβ in BBB, binding Aβ directly. It is reported that there is an impairment of Aβ transportation of BBB in AD. In animal and AD patients, the level of LRP1 is decreased [33, 34]. Studies in AD animals studies have shown the decreased level of LRP1 could affect Aβ efflux across the BBB, leading to an increase of plasma Aβ levels and further aggravating cognitive deficits [35].

In contrast, the level of RAGE is up-regulated in AD. Many studies have demonstrated that RAGE blockade could alleviate amyloidosis and cognitive deficits [32, 36, 37]. Our previous studies indicated that DSS increases the level of LRP1 and decreases the level of RAGE in a vascular cognitive deficit rat [22]. Therefore, we sought to further investigate whether DSS affects RAGE and LRP1 in APP/PS1 mice.

As a result, we found that DSS up-regulated the level of LRP1 and down-regulated RAGE level in APP/PS1 mice. We also examined the Aβ production pathway (including APP, PS1 and BACE1) and Aβ degeneration pathway (including NEP and IDE). However, a two-month DSS treatment showed no effects on protein levels related to Aβ generation and Aβ degeneration in this study. These findings collectively indicate that DSS can alleviate the neuronal degeneration and amyloidosis of AD, which may be through up-regulation of LRP1 and down-regulation of RAGE (Fig. 7).

Due to the lack of effective treatment for AD currently, we selected donepezil, specific central acetylcholinesterase (AChE) inhibitor used in the clinic for AD therapy, as a positive control drug present study. Recently, studies also showed that donepezil is also useful in reducing the amyloidosis and Aβ accumulation of AD [38–40].

We found that DSS effectively alleviates the amyloidosis and neuronal degeneration in the APP/PS1 transgenic mouse, which could achieve the same effect as Donepezil. This indicates that DSS can be used in the clinical treatment
of AD. However, there are some limitations. First, due to the lack of RAGE overexpression or LRP1 knockout, it is unclear whether DSS ameliorates the neuronal injury and amyloidosis of AD directly via the upregulation of LRP1 and downregulation of RAGE. Second, apart from neurons, RAGE and LRP1 are extensively expressed in microglia and endothelial cells [41]. To further understand and explore the therapeutic targets of DSS for modulating the transport of $\text{A}_\beta$ in AD, future studies should focus on the co-expression of RAGE and LRP1 with $\text{A}_\beta$ with microglia and endothelial cells.

5. Conclusions
In conclusion, our findings demonstrated that DSS could ameliorate neuronal injury and amyloidosis related to AD. Also, the effects of DSS on amyloidosis in AD may be mediated via up-regulation of LRP1 and down-regulation of RAGE. Our findings indicate that DSS is an effective AD treatment, and increased focus on regulating $\text{A}_\beta$ transport signaling may be productive.

Author contributions
QW and QD designed the present research. CY and YSM wrote the manuscript. CY, YSM, HFC, YHH, SLL conducted the experiments. XC conducted the data analysis. HW and SQH revised the grammar of the manuscript.

Ethics approval and consent to participate
The present research was approved by the Ethics Committee of laboratory animals of Guangzhou University of Chinese Medicine (No.00107331).

Acknowledgment
We thank two anonymous reviewers for excellent criticism of the article.

Funding
This work was supported by Key laboratory project of colleges and universities in Guangdong province (No. 2019KSYS005), Guangzhou Science Technology and Innovation Commission Technology Research Projects (No. 201805010005, No. 201803010047), Guangdong province science and technology plan international cooperation project (No.2020A0505100052) and National Natural Science Foundation of P.R. China (No.81673627, No.81704131, No.81904168).

Conflict of interest
The authors declare that there is no conflict of interest.

References


