Original Article

Shenqi Xingnao Granules ameliorates cognitive impairments and Alzheimer's disease-like pathologies in APP/PS1 mouse model

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ABSTRACT

Objective: Alzheimer's disease (AD) is along with cognitive decline due to amyloid-β (Aβ) plaques, tau hyperphosphorylation, and neuron loss. Shenqi Xingnao Granules (SQXN), a traditional Chinese medicine, significantly ameliorated the cognitive function and daily living abilities of patients with AD. However, till date, no study has investigated the mechanism of action of SQXN on AD. The present study aimed to verify the effects of SQXN treatment on cognitive impairments and AD-like pathologies in APP/PS1 mice.
Methods: Four-month-old APP/PS1 transgenic (Tg) mice were randomly divided into a model group and SQXN-treated (3.5, 7, 14 g/kg per day) groups. Learning-memory abilities were determined by Morris water maze and object recognition test. All mice were sacrificed and the brain samples were collected after 75 d. The soluble Aβ contents were detected by Elisa kit; The levels of expression of NeuN, APP, phosphorylated tau and related protein were measured by Western blotting; The inflammation factors were detected by the proinflammatory panel kit.
Results: Four-month-old APP/PS1 mice were administered SQXN by oral gavage for 2.5 months. Using the Morris water maze tests and Novel object recognition, we found that SQXN restored behavioral deficits in the experimental group of Tg mice when compared with the controls. SQXN also inhibited neuronal loss (NeuN marker). SQXN treatment decreased soluble Aβ42 through inhibiting the expression of sAPPβ and BACE-1 without regulating full-length amyloid precursor protein (FL APP). Insulin degrading enzyme (IDE), the Aβ degrading enzyme, were increased by SQXN. In addition, SQXN reduced hyperphosphorylated tau protein levels and prevented excessive activation of p-GSK-3β in the brain of APP/PS1 mice. Compared with APP/PS1 transgenic negative mice, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12p70, KC/GRO and TNF-α were not obviously changed in the brain of 6.5-month-old APP/PS1 transgenic (Tg) mice. However, SQXN could inhibited the expression of IL-2.
Conclusion: These results demonstrate that SQXN ameliorates the cognitive impairments in APP/PS1 mice. The possible mechanisms involve its inhibition of neuronal loss, soluble Aβ deposition, tau hyperphosphorylation and inflammation.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disease that impairs memory and cognitive functions and it may cause dementia in the late stages of life (Gerlach and Chaney, 2018) (Selkoe, 2001). About 46 million people worldwide suffer from AD and the number of AD cases is expected to exceed 131 million by 2050 (Espargaró, Medina, Di Pietro, Muñoz-Torrero, & Sabate, 2016). The main pathological findings of AD are senile plaques, neurofibrillary tangles (NFTs), destruction of synapse structure stability, and neuronal death (Gao et al., 2016). Studies have shown that the abnormal accumulation of incorrectly folded Aβ in amyloid plaques and hyperphosphorylated tau proteins in NFTs are causally related to the neurodegenerative processes in patients' brains Karran, Mercken, & Strooper, 2011; Vemuri & Scholl, 2017. In addition, both amyloid plaques and NFTs are responsible for the hyperphosphorylation of tau pro-

tein, leading to neuronal cell loss and death Amatsubo, Yanagisawa, Morikawa, Taguchi, & Tooyama, 2010. AD is a multifactorial and a highly complex process and its pathogenesis involves multiple mechanisms (Iraji et al., 2018); Therefore, the single modality of "One-molecule-one-target" strategy for its treatment has failed. Future therapies relying on the "Combination-drugs-multi-targets" (CDMT) strategy are needed to target the multiple contributing factors and, eventually, block the progression of AD (Sahoo et al., 2018).

Recent research has revealed that traditional Chinese medicine (TCM) may help in the treatment of patients afflicted with AD and could eventually help in the discovery of a cure (Jarrell, Gao, Cohen, & Huang, 2018). However, more research is needed to elucidate the underlying mechanisms by which specific TCM works in ameliorating symptoms of AD (Fang et al., 2017). According to the TCM theory, *qi* and blood deficiencies and toxins hurting brain collaterals are the main causes of dementia. Shenqi Xingnao Granules (SQXN), a Chinese prescription developed by Yong-yan Wang, containing main ingredients of *Panax ginseng* and *Astragali Radix*. SQXN has been shown to significantly improve the cognitive function and daily functionality of patients with AD (Wang et al., 2017). *P. ginseng,* the key herb of SQXN, has been shown to restore the dysfunction of various neurotransmitters and improve the cognitive function and recognition skills in patients with AD (Jiang et al., 2017). *Astragali Radix*, the other key herb of SQXN, has been proved to relieve cognitive impairment induced by diabetes mellitus, suggesting that calycosin (the active *O*-methylated isoflavone isolated from *Astragali Radix*) may improve the cognitive function of patients with AD Song, Li, Bai, Gao, & Wang, 2017.

In our previous study, we showed that SQXN decreased the expression of acetylcholinesterase (AChE) in the hippocampus and significantly improved the learning and memory ability of scopolamine-induced AD mouse model (Jia et al., 2018). In the present study we observed whether administration of SQXN could reduce Aβ deposition, tau hyperphosphorylation and inflammation in the amyloid precursor protein/presenilin-1 (APP/PS1) mouse model of AD and explored the possible molecular mechanisms.

2. Materials and methods

2.1. Drugs

Shenqi Xingnao Granules (SQXN), including *Panax ginseng* C. A. Mey (Anguo Changda Traditional Chinese Medicine Pieces Co., Ltd., China), *Astragalus membranaceus* (Fisch.) Bge. (Anguo Changda Traditional Chinese Medicine Pieces Co., Ltd. China), *Salvia miltiorrhiza* Bge. (Anguo Changda Traditional Chinese Medicine Pieces Co., Ltd., China), *Scutellaria baicalensis* Georgi, (Anguo Changda Traditional Chinese Medicine Pieces Co., Ltd., China), etc., was extracted by the water-extraction and alcohol-precipitation method. The content of the crude drug was 2.24 g/mL. It was provided by the Institute of Chinese Materia, Medica China Academy of Chinese Medical Sciences and stored at 4 °C. Donepezil hydrochloride was purchased from Eisai Pharmaceutical Co., Ltd. (China).

2.2. Animals

Four-month-old male APP/PS1 transgenic (Tg) mice [B6C3-Tg (APPswe, PS1dE9)] were purchased from the model animal research center of Nanjing University (Nanjing, China). These mice were fed following standard conditions of 12 h of light/day at a room temperature of 22 °C with free access to water and food. All experimental procedures involving animals were approved by the Animal Care Committee of Nanjing University and conformed to the ethical standards for the use of animals in research.

2.3. Grouping and treatment

Animals were genotyped by PCR using genomic DNA extracted from mice tails. The mice were divided into the following groups (n = 14–17/group): (1) APP/PS1 Tg negative mice [Tg(-)], Tg (-) + SQXN-M group (7 g/kg), (2) APP/PS1 Tg(+), APP/PS1 Tg + SQXN-L (3.5 g/kg), (3) APP/PS1 Tg + SQXN-M (7 g/kg), (4) APP/PS1 Tg + SQXN-H (14 g/kg, n = 17), and (5) APP/PS1 Tg + Donepezil group (1 mg/kg).

SQXN or vehicle (distilled water) was administered via oral gavage once daily for 75 d. At the end of the 75th day, the Morris water maze and Novel Object Recognition tests were used to assess cognitive behavioral performance. Then, the mice were anesthetized with 10% chloral hydrate (4 mL/kg) and decapitated while in the unconscious state. The hippocampus of brains in different groups were dissected and stored at − 80 °C.

2.4. Morris water maze assay

The Morris water maze (MWM) was used to test the spatial learning and memory of the mice. This device is a large circular pool with a diameter of 100 cm and a black inner surface. The pool was randomly divided into four quadrants. The escape platform was placed 1–1.5 cm below the water surface in the second quadrant center, and the platform position was fixed during the test. The mice were placed respectively in the pool from the first quadrant and the fourth quadrant. The acquisition phase lasted for six training days (days 1–6). Time to reach the platform was recorded as escape latency. On day 7, we removed the platform from the pool and allowed mice to swim freely for 1 min. The swimming traces of mice were monitored by a video camera above the center of the pool to capture images and record the escape latencies automatically.

2.5. Novel object recognition assay

The novel object recognition (NOR) test is widely used to measure recognition memory in mice (Antunes & Biala, 2012). Mice were placed in the center of an empty testing arena (250 mm × 250 mm × 250 mm) on the first day and allowed to explore for 5 min. In the training phase, the mice were presented with two similar objects to explore for 5 min on the second day. In the testing phase, one of the two objects were replaced by a new object on the third day. During the whole trial, to eliminate olfactory cues, the arena and objects were cleaned thoroughly with 10% ethanol. The discrimination index (DI) was calculated as (time exploring novel object – time exploring familiar object)/total time spent with both objects.

2.6. Western blotting analysis

Equal volumes of protein were separated by dodecyl sulfate, sodium salt polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels. The separated proteins were transferred to polyvinylidene fluoride (PVDF) membranes. Non-specific binding sites were blocked by incubating the membrane with 5% non-fat milk for 1 h and then incubated overnight at 4 °C with primary antibodies as follows: anti-NeuN (Abcam, Cambridge, UK); anti-P-S396-Tau and anti-P-T216-GSK-3β were purchased from Cell Signaling Technology (Danvers, MA, USA); anti-β-actin antibody was diluted 1:1000 (ZSGB-Biotechnology Company, Beijing, China). Membranes were washed with Tris-buffered saline with tween 20 (TBST) before incubation with the secondary antibody. Blots were then incubated with goat anti-rabbit or anti-mouse secondary antibodies conjugated to horseradish peroxidase for 2 h. Membranes were then rinsed three times with TBST. Protein signals were visualized with the chemiluminescence reagents in the ECL

kit (Millipore, California, USA) and quantitation of proteins was determined by densitometric analysis using a gel image processing system (Alpha View SA software, USA).

2.7. Aβ ELISA

The brain tissues were homogenized in Tris-buffered saline (TBS) containing a protease inhibitor cocktail and then centrifuged at 35 0000 × g for 20 min at 4 °C. The concentration of $Aβ_{42}$ was measured according to the protocol found in the β-Amyloid x-42 ELISA Kit (Biolegend, California, USA). The supernatant (TBS-soluble fraction) was collected and stored at − 20 °C as the soluble fraction of $Aβ_{42}$ (S1). The pellets were homogenized in TBS plus 1% Triton X-100 and 0.2% SDS (solution 2) containing a protease inhibitor cocktail (solution1) and then centrifuged at 35 0000 × g for 20 min at 4 °C. The resulting supernatant was collected and stored at − 20 °C as the insoluble fraction (E1). Protein standards used for generating the standard curve in the ELISA were prepared following standardized protocols. The prepared incubation buffer was mixed in a one-to-one ratio with the HRP detection antibody and the ELISA was set up and run following instructions provided in the kit. Protein concentrations were then calculated using the standard curve.

2.8. Cytokine quantification

Proteins were extracted from frozen organs using lysis buffer (25 mmol/L Tris-Cl (pH 7.4), 100 mmol/L NaCl, 1 mmol/L EDTA, 1% Triton X-100, 0.5% Nonidet P40 and protease inhibitors (Roche Diagnostics, Meylan, France)). Protein concentration was evaluated by BCA assay (Sigma-Aldrich Chimie, Saint-Quentin Fallavier, France). Proteins (10 μg) were used to evaluate cytokine concentration. Ten cytokines were analysis following manufacturer's instructions using the Proinflammatory Panel 1 Kit for mouse (Meso Scale Discovery, # K15048D) on a QuickPlex SQ 120 apparatus (Meso Scale Discovery) (Yang et al., 2018).

2.9. Statistical analysis

Statistical analysis was performed using the software package SPSS 16.0 (SPSS, Inc., Chicago, IL, United States). Data were expressed as mean ± standard error of the mean (SEM) of separate experiments ($n \geq 3$) after comparison with One-way analysis of variance (ANOVA) followed by Tukey's *post hoc test* were done.

3. Results

3.1. Effects of SQXN treatment on learning and memory deficits of transgenic APP/PS1 mice

The Morris water maze (MWM) test was done to assess the effect of SQXN treatment on spatial learning and memory ability

of transgenic APP/PS1 mice (Table 1). When compared with the APP/PS1 transgenic-negative [Tg(-)] control group, the escape latency of the Tg(+) mice were significantly prolonged at day 6 ($P < 0.001$). There was a significant difference between APP/PS1 and SQXN groups ($P < 0.001$), indicating that the cognitive function of spatial memory was significantly improved after treatment with SQXN.

To further evaluate the learning and recognition memory of AD mice, we performed the Novel Object Recognition test (NOR) (Fig. 1). Fig. 1A showed the diagram of NOR. It was found that the discrimination index of the APP/PS1 Tg(-) mice were higher than that of the Tg(+) mice ($P < 0.05$) (Fig. 1B). SQXN could increase the discrimination index with statistical significance ($P < 0.001$). These results suggested that SQXN-treatment efficiently improved memory retention in APP/PS1 mice.
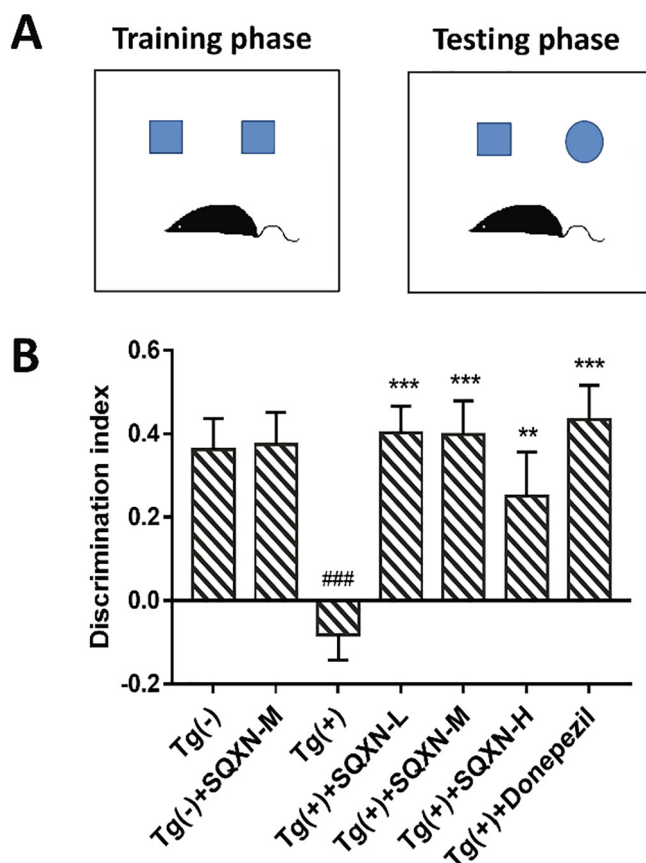

Fig. 1. SQXN treatment improved the ability of learning and memory of transgenic APP/PS1 mice (mean ± SEM, n = 14–17). (A): Novel object recognition (NOR) analysis. (R) Discrimination index during the acquisition memory phase in the novel object recognition test. ###$P < 0.001$ compared with APP/PS1 Tg(-) mice; **$P < 0.01$, ***$P < 0.001$, compared with Tg(+) mice.

Table 1
SQXN improved learning ability and memory of transgenic APP/PS1 mice in Morris water maze test.

Groups	n	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Tg(-)	16	93.06 ± 6.89	76.63 ± 9.81	65.41 ± 10.59	56.36 ± 9.78	61.39 ± 9.40	58.80 ± 11.21
Tg(-) + SQXN-M	15	83.25 ± 7.97	80.63 ± 10.51	61.25 ± 10.83	52.03 ± 11.85	46.56 ± 9.37	45.79 ± 10.63
Tg(+)	17	99.90 ± 6.61	89.96 ± 8.81	94.71 ± 8.73#	95.40 ± 7.01#	92.87 ± 8.22#	112.17 ± 3.96###
Tg(+) + SQXN-L	16	118.16 ± 0.58	107.03 ± 6.44	92.50 ± 9.99	73.08 ± 10.03	79.36 ± 7.67	82.07 ± 9.07*
Tg(+) + SQXN-M	16	98.27 ± 7.78	92.66 ± 7.10	84.02 ± 10.56	63.65 ± 8.65*	67.38 ± 7.86*	47.04 ± 9.93***
Tg(+) + SQXN-H	14	91.80 ± 9.36	87.74 ± 8.12	68.21 ± 7.95	73.13 ± 10.14	65.45 ± 10.63*	67.15 ± 9.80**
Tg(+) + Donepezil	16	103.02 ± 5.72	90.27 ± 9.29	80.16 ± 9.43	71.79 ± 10.62	73.84 ± 10.01	75.11 ± 9.32**

Escape latency for an escape to the submerged platform in the probe trails (day 1–6). All values are expressed as mean ± SEM. #$P < 0.05$, ###$P < 0.001$, compared with the APP/PS1 transgenic-negative [Tg(-)] mice; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with Tg mice.
Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg(+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7, and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg)

3.2. Effects of SQXN treatment on mature neurons in transgenic APP/PS1 mice

Since neuron-specific nuclear protein (NeuN) is the bona fide marker for mature neurons, its levels of expression after SQXN treatment were tested. Fig. 2A showed the representative image of NeuN deteted by Western blotting. We found that the decreased expression of NeuN in APP/PS1 mice ($P < 0.05$, Fig. 2B) were restored to normal levels in the SQXN-treatment and Donepezil-treatment groups ($P < 0.001$). This indicated that SQXN treatment might increase the number of neurons in APP/PS1 mice.

3.3. Effects of SQXN treatment on soluble Aβ42 level in transgenic APP/PS1 mice

We performed ELISA to quantify soluble Aβ42 levels in the brains of these mice. Results showed that the soluble Aβ42 levels were high in the brains of APP/PS1 transgenic mice ($P < 0.001$, Fig. 3). SQXN treatment had a trend to reverse its high expression.

3.4. Effects of SQXN treatment on APP processing in transgenic APP/PS1 mice

In order to make clear the mechanism of SQXN on Aβ reduction, we firstly investigated the pathways of Aβ production. To determine the impact of SQXN treatment on the level of full length APP (FL APP), a Western blot assay were performed, as shown in Fig. 4A. Notably, we found that there was a significant increase in the APP but not PS1 protein in the APP/PS1 mouse model groups
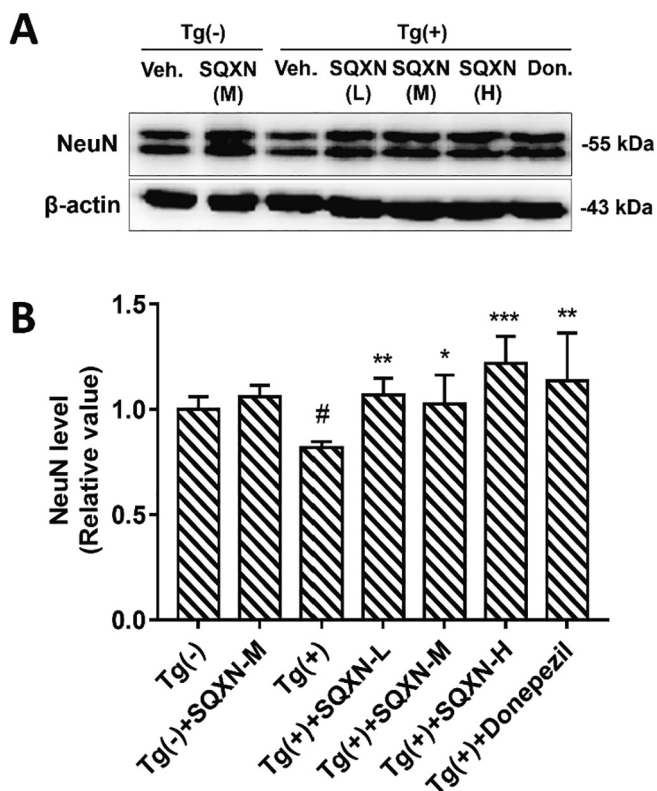
Fig. 3. SQXN treatment decreased soluble Aβ level in transgenic APP/PS1 mice (mean ± SEM, n = 5). An ELISA assay was used to evaluate the effect of SQXN on soluble Aβ$_{42}$ levels in the APP/PS1 mice. β-actin was used as a loading control. $^{###}P < 0.001$, compared with Tg(-) mice. Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg(+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7, and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg).
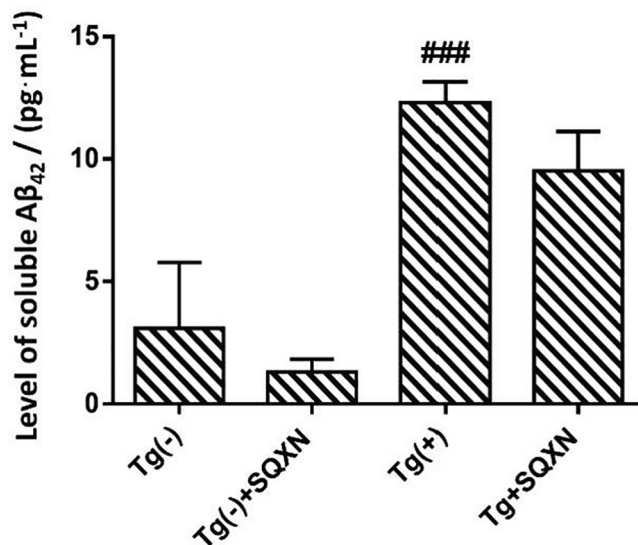
($P < 0.001$, Fig. 4B and C). However, SQXN treatment did not alter the expression of APP. β-site amyloid precursor protein cleaving enzyme 1 (BACE1) cleavage of APP is mediated in Aβ generation. sAPPβ is a direct product of BACE1 enzymatic activity (Kennedy et al., 2016). Administration of SQXN in APP/PS1 mice led to the downregulation of BACE-1 and sAPPβ ($P < 0.001$, Fig. 4D and E).

3.5. Effects of SQXN treatment on Aβ-degrading enzymes IDE in transgenic APP/PS1 mice

Insulin-degrading enzyme (IDE) is one of Aβ-degrading enzymes, which plays an important role in Aβ accumulation. Results in Fig. 5 showed that SQXN elevated the expression of IDE in the brain of APP/PS1 mouse ($P < 0.01$), indicating that SQXN could enhance the clearance of Aβ.

3.6. Effects of SQXN treatment on phosphorylation of tau and GSK-3β in transgenic APP/PS1 mice

We also performed a tau-related assay in the transgenic mice. When compared with the APP/PS1 Tg(-) mice, there was an increase in the level of tau phosphorylation at Ser396 (PS396) in the Tg(+) group ($P < 0.001$, Fig. 6A and C). SQXN treatment could inhibit tau hyperphosphorylation in vivo ($P < 0.01$). We also investigated the effect of SQXN treatment on the phosphorylation of GSK-3β at Tyr216 (pGSK-3β) in the Tg mice. As shown in Fig. 6B and D, SQXN treatment could inhibit the active form of GSK-3β (phosphorylated at Tyr216) in the APP/PS1 Tg mice, suggesting that SQXN can inhibit GSK-3β activity in vivo.

3.7. Effects of SQXN treatment on inflammatory factors in transgenic APP/PS1 mice

We next evaluated the effect of SQXN on pro- and anti-inflammatory cytokines in the APP/PS1mice. We detected IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-12p70, KC/GRO and TNF-α. However, no change was found for the ten cytokines between APP/PS1 Tg(-) and Tg (+) mice. SQXN treatment reduced the level of IL-2 which is implicated in AD ($P < 0.05$, Fig. 7).
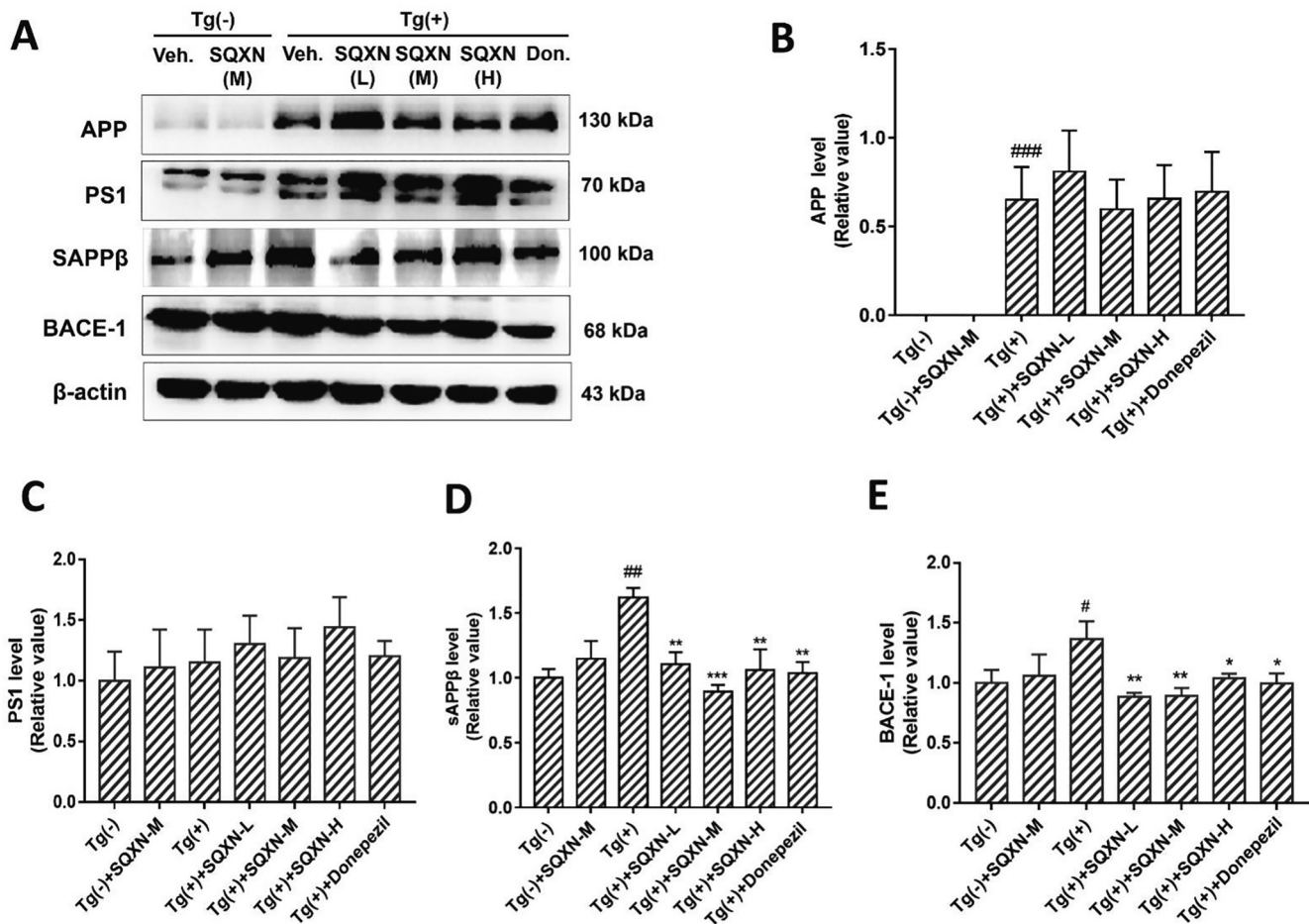
Fig. 2. SQXN treatment regulated protein expression of NeuN in transgenic APP/PS1 mice (mean ± SEM, n = 4). The levels of NeuN in different groups (a – b) were analyzed by Western blotting. β-actin served as a loading control. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, compared with Tg(+) mice. $^{#}P < 0.05$ compared with APP/PS1 Tg(-) mice. Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg (+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7 and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg).

Fig. 4. SQXN could regulate the APP processing in transgenic APP/PS1 mice (mean ± SEM, n = 4). (a) Representative western blot scan showing the expression levels of APP, PS1, BACE1 and sAPPβ. (b) The quantitative analysis of APP and PS1. (c) The quantitative analysis of BACE1 and sAPPβ. β-actin was used as a loading control. [#]$P < 0.05$, [###]$P < 0.001$ compared with Tg(-) mice; [*]$P < 0.05$, [**]$P < 0.01$, [***]$P < 0.001$ compared with Tg(+) mice. Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg(+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7, and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg).

4. Discussion

Alzheimer's disease (AD) is a chronic neurodegenerative disorder in the elderly characterized by progressive deterioration of cognition and memory (Cellai et al., 2018) Wu, Li, Li, Zeng, & Wu, 2017. SQXN, a Chinese prescription, is commonly used in clinics in China. The present study performed in APP/PS1 mice showed that SQXN has neuroprotective effects. SQXN effectively ameliorated cognitive impairment and AD-like pathologies.

The histopathological hallmarks of AD are extracellular plaques containing aggregated amyloid β (Aβ) and intracellular tangles containing aggregated tau, along with neuron(s) and synapse(s) loss in selected brain areas (Amadoro et al., 2012) (Avila, Lucas, Pérez, & Hernández, 2004). Region-dependent amyloid β deposition has been captured in a number of transgenic mouse lines for familial AD-causing mutations in the genes for amyloid precursor protein (APP), presenilin-1 (PS-1), or in bitransgenics expressing both mutant APP and PS-1 (Malthankar-Phatak et al., 2012). In the present study, the 6-month-old APP/PS-1 transgenic mouse exhibited neuronal loss and impairment of spatial memory in the Morris water maze test. These results are consistent with other investigators' reports (Qin et al., 2017) (Hudry et al., 2010). SQXN treatment could protect neurons and ameliorate cognitive function. In AD, neuronal survival is adversely affected (Chen, 2018) and this is linked to the accumulation of Aβ and tau hyperphosphorylation (Michalski

et al., 2016; Parthsarathy & Hölscher, 2013). APP begins to accumulate from birth in the APP/PS-1 double transgenic AD mice (Harris et al., 2014) and a measurable increase in Aβ occurs at 3 months of age, with plaque deposits by 5–6 months, progressing with age up to 15 months (Malthankar-Phatak et al., 2012). The soluble forms of Aβ, in particular, the Aβ42 peptides, can cause neuronal degeneration/cognitive decline in both rats and mice models (Choi, Lee, & Cho, 2018; Nitta et al., 1994). Here, we showed that the soluble Aβ42 peptides increased in the APP/PS-1 transgenic mice and SQXN treatment reversed these changes. Research results during the last decades have put amyloid-β Aβ peptide, generated by proteolytic processing of APP, at the center of AD (Muresan & Muresan, 2008; Selkoe, 2000;Pensalfini et al., 2014). APP can undergo either non-amyloidogenic or amyloidogenic processing depending on the secretases that cleave the protein (Gautam et al., 2015). In the amyloidogenic processing, APP is cleaved by either BACE-1 enzyme generating extracellular soluble APP-β (sAPPβ) (Dobrowolska et al., 2014). In the current study, we found SQXN could inhibit the expression of BACE-1 to reduce the sAPPβ level, demonstrating SQXN downregulated APP processing to decrease Aβ production. We next detected the expression of insulin-degrading enzyme (IDE) which could clear/degrade Aβ (Carrasquillo et al., 2010;) (Vepsalainen et al., 2008). In this study, we observed that expression of IDE was elevated by SQXN administration, indicating that SQXN might regulate Aβ degradation pathways.
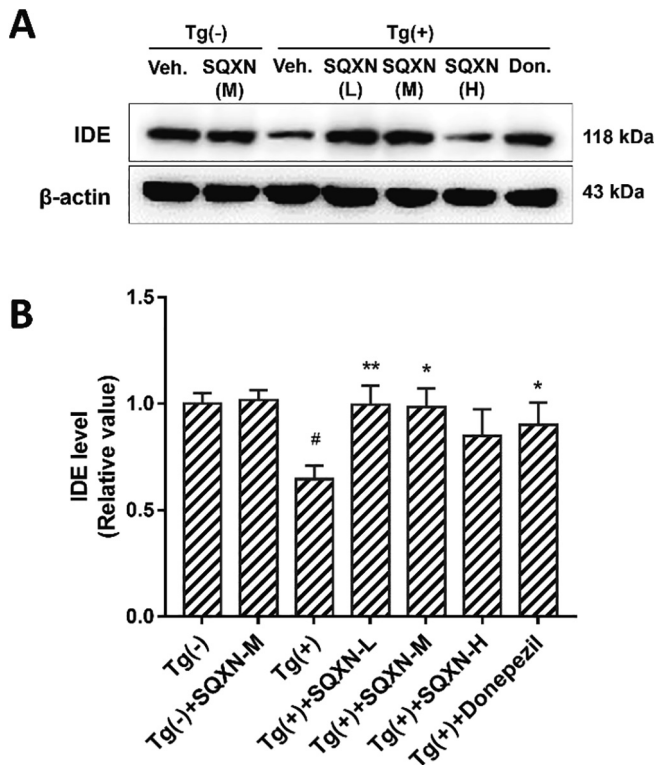
Fig. 5. SQXN treatment increased Aβ-degrading enzymes IDE in transgenic APP/PS1 mice (mean ± SEM, n = 4). The levels of the IDE in different groups (A − B) were analyzed by Western blotting. β-actin served as a loading control. *P < 0.05, **P < 0.01, compared with Tg(+) mice. #P < 0.05 compared with APP/PS1 Tg(-) mice. Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg(+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7, and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg).

Dysregulation of tau, including tau hyperphosphorylation and formation of neurofilament tangles, contributes to neuronal dysfunction and cognitive impairments in AD patients (Ma et al., 2017; Wu, Pina-Crespo, Zhang, Chen, & Xu, 2017). To determine whether tau hyperphosphorylation was involved in the protective effect of SQXN on cognitive function in APP/PS1 mice, Takeshi found that tau phosphorylation at Ser396 in the 5XFAD mouse model was significantly increased before spatial learning and memoryimpairments were noted, showing that hyperphosphorylation of tau at Ser396 occurs before the appearance of learning and memory disorders in these 5XFAD mice(Tg6799) (Kanno et al., 2014). In addition, studies have demonstrated tau phosphorylation at Ser396 (p-S396-tau) appeared in senile plaques (SPs) and NFTs in the brains of AD patients (Hu et al., 2002). These findings implicated abnormal phosphorylation of tau at Ser396 as an early key step in the development of NFT pathology and might be a marker of AD progression. Therefore, it suggested p-S396-tau as a diagnostic biomarker for AD (Jhang, Park, Kim, & Chong, 2017). The tau residues, Tyr18, Thr231, and Ser199 can be phosphorylated by Aβ via different kinases like Fyn, glycogen synthase kinase-3β (GSK-3β) (Neddens et al., 2018). We also found in the present study that the levels of p-S396-tau were up-regulated when Aβ was overexpressed in APP/PS1 Tg mice. Our data showed that SQXN alleviated the hyperphosphorylation of tau independent of decreasing Aβ. Next, we investigated the mechanism underlying the SQXN-induced decrease in tau hyperphosphorylation. GSK-3β is a proline-directed serine/threonine protein kinase and is one pivotal phosphorylation enzyme that enables intracellular tau phosphorylation in AD brains. The active form of GSK-3β, phosphorylated at Tyr216, is increased in AD brains. We found that SQXN inhibited the phosphorylation of GSK-3β at Tyr216. This suggests that SQXN might attenuate GSK-3β activity to decrease tau hyperphosphorylation.

It is now known that inflammation plays a central role in Alzheimer's pathology (Heneka et al., 2015). Pro-inflammatory cytoki-
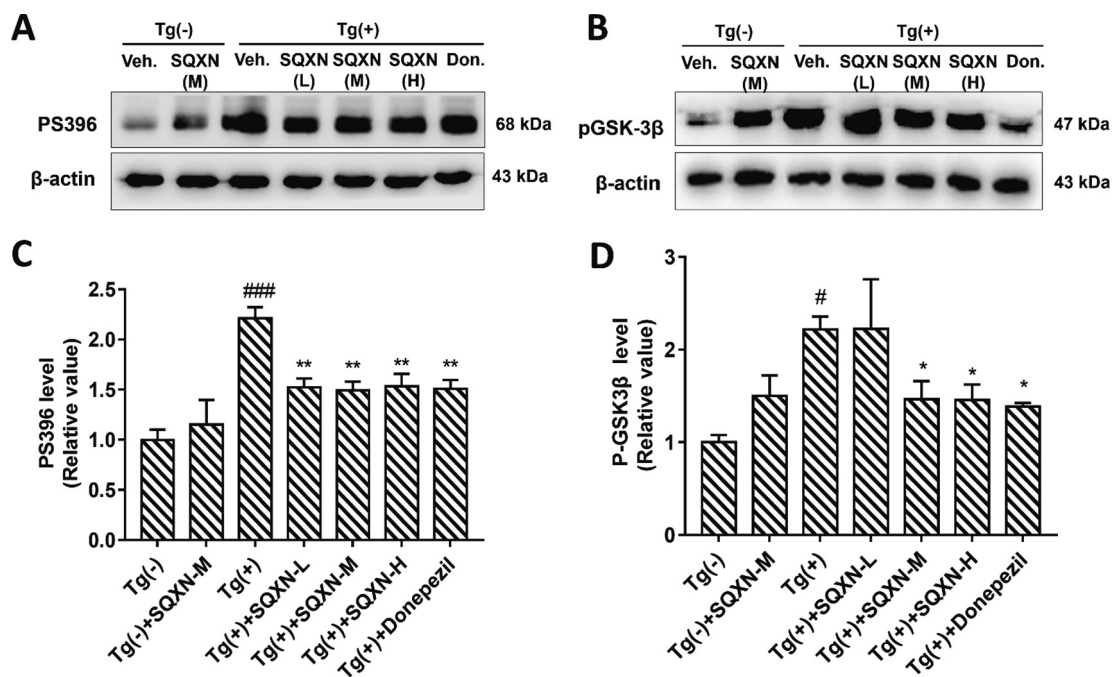

Fig. 6. SQXN treatment reduced tau phosphorylation by inhibiting GSK-3β activity in the APP/PS1 transgenic mice (mean ± SEM, n = 3). (A − B) Representative Western blot scan showing the expression levels of the phosphorylation of tau at Ser396 (PS396) and phosphorylation of GSK-3β at Tyr216 (pGSK-3β). (C − D) Quantitative analysis of PS396 and pGSK-3β. β-actin was used as a loading control. #P < 0.05, ###P < 0.001 compared with Tg(-) mice; *P < 0.05, **P < 0.01, compared with Tg(+) mice. Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg(+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7, and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg).

Fig. 7. Effect of SQXN on inflammatory cytokines in APP/PS1 transgenic mice (mean ± SEM, n = 5). Tg(-): APP/PS1 transgenic negative mice; Tg(-) + SQXN-M (7 g/kg); Tg(+): APP/PS1 transgenic mice; Tg(+) + SQXN(L, M, H): APP/PS1 mice were treated with SQNXN via gastric lavage (3.5, 7, and 14 g/kg); Tg + Donepezil: APP/PS1 mice were treated with Donepezil (1 mg/kg).
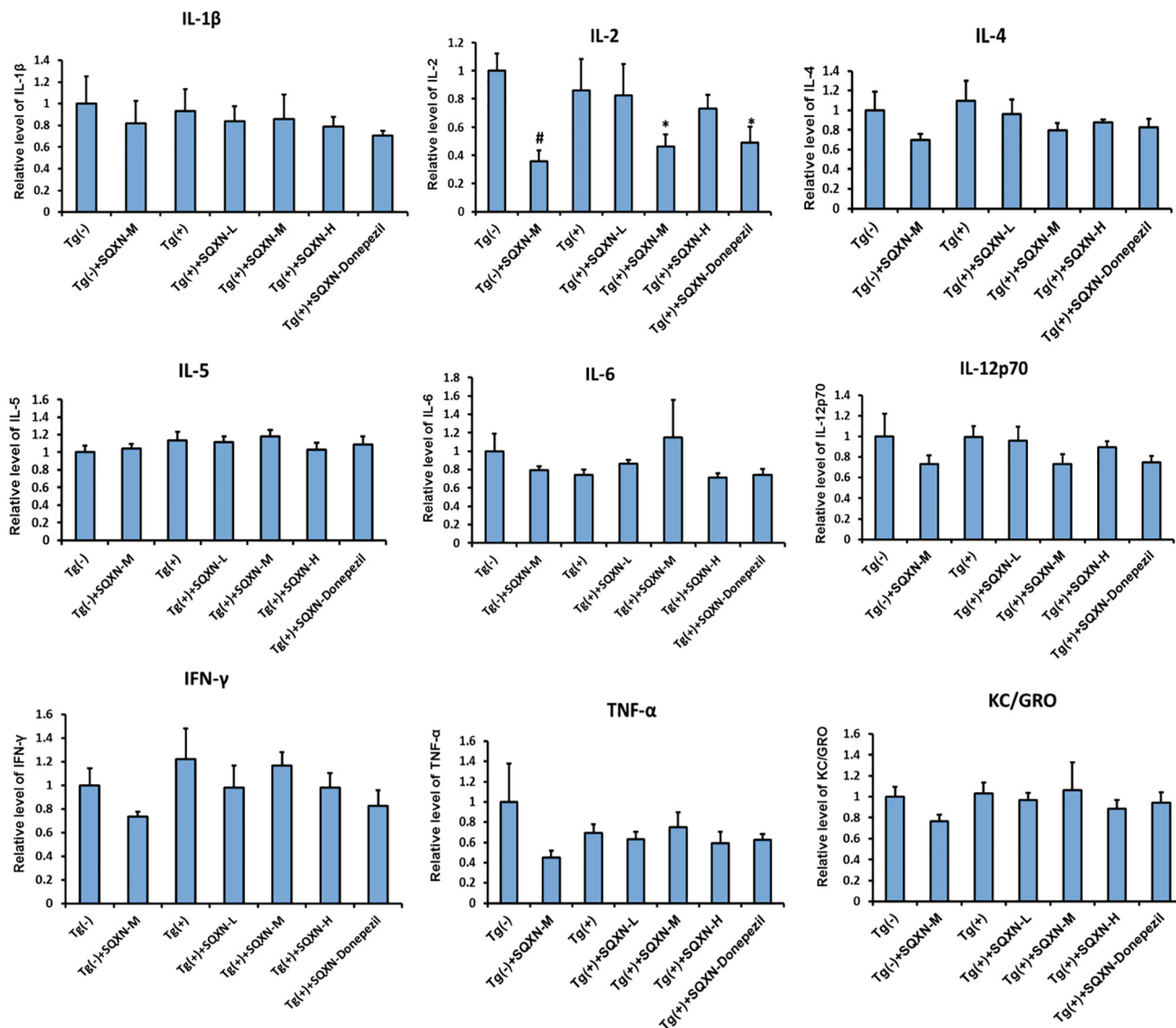
nes such as TNF-α and IL-1β, induce spatial memory impairment (Amani et al., 2019). In the present study, we detected the expression of TNF-α, IL-1β, IL-2, IL-4, IL-5 and so on to investigate the effect of SQXN on neuroinflammation. SQXN could downregulate the expression of IL-2. According to the amyloid cascade hypothesis, Aβ deposits in senile plaques and elicit proinflammatory responses, contributing to oxidative stress, neuronal degeneration and neuroinflammation(Caraci et al., 2013) (Leissring, 2016). In our study, we found the high level of Aβ oligomer was in the brain of 6.5-month old APP/PS1 mice without inflammatory cytokines changing. It demonstrates the appearance of Aβ deposits is earlier than neuroinflammation.

5. Conclusion

In conclusion, we demonstrated that SQXN has positive effects on the prevention of neuron dysfunction and cognitive disorder. This study supported the notion that SQXN could be a potential therapeutic agent for the treatment of AD, based on its effect of reducing the hyperphosphorylation of tau via inhibiting GSK-3β activity. The current study demonstrates the protective effects of SQXN on AD pathology in APP/PS1 mouse model, which suggests that SQXN may serve as a promising therapeutic agent for AD.

Authors' Contribution

Cui-cui Yang was responsible for document preparation, biological experimental set-up, and was a major contributor in writing the manuscript; Xiao-yu Jia contributed to analysis, experimental studies and literature research; Li Zhang and Ya-li Li were responsible for substantial contribution on experimental design, results analysis and technical support during experiment; Lan Zhang, Lin Li and Zhan-jun Zhang provided results analysis, data interpretation, critical review and edition. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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